

Occurrence and antimicrobial susceptibility of *Campylobacter* spp. on fresh and refrigerated chicken meat products in Central Italy

P. Casagrande Proietti,^{*,1} S. Pergola,[†] S. Bellucci,^{*} L. Menchetti,^{*} D. Miraglia,^{*} and M. P. Franciosini^{*}

^{*}Department of Veterinary Medicine, Via S. Costanzo 4, Perugia, Italy; and [†]Department of Life and Environmental Science, Polytechnic University of Marche, P.zza Roma 22, Ancona, Italy

ABSTRACT This study investigated the presence and the level of *Campylobacter* spp. contamination in 41 thigh samples (with skin) and 37 skinless breast samples collected at the end of slaughter (T1) and after 10 day period at refrigeration temperature (4°C) (T2), corresponding to their commercial shelf life. The isolates were phenotypically classified as *Campylobacter* spp. and successively identified by conventional multiplex PCR. The antimicrobial susceptibility of the isolates from fresh thigh and breast samples was also determined via the microdilution method (MIC) in Eucamp microtitre plates with known scalar concentrations of: gentamicin (GEN), streptomycin (ST), ciprofloxacin (CIP), tetracycline (TET), erythromycin (ERY), and nalidixic acid (NA). A greater percentage of positivity for *Campylobacter* spp. ($P < 0.001$) was observed in thighs and *C.jejuni* appeared to be the most common

species identified at this level ($P < 0.001$) followed from its association with *C.coli*. There was a global reduction of *Campylobacter* spp. in both thigh and breast samples at T2 ($P < 0.001$) showing that the refrigeration was able to reduce *Campylobacter* count. The prevalence of resistance to CIP, TET, NA, and ERY was evidenced for *C.jejuni* and *C. coli*. The co (TET-NA, CIP-NA) and multiple resistant (CIP-TET-NA, CIP-TET-NA-ERY) isolates came from the thigh products. It should be highlighted the presence of *Campylobacter* spp. isolates resistant to ST occurred in breast samples, responsible for the ST-CIP co-resistance and ST-CIP-TE multi-resistance profiles, higher in breast than in thigh products ($P > 0.001$). The presence of *Campylobacter* isolates resistant to ST can be further investigated since it is used for therapeutic treatment of several bacterial diseases in humans

Key words: *Campylobacter* spp., count, antimicrobial susceptibility, meat chicken, refrigeration

2018 Poultry Science 97:2895–2901
<http://dx.doi.org/10.3382/ps/pey147>

INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are considered to be the most common agents responsible for acute bacterial diarrhea in humans, though *C. lari*, *C. upsaliensis* and *C. concisus* may also play a role in causing enteritis (Kaakoush and Mitchell, 2012). Several times Campylobacteriosis is seen to pave the way to the onset of Guillain–Barré syndrome, a severe human neuropathy, (Scallan et al., 2011). *Campylobacter* spp. usually colonizes the intestinal mucosa of all animals including humans (Newell and Fearnley, 2003). Although most food producing animals contribute to the dissemination of *Campylobacter* spp. (Stanley and Jones, 2003), birds appear to be the favored hosts (Stephens et al., 1998; Waldenstrom et al., 2001), likely for their body temperature (40–41°C) that permits an optimal growth to the thermotolerant *Campylobacters* (Silva et al., 2011). Moreover the consumption of contaminated

undercooked poultry meat seems to represent the main source of *Campylobacter* infection in humans (Corry and Atabay, 2001). It has also been reported that this bacterium is able to survive refrigeration (Murphy et al., 2006) and that *Campylobacter* population, contaminating meat chicken, can include several strains with different survival degrees at 4°C if tested individually (Chan et al., 2001; Colles et al., 2003; El-Shibiny et al., 2005; Johnsen et al., 2006).

Over the last years the importance of *Campylobacter* as public concern is also due to the fact that a rapid increase of its resistance to antimicrobials has been noticed in several countries (Nachamkin et al., 1993; Lucey et al., 2002; Lubber et al., 2003; Pezzotti et al., 2003; Papavasileiou et al., 2007), in particular toward fluoroquinolones (Ruiz et al., 1998; Blaser et al., 2008). A constant trend for macrolide resistance increase has also been described (Gibreel and Taylor, 2006). *C.jejuni* seemed to exhibit a macrolide prevalence lower than that reported for *C.coli* isolated from chickens and pigs (Papavasileiou et al., 2007; Bardon et al., 2011) as also reported in our previous investigation (Pergola et al., 2017). The aim of this study was to evaluate the presence and the level of *C. jejuni* and *C.coli*

© 2018 Poultry Science Association Inc.

Received December 27, 2017.

Accepted March 22, 2018.

¹Corresponding author: patrizia.casagrandeproietti@unipg.it

contamination in breast (products without skin) and in thigh (products with skin) samples collected at the end of slaughter before packaging and after a period of 10 days under refrigeration condition. Moreover all isolates from fresh breast and thigh products were submitted to phenotypic antimicrobial susceptibility test.

