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Fluoroquinolone-resistant *Campylobacter* in backyard and commercial broiler production systems in the United States

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Objectives: *Campylobacter* spp. are one of the leading foodborne pathogens in the world, and chickens are a known reservoir. This is significant considering broiler chicken is the top consumed meat worldwide. In the USA, backyard poultry production is increasing, but little research has been done to investigate prevalence and antimicrobial resistance associated with *Campylobacter* in these environments.

Methods: Our study encompasses a farm-to-genome approach to identify *Campylobacter* and investigate its antimicrobial resistance phenotypically and genotypically. We travelled to 10 backyard and 10 integrated commercial broiler farms to follow a flock throughout production. We sampled at days 10, 31 and 52 for backyard and 10, 24 and 38 for commercial farms. Bird faecal ($n=10$) and various environmental samples (soil $n=5$, litter/compost $n=5$, and feeder and waterer swabs $n=6$) were collected at each visit and processed for *Campylobacter*.

Results: Our results show a higher prevalence of *Campylobacter* in samples from backyard farms (21.9%) compared to commercial (12.2%). Most of our isolates were identified as *C. jejuni* (70.8%) and the remainder as *C. coli* (29.2%). Antimicrobial susceptibility testing reveals phenotypic resistance to ciprofloxacin (40.2%), an important treatment drug for *Campylobacter* infection, and tetracycline (46.6%). A higher proportion of resistance was found in *C. jejuni* isolates and commercial farms. Whole-genome sequencing revealed resistance genes, such as *tet(O)* and *gyrA_T86I* point mutation, that may confer resistance.

Conclusion: Overall, our research emphasizes the need for interventions to curb prevalence of resistant *Campylobacter* spp. on broiler production systems.

Introduction

Species of the genus *Campylobacter* collectively represent one of the most common foodborne pathogens worldwide.[1] In the USA, *Campylobacter* is the number one cause of bacterial diarrheal related illness, and infections occur so frequently that outbreaks are often not announced by the CDC.[2] Campylobacteriosis was first documented in 1886 and has continued to cause a burden on human health since,[3,4] causing an estimated 1.5 million human illnesses yearly in the USA, according to the CDC.[2] There are two main species responsible for *Campylobacter* infection in humans, *C. jejuni* and *C. coli*; of these, *C. jejuni* is the more frequent culprit.[1,5] These species are known for extensive interspecies recombination, accounting for the majority of their differences evolutionarily, such that a complete picture of the pathogen requires studying both species.[6]

Chickens are a known reservoir for *Campylobacter* and numerous species of this genus naturally colonize the avian (wild and domestic) gastrointestinal tract.[7] For humans, one of the most common sources of *Campylobacter* infections is consuming raw or undercooked poultry.[8] Chicken meat (from broiler-type chickens) is the most consumed meat in the USA (roughly 98.8 pounds per person is consumed yearly).[9,10] The National Antimicrobial Resistance Monitoring System (NARMS) reported that 59% of chicken caecal samples tested positive for *Campylobacter* in

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2021.[11] Backyard owners may be more frequently exposed to the birds and their environment, given their close proximity to the home. However, *Campylobacter* prevalence in backyard broiler flocks has not been well investigated.

The prevalence and identity of *Campylobacter* in US backyard chickens is unknown, which is concerning considering the rise of backyard poultry ownership in the USA.[12] Most of our knowledge of *Campylobacter* in chickens, comes from studies encompassing large-scale commercial flocks or retail meat samples.[7,11,13,14] The microbial ecology of backyard chickens is probably very different from those of commercial birds.[15] For example, backyard chickens are, by definition, maintained in flocks of 1000 birds or fewer, therefore reducing opportunities for infection, given *Campylobacter* can spread very quickly throughout an entire flock after a single bird is infected.[7,14,16,17] However, backyard birds may also have opportunities for infection from wild birds as well as other animal species living on the farm.[2,18] Overall, rates of *Campylobacter* isolation from chicken sources and broiler flocks varies worldwide indicating need for research in small-scale farm environments.[14,19–22]

While the risk of infection itself is concerning, antimicrobial resistance heightens this concern. Antimicrobial resistance is a serious growing public health threat. It has been estimated 1.2 million people died directly because of antimicrobial resistance in 2019.[23] Several studies have already demonstrated the frequent resistance of *Campylobacter* to fluoroquinolones, such as ciprofloxacin.[24–28] This is concerning, given that ciprofloxacin is one of the preferred drugs for clinicians when treating *Campylobacter* infections in humans.[25,29] While most of the time *Campylobacter* infections resolve on their own, serious infections may require drug use.[30,31] A better understanding of the antimicrobial resistance carried by *Campylobacter* in backyard farms is crucial. Backyard production potentially brings risk of infection closer to at-risk individuals who visit with the birds (such as children under the age of five and immunocompromised individuals), as they may need antimicrobial therapy in case of infection.[30,32]

