Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age

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ABSTRACT Thermotolerant *Campylobacter* spp. are a major cause of foodborne gastrointestinal infections worldwide. The linkage of human campylobacteriosis and poultry has been widely described. In this study we aimed to investigate the prevalence, antimicrobial resistance and genetic diversity of *C. coli* and *C. jejuni* in broilers from Ecuador. Caecal content from 379 randomly selected broiler batches originating from 115 farms were collected from 6 slaughterhouses located in the province of Pichincha during 1 year. Microbiological isolation was performed by direct plating on mCCDA agar. Identification of *Campylobacter* species was done by PCR. Minimum inhibitory concentration (MIC) values for gentamicin, ciprofloxacin, nalidixic acid, tetracycline, streptomycin, and erythromycin were obtained. Genetic variation was assessed by RFLP-*flaA* typing and Multilocus Sequence Typing (MLST) of selected isolates. Prevalence at batch level was 64.1%. Of the positive batches 68.7% were positive for *C. coli*, 18.9% for *C. jejuni,* and 12.4% for *C. coli* and *C. jejuni.* Resistance rates above 67% were shown for tetracycline, ciprofloxacin, and nalidixic acid. The resistance pattern tetracycline, ciprofloxin, and nalidixic acid was the dominant one in both *Campylobacter* species. RFLP-*flaA* typing analysis showed that *C. coli* and *C. jejuni* strains belonged to 38 and 26 profiles respectively. On the other hand MLST typing revealed that *C. coli* except one strain belonged to CC-828, while *C. jejuni* except 2 strains belonged to 12 assigned clonal complexes (CC**s**). Furthermore 4 new sequence types (STs) for both species were described, whereby 2 new STs for *C. coli* were based on new allele sequences. Further research is necessary to estimate the impact of the slaughter of *Campylobacter* positive broiler batches on the contamination level of carcasses in slaughterhouses and at retail in Ecuador.

Key words: *Campylobacter*, Ecuador, genetic types, antimicrobial resistance, broilers

INTRODUCTION

Thermotolerant *Campylobacter* spp. are a major cause of foodborne gastrointestinal infections worldwide. Human campylobacteriosis in its acute phase is characterized by diarrhea, fever, abdominal cramps, and vomiting and has been linked to the development of Guillain-Barré syndrome, reactive arthritis,

and irritable bowel syndrome as complications after the acute phase of the disease (Loshaj-Shala et al., 2015). The WHO (2015) estimated that *Campylobacter* caused 37.600 deaths per year worldwide. For 2014, 237,642 campylobacteriosis cases were registered in the European Union (EFSA and ECDC, 2015). However it has been estimated that the real number of cases occurring yearly may be 9 million (Havelaar et al., 2009). Diarrheal illness caused by these pathogens are especially important in developing countries where the infection in children under the age of two years is frequent and may lead to death (WHO, 2011). *Campylobacter* has been associated to 11.3 to 21% of diarrhea episodes in children from low-income countries (Platts-Mills and Kosek, 2014). However, the lack of studies on the epidemiology of *Campylobacter* in developing countries could lead to the underestimation of the burden of *Campylobacter* infections in these regions (Platts-Mills

and Kosek, 2014). In Ecuador data about *Campylobacter* infections in humans is very limited. *Campylobacter* has been reported in Ecuadorian low income communities as a possible cause of diarrhea in humans (Vasco et al., 2014). Furthermore, it has been estimated that 50 to 80% of campylobacteriosis cases may be attributed to the chicken reservoir as a whole being poultry the main source of *Campylobacter* transmission within the European Union (Skarp et al., 2015).

In general, *Campylobacter* infections do not require antibiotic treatment, however the use of erythromycin, tetracycline, and quinolones is recommended in severe cases (WHO, 2011).

Worldwide the use of antibiotics in husbandry practices is a major concern since this may promote the development of resistant and even multidrug-resistant bacteria. Antibiotics in poultry production systems are widely used to prevent, control, and treat bacterial infections as well as growth promoters in a large number of countries (Seiffert et al., 2013). These facts are of special relevance in developing countries where misuse of antibiotics and the lack of control over their usage is a problem to be addressed (Reardon, 2014). In Latin-American countries, increased rates of antimicrobial resistant *Campylobacter* have been reported (Pollett et al., 2012; Sierra–Arguello et al., 2016).