MATERIAL AND METHODS

Samplings

Between 2015 and 2016, a total of 78 samples without and with skin, 37 from breasts and 41 from thighs respectively, were randomly collected from a commercial processing plant at the end of the slaughter (T1) and submitted to microbiological examinations for *Campylobacter* spp detection within 24 h of the sampling. Successively the same products were packed by plastic film and stored under refrigeration conditions (4°C) for 10 days corresponding to end of their commercial shelf life (T2) and they were further submitted to the microbiological examinations for *Campylobacter* spp

Isolation, Count and Identification of *Campylobacter* spp

A 25 g of the thigh skin and a 25 g of the external breast surfaces without skin were cut and aseptically put into a sterile bag and diluted with a ratio of 1:10 in Buffer Peptone Water (BPW) solution. The mixture was then homogenized for 1 min in a peristaltic homogenizer. One mL of a 10^{-1} dilution was spread on three modified Cefoperazone Charcoal Desoxycholate agar plates (mCCDA) (Thermofisher Scientific, Milan, Italy). In addition, 0.1 mL of samples from 10^{-1} dilution was inoculated and spread in mCCDA plates and incubated in microaerophilic conditions at $41.5 \pm 0.5^\circ\text{C}$ for 48 h. Presumptive *Campylobacter* spp. colonies with the typical morphology were counted according to the method described in ISO 10272:2006 and the results were expressed as colony forming units per gram of sample (cfu/g). On the basis of the *Campylobacter* spp. enumeration the samples were categorized as follows: no presence of *Campylobacter* spp (0 category), $<1.0 \times 10^2$ cfu/g (1st category); $1.0 \times 10^2 - 5.0 \times 10^2$ cfu/g (2nd category); $5.0 \times 10^2 - 1.0 \times 10^3$ cfu/g (3rd category) $> 1.0 \times 10^3$ cfu/g (4th category). For each positive plate, up to five typical *Campylobacter* colonies were subcultured in blood agar plates and incubated under microaerophilic conditions at $41.5 \pm 0.5^\circ\text{C}$ for 48 h for further characterization according to ISO 10272:2006. Isolates were confirmed by biochemical tests (oxidase test, catalase test, hippurate hydrolysis test), microscopic examination and gram staining. All strains were stored at -80°C in Brucella broth (Thermofisher Scientific, Milan, Italy). Chromosomal DNA was extracted from colonies using an Ultraclean Microbial DNA Isolation kit (MO BIO Laboratories,

Milan, Italy). *C. coli* and *C. jejuni* were identified by conventional multiplex PCR assay, as elsewhere described (Wang et al., 2002).

Antimicrobial Susceptibility Testing

Twelve *C. coli* and 10 *C. jejuni* isolates from breasts (without skin) and 15 *C. coli* and 31 *C. jejuni* isolates from thighs (with skin) were tested as well as the two *Campylobacter* not identified, isolated from breast samples. The *Campylobacter* susceptibility to antibiotics was evaluated with the microdilution method (MIC). Colonies were picked and grown on Columbia agar (Becton Dickinson, Buccinasco, Milan, Italy) in microaerophilic atmosphere at 42°C for 24 h. The colonies were then seeded in Mueller Hinton Broth (Merck SpA, Serono, Rome, Italy) supplemented with blood and dispensed into Eucamp microtitre plates (Thermofisher Scientific, Rodano, Milan) with scalar concentrations of the following antibiotics: gentamicin (GEN) ($0.12\text{--}16 \mu\text{g/mL}$), streptomycin (ST) ($1\text{--}16 \mu\text{g/mL}$), ciprofloxacin (CIP) ($0.06\text{--}4 \mu\text{g/mL}$), tetracycline (TET) ($0.25\text{--}16 \mu\text{g/mL}$), erythromycin (ERY) ($0.5\text{--}32 \mu\text{g/mL}$), nalidixic acid (NA) ($2\text{--}64 \mu\text{g/mL}$). After inoculation, the plates were incubated at 42°C in microaerophilic atmosphere for 24 h and then screened. *C. jejuni* strain NCTC 11,351 was used as the control. The results related to ciprofloxacin, tetracycline and erythromycin were evaluated according to the breakpoints established from EUCAST (European Committee on Antimicrobial Susceptibility Testing, 2013) for *Campylobacter* spp. For gentamicin, streptomycin, and nalidixic acid the Enterobacteriaceae breakpoints (EUCAST, 2013) were applied.

Statistical Analysis

The antimicrobial resistance of *Campylobacter* was compared by the Chi-square, Fisher's and z-test. Chi-square goodness-of-fit test was used to evaluate distribution within each category (equal distribution of categories assumed). A value of $P < 0.05$ was considered statistically significant. Data were expressed as number (n) and percentage (%) and analyzed using SPSS Statistics, version 23 (IBM, SPSS Inc., Chicago, IL).

RESULTS

Count and Identification of *Campylobacter* spp.

Most of the fresh samples collected at T1 were positive for *Campylobacter* (52 out of 78, 66.7%; $P < 0.01$). The number of fresh products with skin positive for *Campylobacter* (34 out of 41, 82.9%) was higher than that observed for skinless products (18 out of 37, 48.6%; $P < 0.001$).

