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Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* identified in a slaughterhouse in Argentina

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ABSTRACT

The aim of this study was to evaluate the percentage of *Campylobacter* (*C. jejuni* and *C. coli*) from samples collected at the slaughterhouse to describe the prevalence of resistance to selected antimicrobials, and to characterize the genetic determinants. In total, from 333 samples analyzed, 31% were positive for *Campylobacter*. More positive samples were detected before the chiller (46%) than after the chiller (16%). *C. coli* (59%) was more prevalent than *C. jejuni* (41%). Antimicrobial resistance differences between *C. jejuni* and *C. coli* were found ($p < 0.001$). Multidrug resistance was found in 72% of *C. coli* isolates and 69% of *C. jejuni* isolates ($p < 0.001$). Most *C. jejuni* isolates (57%) had the three genes of the *cmeABC* efflux pump. The *tet(O)* gene and resistance-associated point mutations within both the *gyrA* and 23S rRNA genes were detected in 100% of *C. coli* isolates. On the other hand, *C. jejuni* only had more prevalence of the bla_{OXA-61} gene than *C. coli* ($p < 0.001$), and most of the *C. jejuni* isolates (70–80%) had the *tet(O)* and *gyrA* point mutation. These results could contribute to knowledge about the status of thermotolerant *Campylobacter* resistant to antimicrobials isolated from food animals in Argentina and to develop an antimicrobial resistance surveillance system.

1. Introduction

The World Health Organization considered that thermotolerant *Campylobacter* is one of the main causes of enteric infection due to food consumption in developed and developing countries (World Health Organization, 2017; Natsos et al., 2016; Pedersen et al., 2018). In developing countries like Argentina, information on food-borne disease is scant due to the inadequate data provided by the surveillance systems. Additionally, outbreak information is frequently unsubstantial because health authorities lack the capabilities or resources for detection of diarrheal diseases (Zaidi et al., 2008).

C. jejuni and *C. coli* are the most important species of thermotolerant *Campylobacter* and they are enteric commensal bacteria of poultry (Kaakoush et al., 2015). Broilers are the main reservoir of *Campylobacter* spp., and colonization in broiler ceca can reach 10^9 cfu/g of cecal content (Stern et al., 2008; EFSA and ECDC, 2016).

In Argentina, the production of chicken meat has grown substantially during the last years, reaching a total of over 757,9 million chickens processed in 2020 and producing 1,779,000 tonnes of poultry meat, which is approximately 0,13% higher than the figures for 2019. The apparent per capita consumption of chicken meat has increased by 1,2% in the last year, reaching 45.9 kg/inhabitant/year in 2020 (MAGYP, 2020).

Poultry production in Argentina is concentrated within 3800 farms and there are 54 slaughterhouses in the country. During the slaughter process, broiler carcasses may be contaminated with thermotolerant *Campylobacter*. As a common inhabitant of the gastrointestinal tract of warm-blooded animals, *Campylobacter* can be expected to contaminate meat during slaughter and evisceration as a result of fecal contamination (García Sanchez et al., 2018; Osimani et al., 2017). The improper handling or consumption of raw or undercooked meat and meat products are the main risk factors associated with campylobacteriosis in

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humans (Damjanova et al., 2011).

The increase of global antimicrobial resistance threatens human and animal health. Although human campylobacteriosis is self-limiting, ciprofloxacin (fluoroquinolone) and erythromycin (macrolide) are being used as the first line antimicrobial therapy to treat this disease (World Health Organization, 2011; Wieczorek et al., 2013). However, the increase of *Campylobacter* antimicrobial resistance, mainly against fluoroquinolones, has been demonstrated by numerous studies (Wieczorek et al., 2013; EFSA and ECDC, 2016). In this sense, the use of antimicrobial agents in veterinary medicine as a growth promotor, preventive treatment or as clinical therapy was related to the increase in antimicrobial resistance in microorganisms (Radostits and Rubinstein, 2002; Ventola 2015). Therefore, thermotolerant *Campylobacter* resistant isolates could spread throughout the food chain, posing a risk to public health (CDC, 2014; World Health Organization, 2017).

The emergence of multidrug resistance may reflect the acquisition of different resistance determinants, and the mechanisms of genetic resistance might be chromosomal or plasmid-borne, representing a combination of endogenous and acquired genes (Whyte et al., 2011; Nguyen et al., 2016). Different genes related to antimicrobial resistance in *Campylobacter* were described (Tang et al., 2017; Iovine 2013). These mechanisms include restricting antimicrobial access to their targets (efflux pumps), antimicrobial target modification or antimicrobial inactivation. Also, these mechanisms may act together in resisting different classes of antimicrobials (Tang et al., 2017).