In Ecuador chicken meat is frequently consumed and its demand increased over the years (CONAVE, 2014). Although Ecuadorian poultry industry only provides chicken meat for local consumption up to now, it is expected that in the future it can have access to international markets once sanitary conditions are better understood and controlled. Despite of the importance of *Campylobacter* as a foodborne pathogen, little is known about its epidemiology in poultry farms, slaughterhouses, and retail stores in the main centers of production and consumption of poultry products in Ecuador. This information may help to establish surveillance programs and intervention measures regarding to the presence and antimicrobial resistance of *Campylobacter* in Ecuadorian poultry.

The aim of this study was to investigate the prevalence, antimicrobial resistance and genetic profiles of *Campylobacter* in broilers slaughtered in industrial facilities in the province of Pichincha in Ecuador.

MATERIALS AND METHODS

Study Design and Sampling

Pichincha, the province where Quito, the capital city of Ecuador, is located, was selected as the area for the collection of samples since in this province and the surrounding ones 36% of the total Ecuadorian broiler production is located (CGSIN and MAGAP, 2015).

Eight large slaughterhouses are located in Pichincha (CGSIN and MAGAP, 2015). All of them were contacted and asked for their willingness to cooperate in the study. Based on these results sampling was performed in 6 slaughterhouses. From June 2013 to July 2014, a total of 379 batches (birds coming from one house and slaughtered on the same day) were sampled. All sampled batches from a same farm originated from different houses or birds reared on different periods in the same house. In Ecuador commercial broiler management at the farm includes total depopulation of houses, removal of the litter after every reared batch, cleaning and disinfection of the house followed by a down period of 8 to 15 days. All sampled batches were commercially reared and slaughtered at the age of 6 to 7 weeks. From each batch, one caecum from 25 randomly selected chickens was collected, and transported in an ice box within 1 hour to the laboratory for bacteriological analysis.

Isolation and Identification of Campylobacter *spp.*

The content from the 25 collected caeca was aseptically pooled. Therefore, all caeca were immersed in ethanol, and after evaporation of the ethanol approximately 1 g content/cecum was collected in a sterile plastic bag. The pooled sample was homogenized by hand during 1 min. after the addition of 225 mL buffered peptone water (218103, Difco, BD, Sparks, MD) and a loopful (10 μL) from each homogenate was directly streaked onto a modified Charcoal Cefoperazone Deoxycholate Agar (**mCCDA**) (*Campylobacter* blood free selective medium CM0739 plus selective supplement SR0155H [Oxoid, Cheshire, UK]). Plates were incubated under microaerobic conditions at 41.5°C for 48 h. Three presumptive *Campylobacter* colonies were confirmed by Gram staining and microscopic observation. Colonies containing bacteria with a typical shape were subcultured on mCCDA. After incubation under microaerobic conditions at 41.5°C for 48 h the DNA of one colony per plate was extracted by boiling for 10 minutes in 100 μL of DNA free water. The rest of the culture was transferred into sheep blood and stored at −80°C. Multiplex PCR described by Vandamme et al. (1997) was performed for identification of *Campylobacter* species. PCR results indicating the presence of both *C. jejuni* and *C. coli* were retested after sub-culturing of one colony on mCCDA until only one species was detected.

From each positive batch one isolate was randomly selected for further characterization.

Antimicrobial Resistance

Antimicrobial resistance was evaluated in one isolate per sample. The minimum inhibitory concentration (**MIC**) was determined using the EUCAMP2 plates (Thermo Scientific, West Palm Beach, FL). The tests were performed according to the manufacturer instructions. The following antibiotics were evaluated: gentamicin, ciprofloxacin, nalidixic acid, tetracycline, streptomycin, and erythromycin. *Campylobacter jejuni* ATCC

Table 1. *Campylobacter* positive batches in relation to the number of tested batches per farm.

Number of sampled batches/farm	Number of farms	Number of farms with 0 to 6 positive batches						
		0	1	2	3	4	5	6
1	31	15	16					
2	19	2	7	10				
3	12	1	2	8	1			
4	18		1	3	9	5		
5	15		2	3	1	5	4	
6	16			4	4	4	3	1
7	2		1			1		
8	1							1
9	1							1
Total	115	18	29	28	15	15	7	3

33560 was used as the quality control strain. Epidemiological breakpoint values from the European Committee on Antimicrobial Susceptibility Testing were considered to determine bacterial antibiotic resistance (EUCAST, 2015).