Table 1. *Campylobacter* isolates in breast and thigh samples at T1 and T2.

Products		Time		P value ¹
		T1	T2	
Thigh	Negative	7 ^a (17.1%)	20 ^b (48.8%)	0.010
	Positive for <i>C. coli</i>	3 ^a (7.3%)	1 ^a (2.4%)	
	Positive for <i>C. coli</i> and <i>C. jejuni</i>	12 ^a (29.3%)	5 ^a (12.2%)	
	Positive for <i>C. jejuni</i>	19 ^a (46.3%)	15 ^a (36.6%)	
Breast	Negative	19 ^a (51.4%)	36 ^b (97.3%)	<0.001
	Positive for <i>C. coli</i>	6 ^a (16.2%)	1 ^b (2.7%)	
	Positive for <i>C. coli</i> and <i>C. jejuni</i>	6 ^a (16.2%)	0 ^b (0.0%)	
	Positive for <i>C. spp</i>	2 ^a (5.4%)	0 ^a (0.0%)	
	Positive for <i>C. jejuni</i>	4 ^a (10.8%)	0 ^b (0.0%)	

¹estimated by Chi square or Fisher test.
Column proportions within a row lacking a common superscript differ ($P < 0.05$; z test).

Table 2. *Campylobacter* count in breast and thigh samples collected at T1 and T2.

Product	Category (cfu/g)		Time		P value ¹
			T1	T2	
Thigh	0	0 CFU	7 ^a (17.1%)	20 ^b (48.8%)	<0.001
	1th	<100 CFU	16 ^a (39.0%)	18 ^a (43.9%)	
	2nd	100–500 CFU	13 ^a (31.7%)	3 ^b (7.3%)	
	3rd	500–1000 CFU	1 ^a (2.4%)	0 ^a (0.0%)	
	4th	> 1000 CFU	4 ^a (9.8%)	0 ^b (0%)	
Breast	0	0 CFU	19 ^a (51.4%)	36 ^b (97.3%)	<0.001
	1th	<100 CFU	15 ^a (40.5%)	1 ^b (2.7%)	
	2nd	100–500 CFU	3 ^a (8.1%)	0 ^a (0.0%)	

¹estimated by Chi square or Fisher test.
Column proportions within a row lacking a common superscript differ ($P < 0.05$; z test).

At T1 the prevalence of *C.jejuni* was higher in thigh (with skin) than in breast (skinless) samples (46.3% vs 10.8%; $P < 0.05$).

C.coli/C.jejuni mixed contamination was present respectively in 29.3% and 16.2% of the thigh and breast products. There were also 16.2% of the breast samples *C.Coli* positive (Table 1).

At T2 we observed a reduction of *Campylobacter* in all samples; indeed the negative samples increased from 17.1% to 48.8% ($P < 0.05$), and from 51.4% to 97.3% ($P < 0.001$) for thigh and breast products, respectively (Table 1).

At T1 the *Campylobacter* spp count in positive samples fell mostly in the 1st (39.0%) and 2nd (31.7%) categories for thigh products ($P < 0.001$), and in the 1st category (40.5%) for breast products ($P < 0.01$). Four thigh samples (9.8%) showed count falling into 4th category (Table 2).

At T2 a reduced count was seen in breast samples with count $<1.0 \times 10^2$ cfu/g (40.5% vs 2.7%; $P < 0.001$), as well as in thigh samples with count 1.0×10^2 - 5.0×10^2 cfu/g (31.7% vs 7.3%; $P < 0.001$). No samples with count $> 1.0 \times 10^3$ cfu/g. could be also seen at T2 (Table 2).

Antimicrobial Susceptibility Testing

High prevalence of resistance to CIP and TET was observed in skinless breast and with skin thigh products for both species ($P < 0.001$) (Table 3). *C.coli* and

C.jejuni from thigh products were also resistant to NA (93.3% and 90.3% respectively) and to ERY (46.7% - 19.4% respectively).

All isolates from breast samples were susceptible to NA and ERY and showed prevalence of resistance to ST (100.0%), higher than that evidenced in thighs (6.7% and 6.5% for *C.coli* and *C.jejuni*, respectively; $P < 0.001$) (Table 3). The co (TET-NA, CIP-NA) and multiple resistant (CIP-TET-NA, CIP-TET- NA-ERY) isolates came from the thigh products. The presence of *Campylobacter* spp. isolates resistant to ST was responsible for the ST-CIP co-resistance and ST-CIP-TET multi-resistance profiles, higher in breast than in thigh products ($P < 0.001$) (Table 4).

DISCUSSION

Campylobacter contamination of broiler flocks in farms seems to be a focal point in its spread in the poultry production processing plant (Skarp et al., 2016). A recent survey, performed in three Italian slaughterhouses, showed a 60% of *Campylobacter* prevalence from broiler carcasses (Comin et al., 2014). In the current study a great percentage of positivity for *Campylobacter* spp. was evidenced in fresh chicken products as already reported (Fraqueza et al., 2014).

