FOODBORNE PATHOGENS AND DISEASE
Volume 11, Number 11, 2014

DOI: 10.1089/fpd.2014.1794

Phenotypic and Genotypic Diversity of Thermophilic *Campylobacter* spp. in Commercial Turkey Flocks: A Longitudinal Study

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Abstract

Poultry are recognized as a main reservoir of *Campylobacter* spp. However, longitudinal studies investigating the persistence of *Campylobacter* on commercial meat turkeys are rare. The objectives of this study were to determine the prevalence, antimicrobial susceptibility, and persistence of genotypically related strains of *Campylobacter* spp. recovered from three commercial turkey farms in Ohio belonging to a single producer. Eight hundred ten samples were collected from birds aged 1 week to slaughter, consisting of 750 fecal droppings and 60 ceca at slaughter. Overall *Campylobacter* prevalence was 55.9%. Multiplex polymerase chain reaction (PCR) confirmed 72.3% of all isolates as *C. coli*, 5.3% as *C. jejuni*, 10.6% as both, and 11.9% as other *Campylobacter* spp. PCR restriction fragment length polymorphism of the *flaA* gene subtyping detected 70 types—62 for *C. coli* and 8 for *C. jejuni* isolates—with most (80%) of *flaA*-types constituting farm homogeneous groups. Multilocus sequence typing of 99 selected *Campylobacter* isolates resulted in 23 sequence types (STs), consisting of 8 STs for *C. jejuni* and 15 STs for *C. coli* isolates. Six novel STs—four for *C. jejuni* and two—for *C. coli*, were detected. In a subset of isolates ($n = 98$) tested for antimicrobial resistance, the most common resistance was to tetracycline (95%), followed by azithromycin (43%), while 42% and 18% of the isolates were resistant to ciprofloxacin and erythromycin, respectively. All isolates were susceptible to florfenicol. *C. coli* isolates displayed a higher proportion of resistance than *C. jejuni* to most antimicrobials. This study highlights the high prevalence, genotypic diversity, and antimicrobial resistance of *Campylobacter* spp. in commercial turkey from farm to slaughter.

Introduction

Campylobacter is a leading cause of bacterial foodborne diarrheal disease worldwide (Humphrey *et al*., 2007), with over 1 million cases of campylobacteriosis in the United States annually (Scallan *et al*., 2011), 48 cases per 100,000 population in European countries (Anonymous, 2012), and affects 5–20% of children in developing regions including Asia, Africa, and Latin America (Oberhelman and Taylor, 2000). *Campylobacter* spp. in rare cases (1 in 1000) are associated with severe autoimmune-mediated demyelinating neuropathies such as Guillain-Barré and Miller–Fisher syndromes (Skirrow and Blaser, 2000). Antimicrobial therapy is required for severe, prolonged, or systemic infections or to control infections in high-risk groups. Fluoroquinolones (ciprofloxacin), and macrolides (azithromycin and erythromycin) are the antibacterials of choice (Allos and Blaser, 2009). However, antimicrobial use correlates with the emergence of resistant strains (Zhu *et al*., 2006). Ninety percent of the human *Campylobacter* infections are attributed to *C. jejuni*, 6% to *C. coli*, and the rest are due to *C. upsaliensis, C. lari, C. fetus,* and *C. mucosalis* (Friedman *et al.*, 2004). Despite the important public health and socioeconomic impacts of this organism, limited progress has been made in defining routes of infection and reducing associated illness.

Handling and consumption of contaminated raw or undercooked poultry has long been recognized as a major source of human *Campylobacter* enteritis (Rivoal *et al*., 2005; Mazick *et al*., 2006; Lyhs *et al*., 2010). Though chicken

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serves as a major source (Horrocks *et al*., 2009) for human *Campylobacter* infections, turkey is also an important reservoir of *Campylobacter*. In recent years there has been a worldwide increase in consumption of turkey products (USDA, 2012). Studies have reported 65–95% prevalence in U.S. turkeys at production (Cox *et al*., 2000; Luangtongkum *et al*., 2006; Wesley *et al*., 2009), with 34.9% from turkey-processing plants (Logue *et al*., 2003) and 15% in retail turkey meat (Zhao *et al*., 2010). Considering the significance of these infections, in the United States, mandatory testing of turkey carcasses at slaughter has been instituted by the USDA Food Safety Inspection Services (FSIS, 2010). The current situation prompts the need for strong data on the occurrence and genetic diversity of *Campylobacter* in the turkey production chain in order to develop effective prevention and control strategies. The purpose of this study was to longitudinally (from farm until slaughter) define the prevalence, genotypes, and antimicrobial resistance of *Campylobacter* strains isolated from turkeys in order to obtain a better understanding of the epidemiology of the pathogen.

Materials and Methods

Turkey flocks

Between October 2011 and March 2012, three commercial meat turkey farms (A, B, and C) belonging to a single producer were monitored. All farms were located in Ohio, and were within the same climatic zone (within a 10-mile radius from one another). All farms followed similar biosecurity protocols. Farms used an ''all in, all out'' management system. Each farm had approximately 11,000 turkey males coming from the same hatchery that were reared in 2 barns, each with 5500 turkeys. At the completion of the production cycle (21 weeks), birds from each flock were processed separately in the same processing plant.

Sample collection

Six samplings were made starting with 1-week-old poults to 21-week-old toms. The sample size was calculated according to Fosgate (2009) for a large population size allowing the detection of *Campylobacter* spp. at a 95% confidence level and considering a within-flock prevalence of 5% or higher. At 1 week of age, 50 fresh fecal droppings from each farm (25/barn) (total of 150 samples/sampling time) were randomly collected from brooder barns. Subsequently, 50 fecal samples were randomly collected in each farm at monthly intervals starting from 4- to 19-week-old birds. At 21 weeks of age, for logistic reasons, 20 viscera from each farm (total 60 samples) were aseptically collected at slaughter from randomly chosen carcasses immediately following evisceration. Samples were placed in sterile polypropylene tubes (fecal droppings) or sterile plastic bags (slaughter materials) and transported to the laboratory on ice and processed within 12 h.

