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FOOD MICROBIOLOGY - RESEARCH PAPER

SVR-flaA typing of erythromycin- and ciprofloxacin-resistant *Campylobacter jejuni* strains isolated from poultry slaughterhouses in southern Brazil

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Abstract

The emergence of fluoroquinolone and macrolide resistance in *C. jejuni*, a recognized zoonotic pathogen, has increased worldwide. This study aimed to investigate phenotypic resistance to ciprofloxacin and erythromycin, the molecular mechanisms involved, and the strain of *C. jejuni* isolated from broiler carcasses. Eighty *C. jejuni* isolates from broiler carcasses in southern Brazil were investigated for their susceptibility to ciprofloxacin and erythromycin at minimal inhibitory concentrations. Mismatch amplification mutation assay–polymerase chain reaction (MAMA-PCR) was performed to detect substitutions of Thr-86-Ile, A2074C, and A2075G of domain V in the 23S *rRNA*. The presence of *ermB* gene and *CmeABC* operon were investigated by PCR. DNA sequencing was used to detect substitutions in the L4 and L22 proteins of the erythromycin-resistant strains. The Short Variable Region (SVR) of *fla*A was used to type all the strains resistant to both antimicrobials. Ciprofloxacin and erythromycin resistance were detected in 81.25% and 30.00% of the strains, respectively, and minimal inhibitory concentration values ranged from ≤ 0.125 to 64 µg/mL for ciprofloxacin and 0.5 to > 128 µg/mL for erythromycin. The Thr-86-Ile mutation in *gyrA* was observed in 100% of the ciprofloxacin-resistant strains. Mutations in both the A2074C and A2075G positions of 23S *rRNA* were observed in 62.5% of the erythromycin-resistant strains, while 37.5% had only the mutation A2075G. None of the strains harbored *Cme*ABC operon, and *ermB* was not detected. Using DNA sequencing, the amino acid substitution T177S was detected in L4, and substitutions I65V, A103V, and S109A were detected in L22. Twelve *fla*A-SVR alleles were identified among the strains, with the most common SVR-*fla*A allele, type 287, covering 31.03% of ciprofloxacin- and erythromycin-resistant isolates. The present study revealed a high incidence and high levels of resistance to ciprofloxacin and erythromycin, as well as broad molecular diversity in *C. jejuni* isolates from broiler carcasses.

Keywords Antimicrobial resistance · Broiler · *Campylobacter* · *flaA* typing

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Introduction

Campylobacteriosis is the most reported gastrointestinal bacterial infection in humans in the European Union [1] and the third in the USA [2] and *Campylobacter jejuni* is the species commonly associated with human cases. Chicken meat is a major source of *Campylobacter* infection in humans [3] and is recognized as a significant risk factor for acquiring this disease [4]. Human campylobacteriosis is a self-limiting illness that does not require antimicrobial treatment. However, in cases of invasive diseases and infections in immunosuppressed and young individuals, antibiotic therapy can be performed using erythromycin and ciprofloxacin as alternative drugs [5]. In

Brazil, there is little information on human campylobacteriosis because this bacterium has not been routinely investigated in human diarrhea. However, an outbreak of *Escherichia coli* O:157 infection associated with *C. jejuni* that resulted in the death of two children has been reported in southern Brazil. The authors reported that the deaths were attributed to *E. coli* O157, but that co-infection with *C. jejuni* could contribute to the severity of the symptoms [6].

Since the 1980s, an increase in *Campylobacter* fluoroquinolone-resistant strains has been reported in many countries [7–9], coinciding with the introduction of fluoroquinolones in veterinary medicine [10]. Currently, fluoroquinolone-resistant *Campylobacter* is classified as a high-priority pathogen in the Global Priority Pathogens List of Antibiotic-Resistant Bacteria by the World Health Organization [11] and fluoroquinolone- and macrolide-resistant *Campylobacter* are listed as serious threats to public health by the Centers for Disease Control and Prevention [12]. The main mechanism of resistance to fluoroquinolones in *Campylobacter* is a mutation in the Quinolone Resistance Determinant Region (QRDR) of the *gyrA* gene [5]. Resistance to macrolides is related to mutations in positions 2074 and 2075 of domain V of *23 s rRNA*. In both cases, the efflux pump *CmeABC* has been described as a secondary mechanism that acts synergistically by expelling toxic compounds such as antimicrobials, metals, and bile. Other mechanisms, such as *ermB* gene, which encodes a methylase that confers a high level of resistance to this class [13], and mutations in the L4 and L22 ribosomal proteins, have been reported in *Campylobacter* strains and are associated with low levels of resistance [14].