Our study aims to characterize *Campylobacter* species in backyard flocks and compare them to integrated commercial farms. We assess antimicrobial resistance (AMR) profiles phenotypically, through antimicrobial susceptibility testing, and genotypically, through whole-genome sequencing. Our study will help us better understand this pathogen that affects humans and poultry worldwide in an effort to improve production safety.

Methods

Ten backyard and 10 commercial broiler farms were visited at three timepoints throughout flock production. The location of the backyard farms spanned six counties and commercial farms spanned over five counties. For this study, backyard farms were considered to be farms with 22–1000 birds in a flock raised in a residential location.[17] The mean flock size for our backyard flocks sampled was 280 birds (median: 99.5). All backyard birds were Cornish Cross and were raised for meat consumption (either by the family or sold to the public). All owners reported not using antibiotics on their birds. Two pairs of flocks were sampled from the same farm location (1 and 7 as well as 5 and 9). These flocks were sampled in a different season than when the first sampling occurred (autumn/fall versus spring). Commercial flocks for our study are considered birds reared in indoor production facilities as part of a large company. Mean flock size for commercial farms was 20 630 (median 17 900, range 13 500–30 900) birds. Of our commercial farms, two reported using antibiotics in case of illness and eight reported never using antibiotics. All commercial farms reported Ross 708 birds. Commercial farms were sampled by sampling within one house to sample flocks at a farm location. We only sampled one flock on a property. Rarely was there more than one broiler flock simultaneously on a backyard property.

We aimed to sample each flock at 10 days of age, mid-production and processing to account for all stages of production. Backyard farms were visited at 10, 31 and 52 days of production and commercial farms were visited at 10, 24 and 38 days of production. The discrepancy between days visited is due to commercial farm birds for this study being processed at an earlier age than the backyard birds sampled. At each visit, faecal ($n=10$), soil ($n=5$), litter/compost ($n=5$) and swabs of feeders and waterers ($n=6$, three per type) were collected at each farm visit. Faecal samples were collected by scooping faeces from the ground. For commercial farms, soil samples were taken directly outside the poultry house. For backyard farms, litter and compost samples were taken and split among the five samples collected for this category, given the uncertainty of what is present at each visit. Litter was typically available on the first visit, but birds typically moved to pastures for the second and third visits, in which compost samples would be taken instead. Samples were collected in sterile closure bags and were directly transported back to the laboratory, where the samples were immediately processed.

Processing

Processing samples began the same day as sample collection on the farm. Processing protocols were adopted from NARMS Retail Meat Surveillance Laboratory Protocol.[33] After collection, samples were transported to the laboratory. Next, 90 mL of buffered peptone water was added to each sample. Bags were then placed on an automatic shaker at 200 rpm for 15 minutes. After shaking, 20 mL of the buffered peptone water and sample mixture was added to 20 mL of double strength Bolton broth in a 50 mL Falcon tube. The tubes were lightly closed and placed in a microaerophilic (85% nitrogen, 10% carbon dioxide and 5% oxygen) incubator at 42°C for 24 hours.

After incubation, the Bolton broth and sample mixture was gently inverted a few times. A 10 μL inoculation loop was then used to t-streak the mixture onto Campy cefex agar (BD 292487) to get a single isolated colony. The plates were then placed under microaerophilic conditions at 42°C for 48 hours. Plates were assessed the next day for *Campylobacter* characteristic colonies. Any suspected colonies were streaked onto blood agar and allowed to incubate for 24–48 hours. A multiplex PCR was then used to confirm *Campylobacter* presence as well as characterization of species (*coli* or *jejuni*).

