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Prevalence and Antimicrobial Susceptibility of *Campylobacter spp.* in Oklahoma Conventional and Organic Retail Poultry

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Abstract: *Campylobacter* is one of the most important foodborne pathogens that cause bacterial gastroenteritis.This study was conducted to investigate the prevalence and antimicrobial resistance of *Campylobacter* in conventional and organic retail poultry samples purchased from grocery stores in Tulsa, Oklahoma.One hundred and fifty six chilled retail chicken samples (85 conventional and 71 organic) and 65 chilled retail conventional turkey samples were collected in this study. The prevalence of *Campylobacter* in the conventional chicken samples 32/85 (38%) was higher than in the organic ones 21/71 (30%). The prevalence of *Campylobacter* in the conventional turkey samples was 11/65 (17%). Of the 53 positive chicken samples, 42 were *C. jejuni*, 8 were *C. coli* and three isolates were contaminated with both species. Of the 11 positive turkey samples, 8 contained *C. jejuni* and 3 harbored *C. coli* isolates. The antimicrobial susceptibility of one hundred and forty nine recovered *Campylobacter* isolates (130 chickens and 19 turkeys) towards sixteen antimicrobials was determined. The majority of the recovered turkey isolates (13/19) showed resistance to more than 7 antimicrobials while most of the recovered chicken ones (82/130) were resistant to 5 to 7 antimicrobials. Multidrug resistance was not limited to isolates from conventional sources but was also available in isolates of an organic background and was generally lower in *C. jejuni* isolates when compared to the *C. coli* ones.

Keywords: Antibiotic Resistance, *Campylobacter*, Chicken, Food borne Pathogens, Organic, Prevalence, Retail Poultry, Turkey.

INTRODUCTION

Campylobacter is the third bacterial cause of food borne infections in the US [1], and is known to cause gastrointestinal illness [2]. The dangerous Guillain-Barré syndrome can be in rare cases a result of Campylobacteriosis [3]. Improper handling or consumption of raw or undercooked meat is the main cause of the majority of *Campylobacter* infections [3]. Most human *Campylobacter* infections are caused by either *C. jejuni* or *C. coli* [4]. Chicken and turkey products are considered the most common sources of *Campylobacter* [5]. Birds are an important reservoir of *Campylobacter* due to their high body temperature which provides an optimum growth temperature for these thermotolerant species. In a farm, the spread of *Campylobacter* occurs through contaminated food and water as well as feces [6].

The macrolide erythromycin is known to be used in the treatment of Human campylobacteriosis. Fluoroquinolones like ciprofloxacin are used to treat enteritis, while aminoglycosides are commonly prescribed for the treatment of systemic infections [7]. Increase in the resistance to these antimicrobials poses a risk to human health. The improper use of antimicrobials leads to the appearance and persistence of resistance [8, 9]. The rise of resistance has been linked to the overuse of antimicrobials in feed supplements in conventional farming, which may select for more microbial isolates that are resistant and can be health risk to humans if they can reach the food chain [8]. The drugs are administered to the whole flock rather than the individual bird and hence this produces a risk especially when the antimicrobial is extremely important for treating human infections [10]. Organically-produced poultry is an important market in the retail industry especially as they are fed organic feed and supplements with no antimicrobials [11]. This has led to the belief that these animals are healthier and safer to the consumer although the opposite might be true due to the restriction in the use of antimicrobials [12, 13].

This study was conducted to investigate the prevalence and antimicrobial resistance of *Campylobacter* in conventional and organic retail poultry samples purchased from grocery stores in Tulsa, Oklahoma.

MATERIALS AND METHODS

Poultry Samples Collection and Bacterial Isolation

One hundred and fifty six chilled retail chicken and sixty five turkey samples were purchased weekly from several grocery stores in Tulsa, Oklahoma starting in January of 2010 and for a period of approximately six months. Samples were transported to the laboratory on ice and were timely processed once arrived. Care was taken when choosing the samples so that it will be as variable as possible in regards to

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production patches, expiration dates, different poultry cuts, etc. Enrichment and isolation of *Campylobacter* was performed as described previously [14]. Four suspected *Campylobacter* colonies of each poultry sample were purified through sub culturing and subjected to molecular identification by PCR.

