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Occurrence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from domestic animals from Southern Ecuador

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ABSTRACT: *Occurrence and antimicrobial resistance profiles of Campylobacter jejuni and C. coli strains isolated from fecal samples of dogs, pigs, cows and hens in southern Ecuador were studied. Of the 250 samples studied, 84 (33.6%) were Campylobacter positive, with C. jejuni being more frequent (78.6%) than C. coli (21.4%), with the exception of porcine samples, from which C. coli was the only species isolated. Multidrug resistance was reported in 10 Campylobacter strains (11.9%), four C. jejuni and six C. coli, and was always associated with nalidixic acid and ciprofloxacin resistance. All of the amoxicillin-resistant strains were susceptible to amoxicillin-clavulanic acid and were therefore beta-lactamase producers. However, one strain of C. jejuni remained resistant with additional resistance to gentamycin. This is an uncommon resistance pattern in Campylobacter and could reflect different resistance mechanisms.*
Key words: *Campylobacter, reservoirs, domestic animals, antimicrobial resistance.*

Ocorrência e comportamento antimicrobiano de espécies termotolerantes de *Campylobacter* isoladas de animais domésticos no sul do Equador

RESUMO: *A ocorrência e resistência antimicrobiana de cepas de Campylobacter jejuni e C. coli isoladas de amostras fecais obtidas de cães, suínos, vacas e galinhas no sul do Equador foram determinadas. Das 250 amostras estudadas, 84 (33.6%) foram Campylobacter positivas, sendo C. jejuni mais frequente (78.6%) que C. coli (21.4%) com a exceção das amostras de suínos, das quais só fora isolada a espécie C. coli. Multirresistência foi encontrada em 10 cepas (11,9%), quatro C. jejuni e 6 C. coli, sempre associada* à resistência ao ácido nalidíxico e à ciprofloxacina. Todas as cepas resistentes à ampicilina foram susceptíveis para amoxicilina-ácido clavulánico demonstrando que foram produtoras de *beta-lactamase. Porém, uma cepa de C. jejuni permaneceu sendo resistente, apresentando, também, resistência à gentamicina. Este é um padrão de multirresistência incomum em Campylobacter e pode refletir diferentes mecanismos de resistência.*
Palavras-chave: *Campylobacter, reservatórios, animais domésticos, resistência antimicrobiana.*

At present, 27 species and 12 subspecies are included within genus *Campylobacter* (LPSN BACTERIO.NET, 2016; PICCIRILLO et al., 2016). They are found colonizing the intestinal tract of a wide range of animals, mainly birds and mammals, recognized as their reservoir. Within these species, *Campylobacter jejuni* and *C. coli* have acquired great importance from a public health perspective, since they are considered as frequent zoonotic agents of diarrhea for human beings worldwide (KAAKOUSH et al., 2015; NARVAEZ-BRAVO et al., 2017).

Commonly, animals that harbor *Campylobacter* in their intestinal tract are asymptomatic carriers and can be source of contamination for humans, other animals, the environment and even food of animal origin like meat and milk (FERNÁNDEZ, 2011; EPPS et al., 2013; NARVAEZ-BRAVO et al., 2017).

C. jejuni and *C. coli* have been isolated from different animals in several South American countries, with frequencies ranging from 16.96% in dogs to 94% in chickens (FERNÁNDEZ, 2011). In Ecuador, the information about these bacteria is scarce, with a limited number of publications available. VASCO et al. (2016) reported *C. jejuni* and *C. coli* in samples from chickens (76.2%), dogs (27.5%), pigs (47.2%) and cattle (28.6%) in a semirural community East of Quito; whereas VINUEZA et al. (2017) reported that the prevalence at a batch level was 64.1%, while studying 379 broiler batches from 115 farms located

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in Pichincha province. In Southern Ecuador, these bacteria were previously isolated from fecal samples of dogs (10%) (TOLEDO et al., 2015), chicken livers used for human consumption (62.7%) (SIMALUIZA et al., 2015) and domestic backyard chickens (41.7%) (OCHOA et al., 2016).

With the goal to establish, in the southern region of Ecuador, the occurrence and the antimicrobial resistance of *C. jejuni* and *C. coli* isolated from a reduced sample of domestic animals (dogs, pigs, cows and hens) commonly considered as reservoirs for these bacteria.

Fecal samples, obtained by spontaneous emission from 250 domestic animals raised around Loja city (3°59' Lat S; 79°12' Long W) were studied. Taking into consideration that the owners only allowed to sample a limited number of animals, sampling was performed using a non-probabilistic method for convenience. The studied animals were 60 pet dogs, obtaining 20 samples from three different veterinary clinics; 30 fattening pigs obtaining 15 samples from two pigs farms; 100 dairy cows obtaining 25 samples from four different farms and 60 backyard hens obtaining 15 samples from three different farms.