Restriction Fragment Length Polymorphism of the flaA gene (flaA-RFLP)

One *Campylobacter* isolate per positive batch was tested. For the PCR the consensus pair of primers for the *flaA* gene described by Wassenaar and Newell (Wassenaar and Newell, 2000) and the reagents and conditions described by Nachamkin et al. (Nachamkin et al., 1993) were applied. For restriction fragment length polymorphism (**RFLP**) analysis *flaA* PCR amplicons were treated with restriction enzyme *DdeI* (Thermo Scientific, West Palm Beach, FL). PCR amplicons (7 μL) were digested according to the manufacturer's instructions and then separated by electrophoresis for 1:40 hours at 120 V. The gels were stained and photographed. The relatedness among the *flaA*-RFLP profiles was analyzed with GelCompar II software v. 6.6 (Applied Maths, Sint-Martems-Latem, Belgium). Bands representing fragments between 200 bp and 1,100 bp in size were included in the analysis. A similarity dendrogram was constructed by the unweighted pair group method using arithmetic averages algorithm (UPGMA). DICE similarity coefficient with a tolerance position of 1% was calculated. A *flaA*-RFLP genotype was assigned on the basis of the difference in the presence of at least one band in the *DdeI* fingerprint.

Multilocus Sequence Typing

Multilocus Sequence Typing (**MLST**) was carried out on all *C. jejuni* isolates that still could be subcultured (40 isolates). For *C. coli*, 40 randomly selected isolates representing 40 farms were typed by MLST.

MLST was performed by the protocol previously described (PubMLST.org, 2016). Sequence types (**STs**) and clonal complexes (**CCs**) were assigned by submitting DNA sequences to the *Campylobacter* MLST database website (http://pubmlst.org/campylobacter).

Novel alleles and STs were submitted to the MLST database for the assignation of new numbers.

Statistical Analysis

Statistical analysis was carried out with STATA/IC 11.0 (StataCorp LP, College Station, TX). The survey design corrected prevalence estimates of *Campylobacter* at batch level were obtained using the linearized Taylor series method. Farms was identified as first-stage cluster. To determine the prevalence of *Campylobacter* at farm level, a farm was considered positive when at least one of the sampled batches was positive. Farms were assumed to be independent.

Differences of antibiotic resistances between *C. coli* and *C. jejuni* were calculated by the chi-square test. Proportions were considered statistical different when the *P* value was below 0.05.

RESULTS

Prevalence of Campylobacter spp

The 379 sampled batches originated from 115 farms (1 to 9 batches per farm). From all tested batches 243 (64.1%; CI$_{95\%}$: 58.7% to 69.6%) were *Campylobacter* positive and originated from 97 farms (84.4%; Confidence Interval (**CI**)$_{95\%}$: 77.6% to 91.1%). From 84 farms, more than one batch was sampled. The number of times that those farms had *Campylobacter* positive batches ranged from 1 to 6 (Table 1). Initial PCR speciation demonstrated that 167 batches (68.7%; CI$_{95\%}$: 62.9% to 74.6%) were positive for *C. coli*, 46 (18.9%; CI$_{95\%}$: 14.0% to 23.9%) for *C. jejuni* and 30 (12.4%; CI$_{95\%}$: 8.2% to 16.5%) for *C. coli/ C. jejuni*. Subculturing of the mixed cultures yielded 22 *C. coli* and 8 *C. jejuni* isolates.

Antimicrobial Resistance

Twenty-five isolates (19 *C. coli* and 6 *C. jejuni*) could not be sub-cultured from $-80°C$ for MIC test; hence 218 isolates were tested (170 *C. coli* and 48 *C. jejuni*). The MIC distributions for the different antibiotics of *C. coli* and *C. jejuni* are shown in Tables 2 and 3

Table 2. Distribution of the minimal inhibitory concentration values for 170 *C. coli* isolates collected from broiler batches.

