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Short communication

Antimicrobial resistance in thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply chain in Argentina

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ABSTRACT

The objective of this study was to investigate the antimicrobial resistance in thermotolerant *Campylobacter* spp. isolated from different stages of the poultry meat supply chain in Argentina. Six poultry meat chains were studied from the reproductive farm to the chicken at the retail. Chickens sampled along each food chain were from the same batch. Samples collected were: a) cloacal samples from hens and chickens on the farm, b) chicken carcasses from the slaughterhouse and retail market. Samples obtained were examined for *Campylobacter* spp. Antimicrobial resistance was evaluated using the disk diffusion method. Almost all isolates were resistant to nalidixic acid (91.2%) and ciprofloxacin (88.2%). A large proportion of thermotolerant *Campylobacter* isolated from hens and broilers <1 wk showed resistance to erythromycin in comparison with the rest of the stages of the poultry meat supply chain ($P = 0.031$). *Campylobacter* isolated from broilers (both <1 wk and >5 wk) and carcasses at slaughterhouse and at retail showed a proportion of resistance to ciprofloxacin and enrofloxacin higher than isolates from hens ($P = 0.015$ and $P = 0.031$, respectively). One strain was resistant to all the antibiotics analyzed, and 46.1% of the isolates were resistant to three or more drug classes. Almost 50% of the isolates were resistant to all quinolones tested (ciprofloxacin, nalidixic acid, and enrofloxacin), and 13.2% were resistant to all quinolones and erythromycin. *Campylobacter* strains isolated from carcasses at retail showed higher resistance to all quinolones than strains isolated from hens ($P = 0.016$). These results reflect an alarming situation with potential serious consequences to the public health.

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1. Introduction

Diarrheal diseases are leading causes of childhood illness and death in developing countries (Zaidi et al., 2008). Thermotolerant *Campylobacter*, especially *Campylobacter jejuni*, is recognized as responsible for food-borne gastro-enteritis and diarrheal diseases worldwide (Avrain et al., 2003). In Argentina thermotolerant *Campylobacter* was found as the most important gastrointestinal pathogen in humans (with an incidence rate of 22.4% and 13.6% for children under 3 years and adults, respectively) (Fuentes, 2010). These pathogens are frequently found in the intestinal tract of a wide variety of wild and domesticated animals, especially birds and animal origin foods, in particular poultry meat, are known to be the most important source of human campylobacteriosis (EFSA., 2010; Signorini et al., 2013).

Although most cases of campylobacteriosis may be asymptomatic or may cause diarrheas and other serious sequels such as Guillain-Barré syndrome (Avrain et al., 2003). Antimicrobial treatment is only necessary for systemic infections (or in immune-compromised patients) and severe or long-lasting *Campylobacter* infections (Luber, Wagner, Hahn, & Bartelt, 2003). Macrolides and fluoroquinolones (particularly erythromycin and ciprofloxacin) are

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the first- and second-choice antimicrobials for that purpose. Intravenous aminoglycosides are also recommended for the treatment of serious *Campylobacter* bacteremia (Kassa, Gebre-Selassie, & Asrat, 2007). Resistance to antimicrobials, particularly to macrolides and fluoroquinolones, has been reported to be increasing (Avrain et al., 2003; Luber et al., 2003). In Argentina, only few studies have evaluated the antimicrobial resistance in *Campylobacter* strains (Pantozzi, Moredo, Vigo, & Giacoboni, 2010; Tamborini et al., 2012) with a low number of strains analyzed. Additionally, no epidemiological studies in Argentina have assessed the prevalence of *Campylobacter* resistant to antibiotics on the whole food chain from farm to fork. This information is essential to establish a public health program to control the disease and it is considered as a starting point for a future monitoring program.

In poultry meat supply chain there are a variety of environments which thermotolerant *Campylobacter* have to adapt to survive. Biofilm formation is a strategy for *Campylobacter* survival in suboptimal conditions such as temperature variation, aerobic atmosphere, and nutrient starvation (Brown et al., 2014). Biofilm appears to be an important reservoir of viable planktonic cells resistant to antimicrobials (Bae, Oh, & Jeon, 2014).

Regarding the methods to evaluate the antimicrobial resistance, disk diffusion method for aminoglycosides, quinolones, erythromycin, and tetracycline has showed a high-level correlation with agar dilution method. Disk diffusion method was considered as a reliable, easy-to-use and inexpensive method for susceptibility testing of thermotolerant *Campylobacter* spp., especially for screening purposes (Luangtongkum, Morishita, El-Tayeb, Ison, & Zhang, 2007).