Isolation of thermophilic Campylobacter *spp.*

Approximately 2 g of feces were suspended with 9 mL of maximum recovery diluent (MRD) (Neogen, USA). One-mL suspension was added to 9 mL of Preston broth containing *Campylobacter* growth supplements (CM067, SR048, SR117, and SR232; Oxoid, England) and incubated at 42°C for 48 h in a Tri-Gas microaerobic workstation (Microbiology International, USA) (Krause *et al*., 2006). After incubation, 100 μL of culture was spread onto a modified charcoal cefoperazone deoxycholate agar (mCCDA) plate (CM 0739, Oxoid) containing the selective supplement (SR155E, Oxoid) and incubated for 48 h at 42°C microaerobically (Engberg *et al*., 2000). Where available, three presumptive *Campylobacter* colonies from each mCCDA plate were then subcultured onto Muller-Hinton (MH; Difco, MD) agar containing Selective Supplement (SR117, Oxoid) and incubated microaerobically at 42°C for 48 h (Sanad *et al*., 2011). Pure cultures were stored at − 80°C in MH broth supplemented with 30% glycerol (vol/vol) until further identification and characterization. From the slaughter materials, approximately 2 g of cecal homogenates were suspended with 9 mL of MRD and processed for *Campylobacter* isolation as described above.

DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted using either a Bacterial DNA kit (Epicenter, USA) or by boil-prep method (Dingle *et al*., 2005). The genomic DNA was quantified using the NanoDrop 1000 (Fisher Scientific, USA) and stored at − 20°C until use.

Campylobacter isolates were confirmed by a multiplex PCR as described previously (Yamazaki-Matsune *et al*., 2007). Primers for simultaneous identification of *Campylobacter* genus (16S rRNA), *C. coli* (*ceuE*), and *C. jejuni* (*mapA*) have been previously described (Linton *et al*., 1997; Denis *et al*., 1999). *C. jejuni* 81–176 and *C. coli* (ATCC 33559) were used as positive controls.

flaA-*Restriction fragment length polymorphism (*flaA-*RFLP) analysis*

For *fla*A-RFLP typing, the PCR product (1.7-kb fragment of the *flaA* gene) was digested for 5 h at 37°C using *Dde*I (Promega, USA) (Nachamkin *et al*., 1996) and the digested products were separated using 4% agarose gel in TAE buffer at 50 V for 5 h at room temperature. The *flaA*-RFLP profiles were analyzed using BioNumerics V5 (Applied Maths, Belgium). Pairwise comparisons and cluster analysis were made using the Dice correlation coefficient and the unweighted-pair group mathematical average clustering algorithm. The optimization and position tolerance for band analysis were set at 1% and 1.5%, respectively, and a cut-off of 100% was used for the determination of the different *flaA*-RFLP patterns.

Antimicrobial susceptibility testing

A total of 98 isolates (23 *C. jejuni* and 75 *C. coli*) representing different ages, farms, and *fla*A clusters were tested for antimicrobial susceptibility using Sensititre Campy plates (TREK Diagnostic Systems Inc., USA) as previously described (Sanad *et al*., 2011, 2013). Minimum inhibitory concentrations (MICs) were determined based on Clinical and Laboratory Standards Institute (CLSI, 2012) breakpoint interpretative criteria for Campylobacteraceae.

Multilocus sequence typing (MLST) analysis

A subset of 99 isolates (19 *C. jejuni* and 80 *C. coli*) representing different *flaA* subtypes, farm source, and ages of turkeys within the farms were analyzed by MLST as

described (Dingle *et al.*, 2001; Sanad *et al.*, 2011). PCR products were purified using ExoSAP (Affymetrix Inc., USA) prior to sequencing at GENEWIZ DNA sequencing facility (http://www.genowiz.com). Allele numbers and sequence types (STs) were then generated by comparing the sequences to the *Campylobacter* MLST database (http://pubmlst.org/*Campylobacter*).

Statistical analysis

The chi-square test was used to analyze the significance of the difference in prevalence of *C. jejuni* compared to *C. coli* between farms, between age groups within the farms, and in antimicrobial resistance between farms. $P < 0.05$ was considered statistically significant for univariate comparisons. In cases of multiple comparisons, the conservative Bonferroni corrected *p*-value was used as a cut-off for significance.

Simpson's index of diversity was calculated to compare the discriminatory power of *flaA* and MLST genotyping (Hunter and Gaston, 1988).

The evolutionary relationships of the *C. jejuni* and *C. coli* STs were elaborated using minimum spanning tree (Bionumerics V5). Correlations among *flaA*, MLST, and antimicrobial resistance patterns (R-types) as well as relationships of these profiles to age and farm were examined for each *Campylobacter* species using cross-tabulation, Fisher's exact test, uncertainty coefficients, and chi-square tests in IBM SPSS statistical software v9 (SPSS Inc., 2010).

Results

Occurrence and distribution of Campylobacter *spp. in turkey farms*

The overall *Campylobacter* prevalence was 55.9% (453/810), with 56% (420/750) in turkey feces and 55% (33/60) from cecal contents (Table 1). PCR revealed that the vast majority was *C. coli* (72.1%; 327/453), whereas 5.3% (24/453) were *C. jejuni*. In addition, we also identified 10.6% (48/453) isolates that were positive for both *ceuE* and *mapA* PCR, and 11.9% (54/453) of isolates were *Campylobacter* spp., other than *C. jejuni* or *C. coli*. The frequency of *Campylobacter* isolation varied by farm and by age within the farm (Table 1). There was no significant difference in *Campylobacter* prevalence between farm B and C ($p > 0.05$), but farm A had a significantly higher overall *Campylobacter* prevalence ($p < 0.05$) than farms B and C. Differences in *Campylobacter* spp. prevalence were significant between weeks 1 and 4 ($p = 0.00001$), and weeks 19 and 21 ($p = 0.002$) on farm A. Difference in *Campylbacter* spp. prevalence on farm C varied

Table 1. Prevalence of *Campylobacter* Species Isolated from Turkey Droppings and Viscera That Were Collected from Three Turkey Farms Located in Northwest Ohio, United States