Antibiotic	Number of *C. coli* isolates with minimal inhibitory concentrations (μg/μL)[1]										
	0,12	0,25	0,5	1	2	4	8	16	32	64	128
Gentamicin	106	49	8	5	1		1				
Streptomicyn			1	120	30			19			
Erythromycin			38	33	33	22	6		31		7
Tetracycline		34	15	6		1	1	89		24	
Ciprofloxacin			1		2	134	5	28			
Nalidixic acid						1		1	11	157	

[1]Full vertical lines indicate epidemiological break points for resistance described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

Table 3. Distribution of the minimal inhibitory concentration values for 48 *C. jejuni* isolates collected from broiler batches.

Antibiotic	Number of *C. jejuni* isolates with minimal inhibitory concentrations (μg/μL)[1]										
	0,12	0,25	0,5	1	2	4	8	16	32	64	128
Gentamicin	42	3	2			1					
Streptomicyn		2	5	35	2	2		2			
Erythromycin			23	21	2				1		1
Tetracycline		6	2		1		1	28		10	
Ciprofloxacin	1				2	33	3	9			
Nalidixic acid								1	1	46	

[1]Full vertical lines indicate epidemiological break points for resistance described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

respectively. *C. coli* and *C. jejuni* showed very low resistance rates for gentamicin and the resistance rate was not statistically different between both species ($P = 0.752$). For streptomycin the resistance rates were 11.2% and 8.3% for *C. coli* and *C. jejuni* respectively ($P = 0.199$). Resistance rate for erythromycin was statistically higher for *C. coli* (25.9%) compared to *C. jejuni* (4.2%) ($P = 0.024$). In contrast the resistance rates for tetracycline was statistically higher for *C. jejuni* (83.3%) than for *C. coli* (67.6%) ($P = 0.016$). Resistance rates of *C. coli* for ciprofloxacin and nalidixic acid were 100% and 99.4% respectively ($P = 0.086$). Similarly, *C. jejuni* presented resistance rates of 97.9% and 100% for ciprofloxacin and nalidixic acid respectively ($P = 0.558$).

C. coli and *C. jejuni* isolates showed 8 and 6 different resistance patterns respectively. *C. coli* presented resistance against 1 up to 6 antibiotics, whereas for *C. jejuni* resistance against 2 up to 6 antibiotics were involved. The resistance pattern 5 (*C. coli*: 42.9%; *C. jejuni*: 72.9%) was the most frequent one for both species (Table 4). Pattern 3 and, patterns 1, 4, and 6 were presented exclusively for *C. jejuni* and *C. coli* respectively.

RFLP-flaA *Typing*

For RFLP-*fla*A typing 38 isolates (26 *C. coli* and 12 *C. jejuni*) could not be sub-cultured from −80°C; hence 163 *C. coli* and 47 *C. jejuni* isolates were tested. From all tested isolates *1 C. coli* and 7 *C. jejuni* did not present bands in RFLP-*fla*A typing. For *C. coli* 38 profiles were obtained, from which 19 profiles contained more than one strain. Each of the later profiles con-

Table 4. Antibiotic resistance patterns of *C. coli* and *C. jejuni* isolates.

Pattern	Resistance pattern[1]	*C. coli* (%)	*C. jejuni* (%)
1	C	1(0.6)	0
2	CN	49(28.8)	8(16.7)
3	TN	0	1(2.1)
4	CEN	5(2.9)	0
5	CTN	73(42.9)	35(72.9)
6	CTEN	23(13.5)	0
7	SCTN	3(1.8)	2(4.2)
8	SCTEN	14(8.2)	1(2.1)
9	GSCTEN	2(1.2)	1(2.1)
Total		170(100)	48(100)

[1]C, Ciprofloxacin; E, erythromycin; G, Gentamicin; N, Nalidixic; S, Streptomycin; T, Tetracycline.

tained 2 up to 25 strains. For *C. jejuni* 26 profiles were obtained, from which 7 profiles contained 2 to 7 strains. Most of the strains within a RFLP-*flaA* profiles originated from different farms. However, for profile 5, 9, 18, 19, 21, 22 (C. *coli*), and 20 (*C. jejuni*) two strains were found in a single farm, and for profile 16 (*C. coli*) two farms yielded 2 and 3 strains respectively (Table 5).