The objective of this study was to investigate the antimicrobial resistance in thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply chain in Argentina. The criteria used to interpret susceptibility testing results were only for epidemiological monitoring purposes.

2. Materials and methods

2.1. Collection of Campylobacter isolates

A total of 152 thermotolerant *Campylobacter* were isolated from six poultry meat supply chains in Santa Fe region in Argentina (Zbrun et al., 2013). The stages sampled in each poultry meat chain were: a) hens from breeder flocks, b) broilers in flocks (aged <1 wk and >5 wk), c) chickens at the slaughterhouse, and d) chicken meat at the retail market. The chickens sampled along the meat supply chain were from the same batch (defined as a group of chickens from the same flock, sent to the same slaughterhouse at the same time, and sold together at the same retail market). A total of 555 samples were collected from: a) cloacal samples from hens (n = 75) and chickens <1 wk (n = 180) and >5 wk (n = 180) on the farms, b) chicken carcasses from the slaughterhouse (6–7 wk of age) (n = 60) and the retail market (n = 60).

Campylobacter spp. were isolated using the selective media Bolton Broth and Preston Agar (Bolton & Coates, 1983). All incubations were performed under microaerophilic conditions (5% O_2, 10% CO_2 and 85% H_2). Preliminary identification of thermotolerant *Campylobacter* spp. was based on colony morphology, microscopic appearance (curved Gram-negative bacilli with typical motility), and the following phenotypic characteristics: oxidase and catalase production, and hippurate hydrolysis reaction (Lior, 1984). All presumptive *Campylobacter* isolates were identified to the species level (*C. jejuni* and *Campylobacter coli*) by multiplex PCR, as proposed by Vandamme et al. (1997). The negative isolates were tested by PCR for *Campylobacter lari* (Klena et al., 2004). The positive isolates were subsequently purified on Columbia blood agar and stored in glycerol broth (15% glycerol and 85% serum broth) at −80 °C (Terzolo, Lawson, Angus, & Snodgrass, 1987).

2.2. Antimicrobial susceptibility testing

The antimicrobial sensitivity of *Campylobacter* isolates was tested by the disk diffusion assay as recommended by the Clinical and Laboratory Standards Institute in the standard M100-S23 (CLSI, 2013). Strains were removed from the freezer and streaked onto Columbia blood agar and then incubated for 48 h at 42 °C under microaerophilic conditions. Several colonies were transferred to a tube with 5 ml of Mueller-Hinton broth to reach a standard inoculum adjusted to 0.5 McFarland. Sterile cotton-tipped swabs were used to transfer the inocula onto Mueller-Hinton agar supplemented with 5% sheep blood. Antimicrobial disks were added after drying the plates for 5 min. The antimicrobials tested were: ampicillin (10 μg) (Britania), erythromycin (15 μg) (Britania), tetracycline (30 μg) (Britania), nalidixic acid (30 μg) (Britania), enrofloxacin (5 μg) (Oxoid), ciprofloxacin (5 μg) (Britania), and gentamicin (10 μg) (Britania). The plates were incubated for 24 h at 42 °C under microaerophilic conditions. *C. jejuni* ATCC 33560 was used as a reference strain. The zones of growth inhibition were evaluated according to the standards of the National Committee for Clinical Laboratory Standards (NCCLS) (Nobile, Costantino, Bianco, Pileggi, & Pavia, 2013). If an isolate was resistant to three or more drug classes, it was considered multi-drug resistant.

2.3. Statistical analysis

Chi-squared and Fisher's exact two-tailed test were used to compare, for each antibiotic, the distributions of resistant *Campylobacter* isolates according to the stages of the poultry meat supply chain, using Infostat software (Universidad Nacional de Córdoba, Argentina).

3. Results

Table 1 shows the frequency of isolation of thermotolerant *Campylobacter* species according to the stage of the poultry meat chain. Results showed the following distribution: 69.1% (n = 105) was *C. jejuni*, 28.3% (n = 43) was *C. coli*, and 2.6% (n = 4) of positive samples were not possible to identify to species level. *C. jejuni* represented at least 68.2% of the *Campylobacter* species in the

Table 1
Distribution of thermotolerant Campylobacter spp. according to stage of poultry meat supply.