Farm ID	*Age of turkeys (wks)*	*Total isolates no. (%)*	C. coli *no. (%)*	C. jejuni *no. (%)*	C. coli/C. jejuni *no. (%)*	*Other* Campy. *spp no. (%)*
A	1	15 (30)[a]	12 (24)	0	1 (2)	2 (4)
	4	44 (88)[b]	27 (54)	8 (16)	8 (16)	1 (2)
	9	39 (78)[b]	26 (52)	6 (12)	4 (8)	3 (6)
	13	42 (84)[b]	26 (52)	5 (10)	9 (18)	2 (4)
	19	40 (80)[b]	31 (62)	0	0	9 (18)
	21	7 (35)[c]	7 (35)	0	0	0
	Subtotal	187 (69.23)	129 (47.78)	19 (7.04)	22 (8.15)	17 (6.30)
B	1	6 (12)[a]	2 (4)	0	0	4 (8)
	4	6 (12)[a]	5 (10)	1 (2)	0	0
	9	14 (28)[a]	6 (12)	1 (2)	4 (8)	3 (6)
	13	42 (84)[b]	24 (48)	0	15 (30)	3 (6)
	19	41 (82)[b]	40 (80)	0	0	1 (2)
	21	12 (60)[b]	12 (60)	0	0	0
	Subtotal	121 (44.8)	89 (32.96)	2 (0.74)	19 (7.04)	11 (4.07)
C	1	2 (4)[a]	0	0	0	2 (4)
	4	9 (18)[b]	8 (16)	0	0	1 (2)
	9	23 (46)[c]	19 (36)	0	0	4 (4)
	13	47 (94)[d]	41 (82)	0	3 (6)	3 (6)
	19	50 (100)[d]	30 (60)	3 (6)	4 (8)	13 (26)
	21	14 (70)[d]	11 (55)	0	0	3 (15)
	Subtotal	145 (53.70)	109 (40.37)	3 (1.11)	7 (2.59)	26 (9.63)
Overall prevalence	1	23 (15.3)	14 (9.3)	0	1 (0.7)	8 (5.3)
	4	59 (39.3)	40 (26.7)	9 (6.0)	8 (5.3)	2 (1.3)
	9	76 (50.7)	51 (34.0)	7 (4.7)	8 (5.3)	10 (6.7)
	13	131 (86.7)	91 (60.7)	5 (3.3)	27 (18.0)	8 (5.3)
	19	131 (87.3)	101 (67.3)	3 (2.0)	4 (2.7)	23 (15.3)
	21	33 (55.0)	30 (60.0)	0	0	3 (5.0)
	Grand total	453 (55.9)	327 (40.37)	24 (2.96)	48 (5.92)	54 (6.67)

A total of 50 fresh fecal droppings were collected from each farm in the five samplings (week 1, 4, 9, 13, and 19) and 20 viscera/ceca were collected at slaughtering plant from each farm.

For each farm prevalence, numbers in the same column with different letters in the superscript were significantly different ($p < 0.05$), while numbers with the same letters did not differ significantly (chi-square test and Fisher's exact two-tailed test).

with age between weeks 1 and 4 ($p = 0.0317$), weeks 4 and 9 ($p = 0.0110$), and between weeks 9 and 13 ($p = 0.0029$). Though these *p*-values suggest significant differences for all ages, when the stricter Bonferroni-corrected *p*-value cut-off for significance (0.004) was used, only the weeks 9 and 13 difference was significant with this sample size for farms B and C.

At week 1, farm A had higher prevalence (30%) than farms B (12%) and C (4%). Prevalence increased rapidly in farm A to 88% by week 4. In farm B, prevalence was highest (84%) by week 13, while in farm C the prevalence was highest (100%) by week 19. The overall prevalence of *Campylobacter* spp. declined to 55% at slaughter for all farms combined.

flaA-*RFLP polymorphism*

The *flaA* typing was successfully performed on 350/351 (99.7%) isolates (*C. jejuni* [$n = 23$] and *C. coli* [$n = 327$]). A total of 7 different banding profiles (~35–1110 bps) were obtained. Using a similarity cut-off value of 100%, 70 main types were identified (62 for *C. coli* and 8 for *C. jejuni*) (Figs. 1 and 2). Overall, *flaA* types were associated with individual farms (Fisher's exact *p*-value < 0.000; uncertainty coefficient = 0.353). This result was true for both *C. coli* (*p*-value < 0.000) and *C. jejuni* but slightly less significantly (*p*-value = 0.057), which is reasonable given the differences in sample size. Uncertainty coefficients suggest that *flaA* type is a good indicator of which farm a sample originated from and predicts between 70% (coefficient = 0.709 for *C. jejuni*) and 80% (coefficient = 0.809 for *C. coli*) of variability in farms.

MLST of C. jejuni *and* C. coli *isolates*

C. jejuni isolates showed high genetic diversity by MLST analysis (Table 2). A total of 8 different STs were found among 19 *C. jejuni* isolates, of which 4 (STs 6350, 6351, 6352, and 6353) were identified as novel. ST 2934 was the most predominant, detected in 42.1% (8 of 19) of the isolates. This ST was commonly identified from turkeys of different ages. Six STs were assigned to 2 previously described clonal complexes (CC 353, 11 isolates; and CC 828, 5 isolates), while 2 new STs belonged to undefined CC. Overall, the findings imply the occurrence of spatially diverse strains, while at the same time some clonal strains appear to be limited to specific farms.