DNA Extraction and PCR Identification

DNA was extracted from *Campylobacter* cultures using the single cell lysing buffer (SCLB) method [15] and as describer in details previously [14]. Suspected *Campylobacter* isolates were screened for the presence of *Campylobacter* genes by multiplex PCR using primers specific for *C. jejuni* and *C. coli* [16] (Table **1**). The multiplex PCR reaction and cycling protocol was carried out using primers in Table **1** as detailed previously [20]. As shown in Table **1** the presence of the *cadF* gene along with the *C-1* gene in a strain will make it identified as *C. jejuni* while the presence of *cadF* along with the *ceuE* gene will reveal a *C. coli* strain. *C. jejuni* ATCC #33560, and *C. coli* strain # 96121033 (Oklahoma Animal Disease Diagnostic Laboratory, OSU) were used as positive controls.

Antimicrobial Susceptibility Testing

The agar dilution method was used to test for the antimicrobial susceptibility of the recovered 149 *Campylobacter* isolates against sixteen antimicrobials of eight antibiotic classes (Table **2**) as described previously [20]. Table **2** also includes the breakpoints used to determine the resistance of each of the 16 tested antimicrobials

Table 1. A list of PCR primers used for *Campylobacter jejuni* and *Campylobacter coli* identification and their corresponding amplicon sizes and references.

Gene	Size (bp)	Primer sequences	Species	References
cadF	400	F 5′-TTGAAGGTAATTTAGATATG-3′ R 5′-CTAATACCTAAAGTTGAAC-3′	*C. coli &* *C. jejuni*	[16] [17]
ceuE	894	F 5′-ATGAAAAAATATTTAGTTTTTGCA-3′ R 5′-ATTTTATTATTTGTAGCAGCG-3′	*C. coli*	[16] [18]
C-1	160	F 5′-CAAATAAGTTAGAGGTAGAATGT-3′ R 5′-GGATAAGCACTAGCTAGCTAGCTGAT-3′	*C. jejuni*	[16] [19]

Table 2. A list of the sixteen tested antimicrobials, their classes, the concentrations range used for susceptibility testing, and the breakpoints used for each antimicrobial.

Antimicrobial Class	Antimicrobial	MIC Range (µg/ml)	Breakpoint (µg/ml)
Aminoglycosides	Gentamicin	4 - 64	8
	Kanamycin	32 - 512	64
	Streptomycin	48 - 512	64
Beta-lactams	Amoxicillin	16 - 256	32
	Ampicillin	16 - 256	32
	Cephalothin	16 - 256	32
Fluoroquinolones	Ciprofloxacin	2 - 32	4
Lincosamides	Clindamycin	4 - 64	8
Macrolides	Azithromycin	4 - 64	8
	Erythromycin	16 - 256	32
	Tilmicosin	4 - 64	8
Phenicols	Chloramphenicol	16 - 256	32
Quinolones	Nalidixic Acid	32 - 512	64
Tetracyclines	Doxycycline	4 - 64	8
	Oxytetracycline	1 - 16	2
	Tetracycline	8 - 128	16

including those established according to the Clinical and Laboratory Standards Institute (CLSI) when available [21]. *C. jejuni* ATCC #33560 was used as a quality control strain. Antimicrobial susceptibility testing was performed at 42 °C for 48 h under microaerophilic conditions.

RESULTS

Prevalence of *Campylobacter* in Chicken and Turkey Meat

One hundred and fifty six chilled retail chicken and sixty five turkey samples were purchased weekly from several grocery stores in Tulsa, Oklahoma starting in January of 2010 and for a period of approximately six months.

Selection of the retail poultry samples was as variable as possible in regards to production patches, expiration dates, different poultry cuts, etc. (Table **3**). The 156 samples of retail chicken included 85 conventional and 71 organic samples (Table **3**).