The multiplex PCR protocol was taken from Cloak and Fratamico, 2002.[34] Briefly, a colony PCR was conducted using the following genes *cad* (*C. coli* and *C. jejuni*), *ceu* (*C. coli*) and an undefined gene (*C. jejuni*). Mastermix was created in the following proportions per one reaction: 28 μL of water, 5 μL of 10× ammonium buffer (without $MgCl_2$), 5.5 μL of 50 mM $MgCl_2$, 1 μL of dNTPs (2.5 mM), 0.5 μL of BSA, 0.5 μL of 1 U/μL Taq DNA Polymerase, 1.25 μL of each forward primer and 1.25 μL of each reverse primer. Samples were run in a thermocycler with denaturation at 94°C for 4 minutes, then 40 cycles of 94°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute. Then a final step of 72°C for 5 minutes before then being held at 12°C until removed from the thermocycler. Samples were placed in a 1.5% agarose

ethidium bromide gel and run for 40 minutes via gel electrophoresis. Species confirmation was conducted with Bacterial and Viral Bioinformatics Resource Center for the sequenced isolates using the Taxonomic Classification tool.[35]

Antimicrobial susceptibility testing

The protocol for broth microdilution antimicrobial susceptibility testing was taken from the 2020 National Antimicrobial Resistance Monitoring System Manual of Laboratory Methods.[36] Briefly, a McFarland suspension was created with the *Campylobacter* isolate and 5 mL of Mueller–Hinton broth. Then, 100 μL of the suspension was then placed in 11 mL of Mueller–Hinton broth with lysed horse blood and vortexed well. Next, 100 μL of the mixed solution was dispensed using a ThermoFisher Sensititre AIM™ into each well of a *Campylobacter* sensititre plate (CMVCAMPY). The antimicrobials tested were: azithromycin (0.015–64 μg/mL), ciprofloxacin (0.015–64 μg/mL), clindamycin (0.03–16 μg/mL), gentamicin (0.12–32 μg/mL), erythromycin (0.03–64 μg/mL), meropenem (0.004–16 μg/mL), tetracycline (0.12–64 μg/mL), florfenicol (0.12–64 μg/mL) and nalidixic acid (4–64 μg/mL). Plates were then sealed with a perforated plastic sticker and placed in a microaerophilic incubator at 42°C for 48 hours. Plates were read using a VisionBox. NARMS breakpoints were used for all antimicrobials, except meropenem that came from CLSI breakpoints.[37,38] These breakpoints were used to determine susceptible, resistant and intermediate isolates.

DNA isolation and whole-genome sequencing

Bacterial genomic DNA was extracted using the Qiagen DNeasy PowerLyzer Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. The quality and concentration of the extracted DNA were determined using a NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit Flex Fluorometer (Thermo Fisher Scientific). DNA libraries were prepared using the Illumina DNA Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The resulting DNA libraries were re-quantified using the Qubit Flex Fluorometer. Sequencing was performed on the Illumina MiSeq System using MiSeq Reagent Kit v.3 600 cycles (Illumina).

Bioinformatic pipeline and plasmid detection

The bioinformatic pipeline used is described in Hull *et al.*[39] Briefly, whole-genome sequence forward and reverse reads (fastq) were assembled (fasta) *de novo* with Shovill v.1.1.0 using SPAdes v.3.15.1.[40] *De novo* assembly of forward and reverse reads was repeated for plasmid detection using plasmidSPAdes genome assembler v.3.15.1. Plasmid contigs (in fasta file format) were further analysed for antimicrobial resistance genes, virulence factors and plasmid replicons using ABRicate v.1.0.1.[41] Abricate databases include: National Center for Biotechnology Information (NCBI),[42] AMRFinderPlus,[43] Comprehensive Antibiotic Resistance Database (CARD),[44] Resfinder,[45] Virulence Factor Database (VFDB),[46] PlasmidFinder[47] and MEGARES v.2.00.[48] Python code was developed separately to run our isolates through PLSDB using the mash_screen option in Mash v.2.3.[49,50]

Table 1. Individual farm positive sample distribution

Farm	Positive *Campylobacter* samples (*n*/78)	Visit 1	Visit 2	Visit 3
PF1	19	4	3	12
PF2	33	13	10	10
PF3	18	14	1	3
PF4	6	0	3	3
PF5	34	7	15	12
PF6	9	0	2	7
PF7	9	0	0	9
PF8	11	1	1	9
PF9	16	0	1	15
PF10	16	0	7	9
CF1[a]	0	0	0	0
CF2[a]	0	0	0	0
CF3	0	0	0	0
CF4	7	0	7	0
CF5	19	0	5	14
CF6	10	0	0	10
CF7	7	0	0	7
CF8	20	0	6	14
CF9	13	0	0	13
CF10	19	0	10	9

Displays the number of positive samples collected from each farm across the three visits.
There were 78 samples collected total from each farm. PF# stands for each backyard flock and CF# stand for commercial flock.
[a]Antibiotics used on broilers in case of illness.