Among 80 *C. coli* isolates, 15 unique STs were identified. Three isolates assigned to 2 new STs (STs 6348 and 6349). The most common ST was ST 825, which was represented by 24 isolates, with 6 STs occurring only once in the data set. The four most predominant STs (STs 825, 5844, 1119, and 5773) represented more than half of the isolates (56/80 isolates; 70%). All *C. coli* isolates were assigned to a single previously described CC 828 (Table 2). The discriminatory power of *flaA* typing (DI = 0.78) was greater than MLST

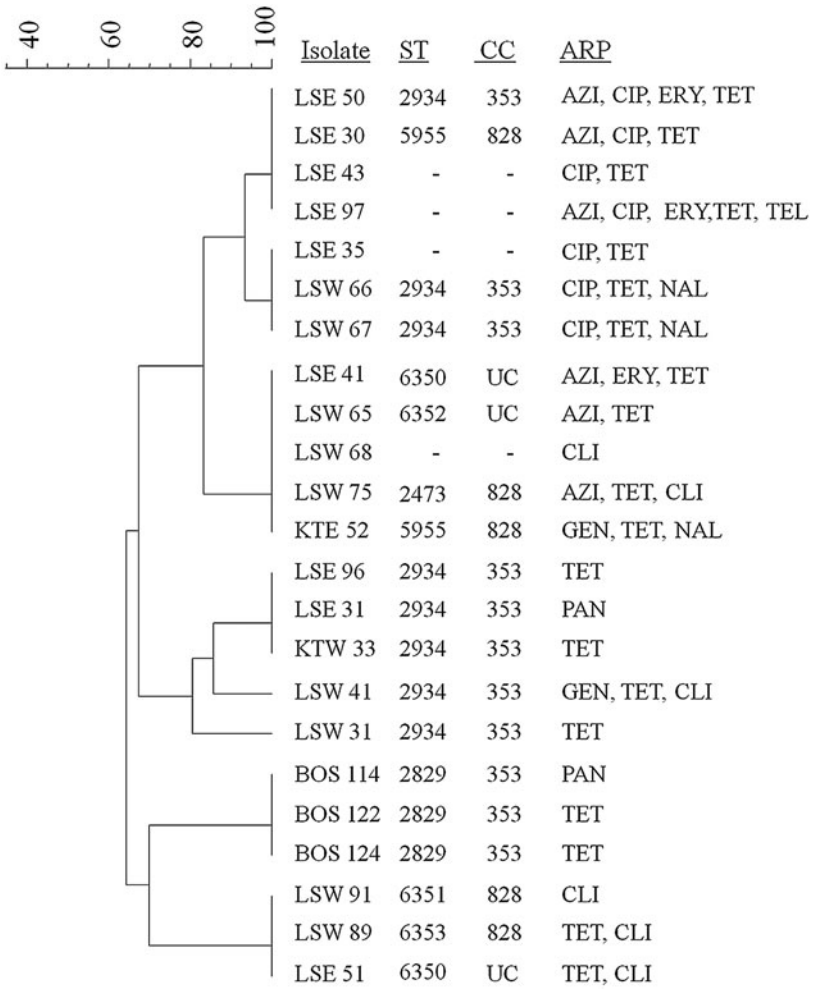

FIG. 1. Dendrogram showing the relationship of *flaA* restriction fragment length polymorphism (*flaA*-RFLP) types of *Campylobacter jejuni* isolates from turkeys. Similarity and clustering analysis of the polymerase chain reaction–RFLP patterns were performed using the Dice coefficient with unweighted pair-group method with arithmetic averages with optimization of 1% and position tolerance of 1.5%. Multilocus ST and antimicrobial resistance patterns (ARP) of *C. jejuni* isolates were combined to the tree. UC, undefined clonal complexes; ST, sequence type; CC, clonal complex; ARP, antimicrobial resistance pattern; LSW/LSE, Farm A; KTW/KTE, Farm B; BOS, Farm C; AZI, azithromycin; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; TEL, telithromycin; PAN, pansusceptible; CLI, clindamycin; GEN, gentamicin; NAL, nalidixic acid.