In Argentina, only a few studies have evaluated antimicrobial resistance in *Campylobacter* strains (Pantozzi et al., 2010; Tamborini et al., 2012; Zbrun et al., 2015). Additionally, no epidemiological studies in Argentina have assessed the prevalence of *Campylobacter* resistant to antimicrobials throughout the poultry meat chain in general and at slaughterhouses in particular. This information is essential to establish a public health program to control the disease and it is fundamental for the creation of a surveillance program to monitor resistance. Because of the importance of *Campylobacter* as regards food safety and public health, the aim of this study was to evaluate the percentage of *Campylobacter* (*C. jejuni* and *C. coli*) from samples collected at the slaughterhouse, to describe the prevalence of resistance to selected antimicrobials, and to characterize the genetic determinants.

2. Materials and methods

2.1. Sample collection and Campylobacter isolation

Samples were taken from different areas of the slaughterhouse. The slaughterhouse belongs to a company with 35 chicken farms and 70 retail markets in Argentina. In the slaughterhouse, 600.000 chickens were slaughtered per month. Sampling was performed in nine visits, one per month during 2015 (from April to December). The slaughterhouse was divided in two areas, before and after the chiller. The samples before the chiller were taken from: cecum (n = 90), evisceration knives (n = 27), processing line surfaces (n = 18), workers' hands (n = 27); and after the chiller: processing line surfaces (n = 27), workers' hands in the packing area (n = 27), packing area surfaces (n = 27), and carcasses (n = 90). *Campylobacter* spp. were isolated using selective media Bolton Broth and Modified Charcoal Cefoperazone Deoxycholate (mCCDA) agar plates (ISO 10272–1). The cecum and carcasses were processed as described previously by Zbrun et al. (2017). The knives and surfaces were sampled using sterile cotton swabs. Each cotton was immersed in 5 mL Bolton Broth and incubated for 24 h at 42 °C under microaerobic conditions (5% O_2, 10% CO_2 and 85% H_2). Then, the same procedure described by Zbrun et al. (2017) was used for *Campylobacter* isolation. The workers' hands were washed with 200 ml of sterile PBS 1X, and the buffer was collected in a sterile screw flask. Then, the procedure employed was the same as for carcasses (Zbrun et al., 2017).

2.2. Identification of Campylobacter species

Preliminary identification of thermotolerant *Campylobacter* isolates was based on colony morphology, microscopic appearance (curved Gram-negative bacilli with typical motility), and the following phenotypic characteristics: oxidase and catalase production. All presumptive *Campylobacter* spp. isolates were identified to the species level (*C. jejuni* and *C. coli*) by multiplex PCR, as proposed by Vandamme et al. (1997). DNA was extracted using a Wizard genomic DNA purification kit (Promega®), and PCR products were analyzed on 1.5% agarose gels and stained with GelRed (Biotium®). Positive isolates were sub-cultured on Columbia blood agar and stored in glycerol broth (15% glycerol and 85% serum broth) at −80°*C*.

2.3. Antimicrobial susceptibility testing and determination of MICs

The antimicrobial sensitivity of *Campylobacter* isolates was tested by agar dilution assay as recommended by the Clinical and Laboratory Standards Institute in the standard M100-S23 (CLSI, 2013). *C. jejuni* and *C. coli* isolates were tested with eight antimicrobial agents: erythromycin (ERY), ciprofloxacin (CIP), gentamicin (GEN), streptomycin (STR), tetracycline (TET), enrofloxacin (ENR), chloramphenicol (CLO), and ampicillin (AMP) (Table 1). The strains were removed from the freezer and streaked onto Columbia blood agar and then incubated for 48 h at 42 °C under microaerobic 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. Approximately 10^4 cfu of these suspensions was inoculated onto Mueller-Hinton agar containing a two-fold dilution series of antimicrobials and supplemented with 5% defibrinated sheep blood using a multipoint inoculator (a Steers replicator system) with 1-mm pins. The plates were incubated for 24 h at 42 °C under microaerobic conditions. *C. jejuni* ATCC 33560 was used as a reference strain. The inhibition was evaluated according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2010). If an isolate was resistant to three or more antimicrobial classes, it was considered to be a multi-resistant profile.