C.jejuni was dominant in thighs products whereas *C.Coli* in skinless breast. The association between the two species was also common in both kind of samples according to other studies (Saiyudthong et al., 2015).

Table 3. *Campylobacter* isolates resistant in thigh and breast samples.

ANTIMICROBIALS		PRODUCT		P value ¹
		Thigh (n = 46)	Breast (n = 24)	
GEN	<i>C. coli</i>	1 (6.7%)	0 (0.0%)	1.000
	<i>C. jejuni</i>	0 (0.0%)	0 (0.0%)	₃
	<i>C. spp</i> ²	-	0 (0.0%)	₂
	Total	1 (2.2%)	0 (0.0%)	1.000
ST	<i>C. coli</i>	1 ^a (6.7%)	12 ^b (100.0%)	< 0.001
	<i>C. jejuni</i>	2 ^a (6.5%)	10 ^b (100.0%)	< 0.001
	<i>C. spp</i> ²	-	2 (100.0%)	₂
	Total	3 ^a (6.5%)	24 ^b (100.0%)	< 0.001
CIP	<i>C. coli</i>	11 (73.3%)	12 (100.0%)	0.106
	<i>C. jejuni</i>	31 ^a (100.0%)	7 ^b (70.0%)	0.011
	<i>C. spp</i> ²	-	2 (100.0%)	₂
	Total	42 (91.3%)	21 (87.5%)	0.684
TET	<i>C. coli</i>	15 (100.0%)	12 (100.0%)	₄
	<i>C. jejuni</i>	24 (77.4%)	10 (100.0%)	0.164
	<i>C. spp</i> ²	-	2 (100.0%)	₂
	Total	39 ^a (84.8%)	24 ^b (100.0%)	0.087
NA	<i>C. coli</i>	14 ^a (93.3%)	0 ^b (0.0%)	< 0.001
	<i>C. jejuni</i>	28 ^a (90.3%)	0 ^b (0.0%)	< 0.001
	<i>C. spp</i> ²	-	0 (0.0%)	₂
	Total	42 ^a (91.3%)	0 ^b (0.0%)	< 0.001
ERY	<i>C. coli</i>	7 ^a (46.7%)	0 ^b (0.0%)	0.008
	<i>C. jejuni</i>	6 (19.4%)	0 (0.0%)	0.307
	<i>C. spp</i> ²	-	0 (0.0%)	₂
	Total	13 ^a (28.3%)	0 ^b (0.0%)	0.007

¹estimated by Chi square or Fisher test. Values in bold are statistically significant at the 0.05 level.

Column proportions within a row lacking a common superscript differ ($P < 0.05$; z test).

²No statistics are computed because only breast products were present.

³No statistics are computed because Susceptibility is a constant.

⁴No statistics are computed because Resistance is a constant

n = number.

Campylobacter is part of the intestinal microbiota in animals and its ability to colonize several host largely influences the epidemiology of the infection. Several authors reported that *C.jejuni* was the most representative species isolated in both commercial broiler farms and abattoirs (Moran et al., 2009; di Giannatale et al., 2014), but in our previous study on 140 isolates of thermotolerant *Campylobacter* collected in farms and in slaughterhouse, 99 resulted to be *C.coli* (Pergola et al., 2017). A higher incidence of *C.coli* compared to *C.jejuni* was seen in trials performed in organic and free range chickens. It could be due to the extended periods of rearing, as *C.jejuni* seemed to be the first to colonize the intestine in both rearing systems, but successively *C.coli* became prevalent (El-Shibiny et al., 2005; El-Shibiny et al. 2007).

C.jejuni is also the agent mostly involved in human foodborne poisoning. Friedman et al. (2000) reported that it was responsible for more than 12 times the number of human Campylobacteriosis occurrence if compared to *C. coli*.

In our study *Campylobacter* spp. count fell frequently in the 1st category in breast and thigh fresh products suggesting the presence of contamination. The breast samples was less contaminated than to thighs in agreement with other investigations confirming that the skin less products could be safer from a consumer

standpoint (Luber and Bartelt, 2007; Peyrat et al., 2008; Skarp et al., 2016).

Several studies showed the recovery of *Campylobacter* spp by swabbing the skin before the entry in scalding tank (Kotula and Pandya, 1995, Stern et al., 1995). Moreover, crates insufficiently cleaned may be a source of skin contamination during the transport to abattoir (Slader et al., 2002). The consistent presence of *Campylobacter* spp in crop and in cecum was observed in chickens before the transport to processing plant (Hargis et al., 1995; Byrd et al., 1998). It has been speculated that a possible contamination by feces during the transport might be more frequent than cross-contamination in slaughterhouse (Rasschaert et al., 2006). Pergola et al. (2017) reported that the genotypes isolated from the cloacal swabs collected in farms matched to those detected at slaughter from the cutaneous samples, coming from the same subjects previously tested, highlighting the importance of the housing environment in the contamination. It is highlighted by several authors that a focal point for the dissemination of the *Campylobacter* along the slaughter line is represented by the entry of infected or contaminated chickens (Reich et al., 2008; Hue et al., 2010).