Campylobacter species	Stage of poultry meat supply n (%)				
	Hens	Broilers (<1 wk)	Broilers (>5 wks)	Carcass at slaughterhouse	Carcass at retail
Campylobacter spp.	2 (5.6)	–	1 (2.5)	1 (2.3)	–
C. jejuni	17 (47.2)	9 (81.8)	32 (80.0)	32 (74.4)	15 (68.2)
C. coli	17 (47.2)	2 (18.2)	7 (17.5)	10 (23.3)	7 (31.8)
TOTAL	**36 (23.7)**	**11 (7.2)**	**40 (26.3)**	**43 (28.3)**	**22 (14.5)**

samples collected from broilers in the farm (at both ages) and carcasses at the slaughterhouse and the retail market. In contrast, the proportions of *C. coli* and *C. jejuni* in the samples collected from hens were similar (47.2%).

The lowest resistance was observed for gentamicin (7.9%) and erythromycin (28.3%), while almost all isolates were resistant to nalidixic acid (91.2%) and ciprofloxacin (88.2%). Table 2 shows the frequency of *C. jejuni* and *C. coli* isolates resistant to the antimicrobials tested. *C. coli* showed higher resistance for tetracycline than *C. jejuni* ($P = 0.039$). For the other antibiotics, the proportions of resistance were similar for both species ($P > 0.05$) (Table 2). Regarding the four isolates of *Campylobacter* spp., that could not be identified to species level, the four were resistant to ampicillin, two to erythromycin, one to gentamicin, three to ciprofloxacin, three to nalidixic acid, three to tetracycline, and two to enrofloxacin.

The proportion of thermotolerant *Campylobacter* isolates resistant to ampicillin, gentamicin, nalidixic acid, and tetracycline was similar for all the stages of the poultry meat supply chain ($P > 0.05$). Almost 50% of the thermotolerant *Campylobacter* spp. isolated from hens were resistant to erythromycin, whereas the proportion of resistant isolates from broilers >5 wk and carcasses at the slaughterhouse and the retail was lower than 21.4% ($P = 0.010$, $P = 0.019$, and $P = 0.048$, respectively). On the other hand, the proportion of thermotolerant *Campylobacter* spp. isolated from broilers >5 wk and carcasses at the slaughterhouse and at retail resistant to ciprofloxacin was higher than that of isolates from hens ($P = 0.018$, $P = 0.013$, and $P = 0.009$, respectively). Additionally, the proportion of thermotolerant *Campylobacter* strains isolated from carcasses at slaughterhouse and at retail resistant to enrofloxacin was higher than that of isolates from hens ($P = 0.011$ and $P = 0.052$, respectively). *Campylobacter* strains isolated from broilers >5 wk and carcasses at slaughterhouse and at retail showed non-significant differences in the proportion of resistance to the antibiotics tested ($P > 0.05$) (Table 2).

Only two isolates were sensitive to all the antibiotics tested and one was resistant to all the antibiotics analyzed. Thirty-one different resistance patterns were identified, being ampicillin, ciprofloxacin, nalidixic acid, and tetracycline (n = 17; 11.2%), ampicillin, ciprofloxacin, nalidixic acid, and enrofloxacin (n = 15; 9.9%), and ampicillin, ciprofloxacin, nalidixic acid, tetracycline, and enrofloxacin (n = 15; 9.9%), the most common combinations (Table 3). About 46.1% of the isolates were resistant to three or more drug classes. Multidrug-resistant isolates were associated with the poultry meat supply chain stage ($P = 0.013$) but not with the *Campylobacter* species ($P = 0.099$).

Quinolones and macrolides are the most important antibiotics used for the treatment of human campylobacteriosis. Considering all the isolates studied, 44.7% (n = 68) were resistant to all quinolones tested (ciprofloxacin, nalidixic acid, and enrofloxacin), and 13.2% (n = 20) were resistant to all quinolones and erythromycin (Table 3). The thermotolerant *Campylobacter* spp. isolated from carcasses at retail showed higher resistance to all quinolones than those isolated from hens ($P = 0.016$). The other poultry meat supply stages had a frequency of isolates resistant to quinolones ($P = 0.187$) similar to that found in hens. Additionally, we found no association between the frequency of isolates resistant to quinolones and quinolones + erythromycin and the *Campylobacter* species ($P = 0.326$ and $P = 0.368$, respectively).