Phylogenetic tree

REALPHY v.1.13 was used to assemble the phylogenetic tree.[51] REALPHY uses SNPs to determine phylogenetic relatedness.[51] The output from REALPHY was put into Interactive Tree of Life (iTOL) for visualization.[52]

Statistics

Fisher's Exact two-tailed *t*-test was conducted by using CDC EpiCalc Info StatCalc v.5.5.11 software and R Studio.[53,54]

Results

Overall, *Campylobacter* was twice as common in backyard farms as in commercial farms. We detected *Campylobacter* in 21.9% ($n = 171/780$) of backyard samples and 12.1% ($n = 95/780$) of commercial farm samples (Fisher's Exact test; *P* value: 2.785e-07) (Table 1). Most positive samples were found on visit 3 for both backyard (52.1%) and commercial farms (70.5%). The most common *Campylobacter* species in both backyard and commercial farms was *C. jejuni* (70.8%, $n = 186/266$). All other *Campylobacter* were of the species *C. coli* (30%, $n = 80/266$).

Our positive samples came from both bird faecal and environmental samples, although most were faecal samples. The breakdown of positive sample locations in backyard farms was 70.2% ($n = 120/171$) from faecal samples, 6.4% ($n = 11/171$) from soil samples, 3.5% ($n = 6/171$) from litter/compost samples and

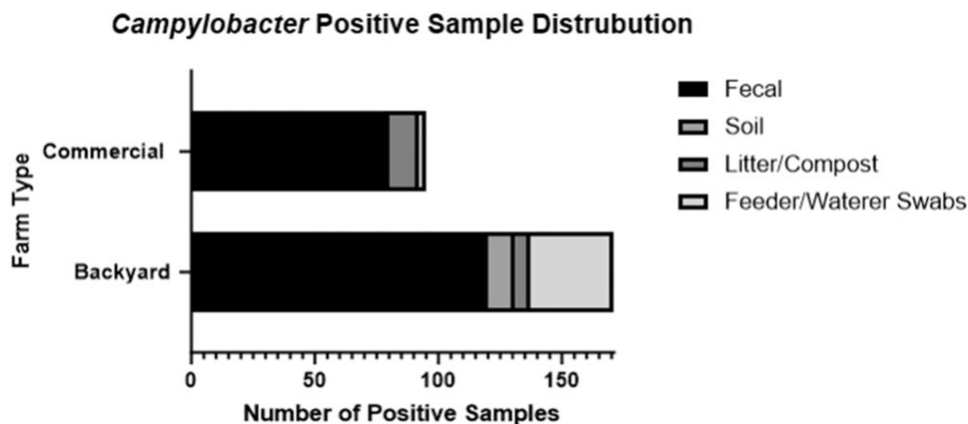

Figure 1. Positive sample distribution, which displays the number of positive *Campylobacter* samples and the sample type they came from in commercial and backyard farms. A total of 10 backyard and 10 integrated commercial farms were sampled three times across the production of a flock (brooding, grow-out and finishing). At each farm visit, 26 samples were collected (faecal, $n=10$; litter/compost, $n=5$; soil, $n=5$; feeder and waterer swabs, $n=6$).

19.9% ($n=34/171$) from swabs of feeders and waterers. For commercial farms, of the positive samples 84.2% ($n=80/95$) came from faecal samples, 12.6% ($n=12/95$) came from litter samples, 3.2% ($n=3/95$) came from swabs of feeders and waterers and no soil samples tested positive. The results are displayed in Figure 1.

Among the *Campylobacter* isolates, we found resistance to mainly three antimicrobials (ciprofloxacin, nalidixic acid, tetracycline); the frequency of resistant isolates was higher among *C. jejuni* than *C. coli*. The number of *Campylobacter* isolates resistant to ciprofloxacin, nalidixic acid and tetracycline was significantly different between farm types (Fisher's exact; *P* value=ciprofloxacin and nalidixic acid, 1.433e-08; tetracycline, <2.2e-16). Among the *Campylobacter* isolates from the backyard samples, 27.3% ($n=47/171$) were resistant to ciprofloxacin and nalidixic acid and 22.7% ($n=39/171$) were resistant to tetracycline. Among isolates from commercial farms, 63.2% ($n=60/95$) were resistant to ciprofloxacin and nalidixic acid and 89.5% ($n=85/95$) of isolates were resistant to tetracycline. One isolate from a backyard farm was resistant to clindamycin. Results for this broken up by species can be seen in Tables 2 and 3. We found a significantly higher frequency of resistant isolates in *C. jejuni* isolates than *C. coli* (Fisher's Exact; *P* value=1.189e-06). All isolates, regardless of production system were susceptible to florfenicol, erythromycin, gentamicin, azithromycin and meropenem.