2.4. Detection of antimicrobial resistance determinants

The efflux pump was evaluated by PCR using different protocols for each component of the pump: *cmeA* (Koolman et al., 2015), *cmeB* (Lin

Table 1
MIC QC ranges and breakpoints used for antimicrobial susceptibility testing by agar dilution.

Antimicrobial groups	Antimicrobial agent	[a]MIC QC range (mg/mL)	[b]MIC breakpoint (mg/mL)		
			S	I	R
Fluoroquinolone	Ciprofloxacin	0.06–0.5	≤1	2	≥4
	Enrofloxacin	N/A	≤0.5	1–2	≥4
Macrolide	Erythromycin	1–8	≤8	16	≥32
Amphenicols	Chloramphenicol	1–4	≤8	16	≥32
Aminoglycoside	Gentamicin	0.4–4	≤2	4	≥8
	Streptomycin	1–4	–	–	[c]4
Tetracycline	Tetracycline	0.25–1	≤4	8	≥16
β-lactam	Ampicillin	N/A	≤8	16	≥32

[a] The QC ranges of *C. jejuni* ATCC 33560 were directly adopted from CLSI (2010). Due to the lack of QC ranges of *C. jejuni* ATCC 33560 for enrofloxacin, we used *E. coli* ATCC 25922 as QC strain for these two antimicrobial agents (CLSI, 2010).

[b] MIC breakpoints for ciprofloxacin, erythromycin, tetracycline, and gentamicin are those recommended by the CLSI (2010). Since standardized MIC breakpoints for enrofloxacin and chloramphenicol are not available for *Campylobacter* spp., we used the breakpoints of Enterobacteriaceae for these four antimicrobial agents, as recommended by CLSI (2010).

[c] Cut off values used for the interpretation of MIC results were in accordance with EUCAST (www.eucast.org).

et al., 2002), and *cmeC* (Fakhr and Logue, 2007). Mutation at position 2075 in domain V of the 23S rRNA gene, associated with high-level erythromycin resistance, was detected by the mismatch amplification mutation assay PCR (MAMA-PCR) (Alonso et al., 2005). For tetracycline resistance, *tet(O)* was detected by PCR assay as described previously by Gibreel et al. (2004). Mutations in the quinolone resistance determining region of *gyrA*, resulting in resistance-associated T86I substitutions, were identified by MAMA-PCR as reported by Zirnstein et al. (2000) and Zirnstein et al. (1999). β-lactamase gene bla_{OXA-61} was detected as described by Obeng et al. (2012). PCR primers are described in Table 2. PCR products were visualized by electrophoresis in 1.5% agarose gels, stained with GelRed® and viewed under UV light.

2.5. Statistical analysis

The resistant frequencies for each class of antimicrobial agents and multidrug resistance (MDR) in *C. jejuni* and *C. coli* isolates, the association between the resistance of each antimicrobial and the presence of genes related to the mechanism of resistance were compared with the chi-square test and Fisher Exact Test using Infostat (Universidad Nacional de Córdoba). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Campylobacter species prevalence in different areas of the slaughterhouse

In total, from 333 samples analyzed, 102 (31%) were positive for *Campylobacter* and were stored in freezer at −80 °C for subsequent studies. The highest *Campylobacter* prevalence was detected in cecum (63%), followed by the evisceration knives (26%), and workers' hands before and after the chiller (26% and 22%). In addition, more positive samples were detected before the chiller (46%) than after the chiller (16%). *C. coli* (18%) was more prevalent than *C. jejuni* (13%) in all samples analyzed. Moreover, *C. coli* was the most prevalent species in cecum and before chilling, but after the chiller its prevalence decreased considerably (Table 3).

Table 3
Prevalence of *C. jejuni* and *C. coli* at the slaughterhouse.

	Sampling location	n samples (% positive)	Isolates of	
			C. jejuni	*C. coli*
Before chiller	Cecum	90 (63%)	15	42
	Evisceration knives	27 (26%)	3	4
	Line processing surfaces	18 (17%)	2	1
	Workers' hands	27 (26%)	1	6
	Total before chiller	162 (46%)	21 (50%)	53 (88%)
After chiller	Line processing surfaces	27 (19%)	4	1
	Workers' hands in packing area	27 (22%)	5	1
	Packing area surfaces	27 (7%)	2	0
	Carcasses	90 (17%)	10	5
	Total after chiller	171 (16%)	21 (50%)	7 (12%)
	Total	**333**	**42 (41%)**	**60 (59%)**