4. Discussion

This study is one of the few reports on antibiotic susceptibility of *Campylobacter* spp. isolated from different stages of the poultry meat supply chain in Argentina. *Campylobacter* strains showed high susceptibility to gentamicin. Messad, Hamdi, Bouhamed, and Ramdani-Bouguessa (2014) also reported this behavior and proposed that it could be the consequence of the limited use of this antibiotic in poultry farms.

The thermotolerant *Campylobacter* strains tested in this study showed high resistance to quinolones, specifically ciprofloxacin, nalidixic acid and enrofloxacin. Similar results have been previously observed in other countries (Luber et al., 2003; Messad et al., 2014; Nobile et al., 2013). The poultry reservoir has a fundamental role in the emergence of quinolone-resistant strains of *Campylobacter* (Giacomelli, Salata, Martini, Montesissa, & Piccirillo, 2014). The frequent use of sub-therapeutic doses of these antibiotics, either as prophylaxis, therapeutic doses or growth promoters in poultry farms, has been proposed as the main reason for this high resistance (Avrain et al., 2003; Messad et al., 2014). The widespread use of antibiotics in poultry selects for antibiotic-resistant mutants which are able to spread throughout the meat supply chain (Desmonts et al., 2004). Australia's government banned the use of quinolones in food-producing animals and currently shows a low frequency of *Campylobacter* strains resistant to fluoroquinolones

Table 2
Antibiotic resistance of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain.

Stage of poultry meat supply	*Campylobacter* spp.	Antibiotic resistance n (%)						
		AMP	ERY	GEN	CIP	NA	TET	ENR
Hens	*C. jejuni* (n = 17)	10 (58.8)	9 (52.2)	4 (23.5)	9 (52.9)	12 (70.6)	8 (47.1)	7 (41.2)
	C. coli (n = 17)	16 (94.1)	8 (47.1)	1 (5.9)	15 (88.2)	15 (88.2)	14 (82.4)	5 (29.4)
	TOTAL (n = 34)	**26 (76.5)**	**17 (50.0)**	**5 (14.7)**	**24 (70.6)**	**27 (79.4)**	**22 (64.7)**	**12 (35.3)**
Broilers (<1 wk)	*C. jejuni* (n = 9)	6 (66.7)	5 (55.6)	2 (22.2)	7 (77.8)	9 (100)	5 (55.1)	6 (66.7)
	C. coli (n = 2)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)
	TOTAL (n = 11)	**8 (72.7)**	**5 (45.5)**	**4 (18.2)**	**9 (81.8)**	**11 (100)**	**7 (63.6)**	**7 (63.6)**
Broilers (>5 wks)	*C. jejuni* (n = 32)	12 (37.5)	3 (9.4)	1 (3.1)	30 (93.8)	31 (96.9)	17 (53.1)	16 (50.0)
	C. coli (n = 7)	4 (57.1)	3 (42.9)	1 (14.3)	7 (100)	7 (100)	6 (85.7)	3 (50.0)
	TOTAL (n = 39)	**16 (41.0)**	**6 (15.4)**	**2 (5.1)**	**37 (94.9)**	**38 (97.4)**	**23 (59.0)**	**19 (50.0)**
Carcasses at slaughterhouse	*C. jejuni* (n = 32)	20 (62.5)	8 (25.0)	2 (6.3)	30 (93.8)	30 (93.8)	15 (46.9)	21 (85.6)
	C. coli (n = 10)	7 (70.0)	1 (10.0)	0 (0.0)	9 (90.0)	10 (100)	5 (50.0)	2 (20.0)
	TOTAL (n = 42)	**27 (64.3)**	**9 (21.4)**	**2 (4.8)**	**39 (92.9)**	**40 (95.2)**	**20 (47.6)**	**23 (54.8)**
Carcasses at retail	*C. jejuni* (n = 15)	9 (60.0)	3 (20.0)	0 (0.0)	15 (100)	13 (86.7)	12 (80.0)	10 (66.7)
	C. coli (n = 7)	3 (42.9)	1 (14.3)	0 (0.0)	7 (100)	7 (100)	5 (71.4)	5 (71.4)
	TOTAL (n = 22)	**12 (54.5)**	**4 (18.2)**	**0 (0.0)**	**22 (100)**	**20 (90.2)**	**17 (77.3)**	**15 (88.2)**
All poultry meat supply	*C. jejuni* (n = 105)	57 (54.3)	28 (26.7)	9 (8.6)	91 (86.7)	95 (90.5)	57 (54.3)	60 (57.1)
	C. coli (n = 43)	32 (74.4)	13 (30.2)	2 (4.7)	40 (93.0)	41 (95.3)	32 (74.4)	16 (38.1)
	P=	0.059	0.739	0.571	0.307	0.411	0.039	0.096

References: AMP, ampicillin; ERY, erythromycin; GEN, gentamycin; CIP, ciprofloxacin; NA, nalidixic acid; TET, tetracycline; ENR, enrofloxacin.