We sequenced the genomes of 200 isolates (124 backyard isolates, 76 commercial isolates; 73 *C. coli*, 127 *C. jejuni*). We picked isolates to be sequenced by choosing representative samples across the categories of: phenotypic resistance, farms and sample type. All 200 sequenced isolates contained AMR genes. All AMR genes, plasmids and phenotypic resistance results for *C. jejuni* and *C. coli* can be found in Figures 2 and 3. Aminoglycoside resistance genes were detected: *aph(3')-IIIa* ($n=14/200$) and *aph(3')-VIIa* ($n=14/200$). The aminoglycoside resistance genes were associated with specific *Campylobacter* species, as *aph(3')-IIIa* was only detected in *C. jejuni* and *aph(3')-VIIa* was only detected in *C. coli*. We detected tetracycline resistance gene *tetO* ($n=85/200$) in both *C. jejuni* ($n=63/85$) and *C. coli* ($n=22/85$). Of the 85 samples in which we detected *tetO*, 83 were also phenotypically resistant to tetracycline. We also detected genes responsible for beta-lactamase enzymes: *OXA-184* ($n=14/200$), *OXA-450* ($n=110/200$) and *OXA-61* ($n=7/200$). We detected these beta-lactamase genes in 65.6% ($n=131/200$) of isolates.

We detected genes responsible for multidrug efflux pump *cmeABC* (*cmeA*, $n=180/200$; *cmeB*, $n=200/200$; *cmeC*, $n=199/200$), which confers resistance to cephalosporins, fluoroquinolones and macrolides were found in a high proportion of both *C. jejuni* and *C. coli*. We also detected the *cmeR* regulator of the cmeABC efflux pump ($n=129/200$), mainly in *C. jejuni* ($n=127/130$).

Through AMRFinder Plus, we detected *gyrA* point mutations in 98.5% ($n=197/200$) of isolates. Of interest is the non-synonomous point mutation, *gyrA_T86I* ($n=68/200$). Almost all isolates containing *gyrA_T86I* displayed phenotypic resistance ($n=67/68$). Of these isolates containing *gyrA_T86I,* 85.3% ($n=58/68$) were *C. jejuni*.

Using Plasmidfinder, we were only able to detect three plasmids among four isolates: Col(MG828)_1 ($n=2/200$), Col440II_1 ($n=1/200$) and ColRNAI_1 ($n=1/200$). We also compared our isolates to the PLSDB database and plasmids were detected in 58% ($n=116/200$) *Campylobacter* isolates, with most being *C. jejuni* ($n=93/118$). We detected plasmids: pCJ3.6K ($n=52/116$), pRM1477 ($n=14/116$), pCOS502 ($n=12/116$), pCCDM18S1 ($n=8/116$), pCC2228-1 ($n=7/116$), pOR12TET ($n=3/116$), pCJ3K-03 ($n=4/116$), pCJDM218 ($n=4/116$), pMTVDSCj13-2 ($n=2/116$), pFORC_083_3 ($n=1/116$), pD6759-1 ($n=1/116$) and eight of the detected plasmids were unnamed. Of these, pCJDM218 and pCOS502 are considered megaplasmids (>100 kb). Through our pipeline, we were able to assemble plasmid data for 114 of the 200 total isolates. Of those plasmid assemblies, 6.14% ($n=6/114$) had AMR genes detected including: *aph(3')-IIIa* ($n=1/7$) and *tetO* ($n=6/7$).

Discussion

Campylobacter spp. are leading foodborne pathogens often associated with consuming raw or undercooked poultry.[8] While poultry are a known reservoir of *Campylobacter*, we still have a poor

Table 2. Minimum inhibitory concentration (MIC) for *C. jejuni* isolates

			C. jejuni distribution of MIC (μg/mL) (no. of isolates)																
Antimicrobial	Source	Percentage resistant (%)	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
AZI	Commercial	0			8	44	17	3											
	Backyard	0			28	31	48	6	1										
ERY	Commercial	0						1	28	34	9								
	Backyard	0						12	28	44	28	2							
GEN	Commercial	0						5	49	18									
	Backyard	0						7	78	26	2	1							
CLI	Commercial	0				4	25	30	10	3									
	Backyard	0.9				9	24	44	33	3	**1**								
TET	Commercial	90.3						1	6				**1**		**2**	**15**	**14**	***33***	
	Backyard	33.3						32	42	2			**2**		**8**	**14**	**14**		
FFN	Commercial	0							1	12	55	4							
	Backyard	0							7	42	63	2							
NAL	Commercial	81.9											13			**15**	**41**	***3***	
	Backyard	33.3											62	14		**20**	**16**	***2***	
CIP	Commercial	81.9					2	9	2				**2**	**21**	**32**	**4**			
	Backyard	33.3				2	27	43	4				**5**	**30**	**3**				
MERO	Commercial	0		1	19	35	17												
	Backyard	0		2	63	22	7	18			1	1							