From Table **3**, one can conclude that *Campylobacter* prevalence in chicken samples was 53/156 (34%). Thirty percent of the chicken samples (42/156) were contaminated with *C.jejuni*, while only five percent (8/156) showed *C. coli* contamination. Three samples (2%) were contaminated with both *C. jejuni* and *C. coli*. *Campylobacter* prevalence in turkeys was approximately 17% (11/65).Twelve percent of the turkey samples (8/65) showed contamination with *C. jejuni*, while only five percent (3/65) were contaminated with *C. coli* (Table **3**). In conventional chicken, the prevalence of *C. jejuni* (31%) was somewhat higher than in the organic ones (23%). *C. coli* prevalence was not much different between the organic (6%) and the conventional samples (4%). Two of the co-infected samples were organic and one was a conventional chicken (Table **3**). When combined, there was a higher prevalence of *C. jejuni* (23%) among the poultry isolates than *C. coli* (5%) (Table **3**).

Antimicrobial Resistance Profiling

One hundred and forty nine recovered *Campylobacter* isolates (130 from chicken and 19 from turkey) were tested for their antimicrobial resistance towards eight different antibiotic classes (16 antimicrobials) (Table **2** and Table **4**). From Table **4**, one can conclude that the percentage of resistance to the 16 tested antimicrobials of the one hundred and thirty *Campylobacter* isolates (107 *C. jejuni* and 23 *C. coli*) isolated from chicken were as follow: ampicillin (40.6%), erythromycin (4.6%), nalidixic acid (20.8%), tetracycline (50.8%), streptomycin (6.9%), kanamycin (50.0%), oxytetracycline (96.2%), amoxicillin (99.2%), gentamicin (6.9%), ciprofloxacin (28.5%), clindamycin (7.7%), azithromycin (9.2%), doxycycline (81.5%), chloramphenicol (4.6%), tilmicosin (10.0%), and cephalothin (97.7%).

The percentage of resistance of the nineteen *Campylobacter* isolates (13 *C. jejuni* and 6 *C. coli*) isolated from turkey to the 16 antimicrobials were as follows: ampicillin (84.2%), erythromycin (21.1%), nalidixic acid (47.4%), tetracycline (100%), streptomycin (15.8%), kanamycin (84.2%), oxytetracycline (94.7%), amoxicillin (100%), gentamicin (5.3%), ciprofloxacin (73.7%), clindamycin (10.5%), azithromycin (21.1%), doxycycline (100%), chloramphenicol (10.5%), tilmicosin (15.8%), and cephalothin (100%) (Table **4**).

There was an apparent variation in antimicrobial resistance between *C. jejuni* and *C. coli* (Table **4**). For the one hundred and seven *C. jejuni* and the twenty three *C. coli* chicken isolates resistance percentages were generally higher in *C. coli* for ciprofloxacin, tilmicosin, chloramphenicol, erythromycin, clindamycin, azithromycin, streptomycin, gentamicin, nalidixic acid, kanamycin, ampicillin, while it was higher in *C. jejuni* for doxycycline and tetracycline (Table **4**).

Resistance percentages of the thirteen *C. jejuni* and six *C. Coli* turkeys isolateswere consequently as follow: amoxicillin (100%, 100%), ampicillin (77%, 100%), azithromycin (0%, 67%), cephalothin (100%, 100%), chloramphenicol(0%, 33%), ciprofloxacin (69%, 83%), clindamycin (0%, 33%), doxycycline (100%, 100%), erythromycin (0%, 67%), gentamicin (8%, 0%), kanamycin (77%, 100%), nalidixic acid (54%, 33%), oxytetracycline

Table 3. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in the 221conventional and organic poultry samples.

Prevalence of *Campylobacter* in chicken & turkey isolates									
Species	Chicken			Turkey			Poultry		
	Conventional	Organic	Total	Conventional	Organic	Total	Conventional	Organic	Total
	np/n (%)	np/n (%)	np/n (%)	np/n (%)	np/n (%)	np/n (%)	np/n (%)	np/n (%)	np/n (%)
C. jejuni	26/85 (31)	16/71 (23)	42/156 (30)	8/65 (12)	0	8/65 (12)	34/150 (23)	16/71 (23)	50/221 (23)
C. coli	5/85 (6)	3/71 (4)	8/156 (5)	3/65 (5)	0	3/65 (5)	8/150 (5)	3/71 (4)	11/221 (5)
Both	1/85 (1)	2/71 (3)	3/156 (2)	0/65 (0)	0	0/65 (0)	1/150 (0.7)	2/71 (3)	3/221 (1)
Total *Campylobacter*	32/85 (38)	21/71 (30)	53/156 (34)	11/65 (17)	0	11/65 (17)	43/150 (29)	21/71 (30)	64/221 (29)

*np: no. of positive samples, n: no. of samples collected.