Globally we observed a reduction in both thigh (with skin) and breast (skinless) products at T2, testifying that the refrigeration can influence the *Campylobacter*

Table 4. Resistance profiles in *Campylobacter* isolated from breast and thigh samples.

RESISTANCE PROFILE	SPECIES	PRODUCTS		P value ¹
		Thigh (n = 46)	Breast (n = 24)	
TET-NA	<i>C. coli</i>	14 ^a (93.3%)	0 ^b (0.0%)	<0.001
	<i>C. jejuni</i>	21 ^a (67.7%)	0 ^b (0.0%)	<0.001
	<i>C. spp</i> ²	-	0 (0.0%)	- ²
	Tot	35 ^a (76.1%)	0 ^b (0.0%)	<0.001
CIP-NA	<i>C. coli</i>	10 ^a (66.7%)	0 ^b (0.0%)	<0.001
	<i>C. jejuni</i>	28 ^a (90.3%)	0 ^b (0.0%)	<0.001
	<i>C. spp</i> ²	-	0 (0.0%)	- ²
	Tot	38 ^a (82.6%)	0 ^b (0.0%)	<0.001
ST-CIP	<i>C. coli</i>	1 ^a (6.7%)	12 ^b (100.0%)	<0.001
	<i>C. jejuni</i>	2 ^a (6.5%)	7 ^b (70.0%)	<0.001
	<i>C. spp</i> ²	-	2 (100.0%)	- ²
	Tot	3 ^a (6.5%)	21 ^b (87.5%)	<0.001
CIP-TET-NA	<i>C. coli</i>	10 ^a (66.7%)	0 ^b (0.0%)	<0.001
	<i>C. jejuni</i>	21 ^a (67.7%)	0 ^b (0.0%)	<0.001
	<i>C. spp</i> ²	-	0 (0.0%)	- ²
	Tot	31 ^a (67.4%)	0 ^b (0.0%)	<0.001
ST-CIP-TET	<i>C. coli</i>	1 ^a (6.7%)	12 ^b (100.0%)	<0.001
	<i>C. jejuni</i>	2 ^a (6.5%)	7 ^b (70.0%)	<0.001
	<i>C. spp</i> ²	-	2 (100.0%)	- ²
	Tot	3 ^a (6.5%)	21 ^b (87.5%)	<0.001
CIP-TET-NA-ERY	<i>C. coli</i>	6 ^a (40.0%)	0 ^b (0.0%)	0.017
	<i>C. jejuni</i>	6 (19.4%)	0 (0.0%)	0.307
	<i>C. spp</i> ²	-	0 (0.0%)	- ²
	Tot	12 ^a (26.1%)	0 ^b (0.0%)	0.004
ST-CIP-TET-NA	<i>C. coli</i> [*]	0 (0.0%)	0 (0.0%)	- ³
	<i>C. jejuni</i>	1 (3.2%)	0 (0.0%)	1.000
	<i>C. spp</i> ²	-	0 (0.0%)	- ²
	Total	1 (2.2%)	0 (0.0%)	1.000

¹estimated by Chi square or Fisher test. Values in bold are statistically significant at the 0.05 level (Chi square or Fisher test).

Column proportions within a row lacking a common superscript differ ($P < 0.05$; z test).

²No statistics are computed because only breast products were present.

³No statistics are computed because Susceptibility category is a constant.

n = number.

survival. A decline of *C. jejuni* count in poultry carcasses during refrigeration was also seen by Alter et al. (2005) and Meldrum et al. (2005), but several studies failed to demonstrate the *Campylobacter* reduction in the same conditions (Bhaduri and Cottrell, 2004; Paulsen et al., 2005; Maziero and de Oliveira, 2010). Georgsson et al. (2006) showed that a decrease in count of *Campylobacter* occurred most rapid immediately after the carcasses were frozen and a further loss of viability was limited to the first week of frozen storage.

As regard the antimicrobial susceptibility test, our work confirmed the high prevalence of resistance to CIP and TET for *C.coli* and *C.jejuni* in both thigh and breast samples. The thigh isolates were also resistant to NA. From the 1980s the resistance of *Campylobacter* to quinolones has been reported as result of their large use in metaphylaxis and therapy in industrial farms (Pezzotti et al., 2003; Alfredson and Korolik, 2007; Ge et al., 2013). Resistant *Campylobacter* isolates were isolated in feces of chickens, experimentally infected, already 24 hours after the beginning of enrofloxacin treatment. Moreover the antibiotic was unable to completely eliminate microorganisms but it could cause the

Campylobacter “conversion” to a drug-resistant bacterial population (Griggs et al., 2005).

As regard to ERY the 46.7% and the 19.4% of *C.coli* and *C.jejuni* isolates from thigh samples were respectively resistant. We have already reported in broiler chickens that the 30% of *C.coli* isolates were resistant to ERY whereas all *C.jejuni* isolates were susceptible (Pergola et al., 2017). These results are in agreement with a recent study (Fraqueza et al. 2014) showing that *Campylobacter* isolates from poultry displayed erythromycin resistance, often connected with resistance to other antimicrobial classes. In our work the ERY resistant *Campylobacter* showed the multiresistance pattern only in association with CIP-TET-NA.