MLST Typing

From the 40 *C. coli* isolates selected for MLST 39 belonged to CC-828 and 1 did not have an assigned CC (ST-1581). The most frequent STs were ST-5777 (9 isolates), followed by ST-829 (8 isolates) and ST-828 (6 isolates) (Figure 1). From the 40 *C. jejuni* isolates selected for MLST the most common CCs were CC-574 (9 isolates), CC-257 (7 isolates), CC-353 (5 isolates), and CC-354 (5 isolates) (Figure 2). Two *C. jejuni* isolates did not correspond to an assigned CC. The most

Table 5. *Campylobacter* spp. RFLP-*flaA* profiles with more than one isolate.

Campylobacter spp.	ID of RFLP-*flaA* profiles	Number of isolates within each profile	Number of origin farms
C. coli	22	2	1
	7, 17, 25, 31	2	2
	1, 11, 32	3	3
	21	5	4
	5	6	5
	3, 30	7	7
	6	9	9
	8	10	10
	18	10	9
	9	12	11
	29	12	12
	16	22	19
	19	25	24
C. jejuni	7, 8, 9, 16, 19	2	2
	14	4	4
	20	7	6

ST-diverse CC was CC-353 (4 STs) followed by CC-257 (3 STs), CC-52 (2 STs), CC-354 (2 STs), CC-464 (2 STs), and CC-21 (2 STs).

In total, 9 *C. coli* and 7 *C. jejuni* strains belonged to STs which were not reported previously. Sequence data from those strains were submitted to the *Campylobacter* MSLT database (PubMLST.org, 2016) leading to the assignation of 8 novel ST numbers (4 STs for each species) (Table 6). Two novel STs within *C. coli* (ID PubMLST 48107 and 48108) resulted from novel allele sequences: 5 strains had a novel allele sequence for *aspA*, of which one strain had also a novel allele sequence for *tkt*.

Comparison of RFLP-flaA profiles and MLST Data

When comparing MLST data with RFLP-*flaA* profiles, *C. coli* STs 8315, 8317, 828, 5777, and 829 included 2, 2, 3, 4, and 5 RFLP-*flaA* profiles respectively, while RFLP-*flaA* profiles 18, 21, 16, and 19 included 2, 2, 4, and 4 different ST types. For *C. jejuni* 4 STs (6244, 8308, 8309, and 8310) had two RFLP-*flaA* profiles and only the RFLP-*flaA* profile 14 included 2 ST types. No association of RFLP-*flaA* profiles within STs was found regarding the origin of the isolates.