Table 4. Antimicrobial resistance of the 149 *Campylobacter* poultry isolates against the sixteen tested antimicrobials.

Antimicrobial Resistance									
Antimicrobials	**Chicken**			**Turkey**			**Poultry**		
	***C. jejuni* np/n (%)**	***C. coli* np/n (%)**	**Total np/n (%)**	***C. jejuni* np/n (%)**	***C. coli* np/n (%)**	**Total np/n (%)**	***C. jejuni* np/n (%)**	***C. coli* np/n (%)**	**Total np/n (%)**
Amoxicillin	106/107 (99)	23/23 (100)	129/130 (99)	13/13 (100)	6/6 (100)	19/19 (100)	119/120 (99)	29/29 (100)	148/149 (99)
Ampicillin	39/107 (36)	13/23 (57)	52/130 (40)	10/13 (77)	6/6 (100)	16/19 (84)	49/120 (41)	19/29 (66)	78/149 (52)
Azithromycin	3/107 (3)	9/23 (39)	12/130 (9)	0/13 (0)	4/6 (67)	4/19 (21)	3/120 (3)	13/29 (45)	16/149 (11)
Cephalothin	104/107 (97)	23/23 (100)	127/130 (98)	13/13 (100)	6/6 (100)	19/19 (100)	117/120 (98)	29/29 (100)	146/149 (98)
Chloramphenicol	0/107 (0)	6/23 (26)	6/130 (5)	0/13 (0)	2/6 (33)	2/19 (11)	0/120 (0)	8/29 (28)	8/149 (5)
Ciprofloxacin	21/107 (20)	16/23 (70)	37/130 (28)	9/13 (69)	5/6 (83)	14/19 (74)	30/120 (25)	21/29 (72)	51/149 (34)
Clindamycin	2/107 (2)	8/23 (35)	10/130 (8)	0/13 (0)	2/6 (33)	2/19 (11)	2/120 (2)	10/29 (34)	12/149 (8)
Doxycycline	88/107 (82)	18/23 (78)	106/130 (82)	13/13 (100)	6/6 (100)	19/19 (100)	101/120 (84)	24/29 (83)	125/149 (84)
Erythromycin	1/107 (0.9)	5/23 (22)	6/130 (5)	0/13 (0)	4/6 (67)	4/19 (21)	1/120 (0.8)	9/29 (31)	10/149 (7)
Gentamicin	4/107 (4)	5/23 (22)	9/130 (7)	1/13 (8)	0/6 (0)	1/19 (5)	5/120 (4)	5/29 (17)	10/149 (7)
Kanamycin	46/107 (43)	19/23 (83)	65/130 (50)	10/13 (77)	6/6 (100)	16/19 (84)	56/120 (47)	25/29 (86)	81/149 (54)
Nalidixic Acid	17/107 (16)	10/23 (43)	27/130 (21)	7/13 (54)	2/6 (33)	9/19 (47)	24/120 (20)	12/29 (41)	36/149 (24)
Oxytetracycline	102/107 (95)	23/23 (100)	125/130 (96)	12/13 (92)	6/6 (100)	18/19 (95)	114/120 (95)	29/29 (100)	143/149 (96)
Streptomycin	4/107 (4)	5/23 (22)	9/130 (7)	0/13 (0)	3/6 (50)	3/19 (16)	4/120 (3)	8/29 (28)	12/149 (8)
Tetracycline	56/107 (52)	10/23 (43)	66/130 (51)	13/13 (100)	6/6 (100)	19/19 (100)	69/120 (58)	16/29 (55)	85/149 (57)
Tilmicosin	4/107 (4)	9/23 (39)	13/130 (10)	0/13 (0)	3/6 (50)	3/19 (16)	4/120 (3)	12/29 (41)	16/149 (11)

*np: no. of positive samples, n: no. of samples collected.