The progressive increase of resistance to erythromycin, as well as to all macrolides, can be justified by the use for the therapy of several infectious diseases in industrial poultry over the years (Bradbury et al. 1994; Collier et al. 2003), It should be also reported that the erythromycin is one of most efficient drug used for human Campylobacteriosis (Xia et al., 2013) and, although the prevalence of resistance in human is still low, it could become higher.

In our work we observed the presence of *Campylobacter* isolates resistant to ST in skinless breast, responsible for the co and multiple resistance patterns that were the only ones reported in these samples. It should be highlighted that ST is not largely used in industrial chicken. This datum should be further assessed since this antimicrobial have been used for therapeutic treatment of several bacterial diseases in man and WHO (2013) reported it in the list of “critical antimicrobials”. Recently a new streptomycin resistance gene in *C. coli* has been detected and sequenced by Olkkola et al. (2016). This gene does not belong to a multiple drug resistance plasmid or transposon and appear widely to spread among *C. coli* strains from Europe and the United States.

In our work multiple resistant isolates came from thigh products and they were characterized by multi-resistant patterns as described by others (Kurincic et al., 2005; Wang et al., 2014). The presence of simultaneous resistance profiles are also observed in organic rearing, although the antibiotic use is strictly controlled, but the possibility for birds to stay outdoors exposes them to a greater contact with *Campylobacter* and other microorganisms, eliminated by wild birds or present in the soil (Rosenquist et al., 2013).

The present study evidenced the need to implement the control measure strategy addressed to reduce the level of *Campylobacter* contamination during the slaughter steps because of the consistent counts found from meat chicken products, especially from meat chicken products with skin, as thigh. Refrigeration seemed to be a tool, helpful in reducing the number of *Campylobacter*, though it is not able to eliminate it fully. The antimicrobial resistance evidenced in the isolated strains, supports that it is actually the true concern for Public Health, as seen in our work for the progressive occurrence of erythromycin and streptomycin *Campylobacter* resistant isolates, since these antimicrobials are used in human diseases.

REFERENCES

- Alfredson, D. A., and V. Korolik. 2007. Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. Federation of European Microbiological Societies (FEMS) Microbiol. Lett. 277:123–132.
- Alter, T., F. Gaull, A. Froeb, and K. Fehlhaber. 2005. Distribution of *Campylobacter jejuni* strains at different stages of a turkey slaughter line. Food Microbiol. 22:345–351.
- Bardon, J., M. Kolar, R. Karpiskova, and K. Hricova. 2011. Prevalence of thermotolerant *Campylobacter* spp. in broilers at retail in the Czech Republic and their antibiotic resistance. Food Control. 22:328–332.
- Bhaduri, S., and B. Cottrell. 2004. Survival of cold-stressed *Campylobacter jejuni* on ground chicken and chicken skin during frozen storage. Appl. Environ. Microbiol. 70:7103–7109.
- Blaser, M. J., D. G. Newell, S. A. Thompson, and E. L. Zechner. 2008. Pathogenesis of *Campylobacter fetus*. Pages 401–428 In: I. Nachamkin, C. M. Szymanski, and M. J. Blaser, ed. *Campylobacter*. ASM Press, Washington, DC.
- Bradbury, J. M., C. A. Yavari, and C. J. Giles. 1994. In vitro evaluation of various antimicrobials against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by the micro-broth method, and comparison with a commercially-prepared test system. Avian Pathology. 23:105–115.
- Byrd, J. A., D. E. Corrier, M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. Avian Diseases. 42:802–806.
- Chan, K. F., H. Le Tran, R. Y. Kanenaka, and S. Kathariou. 2001. Survival of clinical and poultry-derived isolates of *Campylobacter jejuni* at a low temperature (4 degrees C). Appl. Environ. Microbiol. 67:4186–4191.
- Colles, F. M., K. Jones, R. M. Harding, and M. C. Maiden. 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl. Environ. Microbiol. 69:7409–7413.
- Collier, C. T., J. D. van der Klis, B. Deplancke, D. B. Anderson, and H. R. Gaskins. 2003. Effects of tylosin on bacterial mucolysis, *Clostridium perfringens* colonization, and intestinal barrier function in a chick model of necrotic enteritis. Antimicrob. Agents Chemother. 47:3311–3317.
- Comin, D., A. Valero, G. Manfreda, R. M. García-Gimeno, A. Paiusco, D. De Medici, P. Terza, S. Ferrarini, and A. De Cesare. 2014. Microbiological criteria for *Campylobacter* in broiler carcasses in Italy: a possible approach to derive them. Int. J. Food Microbiol. 184:64–68.
- Corry, J., and H. Atabay. 2001. Poultry as a source of *Campylobacter* and related organisms. J. Appl. Microbiol. 90:96S–114S.
- Di Giannatale, E., G. Di Serafino, K. Zilli, A. Alessiani, L. Sacchini, G. Garofolo, G. Aprea, and F. Marotta. 2014. Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. Sensors. 14: 3308–3322.
- El-Shibiny, A., P. L. Connerton, and I. F. Connerton. 2005. Enumeration and Diversity of *Campylobacter*s and Bacteriophages Isolated during the Rearing Cycles of Free-Range and Organic Chickens. Appl. Environ. Microbiol. 71:1259–1266.
- El-Shibiny, A., P. L. Connerton, and I. F. Connerton. 2007. *Campylobacter* succession in broiler chickens. Vet. Microbiol. 125:323–332.
- EUCAST. 2013. Breakpoint tables for interpretation of MICs and zone diameters. <http://www.eucast.org>, Version 3.1.
- Fraqueza, M. J., A. Martins, A. C. Borges, M. H. Fernandes, M. J. Fernandes, Y. Vaz, R. J. Bessa, and A. S. Barreto. 2014. Antimicrobial resistance among *Campylobacter* spp. strains isolated from different poultry production systems at slaughterhouse level. Poult. Sci. 93:1578–1586.
- Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe. 2000. “Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations,” Pages 163–189 in I. Nachamkin, C. Szymanski, and M. J. Blaser, ed. *Campylobacter*. Third Edition. ASM Press, Washington.
- Ge, B., F. Wang, M. Sjölund-Karlsson, and P. F. McDermott. 2013. Antimicrobial resistance in *Campylobacter*: Susceptibility testing methods and resistance trends. J. Microbiol. Methods. 95: 57–67.
- Georgsson, F., A. Porkelsson, M. Geirsdóttir, J. Reiersen, and N. J. Stern. 2006. The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. Food Microbiol. 23:677–683.
- Gibreel, A., and D. E. Taylor. 2006. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. 58:243–255.
- Griggs, D. J., M. M. Johnson, J. A. Frost, T. Humphrey, F. Jørgensen, and L. J. Piddock. 2005. Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. Antimicrob. Agents Chemother. 49:699–707.
- Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. Poult. Sci. 74:1548–1552.
- Hue, O., S. Bouquin, M.-J. Laisney, V. Allain, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P.-Y. Gloaguen, M. Picherot, J. Santolini, G. Salvat, S. Bougeard, and M. Chemaly. 2010. Prevalence of