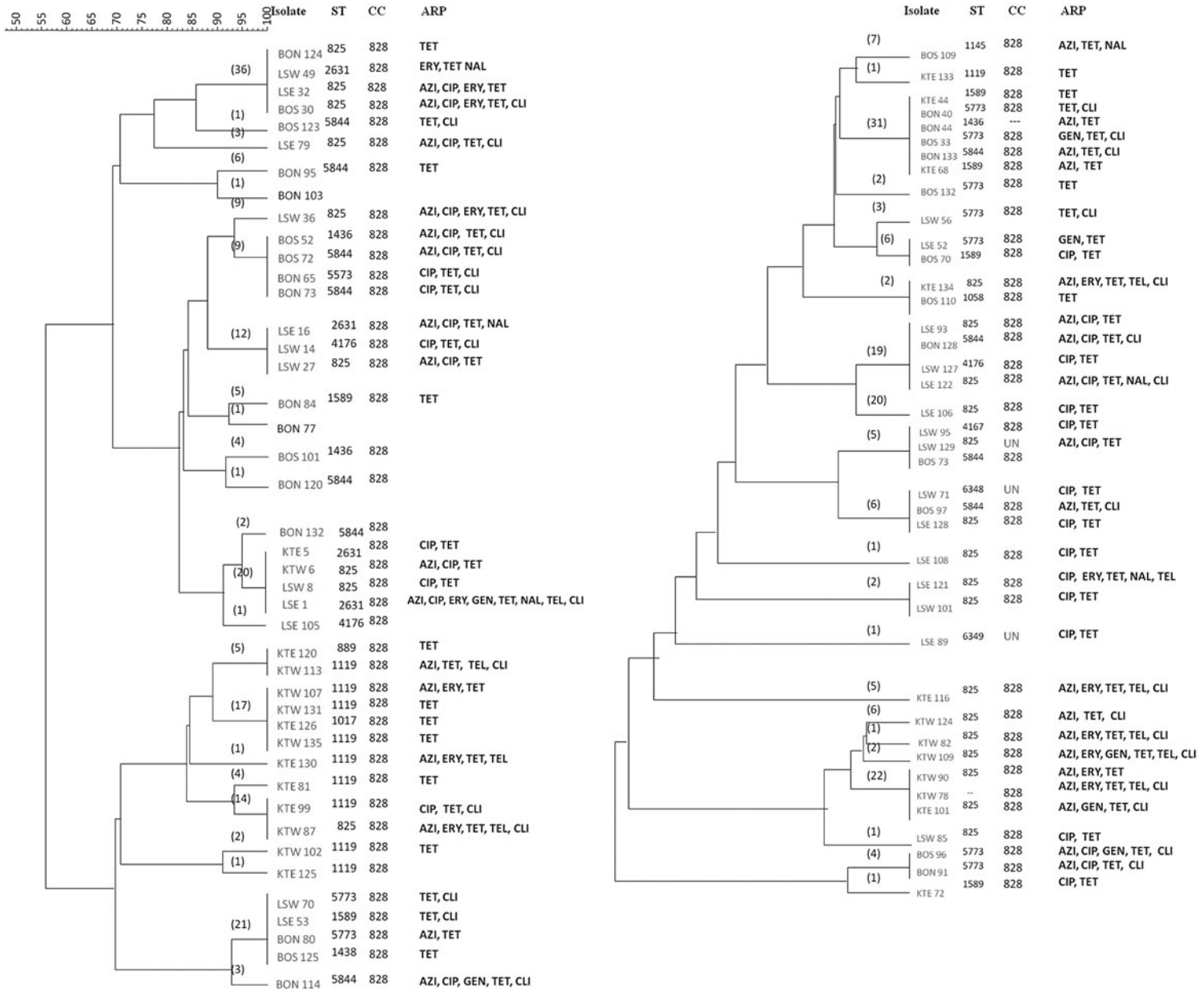

FIG. 2. Dendrogram showing the relationship of *flaA* restriction fragment length polymorphism types of *Campylobacter coli* isolates from turkeys. The cluster analysis was performed as described in Figure 1. Numbers in parentheses denote total number of isolates in each cluster. UC, undefined clonal complexes; ST, sequence type; CC, clonal complex; ARP, antimicrobial resistance pattern; LSW/LSE- Farm A; KTW/KTE- Farm B; BOS/BON-Farm C; NAL, nalidixic acid. For other abbreviations, see Figure 1 legend.

TABLE 2. SEQUENCE TYPES AND ST-COMPLEXES (ST-CC) OF *CAMPYLOBACTER JEJUNI* AND *C. COLI* STRAINS ISOLATED FROM TURKEYS

ST	ST-CC	No. of isolates	Farms			Resistance profiles
			A	B	C	
C. jejuni						
2829	353	3	0	0	3	Pan, T
2934	353	8	7	1	0	Pan, T, CTNa, ACET
6350	UC	2	2	0	0	AET, TCl
2473	828	1	1	0	0	ATCl
5955	828	2	1	1	0	ACT, GTNa
6351	828	1	1	0	0	Cl
6352	UC	1	1	0	0	ATCl
6353	828	1	1	0	0	TCl
C. coli						
825	828	24	14	9	1	ACT, CT, ACET, ACTCl, ACTNaCl, CETTeCl, AEGTTeCl, AETTeCl
889	828	1	0	1	0	T
1017	828	1	0	1	0	T
1058	828	1	0	0	1	T
1119	828	10	0	10	0	T, ATTeCl, AET,CTCl, AETTe
1145	828	1	0	0	1	ATNa,
1436	828	3	0	0	3	AT, ACTCl
1438	828	1	0	0	1	T
1589	828	6	1	3	2	T, TCl, CT, AT
2631	828	4	3	1	0	CT, ETNa, ETNa, ACEGTNaTeCl
4176	828	3	3	0	0	CT, CTCl
5773	828	10	3	0	7	T, TCl, AGT, CTCl, GTCl, ACTCl, ACGTCl
5844	828	12	0	0	12	T, TCl, ATCl, CTCl, ACTCl, ACGTCl
6348	828	2	2	0	0	CTCl, CT
6349	828	1	1	0	0	CT

A, azithromycin; C, ciprofloxacin; Cl, clindamycin; E, erythromycin; G, gentamicin; Na, nalidixic acid; T, tetracycline; Te, telithromycin; Pan, pansusceptible; UC, undefined clonal complex.

(DI = 0.51). However, *flaA* typing did not allow for the clear molecular grouping of the *C. jejuni* and *C. coli* strains. The frequency of STs and *flaA* types are shown in Figure 3.

Phylogenetic analysis of C. jejuni *and* C. coli *STs*

The evolutionary relationships of the 8 *C. jejuni* and 15 *C. coli* STs are represented in 2 minimum spanning trees (Fig. 4). *C. jejuni* isolates tend to be more clustered by farm, with very limited clonality between farms. Among eight unique STs, five were identified from farm A only, while a single ST was identified from farm C and two STs were common to farms A and B. The majority of the *C. jejuni* STs were also limited to one age group except ST 2934, which was identified persistently from three different age groups (4, 9, and 13 weeks). On the other hand, for the *C. coli*, STs showed much more diversity. Though most of the STs were identified from multiple age groups, the STs were found to have multilocus variations, strengthening the highly diverse nature of the *C. coli* population in turkeys. The founding genotype, ST 828, was identified from all three farms as well as all five age groups.

Antimicrobial resistance profiles (ARP) of C. jejuni *and* C. coli *isolates*

Campylobacter isolates displayed resistance most frequently to tetracycline (TET) (95%) and all isolates tested were susceptible to florfenicol (FFN) (Table 3). *C. coli* displayed significantly greater resistance ($p = 0.0346$) than *C. jejuni* to TET regardless of the source of isolation. The occurrence of the multiple drug resistance isolates varied among *C. coli* and *C. jejuni* (Table 3). Approximately 13% (3/23) of *C. jejuni* isolates were resistant to 4 or more antimicrobials. In contrast, 30.6% (23/75) of *C. coli* isolates were resistant to 4 or more antimicrobials. A wider range of MICs was observed for some of the antimicrobials, mainly among *C. coli* isolates (Table 3).

Approximately 26% and 30% of *C. jejuni* strains were resistant to azithromycin (AZI) and ciprofloxacin (CIP), respectively. Similarly, 48% and 45% of *C. coli* strains were resistant to AZI and CIP, respectively (the difference between the *C. coli* versus *C. jejuni* was not significant, Fisher's exact p-value = 0.516). In contrast, the majority of both *C. jejuni* and *C. coli* strains displayed diversity in resistance within the macrolides group of antimicrobials (AZI, erythromycin [ERY], and telithromycin [TEL]) (Table 3). While about 4% and 13% of *C. jejuni* and *C. coli*, respectively, were resistant to TEL (the difference between *C. coli* versus *C. jejuni* was not significant, Fisher's exact p-value = 0.201), 26% and 13% of *C. jejuni* strains were resistant to AZI and ERY and about 48% and 20% *C. coli* strains were resistant to AZI and ERY, respectively (p-values for difference between species 0.197 and 0.516, respectively).