3.2. Antimicrobial resistance and multidrug profiles of Campylobacter isolates

Antimicrobial resistance differences between *C. jejuni* and *C. coli* were found ($p < 0.001$). In general, *C. coli* isolates were more resistant against the antimicrobials tested than *C. jejuni* isolates (Fig. 1). The analysis of *C. coli* antimicrobial resistance shows isolates having higher resistance to ciprofloxacin (100%; 95%CI 94.1%–100.0%), tetracycline (100%; 95%CI 94.1%–100.0%), erythromycin (95%; 95%CI 86.3%–98.2%), enrofloxacin (88%; 95%CI 77.8%–94.2%), and ampicillin (77%; 95%CI 64.5%–85.5%) than *C. jejuni*. Moreover, several *C. coli* isolates were classified as "intermediate" when tested on media amended with erythromycin (5%; 95%CI 1.8%–13.7%), enrofloxacin (12%; 95%CI 5.8%–22.2%), or ampicillin (23%; 95%CI 14.5%–35.5%); thus, all *C. coli* isolates were classified as either "resistant" or "intermediate" with regards to these three antibiotics". *C. jejuni* isolates showed high

Table 2
List of primers and primer sequences used for detection of antimicrobial resistance genes.

Antimicrobial	Gene	*Primer*	Sequence (5′-3′)	Amplicon length (bp)	Reference
Multiple antimicrobials (Efflux pump)	*cmeA*	*cmeA* F	TGTGCATCAGCTCCTGTGTAA	957	Koolman et al. (2015)
		cmeA R	ACGGACAAGCTTTGATGGCT		
	cmeB	*cmeB* F	GGTACAGATCCTGATCAAGCC	820	Lin et al. (2002)
		cmeB R	AGGAATAAGTGTTGCACGGAAATT		
	cmeC	*cmeC* F	AGATGAAGCTTTTGTAAATT	500	Fakhr and Logue (2007)
		cmeC R	TATAAGCAATTTTATCATTT		
Tetracycline	*tet(O)*	*tet(O)* F	GGCGTTTTGTTTATGTGCG	559	Gibreel et al. 2004
		tet(O) R	ATGGACAACCCGACAGAAGC		
Ampicillin	bla_{OXA-61}	bla_{OXA-61} F	AGAGTATAATACAAGCG	372	Obeng et al. (2012)
		bla_{OXA-61} R	TAGTGAGTTGTCAAGCC		
Ciprofloxacin	*gyrA C. jejuni*	*gyrA* F	CAACTGGTTCTAGCCTTTTG	1083	Wang et al., 2016
		gyrA R	AATTTCACTCATAGCCTCACG		
	gyrA C. coli	*gyrA* F	TATGAGCGTTATTATCGGTC	505	Zirnstein et al. (2000)
		gyrA R	GTCCATCTACAAGCTCGTTA		
	Mutation Thr-86-Ile *C. jejuni*	*gyrA* F	CAACTGGTTCTAGCCTTTTG	410	Wang et al., 2016
		MAMA*gyrA*-R	CAAAGCATCATAAACTGCAA		Zirnstein et al., 1999
	Mutation Thr-86-Ile *C. coli*	*gyrA* F	TATGAGCGTTATTATCGGTC	192	Zirnstein et al. (2000)
		MAMA*gyrA*-R	TAAGCCATCGTAAACAGCCA		
Erythromycin	ARNr23S	ARNr23 S F	GTAAACGGCGGCCGTAACTA	699	Jensen and Aarestrup (2001)
		ARNr23 S R	GACCGAACTGTCTCACGACG		
	Mutation A2075-G ARNr23S	ARNr23 S F	GTAAACGGCGGCCGTAACTA	184	Jensen and Aarestrup (2001)
		MAMAARNr 23S-R	TAGTAAAGGTCCACGGGGTCGC		Alonso et al. (2005)