This table displays the number of *C. jejuni* isolates falling into each MIC for all the antimicrobials that isolates were tested against. Numbers in bold indicate resistant isolates as determined by a combination of NARMS and CLSI breakpoints. The resistance breakpoint is represented with a vertical line. All breakpoints came from NARMS except for meropenem, which came from CLSI breakpoints. Italicized numbers mean the true MIC is beyond the measurable range of the Sensititre^{TM} plates.

AZI, azithromycin; ERY, erythromycin; GEN, gentamicin; CLI, clindamycin; TET, tetracycline; FFN, florfenicol; NAL, nalidixic acid; CIP, ciprofloxacin; MERO, meropenem.

understanding of the on-farm prevalence of *Campylobacter* and how this can vary by production system. Filling this knowledge gap is essential to our ability to reduce down-stream retail meat contamination as well as reduce the potential of contracting infection from live birds.[55] The majority of positive samples were found in the final visit, which is not surprising considering previous studies note the highest load of *Campylobacter* is close to slaughter age.[7] The prevalence of *Campylobacter* that we observed (12.18%) among commercial samples is in-line with previous investigations in the USA (Thakur *et al.*, 13.6%; Poudel *et al.*, 18.5%).[56,57] To date, data investigating *Campylobacter* in US backyard broiler systems are limited; however, Pires and colleagues conducted a study investigating small-scale farms in California and found broiler faecal and environmental swabs to be positive for *Campylobacter* at a 9.8% prevalence rate.[58] Our study found a much higher rate in backyard farms (22.1%). These differences emphasize the need for further research investigating other areas of the USA as well as factors leading to a higher rate in backyard broilers. Faecal samples represent the greatest environmental contamination risk; however, they may not be the best indicator for bird colonization compared to caecal samples.[59–62] It is known that the rate of *Campylobacter* shedding can vary, therefore, further research including faecal cfu could provide interesting insight as to the differences in faecal and environmental prevalence not only between farm types (backyard and commercial) but within farm types.[7,63–65] We hypothesize these differences are mainly related to factors beyond bird genetics, given Ross 708 (used in our commercial farms) are a strain of Cornish Cross (used in our backyard farms).

We found phenotypic resistance to ciprofloxacin, one of the main antimicrobials used to treat *Campylobacter* infection in humans.[31] Phenotypic resistance to ciprofloxacin was noted in most isolates carrying *gyrA_T86I* mutation. Previous studies show this point mutation is a reliable indicator of fluoroquinolone resistance.[66–68] We also detected CmeABC multidrug efflux pump genes, also attributed to fluoroquinolone resistance.[44,69] Fluoroquinolone resistance in *Campylobacter* is well-known. The 2019 Integrated NARMS Report described ciprofloxacin-resistant *Campylobacter* (*jejuni* and *coli*) from chicken retail samples and humans has been increasing relative to recent years.[70] The exact reason for why ciprofloxacin resistance continues to rise in *Campylobacter* is unknown, especially given the fact that fluoroquinolones were banned from US poultry production in 2005.[11,70–73] It has been shown previously that even without antimicrobial selective pressure, *Campylobacter* containing the *gyrA* point mutation outcompete those that do not, suggesting that this mutation confers additional benefits beyond those associated with resistance.[74] An observation further supported by our data, especially considering the lack of antimicrobial use on our flocks. Fluoroquinolone resistance has also recently been noted to be associated with increased

Table 3. Minimum inhibitory concentrations for *C. coli* isolates

			C. coli distribution of MIC (μg/mL) (no. of isolates)																
Antimicrobial	Source	Percentage resistant (%)	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
AZI	Commercial	0				1	5	17											
	Backyard	0				10	28	19											
ERY	Commercial	0								3	16	4							
	Backyard	0							12	5	40								
GEN	Commercial	0							5	17	1								
	Backyard	0							2	25	30								
CLI	Commercial	0						9	14										
	Backyard	0					2	9	27	18	1								
TET	Commercial	87							1	2							**1**	***19***	
	Backyard	1.8						14	37	5							**1**		
FFN	Commercial	0									20	3							
	Backyard	0								3	52	2							
NAL	Commercial	4.4											7		15			***1***	
	Backyard	15.8											34	14		**4**	**5**		
CIP	Commercial	4.4						4	10	8				**1**					
	Backyard	15.8					3	16	29					**3**		**6**			
MERO	Commercial	0			1		5		4	13									
	Backyard	0			1		34	22											