(92%, 100%), streptomycin (0%, 50%), tetracycline (100%, 100%), and tilmicosin (0%, 50%) (Table **4**).

From data shown in Table **4** one can conclude that the majority of the recovered turkey isolates (13/19) showed resistance to more than 7 antimicrobials while most of the recovered chicken isolates (82/130) were resistant to 5 to 7 antimicrobials. As shown in Fig. (**1**), multidrug resistance was generally higher among the *C. coli* isolates than *C. jejuni* strains. The 57 isolates in the dendrogram are representatives of all positive samples and was selected by choosing one isolate as a representative of the most common antimicrobial resistance profile among colonies tested out of each positive sample. The highest antimicrobial resistance profile among *C. jejuni* isolates showed resistance to 10 antimicrobials, while the highest one among the *C. coli* ones carried resistance to the 16 antimicrobials tested (Fig. **1**). It is also obvious from the dendrogram that multidrug resistance was not limited to isolates from conventional sources since few of the organic isolates showed resistance to several antimicrobials (Fig. **1**).

DISCUSSION

In this study, 34% of the chicken and 17% of the turkey samples were positive for *Campylobacter*. This is not surprising since other studies reported similar prevalence rates for *Campylobacter* in retail chicken [5, 22, 23]. On the other hand Cui, *et al*. [24] found in their study that 76% of the organic and 74% of the conventional chicken samples was positive for *Campylobacter* spp. Prevalence of *Campylobacter* in turkey was similar to a previous US study [25] while lower than a Canadian study where they reported 46% prevalence in turkey samples [10]. This is obviously different than *Campylobacter* prevalence at the farm level where it is significantly higher [26]. Our findings showed that *C. jejuni* was more prevalent than *C. coli* in poultry. *C. jejuni* has been reported to be the most isolated species in chicken [11, 27-29]. While variations in *Campylobacter* prevalence could be seasonal, Williams and Oyarzabal reported that seasons did not affect much the prevalence of *C. jejuni*, but they did affect *C. coli* prevalence in broiler meat in Alabama [23].