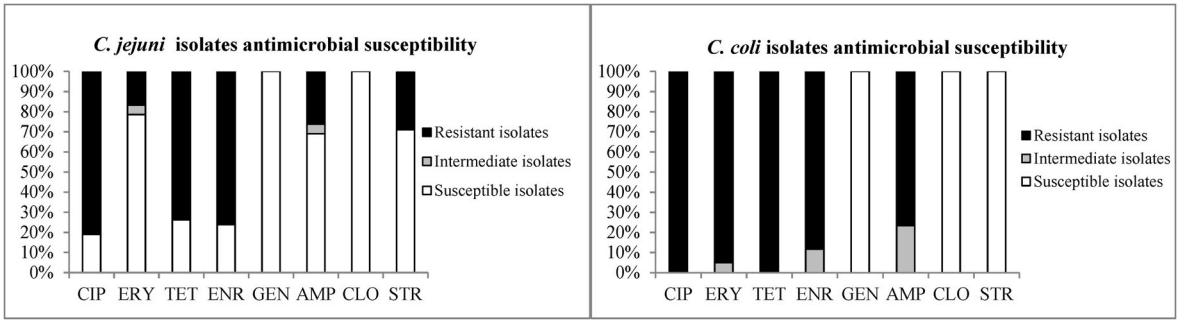

Fig. 1. Thermotolerant *Campylobacter* antimicrobials susceptibility
Reference: ERY = erythromycin, CIP = ciprofloxacin, GEN = gentamicin, STR = streptomycin, TET = tetracycline, ENR = enrofloxacin, CLO = chloramphenicol, AMP = ampicillin.

resistance to ciprofloxacin (81%, 95%CI 66.6%–90.0%), enrofloxacin (76%; 95%CI 61.4%–86.5%) tetracycline (74%; 95%CI 58.8%–84.7%), and a lower proportion of isolates were resistant to streptomycin (29%; 95%CI 17.2%–43.7%), ampicillin (26%; 95%CI 15.3%–41.2%), and erythromycin (17%; 95%CI 8.4%–30.7%). Both *Campylobacter* species were susceptible to gentamicin and chloramphenicol.

Multidrug resistance to three or more classes of antimicrobials was found in 72% (n = 43) of *C. coli* isolates and 69% (n = 29) of *C. jejuni* isolates ($p < 0.001$) (Table 4). The only MDR profile in *C. coli* was quinolone ciprofloxacin and enrofloxacin, tetracycline, ampicillin and erythromycin. For *C. jejuni*, quinolone (ciprofloxacin and enrofloxacin), tetracycline and streptomycin was the more prevalent MDR profile (28%).

3.3. Prevalence of resistant genes of Campylobacter isolates

The genes from three parts of the efflux pump were evaluated. The majority of *C. jejuni* isolates (57%) had the three genes of the efflux pump. In contrast, only one *C. coli* had the three components of the efflux pump. In addition, both species had more prevalence of *cmeC* than *cmeB* and *cmeA*. *C. jejuni* isolates had more prevalence of *cmeA* (43%) and *cmeB* (48%) of the efflux pump ($P < 0.001$) than *C. coli* isolates (*cmeA* 3%, *cmeB* 28%). Both species had a similar prevalence of *cmeC* (88.1% *C. jejuni* and 88.3% *C. coli* ($P = 0.971$).

The presence of the *tet(O)* gene, and resistance-associated point mutations within *gyrA* and the 23S rRNA were detected in 100% of the *C. coli* isolates. The bla_{OXA-61} gene was more prevalent in *C. jejuni* than in *C. coli* ($p < 0.001$), and most of the *C. jejuni* isolates (70–80%) possessed both *tet(O)* and the resistance-associated *gyrA* point mutation (Fig. 2).

3.4. Antimicrobial resistance genes and resistance profile of Campylobacter isolates

The presence of the *cmeA* ($P = 0.573$), *cmeB* ($P = 0.824$), or *cmeC* ($P = 0.343$) genes was not associated with the prevalence of multidrug resistance in *C. jejuni* isolates. All ciprofloxacin resistant *C. jejuni* and *C. coli* had the resistance-associated point mutation in the *gyrA* gene. However, for enrofloxacin, 20% of susceptible isolates of *C. jejuni* and all *C. coli* with intermediate classification harbored the point mutation in the *gyrA* gene.

Table 4
Multi-resistant profiles of thermotolerant *Campylobacter*.

Multi-resistant resistant isolates	Antimicrobial resistance profile	No. of resistant isolates (%)
C. coli (n = 60)	CIP-TET-AMP-ENR-ERY	43 (72%)
C. jejuni (n = 42)	CIP-TET-ENR-STR	12 (28%)
	CIP-TET-AMP-ENR	10 (24%)
	CIP-TET-AMP-ENR-ERY	7 (17%)

In addition, the *C. jejuni* and *C. coli* isolates which were phenotypically resistant to tetracycline harbored the *tet(O)* gene identified by PCR, and all isolates susceptible to tetracycline did not present this gene (Table 5).

The point mutation of the 23S rRNA gene was detected in all *C. jejuni* and *C. coli* isolates classified as resistant and intermediate to erythromycin. Additionally, none of the *C. jejuni* isolates susceptible to erythromycin presented the mutation (Table 5).