This table displays the number of *C. coli* isolates falling into each MIC for all the antimicrobials that isolates were tested against. Numbers in bold indicate resistant isolates as determined by a combination of NARMS and CLSI breakpoints. The resistance breakpoint is represented with a vertical line. All breakpoints came from NARMS except for meropenem, which came from CLSI breakpoints. Italicized numbers mean the true MIC is beyond the measurable range of the Sensititre^{TM} plates.

AZI, azithromycin; ERY, erythromycin; GEN, gentamicin; CLI, clindamycin; TET, tetracycline; FFN, florfenicol; NAL, nalidixic acid; CIP, ciprofloxacin; MERO, meropenem.

virulence and biofilm formation of *C. jejuni*, potentially contributing to its selective advantage.[75] Given ciprofloxacin resistance markers seem to be beneficial to *Campylobacter* fitness, more research needs to be dedicated to finding ways to curb the selection of these resistant populations in antimicrobial-free broilers.

In our study, *C. jejuni* carried a significantly higher proportion of phenotypic resistant isolates to ciprofloxacin, nalidixic acid and tetracycline than did *C. coli*. Historically, *C. coli* has been noted to have a higher proportion of resistance to antimicrobials from poultry related samples than *C. jejuni*. This is particularly the case for ciprofloxacin and nalidixic acid.[11,70,73,76] However, our findings of higher resistance displayed by *C. jejuni* could indicate ramifying effects for public health, given that *C. jejuni* is more commonly associated with human illness.[1,77] Interspecies genetic transfer is a known concern with *Campylobacter* species.[78,79] Previous research in our laboratory found evidence of interspecies gene transfer in *Campylobacter* from poultry samples, raising concern that sharing resistance could increase overall AMR in *Campylobacter* species.[78] Our findings may portent that increased resistance in *C. jejuni* are becoming a reality. NARMS also specifically noted a jump in ciprofloxacin-resistant *C. jejuni* in chicken retail and caecal samples.[70] NARMS Now data report an increase in resistant *C. jejuni* from chicken caecal samples from 2018 to 2019 (20.5% to 25.6%) as well as 2021 to 2022 (18.3% to 25.8%), whereas these increases were not noted for *C. coli*, or not noted to the same magnitude (2018–2019, 19% to 16.7%; 2021–2022 and 16.2%–17.0%).[11] This could indicate that at least with chicken related samples, we are seeing high populations of resistant *C. jejuni* even in antimicrobial restricted production systems.

A known platform for *Campylobacter* to share genetic information between different species is through horizontal gene transfer by plasmids.[80,81] Of all the isolates containing plasmids that were detected on PLSDB, 13.6% ($n=16/116$) were deemed to contain megaplasmids (pCJDM218, $n=4$ and pCOS502, $n=12$). However, we did not see extensive AMR gene carriage associated with our assembled plasmid sequences. Other studies note *Campylobacter* plasmids to play a role in AMR gene prevalence in samples taken from chicken sources.[80,82] Although our data do not reflect high AMR carriage on our plasmids in general, it is consistent with previous findings that megaplasmids may not be associated with AMR carriage.[80] Hull and colleagues noted many megaplasmids may play a more prominent role in interspecies recombination and virulence factors rather than AMR gene carriage.[80] Overall, *Campylobacter* plasmid research is not well established at this point and further investigation of their role in AMR in broiler production environments is needed.

Conclusions

Our research shows *Campylobacter* spp. are prevalent in mostly bird faecal samples, but also various environmental samples of both backyard and commercial broiler production systems.

Figure 2. *C. jejuni* AMR dendrogram, which displays the phenotypic and genotypic AMR profiles for the sequenced *C. jejuni*. A circle indicates phenotypic resistance to the corresponding antimicrobial and isolate, a square indicates containing the corresponding AMR gene and a star indicates carriage of the corresponding plasmid. Farm type and number are indicated on the phylogenetic tree with PF# indicating a backyard farm and CF# being commercial.