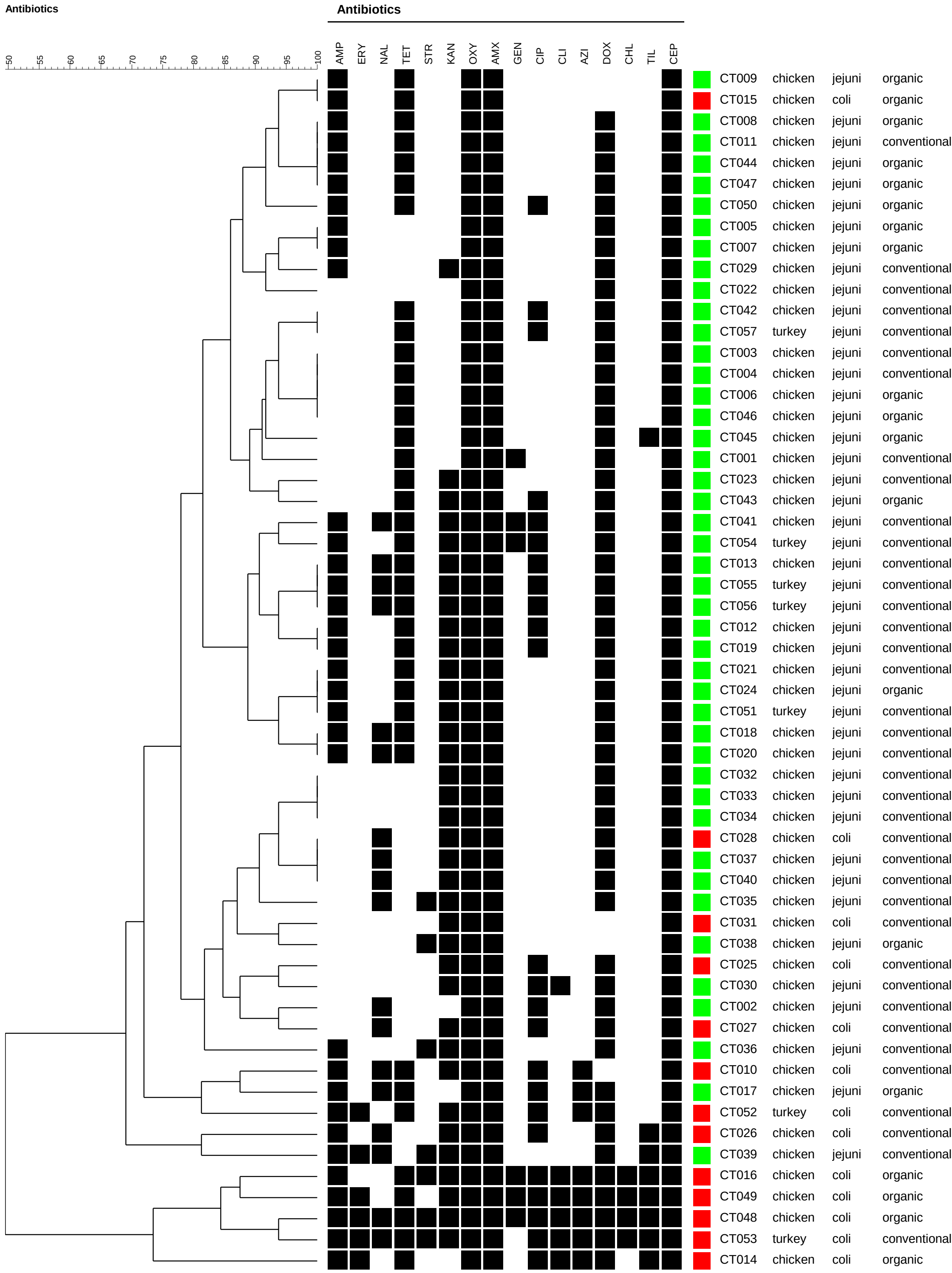

Fig. (1). A simple comparison dendrogram of the antimicrobial resistance profiling for a 57 *Campylobacter* representative isolates of positive samples created using the BioNumerics software. *C. jejuni* and *C. coli* are shown by green and red squares respectively. Meat source is indicated besides each isolate code. The source of the poultry is also denoted on the figure as organic or conventional. The three letter abbreviations representing the sixteen tested antimicrobials are shown on the top of the dendrogram as follows: ampicillin (AMP), erythromycin (ERY), nalidixic acid (NAL), tetracycline (TET), streptomycin (STR), kanamycin (KAN), oxytetracycline (OXY), amoxicillin (AMX), gentamicin (GEN), ciprofloxacin (CIP), clindamycin (CLI), azithromycin (AZI), doxycycline (DOX), chloramphenicol (CHL), tilmicosin (TIL), and cephalothin (CEP).

While our study showed that prevalence of *Campylobacter* specially *Campylobacter jejuni* was somewhat higher in conventional chicken samples than the organic ones, the overall prevalence of *Campylobacter* in the organic ones still troubling. The recent increase in the consumption of organically raised poultry [30] due to the consumer assumption that organic poultry meat is of a better quality and more nutritious than the conventionally raised ones [31] raised concerns about the microbiological safety of organic meat. In a recent study in which the authors were trying to determine the impact of rearing conditions (conventional vs organic) on the microbiological quality of raw retail poultry, *Campylobacter* was highly prevalent on both rearing types and *Salmonella* was even higher under the organic rearing conditions [32]. The authors concluded that organic poultry should not be considered superior to conventionally produced one in relation to microbiological quality. In another recent study from Denmark, organic broiler carcasses were found to be more frequently contaminated with *Campylobacter* spp. than conventional carcasses after chilling [33]. The authors estimated the risk per serving from organic carcasses to be 1.7 times higher than that of conventional ones [33]. Why organic rearing conditions would favor more *Campylobacter* contamination? Possible reasons could be the longer rearing periods under organic rearing conditions which might allow more time for *Campylobacter* colonization [34] or that birds reared under free range or cage free organic conditions might be more likely to come in contact with wild birds which are known sources of *Campylobacter*.