Table 3
Antimicrobial resistance patterns of thermotolerant *Campylobacter* isolates.

N° of antimicrobials	Resistance patterns	Classes of antibiotic in pattern (n)	Strains (n)	Total strains n (%)
Two antibiotics	CIP, NA	1	10	
	AMP, NA	2	3	
	AMP, CIP	2	1	17 (11.2)
	AMP, ERY	2	1	
	ERY, TET	2	1	
	GEN, NA	2	1	
Three antibiotics	CIP, NA, TET	2	14	
	CIP, NA, ENR	1	8	
	AMP, CIP, NA	2	6	
	AMP, CIP, ENR	2	2	34 (22.4)
	ERY, CIP, NA	2	2	
	AMP, CIP, TET	3	1	
	GEN, NA, ENR	2	1	
Four antibiotics	AMP, CIP, NA, TET	3	17	
	AMP, CIP, NA, ENR	2	15	
	CIP, NA, TET, ENR	2	8	
	ERY, NA, TET, ENR	3	3	
	AMP, ERY, TET, CIP	4	2	50 (32.9)
	ERY, CIP, NA, TET	3	2	
	ERY, CIP, NA, ENR	2	2	
	AMP, ERY, CIP, ENR	3	1	
Five antibiotics	AMP, CIP, NA, TET, ENR	3	15	
	AMP, ERY, CIP, NA, TET	4	4	25 (16.4)
	ERY, CIP, NA, TET, ENR	3	3	
	AMP, ERY, CIP, NA, ENR	3	2	
	ERY, GEN, CIP, NA, TET	4	1	
Six antibiotics	AMP, ERY, CIP, NA, TET, ENR	4	12	
	AMP, ERY, GEN, NA, TET, ENR	5	5	20 (13.2)
	AMP, ERY, GEN, CIP, NA, TET	5	2	
	AMP, GEN, CIP, NA, TET, ENR	4	1	
Seven antibiotics	AMP, ERY, GEN, CIP, NA, TET, ENR	5	1	1 (0.7)

References: AMP, ampicillin (β-lactam class); ERY, erythromycin (macrolide class); GEN, gentamycin (aminoglycoside class); CIP, ciprofloxacin (quinolone class); NA, nalidixic acid (quinolone class); TET, tetracycline (tetracycline class); ENR, enrofloxacin (quinolone class).

(Wieczorek, Dykes, Osek, & Duffy, 2013). Additionally, it has been reported that flocks treated with ionophores (as coccidicidals) had a higher proportion of *Campylobacter* strains resistant to multiple antibiotics such as ampicillin, nalidixic acid, and tetracycline (Avrain et al., 2003). All this suggests that the control tools for thermotolerant *Campylobacter* multiresistant to antibiotics cannot be addressed only to the restriction or prohibition of antimicrobial drugs in animal feed. New production systems and new health tools should be designed to address the public health problem of thermotolerant *Campylobacter* and antimicrobial resistance.

Many studies regarding the prevalence of antimicrobial-resistant *Campylobacter* have been performed in different countries. Each of these studies have shown a particular pattern of antimicrobial resistance, which may be explained by the variations in the use of antibiotic, origin of the samples, isolation techniques, and antimicrobial susceptibility tests (Nobile et al., 2013). It is thus difficult to compare the prevalences among countries. In that sense, a standard guideline should be developed to establish a global surveillance and thus allow monitoring this food-borne pathogen both currently and after the implementation of effective control measures to avoid resistance dissemination through the food chain.

The most remarkable result of our study was the high frequency of *Campylobacter* strains with multi-drug resistance, especially to the antibiotics considered as first-choice for serious *Campylobacter* infections (ciprofloxacin and erythromycin). The isolation of a high proportion of *Campylobacter* strains resistant to all quinolones and resistant to quinolones + erythromycin provides evidence of the key role of raw poultry meat in the exposure risk to antibiotic-resistant *Campylobacter* strains in humans. This situation demands the adoption of appropriate risk management measures to control the antibiotic use in food animals (Nobile et al., 2013) and in human therapeutic treatment.