Following collection, each fecal sample was inoculated onto a transport-enrichment medium (FERNÁNDEZ, 1992) consisting of (composition/L) Brucella broth 28g, agar 1.5g, sodium metabisulphite 0.5g, ferrous sulphate 0.5g, sodium pyruvate 0.5g, trimethoprim 10mg, rifampicin 15mg, colistin 10,000IU, amphotericin 10mg and horse blood 30mL, and transported to the laboratory under a microaerobic atmosphere obtained using commercial generator envelopes (FERNÁNDEZ, 1992; TOLEDO et al. 2017). After an enrichment period of 24h at 42°C under microaerobic conditions, aliquots of each enriched sample were seeded on Butzler medium plates and incubated for 48h under the same conditions described above. Suspected colonies were initially identified by phenotypic test (Gram stain, oxidase, catalase, hippurate and indoxylacetate "hydrolysis) and confirmed by the multiplex PCR test as described by YAMAZAKI-MATSUNE et al. (2007).

Susceptibility to nalidixic acid, ciprofloxacin, erythromycin, gentamycin, amoxicillin and amoxicillin/clavulanic acid was determined by the disk diffusion method following the recommendations for *Campylobacter* of the European Committee on Antimicrobial Susceptibility Testing - EUCAST (SIFRÉ et al., 2015). Amoxicillin resistant strains were tested for β-lactamase production using the cefinase β-lactamase detection discs and the disc diffusion susceptibility test for amoxicillin-clavulanic acid (LACHANCE et al., 1991; IOVINE, 2013). The differences between groups was assessed by a Ji-squared test.

As shown in table 1, 33.6% of the samples were positive for *Campylobacter* sp. The highest isolation rate was reported among hens (68.3%), followed by dogs (33.3%), pigs (26.7%) and cows (15%). Similar distribution was reported by VASCO et al. (2016) in Central Ecuador; however, with some differences in the isolation percentages in chickens 76.2%, pigs 47.2%, dogs 27.5% and cattle 28.6%. Both *Campylobacter* species have been isolated from these animals in different South American countries, also with high frequencies (FERNÁNDEZ, 2011), thus indicating that these animals are important reservoirs of the bacteria in South America. Therefore, it would be important to establish the existence of the epidemiological relationship between strains isolated from these animal reservoirs and cases of diarrhea in humans. Previous studies have demonstrated that *Campylobacter* isolates from humans exhibited characteristics identical to those isolated from domestic animals (ISHIHARA et al., 2006; GONZÁLEZ-HEIN et al., 2013).

C. jejuni was most frequently isolated (78.6%) versus *C. coli* (21.4%) from all animals studied, with the exception of pigs, from which the only isolated species was *C. coli*. However, the proportion

Table 1 - *Campylobacter* species distribution among domestic animals from Southern Ecuador.

Source	-----Samples (n)-----	---------Positive samples---------		-------------*C. jejuni*-------------		-----------------*C. coli*---------------	
		(n)	(%)	(n)	(%)	(n)	(%)
Dogs	60	20	33.3	17	85.0	3	15.0
Pigs	30	8	26.7	0	0.0	8	100
Cows	100	15	15.0	12	80.0	3	20.0
Hens	60	41	68.3	37	90.2	4	9.8
Total	250	84	33.6	66	78.6	18	21.4

of *C. coli* isolates, compared to the total isolates of both species, was 21.4%, which is consistent with the *C. jejuni*/*C. coli* ratio (close to 25%) observed in different types of samples in several countries in South America (FERNÁNDEZ, 2011). The isolation of *C. coli* as the only species in pigs is not surprising, since these animals are considered the primary reservoir from which *C. coli* is isolated in high frequency (ALTER et al., 2005; GEBREYES et al., 2005) or as the only species (KEMPF et al., 2017).

Table 2 shows the antimicrobial resistance of the tested *Campylobacter* strains. Multidrug resistance, defined as the resistance to three or more antimicrobials (HAKANEN et al., 2003), was reported in 10 strains (11.9%), four corresponding to *C. jejuni* and six to *C. coli*. Multidrug resistance was always associated with nalidixic acid and ciprofloxacin resistance since all 10 strains exhibited resistance to both antimicrobials. The association of multidrug resistance with fluoroquinolone resistance was described in clinical strains by HAKANEN et al., in 2003. Currently, fluoroquinolone resistance in *Campylobacter* species is an emerging public health problem in several Latin American countries, and is frequently associated with multidrug resistance (FERNÁNDEZ & PÉREZ-PÉREZ, 2016; VINUEZA et al., 2017).

Some peculiarities could be observed from the results shown in table 2. All of the strains of *C. jejuni* and *C. coli* isolated from dogs were susceptible to nalidixic acid and ciprofloxacin. This is in contrast with the previous data from Latin America where *Campylobacter* strains resistant to fluoroquinolones were reported among pet dogs (FERNÁNDEZ & PÉREZ-PÉREZ, 2016; TOLEDO et al., 2015). It is plausible that the results could be associated with the condition of these pets, since they are not exposed with the same frequency to the transmission factors of *Campylobacter* as feral dogs (IANNINO et al., 2017). Besides, fluoroquinolones are not first-line antimicrobials in the treatment of some infections in domestic dogs (GUARDABASSI et al., 2004). All of

Table 2 - Antimicrobial behavior of *Campylobacter* species isolated from domestic animals of southern Ecuador.