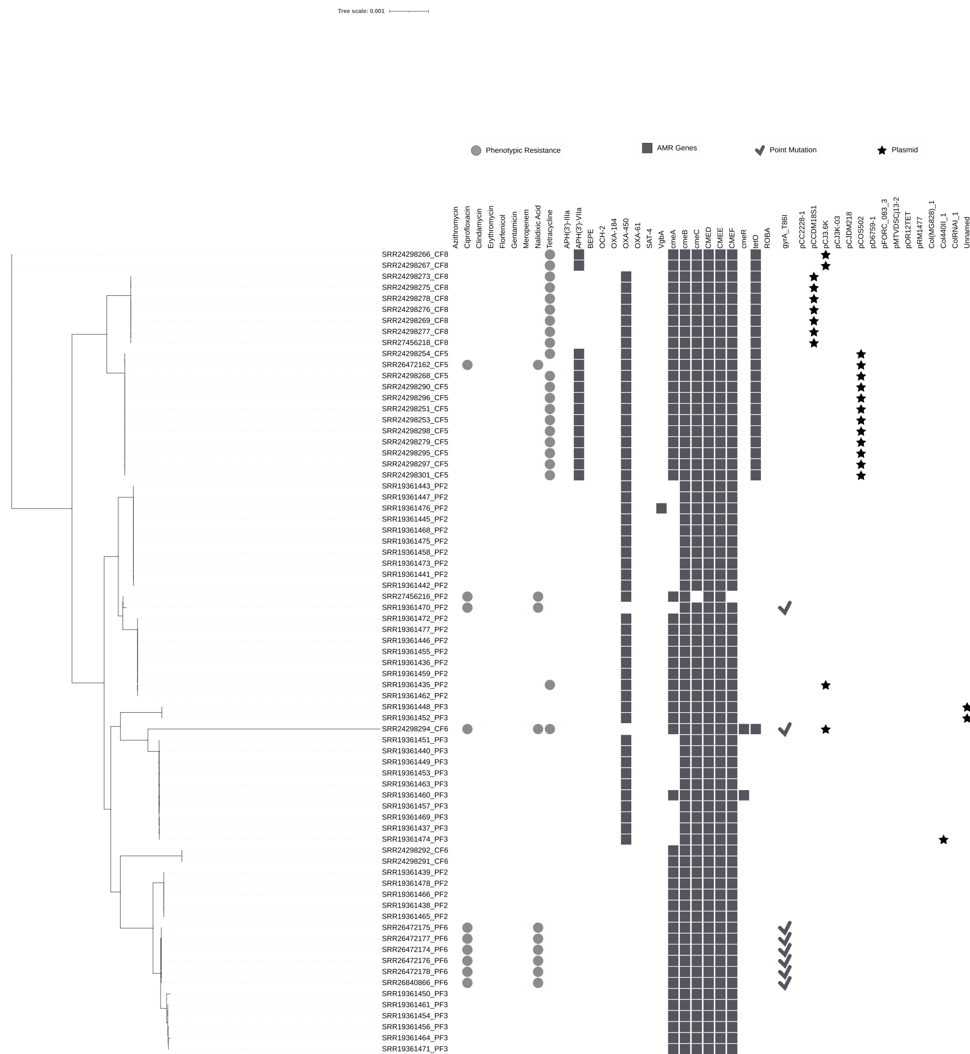

Figure 3. *C. coli* AMR dendrogram, which displays the phenotypic and genotypic AMR profiles for the sequenced *C. coli*. A circle indicates phenotypic resistance to the corresponding antimicrobial and isolate, a square indicates containing the corresponding AMR gene and a star indicates carriage of the corresponding plasmid. Farm type and number are indicated on the phylogenetic tree, with PF# indicating a backyard farm and CF# being commercial.

We detected a higher prevalence of *Campylobacter* in backyard farms, and future studies need to identify the sources of contamination in these birds to understand the discrepancy. Despite higher prevalence in backyard farms, we found a higher proportion of resistant isolates in commercial farms, with unprecedented higher levels in *C. jejuni* versus *C. coli*. Fluoroquinolone resistance is rising in *Campylobacter* despite the lack of antimicrobial use, and our research emphasizes that further research needs to be done to understand the factors involved in creating this selective pressure. We hypothesize various factors could influence these differences, such as exposure before farm arrival, exposure to other reservoir species and overall farm management practices. Overall, our research highlights a need for future studies on broiler supply chains to identify sources of contamination and interventions to lower AMR *Campylobacter*.

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Transparency declarations

The authors do not have competing interests to declare.

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