- and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. *Food Microbiol.* 27:992–999.
- ISO 10272: 2006. Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp.
- Johnsen, G., H. Kruse, and M. Hofshagen. 2006. Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. *J. Appl. Microbiol.* 101:1130–1139.
- Kaakoush, N. O., and H. M. Mitchell. 2012. *Campylobacter concisus*—a new player in intestinal disease. *Front. Cell. Inf. Microbiol.* 2:3–4.
- Kotula, K. L., and Y. Pandya. 1995. Bacterial contamination of broiler chickens before scalding. *J. Food Prot.* 58:1326–1329.
- Kurincic, M., I. Berc, T. Zorman, and S. Smole Mozina. 2005. The prevalence of multiple antibiotic resistance in *Campylobacter* spp. from retail poultry meat. *Food Technol. Biotech.* 43:157–163.
- Luber, P., E. Bartelt, E. Genschow, J. Wagner, and H. Hahn. 2003. Comparison of broth microdilution, E test, and Agar Dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. *J. Clin. Microbiol.* 41:1062–1068.
- Luber, P., and E. Bartelt. 2007. Enumeration of *Campylobacter* spp. on the surface and within chicken breast filets. *J. Appl. Microbiol.* 102:313–318.
- Lucey, B., B. Cryan, F. O'Halloran, P. G. Wall, T. Buckley, and S. Fanning. 2002. Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. *Vet Rec.* 151:317–320.
- Maziero, M. T., and T. C. R. M. de Oliveira. 2010. Effect of refrigeration and frozen storage on the *Campylobacter jejuni* recovery from naturally contaminated broiler carcasses. *Braz. J. Microbiol.* 41:501–505.
- Meldrum, R. J., D. Tucker, R. M. M. Smith, and C. Edwards. 2005. Survey of Salmonella and Campylobacter Contamination of Whole, Raw Poultry on Retail Sale in Wales in 2003. *J. Food Prot.* 68:1447–1449.
- Moran, L., P. Scates, and R. H. Madden. 2009. Prevalence of *Campylobacter* spp. in raw retail poultry on sale in Northern Ireland. *J. Food Prot.* 72:1830–1835.
- Murphy, C., C. Carroll, and K. N. Jordan. 2006. Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *J. Appl. Microbiol.* 100:623–632.
- Nachamkin, I., K. Bohachick, and C. M. Patton. 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* 31:1531–1536.
- Newell, D. G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. *Appl. Environ. Microbiol.* 69:4343–4351.
- Oikkola, S., A. Culebro, P. Juntunen, M. L. Hänninen, and M. Rossi. 2016. Functional genomics in *Campylobacter coli* identified a novel streptomycin resistance gene located in a hypervariable genomic region. *Microbiol.* 162:1157–1166.
- Papavasileiou, E., A. Voyatzi, K. Papavasileiou, A. Makri, I. Andrianopoulou, and S. Chatzipanagiotou. 2007. Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from hospitalized children in Athens, Greece, collected during 2004–2005. *Eur. J. Epidemiol.* 22:77–78.
- Paulsen, P., P. Kanzler, F. Hilbert, S. Mayrhofer, S. Baumgartner, and F. J. M. Smulders. 2005. Comparison of three methods for detecting *Campylobacter* spp. in chilled or frozen meat. *Int. J. Food Microbiol.* 103:229–233.
- Pergola, S., M. P. Franciosini, F. Comitini, M. Ciani, S. De Luca, S. Bellucci, L. Menchetti, and P. Casagrande Proietti. 2017. Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. *J. Appl. Microbiol.* 122:1348–1356.
- Peyrat, M. B., C. Soumet, P. Maris, and P. Sanders. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *Int. J. Food. Microbiol.* 124:188–194.