ANIMALS OF ORIGIN (n)	NAL		CIP		ERY		GEN		AMO		AMO-CLAV	
	S	R	S	R	S	R	S	R	S	R	S	R
DOGS (20)												
C. jejuni (17)	17/17	0/17*	17/17	0/17*	15/17	2/17*	17/17	0/17	14/17	3/17*	17/17	0/17*
	(100%)	(0%)	(100%)	(0%)	(88.2%)	(11.8%)	(100%)	(0%)	(82.4%)	(17,6%)	(100%)	(0%)
C. coli (3)	3/3	0/3*	3/3	0/3*	2/3	1/3	3/3	0/3	3/3	0/3*	3/3	0/3*
	(100%)	(0%)	(100%)	(0%)	(66.7%)	(33.3%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)
PIGS (8)												
C. coli (8)	0/8	8/8*	0/8	8/8*	7/8	1/8*	8/8	0/8*	8/8	0/8*	8/8	0/8*
	(0%)	(100%)	(0%)	(100%)	(87.5%)	(12.5%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)
COWS (15)												
C. jejuni (12)	10/12	2/12*	10/12	2/12*	12/12	0/12*	12/12	0/12	11/12	1/12*	12/12	0/12*
	(83.3%)	(16.7%)	(83.3%)	(16.7%)	(100%)	(0%)	(100%)	(0%)	(91.7%)	(8.3%)	(100%)	(0%)
C. coli (3)	0/3	3/3*	0/3	3/3*	3/3	0/3*	3/3	0/3*	3/3	0/3*	3/3	0/3*
	(0%)	(100%)	(0%)	(100%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)
HENS (41)												
C. jejuni (37)	33/37	4/37*	33/37	4/37*	35/37	2/37*	36/37	1/37*	32/37	5/37*	36/37	1/37*
	(68.2%)	(10.8%)	(68.2%)	(10.8%)	(94.6%)	(5.4%)	(97.3%)	(2.7%)	(86.5%)	(13.5%)	(97.3%)	(2.7%)
C. coli (4)	0/4	4/4*	0/4	4/4*	3/4	1/4	4/4	0/4*	4/4	0/4*	4/4	0/4*
	(0%)	(100%)	(0%)	(100%)	(75%)	(15%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)
TOTAL (84)												
C. jejuni (66)	60/66	6/66*	60/66	6/66*	62/66	4/66*	65/66	1/66*	57/66	9/66*	65/66	1/66*
	(90.9%)	(9.1%)	(90.9%)	(9.1%)	(93.9%)	(6.1%)	(98.5%)	(1.5%)	(86.4%)	(13.6%)	(15.4%)	(84.6%)
C. coli (18)	3/18	15/18*	3/18	15/18*	15/18	3/18*	18/18	0/18*	18/18	0*	18/18	0/18*
	(16.7%)	(83.3%)	(16.7%)	(83.3%)	(83.3%)	(16.7%)	(100%)	(0%)	(100%)	(0%)	(100%)	(0%)

S = susceptible, R = resistant, NAL= nalidixic acid, CIP = ciprofloxacin, ERY = erythromycin, GEN = gentamycin, AMO =amoxicillin, AMO-CLAV = amoxicillin-clavulanicacid, *Difference between groups is statistically significant (P<0.05).

the strains isolated from pigs were *C. coli* and all were resistant to quinolones. High resistance to quinolones has been shown in *C. coli* strains isolated from pigs (THAKUR & GEBREYES, 2005; QIN et al., 2011).

All of the strains, except one strain of *C jejuni,* were susceptible to gentamycin and amoxicillin-clavulanic acid. In previous studies conducted in Southern Ecuador, no resistance was observed in either *C. jejuni* or in *C. coli* strains isolated from dogs (TOLEDO et al., 2015), backyard chickens (OCHOA et al., 2016) and healthy children (TOLEDO et al., 2017). All of the amoxicillin-resistant strains were cefinase positive and susceptible to amoxicillin-clavulanic acid and are, therefore, beta-lactamase producers (IOVINE, 2013), with the exception of one strain of *C. jejuni* that remained resistant. This particular strain was also gentamycin resistant, showing an uncommon resistance pattern whose genetic determinants should be investigated in future studies. Resistance to amoxicillin and amoxicillin/clavulanic acid was tested because these antibiotics are recommended for the treatment of systemic *Campylobacter* infections, similarly to aminoglycosides and fluoroquinolones (SIFRÉ et al., 2015).

The antimicrobial resistance levels reported in this study suggested that prudent measures should be implemented to prevent the emergence, persistence and transmission of antibiotic resistant *Campylobacter* strains between animals, humans, food of animal origin and the environment.

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DECLARATION OF CONFLICTS INTERESTS

There is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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