Our results indicate that, with the exception of tetracycline, both *Campylobacter* species had the same frequency of antibiotic resistance. Tetracyclines are considered the second-choice treatment and high resistance to this antimicrobial may have a negative impact on public health. Many studies (e.g. Luber et al., 2003; Nobile et al., 2013) have reported the influence of *Campylobacter* species on the resistance to antibiotics. In general, *C. coli* shows higher resistance than *C. jejuni* (Giacomelli et al., 2014). Apparently, *C. coli* strains have an intrinsic ability to generate resistance against some antibiotics such as erythromycin (Luber et al., 2003). Wieczorek, Kania, and Osek (2013) found greater resistance to antimicrobials for *C. coli* than for *C. jejuni* for tetracycline. On the other hand, Nobile et al. (2013) found that, in general, *C. jejuni* shows higher resistance to ciprofloxacin, gentamicin, and norfloxacin than *C. coli*.

In the present work, the *Campylobacter* strains isolated from the different stages of the poultry meat supply chain presented the same frequency of antimicrobial resistance. The colonization of broilers with thermotolerant *Campylobacter* seems to be unavoidable. The high prevalence of these microorganisms was spreading throughout the poultry meat supply chain. In this regard, it would be useful to conduct studies in order to investigate the genotypic variation among strains of *Campylobacter* spp. isolated from different stages of the poultry meat supply chain to evaluate whether the bacterial population was a stable single genotype or had a mixed and changing profile according with the stage. However, the strains isolated from hens showed higher resistance to erythromycin than the strains isolated from the remaining stages of the poultry meat supply chain. On the other hand, the frequency of resistance to quinolones (ciprofloxacin and enrofloxacin) was higher in the strains isolated from poultry in the farm and from slaughterhouse and retail carcasses than in the

strains isolated from hens. Four possible explanations might be proposed to clarify this finding: a) Both the use of antimicrobials (frequency, dose and type of antimicrobial used) and other animal husbandry practices are completely different between hens and poultry farms. This may impact on the selection pressure and the emergence of *Campylobacter* strains resistant to specific antibiotics. This different selection pressure is responsible for generating specific clusters of *Campylobacter* strains at these two stages (hens *vs.* the rest of the stages) of the poultry meat supply chain. Argentina does not have a surveillance system on the use of veterinary drugs that allows collecting data on sales of antimicrobial agents. This surveillance system dedicated to monitoring drug on species level is needed to assess the true levels of antimicrobial use and therefore analyze the possible consequences of their use in the emergence of antimicrobial-resistant strains; b) hens have a higher life than poultry and therefore greater potential exposure to antimicrobials applied for prophylactic or therapeutic purposes; c) the biosecurity measures adopted by the parent flocks are much stricter than in poultry farms. Broilers have more possibilities to be in contact with many vectors (such as flies, rodents, and wild birds) which have been identified as reservoirs for the transmission of antibiotic-resistant *Campylobacter* to poultry (Sippy et al., 2012). In addition to the fact that the ecological conditions are different, the transmission of *Campylobacter* spp. from hen farms to poultry farms through eggs is unlikely (Zbrun et al., 2013); and d) poorly sanitized abiotic surfaces and juices released from avian meat, found in slaughterhouse and retail environment, support the biofilms formation, which reintroduce planktonic microorganisms resistant to antimicrobials. Additionally, *C. jejuni* has the ability to form biofilms in the watering supplies and plumbing systems of animal husbandry facilities and animal-processing plants (Bae et al., 2014; Pearson et al., 1993).

The horizontal genes transmission has been identified as a relevant mechanism in the dissemination of antibiotic resistance in *Campylobacter* spp. Mobile genetic elements are commonly involved in the horizontal gene transfer, allowing the bacteria to acquire foreign DNA from the environment. *C. jejuni* has the ability to develops biofilms on different abiotic surfaces as a mechanism to survive in suboptimal conditions, increasing the resistance to antimicrobials. *Campylobacter* biofilms help to resist a variety of stressors throughout poultry meat chain supply and appear to be an important reservoir of antibiotic-resistant *C. jejuni* under normal growth conditions even without antimicrobial pressure (Bae et al., 2014).