Cross-contamination of poultry carcasses during different processing steps in slaughter houses is documented. In a study conducted to check the potential of cross-contamination of *Campylobacter* spp. during slaughter knowing the contamination level at entrance, entrance of a positive flock resulted in contamination of the abattoir environment and the bacteria was isolated throughout the whole processing line [35]. Processing water was contaminated with *Campylobacter i*n some cases before slaughtering and contamination rate was still very high after air cooling [35]. In a more recent study in which *Campylobacter jejuni* isolates from turkey farms and at different stages at slaughter were source tracked using PFGE and *flaA*-short variable region sequencing, contaminating *Campylobacter* free flocks at slaughter was evident [36]. Entering *Campylobacter* free flocks were found to be contaminated with specific PFGE profiles of preceding contaminated flocks [36]. In the same study they even found that the high temperature of defeathering and the drying cool temperature of air chilling did not reduce the contamination by *Campylobacter* [36]. Some of the organic chicken brands used in our study were labeled as air chilled instead of soaking in cold water which was advertised as an effective tool to reduce microbial contamination. Results of the above mentioned study [36] would exclude *Campylobacter* from this claim. This can be explained by proposing that *Campylobacter* spp. might develop an enzymatic protection system against oxidative stress [37]. Following a good hygiene practice by decontaminating and disinfecting the slaughter house environment before the introduction of a new coming flock, while seems difficult, remains of a particular value in reducing *Campylobacter* contamination during slaughter.

Multidrug resistance in our study was generally higher in *C. coli* than in *C. jejuni* isolates. Multidrug resistance was not limited to isolates from conventional sources but was also available in isolates of an organic background. A previous study found a high frequency of tetracycline resistance among both organic and conventional poultry [11]. Most of the Turkey isolates were resistant to more than 7 antimicrobials while most of the recovered chicken isolates were resistant to 5 to 7 antimicrobials. In a study by Cook, *et al*. [10], 13% of their turkey sample recovered strains showed resistance to 5 or more antibiotics. The fact that multidrug resistance (MDR) in our study was generally higher in *C. coli* is not surprising. Ishihara, *et al.* [38] found that their *C. coli* showed resistance to more antimicrobials than the *C. jejuni* isolates.

Among the chicken isolates in our study, the highest antibiotic resistances were to amoxicillin, cephalothin, and doxycycline followed by tetracycline, kanamycin and ampicillin. The lowest resistances were to chloramphenicol, erythromycin, gentamicin and streptomycin (Table **4**). In poultry, resistance to azithromycin, chloramphenicol, ciprofloxacin, erythromycin, gentamycin, kanamycin, streptomycin, clindamycin, nalidixic acid, and tilmicosin was significantly higher in *Campylobacter coli* than *Campylobacter jejuni* (Table **4**). The majority of the recovered turkey isolates (13/19) were resistant to more than 7 antimicrobials. Ge, *et al*. [39] found that the most common resistantce in retail chicken and turkey was to tetracycline (82%), erythromycin (54%), nalidixic acid (41%) and ciprofloxacin (35%). Cui, *et al*. [24] also found the highest resistance among chicken isolates to be to tetracycline (78%), followed by erythromycin (46%), and ciprofloxacin (8%) and that all isolates were susceptible to chloramphenicol. Ge, *et al*. [39] found that turkey isolates showed significantly higher rates of resistance to ciprofloxacin, erythromycin, nalidixic acid, and doxycycline than chicken isolates.

In conclusion, *Campylobacter* was prevalent in retail poultry meat sold in the Tulsa, Oklahoma area with a higher prevalence in chicken than turkey meat. Multidrug resistance was higher in Turkey than chicken isolates and in the recovered *C. coli* strains than in *C. jejuni* ones. Multidrug resistance was not limited to isolates from conventional sources but was also available in isolates of an organic background. The high incidence of *Campylobacter* resistance in retail poultry reported here highlights the danger of the use of antimicrobials as feed additives inpoultry production and its implication on the control of foodborne infections caused by this foodborne bacterium.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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