DISCUSSION

Our findings demonstrated that the prevalence of *Campylobacter* in broiler batches at slaughter age was 64.1%. Studies from other Latin American countries showed different prevalences. From Brazil and Costa Rica, 100.0% respectively 80.0% of the flocks were reported to be positive for *Campylobacter* when ceca samples were studied (Giombelli and Gloria, 2014; Zumbaco-Gutiérrez et al., 2014). On the other hand, in Argentina and Chile *Campylobacter* was found in 33.3% of samples (Rivera et al., 2011; Zbrun et al., 2013a) while in Peru Tresierra-Ayala et al. (1995) reported a prevalence of 35%. Other tropical countries such as Vietnam and South Africa have reported a prevalence of 31.9 and 14.2% respectively (Jonker and Picard, 2010; Carrique-Mas et al., 2014). Although different prevalences are shown in developing countries, it should be keep in mind that differences in methodologies can make direct comparison of results difficult. Moreover,
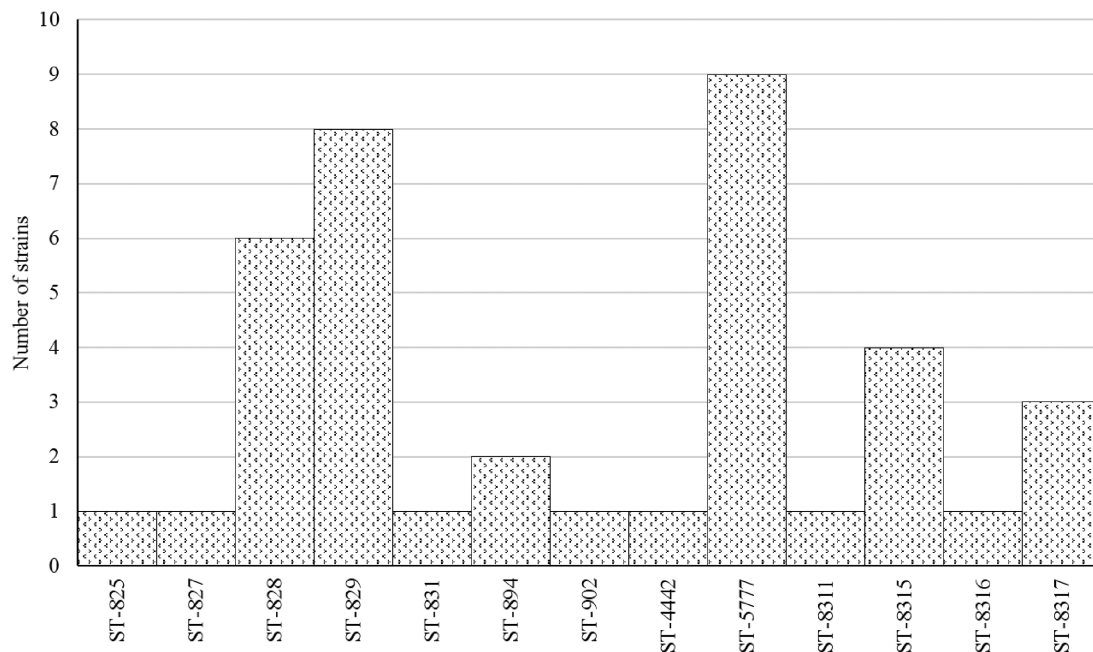
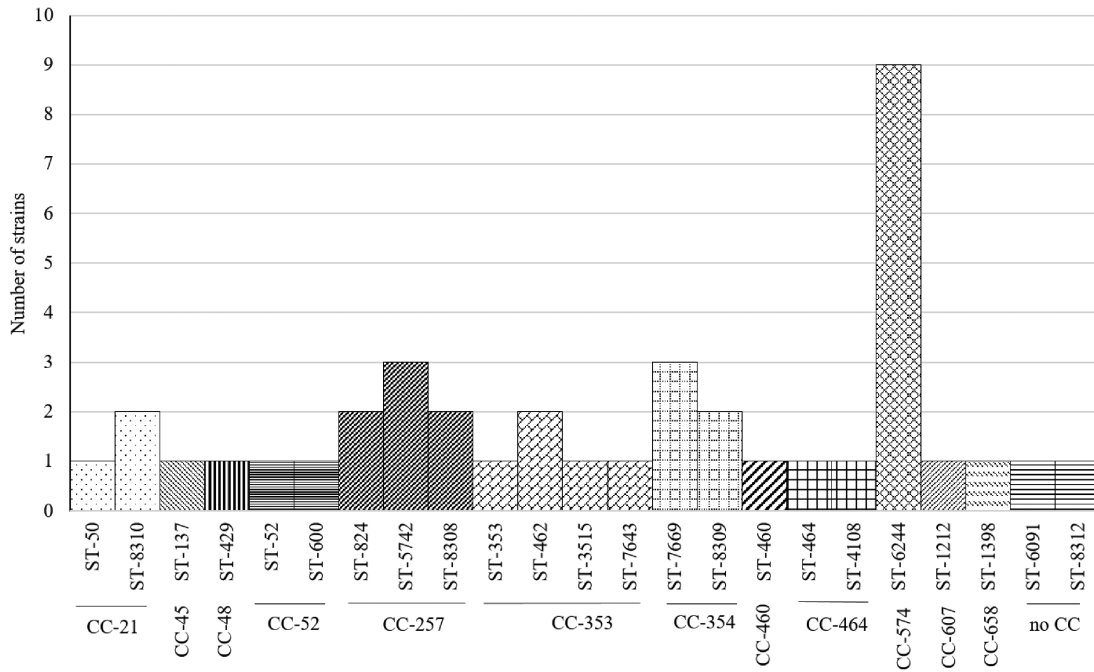**Figure 1.** Distribution of STs among the 39 *C. coli* strains belonging to clonal complex 828.

Figure 2. Distribution of STs and clonal complexes among the 40 *C. jejuni* strains.

Table 6. MLST profiles of novel STs identified in *Campylobacter* strains.