An increase in the presence of antibiotic-resistant strains has also been observed in *Campylobacter* spp. isolated from human cases (WHO, 2014). This emphasizes that these results reflect the behavior of *Campylobacter* spp. along the poultry meat supply chain (Luber et al., 2003). Studies conducted in Argentina testing *Campylobacter* strains isolated from humans have shown remarkable resistance to ciprofloxacin (>70%) and tetracycline (30–40%), but high susceptibility to erythromycin (Fuentes, 2010; Tamborini et al., 2012). Antimicrobials used massively in both human medicine and veterinary medicine can generate a high level of resistance (Wieczorek et al., 2013).

Although the agar dilution technique has been classified as the standard test for *Campylobacter* (McDermott et al., 2004), here we used the disk diffusion test to investigate antimicrobial resistance. Because of its convenience, flexibility, and low cost, disk diffusion has been standardized (EUCAST, 2012) and used widely to test rapidly growing pathogens such as Enterobacteriaceae and also modified to test some fastidious organisms (CLSI, 2013).

5. Conclusions

The finding of antibiotic-resistant (especially multi-drug resistant) *Campylobacter* spp. reflects an alarming situation with potential serious consequences to the health of poultry meat consumers. The frequency of antibiotic resistant *Campylobacter* was variable in the different steps of the poultry meat chain supply. The use of antimicrobials in poultry farms may generate a selection pressure and lead the emergency of *Campylobacter* strains resistant against specific antibiotics. It is necessary to immediately implement husbandry measures with the aim to guide the rational use of antibiotics in poultry production. Argentina should establish a surveillance program to investigate the prevalence and tendency in prevalence of antibiotic-resistance *Campylobacter* throughout the poultry meat supply chain which may be the scientific basis to establish a risk management policy.

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References

Avrain, L., Humbert, F., L'Hospitalier, R., Sanders, P., Vernozy-Rozand, C., & Kempf, I. (2003). Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. *Veterinary Microbiology, 96*, 267–276.

Bae, J., Oh, E., & Jeon, B. (2014). Enhanced transmission of antibiotic resistance in *Campylobacter jejuni* biofilms by natural transformation. *Antimicrobial Agents and Chemotherapy, 58*, 7573–7575.

Bolton, F. J., & Coates, D. (1983). Development of a blood-free *Campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplements to facilitate aerotolerance. *Journal of Applied Bacteriology, 54*, 115–125.

Brown, H. L., Reuter, M., Salt, L. J., Cross, K. L., Betts, R. P., & van Vliet, A. H. (2014). Chicken juice enhaces surface attachment and biofilm formation of *Campylobacter jejuni*. *Applied and Environmental Microbiology, 80*, 7053–7060.

Clinical and Laboratory Standards Institute (CLSI). (2013). *Performance standards for antimicrobial susceptibility testing approved standards M100-S23* (23rd ed.). Wayne, Pa: NCCLS.

Desmonts, M. H., Dufour-Gesbert, F., Avrain, L., & Kempt, I. (2004). Antimicrobial resistance in *Campylobacter* strains isolated from French broilers before and after antimicrobial growth promoter bans. *Journal of Antimicrobial Chemotherapy, 54*, 1025–1030.

EFSA. (2010). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008, Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal, 8*(03), 1503.

EUCAST. (2012). *Clinical breakpoints, epidemiological cut-off (ECOFF) values and EUCAST disk diffusion methodology for Campylobacter jejuni and Campylobacter coli*. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/Campylobacter_wide_consultation_August_2012.pdf Accessed 11.12.14.

Fuentes, L. S. (2010). Prevalencia y perfiles de sensibilidad en cepas de *Campylobacter* spp. aisladas de diarreas en Córdoba, Argentina. *Gastroenterology, 12*, 2–5.

Giacomelli, M., Salata, C., Martini, M., Montesissa, C., & Piccirillo, A. (2014). Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. *Microbiology Drug Resistance, 20*, 181–188.

Kassa, T., Gebre-Selassie, S., & Asrat, D. (2007). Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Veterinary Microbiology, 119*, 82–87.

Klena, J. D., Parker, C. T., Knibb, K., Ibbitt, J. C., Devane, P. M. L., Horn, S. T., et al. (2004). Differentiation of *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, and Campylobacter upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA*. *Journal of Clinical Microbiology, 42*, 5549–5557.

Lior, H. (1984). New, extended biotyping scheme for *Campylobacter jejuni, Campylobacter coli* and *Campylobacter laridis*. *Journal of Clinical Microbiology, 20*, 636–640.