Association of flaA *types and STs with antimicrobial resistance pattern (ARP)*

Some *flaA* types for *C. coli* were more likely to show antibiotic resistance in general (uncertainty coefficient = 1.00; Fisher's exact p-value = 0.003) and specifically for CIP

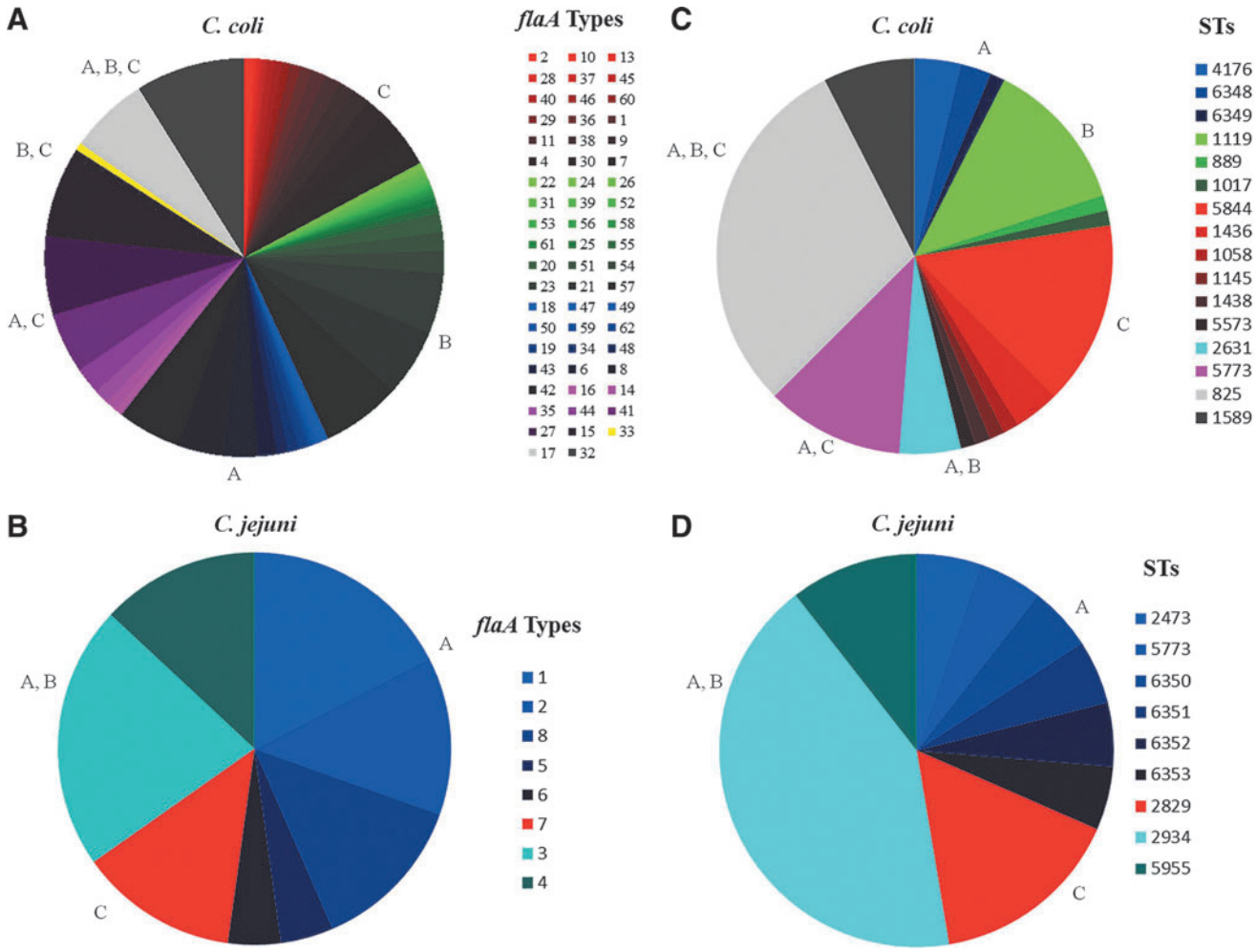

FIG. 3. Frequency of *flaA* types **(A, B)** and sequence types **(C, D)** in isolates of *Campylobacter coli* ($n = 327$) (A, C) and *C. jejuni* ($n = 19$) (B, D) collected from commercial turkeys. Farm of origin for the isolate is indicated by color: blue (A), green (B), red (C), and mixtures of colors for groups containing isolates from different farms. STs, sequence type. Color images available online at www.liebertpub.com/fpd

(uncertainty coefficient = 0.80; Fisher's exact p-value < 0.000), TET (uncertainty coefficient = 1.00; Fisher's exact p-value = 0.003), and ERY (uncertainty coefficient = 0.728; Fisher's exact p-value = 0.049). The relationships between TET and ERY resistance and *flaA* type were not significant for *C. jejuni*, but the relationship with CIP resistance was significant (uncertainty coefficient = 1.00; Fisher's exact p-value = 0.003).

Several *C. coli* STs showed a good correlation with their antimicrobial resistance profiles (Table 2 and Fig. 2). For instance, the majority of *C. coli* isolates (18/24; 75%) in ST 825 were resistant to multiple antimicrobials, with the majority (62.5%) of isolates being resistant to CIP. Almost all isolates in ST 1589 ($n = 6$) were resistant to either 1 or 2 antimicrobials, while isolates in ST 5844 showed diverse resistance profiles to a majority of antimicrobials tested, with most isolates (73%) being resistant to clindamycin. All *C. coli* STs from all farms were pansusceptible to FFN and resistant to TET. In general, STs were significantly associated with ARP (Fisher's exact p-value = 0.009) for *C. coli*, but they accounted for a relatively small portion of the variability in resistance (uncertainty coefficient = 0.344).