In some *C. coli* isolates that were resistant (61%) and intermediate (43%) to ampicillin, the bla_{OXA61} amplicon was detected. In most of the *C. jejuni* resistant isolates (73%) and in the intermediate resistant isolates, the bla_{OXA61} amplicon was detected. However, 83% of *C. jejuni* susceptible isolates harbored this amplicon (Table 5).

4. Discussion

In the present study, the presence in the slaughterhouse, microbial resistance and resistance mechanisms of thermotolerant *Campylobacter* were evaluated. Commercial chickens frequently carry high levels of *Campylobacter* spp. (primarily *C. jejuni* and *C. coli*) in their intestine as part of the normal microbiota (Newell and Wagenaar, 2000; Sahin et al., 2002). While *C. jejuni* is, in general, the most prevalent species of thermotolerant *Campylobacter* isolated at farm (Bull et al., 2006; Rossler et al., 2019), sometimes a predominant proportion of *Campylobacter* isolates from broilers are *C. coli* (Ma et al., 2014; Damjanova et al., 2011; Zbrun et al., 2013, Rossler et al., 2019).

Interestingly, in this study *C. coli* (42/90) was observed to be more prevalent than *C. jejuni* (15/90) in cecum samples. Similar results have been reported previously where *C. coli* was the predominant *Campylobacter* species in broiler intestinal tracts, which seems to depend on several factors as the geographical area evaluated (Hariharan et al., 2009; Henry et al., 2011; Ma et al., 2014), the age of the chicken and antibiotic selection pressure (Wang et al., 2016). However, in this study, the species proportion changed at the end of the slaughter line, where *C. jejuni* (10/90) was more prevalent than *C. coli* (5/90) in broiler carcasses. In this sense, some authors suggest that *C. coli* is less robust and might be more sensitive to the stress conditions found in poultry abattoirs (Peyrat et al., 2008).

Furthermore, thermotolerant *Campylobacter* were isolated from samples taken in the processing line surfaces, knives and workers' hands. Many studies have reported similar results, which can be explained by cross contamination with positive *Campylobacter* carcasses (Ono and Yamamoto, 1999; Chlebicz and Śliżewska, 2018; Zhang et al., 2018). In addition, biofilm formation may be another cause of *Campylobacter* presence on line processing surfaces. In this sense, previous research identified that some strains are able to form biofilms and can survive longer and resist inactivation (García-Sánchez et al., 2019; Lamas et al., 2018).

Regardless of the source of *Campylobacter* spp., a recent meta-

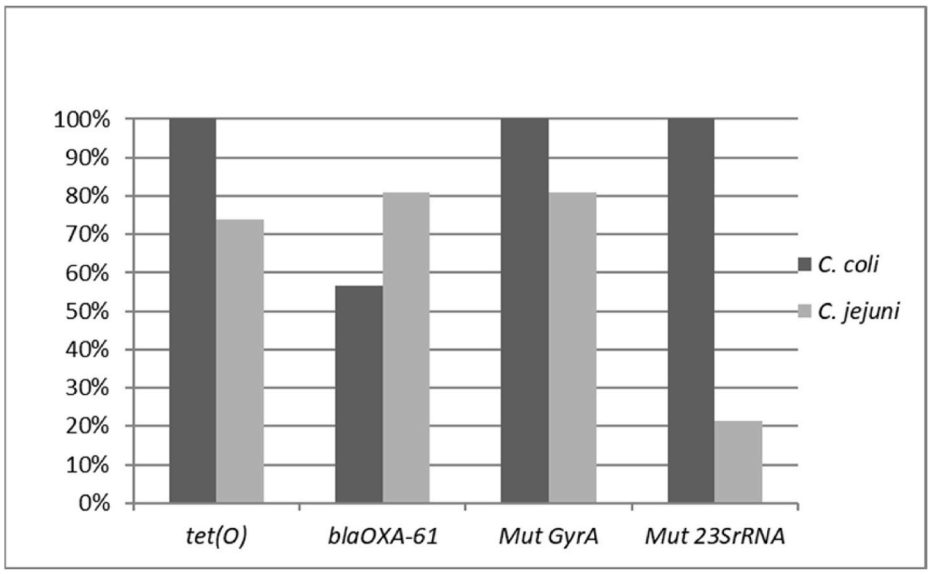

Fig. 2. Prevalence of genetic determinants to resistance of thermotolerant *Campylobacter*.

Table 5
Antimicrobial resistance genes and resistance profile of *Campylobacter* isolates.