Luangtongkum, T., Morishita, T. Y., El-Tayeb, A. B., Ison, A. J., & Zhang, O. (2007). Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. *Journal of Clinical Microbiology, 45*, 590–594.

Luber, P., Wagner, J., Hahn, H., & Bartelt, E. (2003). Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001–2002 from poultry and humans in Berlin, Germany. *Antimicrobial Agents Chemotherapy, 47*, 3825–3830.

McDermott, P. F., Bodeis, S. M., Aarestrup, F. M., Brown, S., Traczewski, M., Fedorka-Cray, P., et al. (2004). Development of a standardized susceptibility test for *Campylobacter* with quality-control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. *Microbial Drug Resistance, 10*, 124–131.

Messad, S., Hamdi, T. M., Bouhamed, R., & Ramdani-Bouguessa, N. (2014). Frequency of contamination and antimicrobial resistance of thermotolerant *Campylobacter* isolated from some broiler farms and slaughterhouses in the region of Algiers. *Food Control, 40*, 324–328.

Nobile, C. G. A., Costantino, R., Bianco, A., Pileggi, C., & Pavia, M. (2013). Prevalence and pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. *Food Control, 32*, 715–718.

Pantozzi, F. L., Moredo, F. A., Vigo, M. G. B., & Giacoboni, G. I. (2010). Antimicrobial resistance in indicator and zoonotic bacteria isolated from domestic animals in Argentina. *Revista Argentina de Microbiología, 42*, 49–52.

Pearson, A. D., Greenwood, M., Healing, D., Rollins, M., Shahamat, M., Donaldson, J., et al. (1993). Colonization of broiler chickens by waterborne *Campylobacter jejuni*. *Applied and Environmental Microbiology, 59*, 987–996.

Signorini, M. L., Zbrun, M. V., Romero-Scharpen, A., Olivero, C., Bongiovanni, F., Soto, L. P., et al. (2013). Quantitative risk assessment of human campylobacteriosis by consumption of salad cross-contaminated with thermophilic *Campylobacter* spp. from broiler meat in Argentina. *Preventive Veterinary Medicine, 109*, 37–46.

Sippy, R., Sandoval-Green, C. M. J., Sahin, O., Plummer, P., Fairbanks, W. S., Zhang, Q., et al. (2012). Occurrence and molecular analysis of *Campylobacter* in wildlife on livestock farms. *Veterinary Microbiology, 157*, 369–375.

Tamborini, A. L., Casabona, L. M., Viñas, M. R., Asato, V., Hoffer, A., Farace, M. I., et al. (2012). *Campylobacter* spp.: prevalence and pheno-genotypic characterization of isolates recovered from patients suffering from diarrhea and their pets in La Pampa Province, Argentina. *Revista Argentina de Microbiología, 44*, 266–271.

Terzolo, H. R., Lawson, G. H. K., Angus, K. W., & Snodgrass, D. R. (1987). Enteric *Campylobacter* infection in gnotobiotic calves and lambs. *Research in Veterinary Science, 43*, 72–77.

Vandamme, P., van Doorn, L.-J., Rashid, S. T. A., Quint, W. G. V., Plas, J.v.d., Chan, V. L., et al. (1997). *Campylobacter hyoilei* Alderton et al. 1995 and *Campylobacter coli* veron and chatelain 1973 are subjective synonyms. *International Journal of Systematic. Bacteriol, 47*, 1055–1060.

WHO (World Health Organization). (2014). *Antimicrobial resistance*. Global report on surveillance http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf.

Wieczorek, K., Dykes, G. A., Osek, J., & Duffy, L. L. (2013). Antimicrobial resistance and genetic characterization of *Campylobacter* spp. from three countries. *Food Control, 34*, 84–91.

Wieczorek, K., Kania, I., & Osek, J. (2013). Prevalence and antimicrobial resistance of *Campylobacter* spp. Isolated from poultry carcasses in Poland. *Journal of Food Protection, 76*, 1451–1455.

Zaidi, M. B., Calva, J. J., Estrada-García, M. T., León, V., Vázquez, G., Figueroa, G., et al. (2008). Integrated food chain surveillance system for *Salmonella* spp. in Mexico. *Emergency Infectious Diseases, 14*, 429–435.

Zbrun, M. V., Romero-Scharpen, A., Olivero, C., Rossler, E., Soto, L. P., Rosmini, M. R., et al. (2013). Occurrence of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain in Argentina. *New Zealand Veterinary Journal, 61*, 337–343.