In general, *C. jejuni* isolates showed higher antimicrobial resistance (R-type) diversity than *C. coli* isolates, which is consistent with the MLST findings. With exception of ST 2829, which contained isolates resistant to either one antimicrobial agent or pansusceptible isolates, the majority of STs contained isolates with different resistance profiles (Table 2 and Fig. 1). For example, ST 2934 contained isolates that were pansusceptible, resistant to one, three, and more than three antimicrobials.

Discussion

In the present study, *Campylobacter* was recovered from the feces starting from 1-week-old birds (14.7%) and continuing to slaughter age (58.3%). The observed timing of colonization of *Campylobacter* in this study is earlier than what was found in previous reports. Smith *et al.* (2004) reported that sibling turkey flocks started to be colonized by *Campylobacter* spp. between weeks 2 and 3 and remained colonized until processing. Similarly, El-Adawy *et al.* (2012) showed that poults were colonized by thermophilic *Campylobacter* spp. on week 2. All 3 flocks in our study remained positive throughout, with

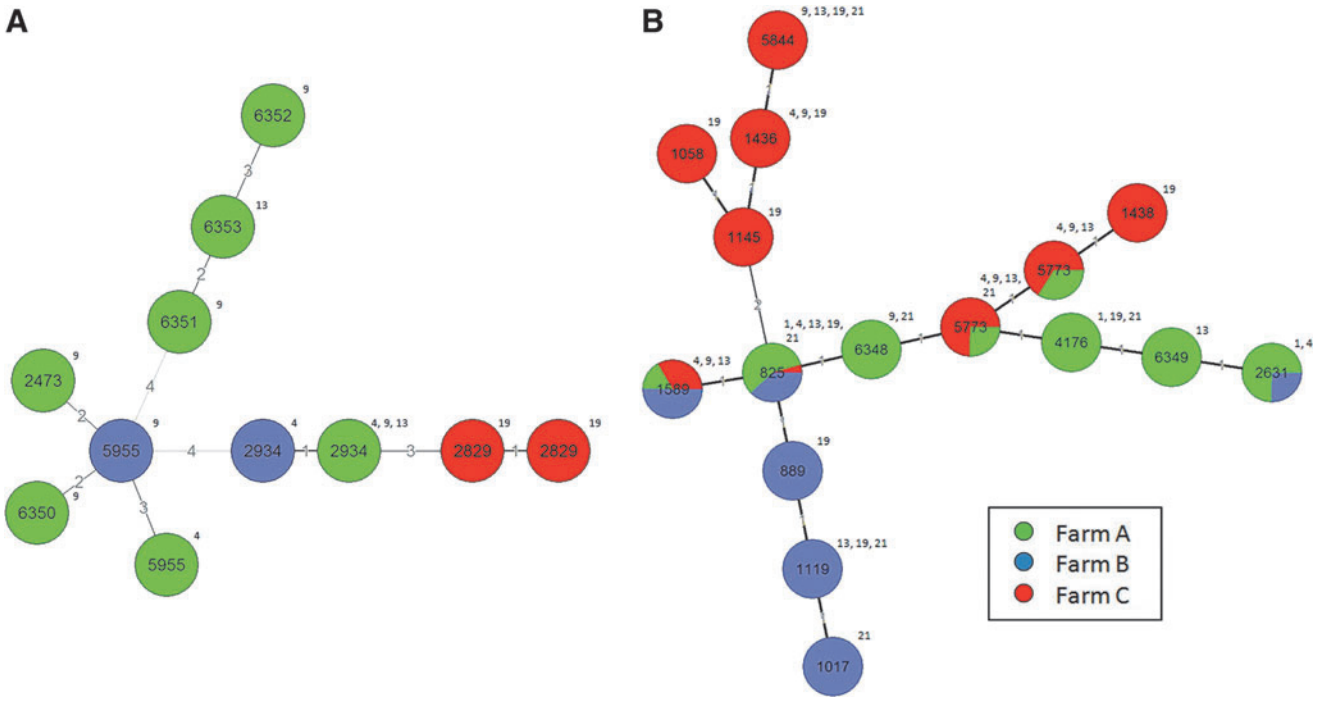

FIG. 4. BioNumerics Minimum Spanning Tree for *Campylobacter jejuni* (A) and *C. coli* (B). Color codes represent the farm origins of the isolates. The sequence types are numbers in the circles and the numbers outside the circles represent the age of the animals in weeks. The branch length is proportional to the locus variations and as also indicted in numbers on each branch. Color images available online at www.liebertpub.com/fpd

TABLE 3. ANTIMICROBIALS RESISTANCE AND MINIMUM INHIBITORY CONCENTRATION (MIC) DISTRIBUTION OF *CAMPYLOBACTER JEJUNI* AND *C. COLI* ISOLATED FROM TURKEYS

		Number of resistant isolates (%) and range of MICs in μg/mL								
Isolates		*AZI*	*CIP*	*ERY*	*GEN*	*TET*	*FFN*	*NAL*	*TEL*	*CLI*
C. jejuni	Total ($n=23$)	6 (26)[a] 64*	7 (30.4)[a] 8–16	3 (13)[a] 64	2 (8.7)[a] 8–16	19 (82.6)[a] 32–64	0	3 (13.0)[a] 64	1 (4.3)[a] 8	7 (30.4)[a] 16
	Farm A ($n=17$)	5(29.4) 64	7 (38.9) 8–16	3 (16.7) 64	1 (5.6) 8	14 (82.3) 32–64	0	1 (5.8) 64	1 (5.6) 8	6 (35.2) 16
	Farm B ($n=3$)	1 (33.3) 64*	0	0	1 (33.3) 16	3 (100) 32–64	0	2 (66.7) 64	0	1 (33.3) 16
	Farm C ($n=3$)	0	0	0	0	2 (66.7) 64	0	0	0	0
C. coli	Total ($n=75$)	36 (48)[a] 8–64	34 (45.3)[a] 8–32	15 (20)[a] 32–64	7 (9.3)[a] 8–32	74 (100.0)[b] 32–64	0	6 (8)[a] 64	10 (13.3)[a] 8	33 (44)[a] 8–16
	Farm A ($n=26$)	10 (38.5)[c] 8–64	21 (80.8)[c] 8–32	5 (19.2)[c] 32–64	2 (7.7)[c] 16–32	26 (100.0)[c] 32–64	0	5 (19.2)[c] 64	2 (7.7)[c] 8	9 (34.6)[c] 8–16
	Farm B ($n=25$)	14 (56.0)[c] 8–64	4 (16.0)[d] 8–32	9 (36.0)[d] 64	2 (8.0)[c] 8–16	25 (100.0)[c] 32–64	0	0[d]	8 (32.0)[d] 8	10 (40.0)[c] 8–16
	Farm C ($n=24$)	12 (50.0)[c] 8–64	9 (37.5)[e] 8–32	1 (4.2)[c] 64	3 (12.5)[c] 16	23 (95.8)[c] 32–64	0	1 (4.2)[c] 64	0[c]	14 (58.3)[c] 8–16

*MIC ranges of antimicrobials for the resistant isolates.