Campylobacter species	Antimicrobial agent	Agar dilution assay			Gene presence			
		Susceptible isolates	Intermediate isolates	Resistant isolates	Gene	Susceptible isolates	Intermediate isolates	Resistant isolates
C. jejuni (n = 42)	Ciprofloxacin	8	0	34	Mut *GyrA*	0	0	34
	Enrofloxacin	10	0	32	Mut *GyrA*	2	0	32
	Erythromycin	33	2	7	Mut *23SrRNA*	0	2	7
	Tetracycline	11	0	31	*tet(O)*	0	0	31
	Ampicillin	29	2	11	bla_{OXA-61}	24	2	8
C. coli (n = 60)	Ciprofloxacin	0	0	60	Mut *GyrA*	0	0	60
	Enrofloxacin	0	7	53	Mut *GyrA*	0	7	53
	Erythromycin	0	3	57	Mut *23SrRNA*	0	3	57
	Tetracycline	0	0	60	*tet(O)*	0	0	60
	Ampicillin	0	14	46	bla_{OXA-61}	0	6	28

analysis has shown that *C. coli* isolates presented a higher prevalence of antimicrobial resistance to most antimicrobials than *C. jejuni* (Signorini et al., 2018). In this study, more than 77% of *C. coli* isolates were resistant to five (CIP, ERY, TET, ENR and AMP) out of eight antimicrobials evaluated (Fig. 1). Also, 74% of the *C. jejuni* isolates showed resistance against three (CIP, TET and ENR) out of eight antimicrobials evaluated (Fig. 1). High resistance in thermotolerant *Campylobacter* was described previously, which may be linked to the use of antimicrobials in food-producing animals in each country (CDDEP, 2015; Lajhar et al., 2015, 255 Unicomb et al., 2006; Cha et al., 2017; Nelson et al., 2007). Antimicrobials used as growth promoters in animals, and the abuse or misuse of those antimicrobials, have many times affected the resistance profile of bacteria isolates (Ventola, 2015). Particularly in Argentina, different government institutions have begun to outline strategies to control the use of antimicrobials. However, nowadays there are no clear regulations for the use of antimicrobials in the different stages of the production of food of animal origin (Lazovski et al., 2017).

Furthermore, results have shown that the MDR rate in *C. coli* and *C. jejuni* isolates was high (Table 4). The main difference between the *Campylobacter* species were erythromycin and ampicillin resistance and might be due to *C. coli* having better adaptation and survivability under antimicrobial selection pressure (Wang et al., 2016), which allows it to develop resistance to these antimicrobials (Chen et al., 2010).

In different countries such as the United States (Tang et al., 2017; Ricotta et al., 2014), China (Li et al., 2017; Wang et al., 2016), Europa (EFSA, 2019), and Guatemala (Benoit et al., 2014); the most common resistance pattern was ciprofloxacin, nalidixic acid, and tetracycline, in concordance with our results.

An intermediate classification of different antimicrobials in thermotolerant *Campylobacter* isolates was detected in this study (Fig. 1). *C. coli* had more prevalence of isolates with an intermediate classification level of resistance; this is a serious public health concern because the “intermediate” category includes isolates which showed reduced susceptibility to antimicrobials in comparison with susceptible isolates (CLSI, 2010). In this sense, most of the *C. coli* isolates were susceptible to streptomycin. Interestingly, the prevalence of *C. jejuni* isolates resistant to streptomycin was higher than *C. coli* isolates. Many studies have found that *C. coli* had higher levels of resistance than *C. jejuni* to streptomycin (Wieczorek et al., 2013; Aarestrup et al., 1997), but a few studies have detected more prevalence in *C. jejuni,* as has been found in this study (Nguyen et al., 2016).

Molecular mechanisms of resistance were also evaluated, and PCR analysis was used to detect *cmeABC* genes. While the *cmeABC* efflux pump is widely distributed in *Campylobacter* and it is constitutively expressed (Lin et al., 2002; Payot et al., 2002), only 57% of the *C. jejuni* isolates tested in this study had the three parts of the *cmeABC* efflux pump. Similar results were found by Olah et al. (2006), and it can be explained by: a) the pump being inactive, having a non-functional role

(Olah et al., 2006); or b) the efflux pump genes sequence variation (polymorphism) (Guo et al., 2004).