Campylobacter species	No. of isolates	Clonal complex	Sequence type	MLST allelic profile[a]							ID on the PubMLST
				aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*	
C. jejuni	2	257	8308	9	2	4	62	606	5	6	48096
C. jejuni	2	354	8309	8	10	95	2	10	12	6	48097
C. jejuni	2	21	8310	2	1	5	672	11	1	5	48113
C. jejuni	1	NA[b]	8312	2	84	5	10	11	3	6	48099
C. coli	1	828	8311	33	39	30	82	373	56	17	48106
C. coli	4	828	8315	**441**	39	30	82	373	47	17	48107
C. coli	1	828	8316	**441**	39	30	82	113	**641**	17	48108
C. coli	3	828	8317	33	39	30	82	373	47	17	48110

[a]New allele sequences are given in bold.
[b]NA, not assigned.

obtained data indicated that at least 84.3% of farms delivered *Campylobacter* positive batches. For farms delivering only *Campylobacter* negative batches only a maximum of 3 batches were tested. For those farms it can be hypothesized that when more batches would be sampled also these farms would deliver *Campylobacter* positive batches for slaughter. On the other hand, the number of positive batches per farm variated considerably which is in concordance with the observations described by Mc-Dowell et al. (2008). This variation may be attributed to different risk factors for the introduction of *Campylobacter* in broilers (Adkin et al., 2006; Torralbo et al., 2014; Sandberg et al., 2015).

Considering *Campylobacter* species, *C. coli* was the dominant species in positive batches. This contrasts with other studies from Latin America where *C. jejuni* has been demonstrated to be the most prevalent species in broilers (Tresierra-Ayala et al., 1995; Rivera et al., 2011; Zbrun et al., 2013b; Giombelli and Gloria, 2014; Zumbaco-Gutiérrez et al., 2014). *C. jejuni* has also been demonstrated as the most common *Campylobacter* species from broilers at slaughter age in China and South Africa (Jonker and Picard, 2010; Ma et al., 2014). Meanwhile, the European baseline study on *Campylobacter* in broilers indicated that the proportion of *C. coli*/*C. jejuni* varied considerable between countries and this proportion was generally higher in southern countries than in northern countries (EFSA, 2010).

In this study, *C. coli* and *C. jejuni* presented high resistance rates to ciprofloxacin, nalidixic acid and tetracycline while erythromycin, gentamicin, and streptomycin showed lower resistance rates. This is in accordance with a study in Brazil where high resistance rates to ciprofloxacin, nalidixic acid, and tetracycline, and low resistance rates to erythromycin and gentamicin were reported (Ferro et al., 2015). Besides, a similar low resistance rate for erythromycin, a low resistance rate for ciprofloxacin (11,8%) was reported from Chile (Rivera et al., 2011).

In contrast with the data reported in European Union, this study showed that *C. jejuni* presented

higher resistance rates for tetracycline than *C. coli* (EFSA, 2015). On the other hand, a higher resistance rate to erythromycin was shown for *C. coli*, which is consistent with data from China and South Africa that showed higher erythromycin resistance rates for *C. coli* (92.0% and 72.7% respectively) than for *C. jejuni* (18.8% and 20% respectively) (Jonker and Picard, 2010; Ma et al., 2014).

High resistance rates for (fluoro)quinolones and tetracycline found in the present study may be explained by the common use of these antibiotics as therapeutics in Ecuadorian poultry farms. However it is not clear why the resistance rate to tetracycline was higher for *C. jejuni* than for *C. coli* in Ecuador. The low antimicrobial resistance rates to aminoglycosides and macrolides for *C. jejuni* found in this study indicates that gentamicin and erythromycin can still be used for the treatment of human campylobacteriosis when necessary (WHO, 2011). However, changes in resistance rates presented in this research have to be monitored by the implementation of antimicrobial resistance surveillance on *Campylobacter* in Ecuador.

Campylobacter typing by RFLP-*fla*A has been used based on the highly conserved character of this gene. It has also been shown to be a cost-effective alternative to more costly methodologies (Djordjevic et al., 2007). The use of RFLP-*flaA* as the only typing method is questioned due to intra- and intergenomic recombination within the flagellin genes (Eberle and Kiess, 2012) which can make the comparison of isolates over time difficult. In contrast MLST typing is a more reliable method since it is based on changes in allele sequences of determined housekeeping genes and a library of MLST types is available to compare results from all over the world (PubMLST.org, 2016). Our results showed that the combination of RFLP-*flaA* and MLST typing led to a further differentiation of a number of isolates. This is in concordance with the results of Duarte et al. (2016) who demonstrated that the combination of both RFLP-*flaA* and MLST had a higher discriminatory power than both methods separately.