For each antimicrobial, numbers in the same row with different superscript letters were significantly different ($p<0.05$), while numbers with the same letters did not differ significantly (chi-square test and Fisher's exact two-tailed test).

Resistance breakpoints: AZI, ≥8 μg/mL; CIP, ≥4 μg/mL; CLI, ≥8 μg/mL; ERY, ≥32 μg/mL; GEN, ≥8 μg/mL; NAL, ≥64 μg/mL; TEL, ≥8 μg/mL; FEN, ≥8 μg/mL; TET, ≥16 μg/mL.

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

the highest prevalence after week 13. This confirms the potential rapid bird-to-bird transmission of *Campylobacter* spp. in commercial meat poultry farms as indicated previously (Newell *et al*., 2003; Horrocks *et al*., 2009).

In this study, *C. coli* was frequently recovered from the turkey fecal droppings. *C. coli* was also frequently identified previously in turkey flocks in the United States (Logue *et al*., 2003; Smith *et al*., 2004; Wesley *et al*., 2005). Proportional higher prevalence of *C. coli* than *C. jejuni* could be due to host adaptation. Resistance to certain antibacterials is reported to be significantly more common in *C. coli* than in *C. jejuni* strains (Aarestrup *et al*., 1997; Ge *et al*., 2003; Ladely *et al*., 2007), which could select for one species versus the other. However, studies from both conventional and organic turkey farms in Ohio reported a high prevalence of *C. coli* (Luangtongkum *et al*., 2006), so other environmental factors may also contribute to the observed differences.

Eight and 62 different *flaA* PCR-RFLP profiles were identified for *C. jejuni* and *C. coli*, respectively. These results are in line with the previous studies in chickens (Behringer *et al*., 2011; Miller *et al*., 2010); however, fewer *flaA*-types (i.e., 6 for *C. jejuni* [$n=35$] and 14 for *C. coli* [$n=65$]) isolated from processed turkeys were reported (Lutgen *et al*., 2009). These discrepancies may be related to animal sources; environmental, management, and husbandry conditions of flocks; primers used; as well as geographic diversity in the distribution of *C. jejuni* and *C. coli* isolates.

Of the eight *C. jejuni* STs, only ST 2934 was previously reported in turkeys (Gu *et al*., 2009). Therefore, further studies are needed to assess possible host associations of this ST with turkeys. The ST 353 complex, the most common complex seen in *C. jejuni* strains in this study, has been reported previously in poultry (Manning *et al*., 2003), and is associated with human gastroenteritis (Dingle *et al*., 2002; Duim *et al*., 2003; Manning *et al*., 2003). Indeed, the ST 353 complex contains a majority of the isolates obtained from human disease. Furthermore, the ST 828 complex, the main group identified in this study for *C. coli* and some *C. jejuni*, is associated with strains that are mainly isolated from agricultural and environmental sources, and some from human clinical cases (Sheppard *et al*., 2010). Also, other researchers have reported the presence of progenitor strains of ST 828 complex in humans, swine, and cattle from different parts of the world (Dingle *et al*., 2005; Miller *et al*., 2006). This indicates that ST 828 has potential to infect both humans and animals.

The majority of the isolates ($n=77$) were resistant to 2 or more antimicrobials, and *C. coli* displayed significantly more ($p=0.0346$) resistance than *C. jejuni* to TET. High occurrences of multidrug-resistant *Campylobacter* isolated from turkeys have also been reported by other researchers. Lutgen *et al*. (2009) examined 801 *Campylobacter* isolates from processed turkey in Midwestern United States and found that *C. coli* were more resistant to CIP (63%) than *C. jejuni* (28%), and a subset ($n=100$) of isolates were resistant to TET (100%) and nalidixic acid (49%). High prevalence of TET resistance in *Campylobacter* isolates from chickens and turkeys has also been reported (Luangtongkum *et al*., 2006; Anderson *et al*., 2006; Gu *et al*., 2009; Zhao *et al*., 2010). Studies have also shown that TET resistance is typically transferable (Taylor *et al*., 1981; Avrain *et al*., 2004). Since TETs have been used as feed additives for livestock and poultry for both therapeutic and subtherapeutic purposes for a long time (Chopra and Roberts, 2001; Fallon *et al*., 2003), it is possible that *Campylobacter* may have become resistant to this class of antimicrobials through selective pressure.

Conclusions

The present study found a high prevalence of *Campylobacter* spp. in commercial turkey farms and at slaughter, suggesting that on farms high prevalence can be a high risk for carcass contamination. The majority of the *Campylobacter* isolates examined were resistant to multiple antimicrobials, including ERY and CIP. We observed that turkeys can be colonized by *Campylobacter* as early as the first week of introduction to the barns; thus, further studies aimed at identifying the sources and vehicles for *Campylobacter* spp. in turkey farms are needed.

Acknowledgments

We thank Zhe Liu, Mary Drozd, Kshipra Chandrashekhar, Serpil Baspinar, Gokben Ozbey, and Ahmed Hikal for assistance with sample collection and processing. Dr. Kashoma is supported by a National Institutes of Health Fogarty fellowship (D43TW008650-01). Dr. Rajashekara's laboratory is supported by the funds from Ohio Agricultural Research and Development Center (OARDC), and the Agriculture and Food Research Initiative (AFRI) grant# 2012-68003-19679, U.S. Department of Agriculture.

Disclosure Statement

No competing financial interests exist.

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