All isolates of this study which were resistant to ciprofloxacin and enrofloxacin carried a substitution of the amino acid 86 as consequence of a mutation in the *gyrA* gene, and this result was in agreement with previous reports (El-Adawy et al., 2015; Nguyen et al., 2016; Whitehouse et al., 2018). Interestingly, in two *C. jejuni* isolates, the *gyrA* point mutation was detected, but said isolates were not resistant to enrofloxacin. It could be suggested that the Thr-86-Ile substitution may not confer universal resistance to all quinolones as has been previously reported (Dionisi et al., 2004; Corcoran et al., 2005; Bolton et al.2013).

In *Campylobacter*, resistance to erythromycin is chromosomally encoded by a point mutation of the 23S rRNA gene. In this study, the mutation was detected by MAMA–PCR in resistant and intermediate isolates. Previously, this point mutation has been associated with high levels of erythromycin resistance (Corcoran et al., 2005; Payot et al., 2004; Taylor and Tracz, 2005).

The results of phenotypic and genetic analyses of resistance to tetracycline were fully concordant. All strains resistant to tetracycline were shown to carry the gene *tet(O)*. A correlation study of susceptibility phenotypes and genotypes using WGS has shown that all tetracycline-resistant isolates (n = 108) carried *tet(O)*, but none of the tetracycline-susceptible isolates had this gene (Zhao et al., 2001).

The mechanisms of resistance to some β-lactams such as ampicillin and some of the expanded-spectrum cephalosporins are variable and not very clearly defined (Lachance et al., 1991; Reina et al., 1994; Tajada et al., 1996). The β-lactamase gene $bla_{OXA\text{-}61}$ has spread widely in *C. jejuni* and *C. coli*, and the prevalence of the $bla_{OXA\text{-}61}$ gene in ampicillin-resistant *Campylobacter* can reach up to 91% (Griggs et al., 2009). The *C. coli* isolates demonstrated to be resistant or intermediate to ampicillin; however, the $bla_{OXA\text{-}61}$ gene was detected in only 57% of them. In *C. jejuni*, we detected the gene in 73% of resistant isolates. Other types of beta-lactamase were described (Lucain et al., 1985), which could explain these results. Lucain et al. (1985) described four enzymes based on their differing activity against eight B-lactams, relative rates of hydrolysis, molecular weight, immunological specificity, and isoelectric point (pI). However, the roles of beta-lactamases in the mechanism of resistance to ampicillin in campylobacters are not yet clear (Griggs et al., 2009).

However, 83% of *C. jejuni* susceptible isolates showed the presence of the $bla_{OXA\text{-}61}$ gene. In this sense, Casagrande Proietti et al. (2020) hypothesize that the blaOXA-61 gene was poorly expressed in the ampicillin-sensitive isolates and, therefore, they produced less β-lactamase than resistant isolates.

In conclusion, although the size of the samples analyzed is limited and they come from a single slaughterhouse, this study has revealed that the slaughter process line and the carcasses are often contaminated with thermotolerant *Campylobacter*, suggesting a possible risk of infection to consumers by improper handling and preparation of poultry meat. Moreover, taking into account the limitations regarding the cut-off points of some of the ATMs used, resistance was detected in most of the antimicrobial agents tested, and many of the *Campylobacter* isolates showed resistance to three or more antimicrobial groups (MDR). Except for ampicillin, all the resistance molecular mechanisms evaluated were detected and correlated with phenotypic resistance. In Argentina, data on the prevalence of *Campylobacter* throughout the agry-food chain and the incidence of human campylobacteriosis are uncertain. In addition, only a few studies have evaluated antimicrobial resistance in *Campylobacter* strains. In 2015, an action plan to optimize the AMR surveillance in Argentina was launched by the National Commission for the Control of Antimicrobial Resistance (CoNaCra), coordinated by the National Directorate of Epidemiology. Thus, this information is essential to establish a public health program to control the disease, and it is fundamental for designing a surveillance program to monitor resistance. Therefore, coordinated actions are recommended to reduce or eliminate the risk of thermotolerant *Campylobacter* at different stages in the slaughterhouse.

CRediT authorship contribution statement

Mariana E. Schreyer: Investigation, Methodology, Writing – original draft. **Carolina R. Olivero:** Methodology. **Eugenia Rossler:** Investigation. **Lorena P. Soto:** Supervision. **Laureano S. Frizzo:** Validation, Data curation, Conceptualization. **Jorge A. Zimmermann:** Investigation. **Marcelo L. Signorini:** Writing – review & editing, Conceptualization, Formal analysis. **Zbrun M. Virginia:** Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zbrun reports financial support was provided by Agencia Nacional de Promoción Científica y Tecnológica. Zbrun reports financial support was provided by National University of the Littoral.

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