Based on one isolate per batch, our results indicated that a large variation of genetic types were present in Ecuadorian broiler batches. Some genetic types seemed to be more widespread than other ones. Additionally, RFLP-*flaA* data suggested that over time the persistence of specific genetic types on farms is limited. Analyses of the variable region in the *flaA* locus (*flaA*-SVR) have demonstrated that more than one *Campylobacter* genotype may be present in the same farm (Jorgensen et al., 2011; O'Mahony et al., 2011; Prachantasena et al., 2016). Moreover, some batches were simultaneously colonized with *C. coli* and *C. jejuni* in the present study.

To the best of our knowledge, this study is the first report that showed *Campylobacter* MSLT types from commercial broiler batches in Andes region of Latin America. In this study 39 out of 40 *C. coli* isolates belonged to CC-828. Predominant distribution of *C. coli* within CC-828 has also been reported in Europe (Levesque et al., 2013; Piccirillo et al., 2014). It is suggested that the low diversity of CCs in *C. coli* can be attributed to the presence of a 3-clade *C. coli* population structure. In this genetic structure, horizontal gene transfer within each clade would be more common than among members of different clades (Sheppard and Maiden, 2015), resulting in a limited number of CCs.

Interestingly, the new reported ST-8315 was present in 4 (10%) *C. coli* isolates. The implication of this ST in the epidemiology of *Campylobacter* needs further research. From the 40 *C. jejuni* isolates tested, the majority belonged to CC-574 (9 isolates), CC-257 (7 isolates), CC-353 (4 isolates), CC-354 (5 isolates), and CC-21 (3 isolates). In Great Britain, an important number of *C. jejuni* strains were grouped in CC-257, CC-353, and CC-574 (Jorgensen et al., 2011). Meanwhile, CC-354 has been found in commercial poultry in Thailand (Prachantasena et al., 2016). This is in accordance with our results where these CCs were found in 72.5% of the tested samples. Additionally, a Canadian study reported CC-353 in *C. jejuni* isolates from chickens originated in Peru, Bolivia and Argentina (Lévesque et al., 2008). Other less common CCs found in this study (CC-45, CC-48, CC-52, CC-460, CC-658, CC-464, and CC-607) have also been reported in poultry from Europe, Africa, Asia, and North America (Lévesque et al., 2008; Griekspoor et al., 2010; O'Mahony et al., 2011; Kittl et al., 2013; Ngulukun et al., 2016; Zeng et al., 2016).

Moreover, a study in Ecuador demonstrated that CC-353, CC-354 and CC-607 were present in *C. jejuni* isolates from backyard poultry and other domestic animals kept in households (Graham et al., 2016). A query in the *Campylobacter jejuni/coli* PubMSLT database (PubMLST.org, 2016) (Last accessed: 21/07/2016) showed that in Latin America, Brazil, and Uruguay reported *Campylobacter* MLST profiles from chicken samples. These isolates belonged to CC-257, CC-52 (*C. jejuni*), and CC-828 (*C. coli*) in Uruguay, while in Brazil a no determined CC (ST-7370) was reported.

Although there are new STs in some of our strains *C. coli* isolates, the most of CCs found in this study have been reported in chicken samples (PubMLST.org, 2016).

This study gives insights on the epidemiology of *Campylobacter* in commercial reared poultry in Ecuador. Since high levels of *Campylobacter* on carcasses has been linked to an increasing risk of *Campylobacter* infections in humans (EFSA, 2011), it would be interesting to collect data about the contamination including contamination levels, of broiler meat and related risk factors for contamination at the following stages of the broiler meat chain. *Campylobacter* types and its antimicrobial resistance have not been studied from humans in Ecuador. Therefore, it is not possible to link human campylobacteriosis to the genotypes found in this study. Therefore further research on *Campylobacter* isolates from the broiler meat chain and humans may give more insights on the epidemiology of *Campylobacter* in Ecuador.

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