- Pezzotti, G., A. Serafin, I. Luzzi, R. Mioni, M. Milan, and R. Perin. 2003. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *Int. J. Food. Microbiol.* 82:281–287.
- Rasschaert, G., K. Houf, J. Van Hende, and L. De Zutter. 2006. *Campylobacter* contamination during poultry slaughter in Belgium. *J. Food Prot.* 69:27–33.
- Reich, F., V. Atanassovaa, E. Haunhorstb, and G. Kleina. 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. *Int. J. Food. Microbiol.* 127:116–120.
- Rosenquist, H., L. Boysen, A. L. Krogh, A. N. Jensen, and M. Nauta. 2013. *Campylobacter* contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat. *Int. J. Food. Microbiol.* 162:226–230.
- Ruiz, J. L., P. Goñi, F. Marco, F. Gallardo, B. Mirelis, T. Jimenez De Anta, and J. Vila. 1998. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of gyrA gene mutations in quinolone-resistant clinical isolates. *Microbiol. Immunol.* 42:223–226.
- Saiyudthong, S., K. Phusri, and S. Buates. 2015. Rapid Detection of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* in Fresh Chicken Meat and By-Products in Bangkok, Thailand, Using Modified Multiplex PCR. *J. Food. Prot.* 78:1363–1369.
- Scallan, E., P. M. Griffin, F. J. Angulo, R. V. Tauxe, and R. M. Hoekstra. 2011. Foodborne Illness Acquired in the United States—Major Pathogens. *Emerg. Infect. Dis.* 17:7–15.
- Skarp, C. P., M. L. Hanninen, and H. I. Rautelin. 2016. Campylobacteriosis: the role of poultry meat. *Clin. Microbiol. Infect.* 22:103–109.
- Silva, J., D. Leite, M. Fernandes, C. Mena, P. A. Gibbs, and P. Teixeira. 2011. *Campylobacter* spp. as a Foodborne Pathogen: A Review. *Front. Microbiol.* 2:200.
- Stanley, K., and K. Jones. 2003. Cattle and sheep farms as reservoirs of *Campylobacter*. *J. Appl. Microbiol.* 94:104–113.
- Slader, J., G. Domingue, F. Jorgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Appl. Environ. Microbiol.* 68:713–719.
- Stephens, C. P., S. L. On, and J. A. Gibson. 1998. An outbreak of infectious hepatitis in commercially reared ostriches associated with *Campylobacter coli* and *Campylobacter jejuni*. *Vet. Microbiol.* 61:183–190.
- Stern, N. J., M. R. S. Clavero, J. S. Bailey, N. A. Cox., and M. C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. *Poult. Sci.* 74:937–941.
- Waldenstrom, J., T. Broman, I. Carlsson, D. Hasselquist, and B. Olsen. 2001. Prevalence of *Campylobacter* spp. in migrating wild birds in Sweden—ecological considerations of carriership. *Int. J. Med. Microbiol.* 291:37–38.
- Wang, G., C. G. Clark, T. M. Taylor, C. Pucknell, C. Barton, L. Price, D. L. Woodward, and F. G. Rodgers. 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J. Clin. Microbiol.* 40:4744–4747.
- Wang, Y., M. Zhang, F. Deng, Z. Shen, C. Wu, J. Zhang, Q. Zhang, and J. Shen. 2014. Emergence of multidrug-resistant *Campylobacter* species isolates with a horizontally acquired rRNA methylase. *Antimicrob. Agents Chemother.* 58:5405–5412.
- World Health Organization (WHO). 2013. The global view of campylobacteriosis: report of expert consultation, Utrecht, Netherlands, 9 to 11 July 2012. World Health Organization, Geneva, Switzerland. Available at: <http://apps.who.int/iris/handle/10665/80751>.
- Xia, Q., W. T. Muraoka, Z. Shen, O. Sahin, H. Wang, Z. Wu, P. Liu, and Q. Zhang. 2013. Adaptive mechanisms of *Campylobacter jejuni* to erythromycin treatment. *BMC Microbiol.* 13:1–16.