Several studies have reported high genetic diversity in *C. jejuni* [15–17]. Short variable regions of *fla*A gene sequence (SVR-*fla*A), Pulsed Field Gel Electrophoresis (PFGE), multilocus sequence typing (MLST), and whole genome sequencing (WGS) [18, 19] are typing methods commonly used to differentiate *Campylobacter* isolates. Although MLST and WGS are more recent typing techniques, SVR-*fla*A typing is considered a very useful tool for epidemiological studies of *Campylobacter* [20], in addition to being more affordable.

When compared to the other countries Brazil, has less data about *Campylobacter jejuni*. In this context, this study aimed to investigate the phenotypic resistance to ciprofloxacin and erythromycin, the molecular mechanisms involved, and the type of *C. jejuni* isolated from broiler carcasses.

Material and methods

Sample collection

A total of 80 *C. jejuni* strains isolated from broiler carcasses in slaughterhouses under federal inspection in three neighboring states (Parana = 28 strains; Santa Catarina and Rio Grande do Sul = 26 strains each) in southern Brazil between 2017 and 2018 [21] were included in this study. After species identification by Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry, the strains were stored in skim milk at − 80 °C for further analysis.

Antibiotic susceptibility test

The Minimum Inhibitory Concentrations (MIC) of erythromycin and ciprofloxacin (Sigma-Aldrich, Brazil) were determined according to the method reported by the Clinical and Laboratory Standards Institute [22]. MIC breakpoints for erythromycin and ciprofloxacin were ≥ 4 µg/mL and ≥ 32 µg/mL, respectively. The concentrations of erythromycin and ciprofloxacin ranged from 0.5 to 128 µg/mL and can be checked in Table 2. *C. jejuni* ATCC 33,560 was used as a quality control strain.

Molecular characterization of antimicrobial resistance

DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, Brazil) following the manufacturer's recommendations. The concentration and quality of genomic DNA were measured using a Nanodrop spectrophotometer (Biodrop®).

The strains were subjected to MAMA-PCR to detect *gyrA* mutation in codon 86 (Thr-Ile) [23] and A2075G/A2074C mutations in the 23S *rRNA* gene [24]. DNA sequencing to evaluate the presence of mutations in the *rplD* and *rplV* genes encoding the L4 and L22 ribosomal proteins was performed in erythromycin-resistant strains, according to Corcoran et al. (2006) [14]. The presence of the *ermB* gene was investigated as described by Wang et al. (2014) [13]. All strains were subjected to PCR to detect the genes *cmeA*, *cmeB*, and *cmeC* following the protocol described by Lin et al. (2002) [25]. The PCR reactions had a final volume of 25 µl containing:1X PCR buffer, 1.5 mM MgCl2, 5µL of DNA, 0.2 µM of each primer. All reactions were performed with GoTaq® G2 Hot Start Taq Polymerase (Promega, Brazil) in a T100 (Biorad) thermocycler. *C. jejuni* strains identified as carriers of the investigated genes and/or mutations were used as positive controls and ultrapure water was used as a negative control. The details of all primers used and their references are listed in Table 1. The obtained amplicons were loaded onto a 1.5% agarose gel (Lwt Biotec, Brazil) stained with ethidium bromide, submerged in Tris-Acetato-EDTA Buffer (Ludwig, Alvorada, Brazil), and subjected to electrophoresis in a horizontal electrophoresis system (Loccus, São Paulo, Brazil) at 90 V for 40 min.

Genetic diversity

Genetic diversity was assessed by DNA sequencing of the Short Variable Region (SVR) of the *flaA* gene according to Meinersmann et al. 1997 [26]. All erythromycin- and ciprofloxacin-resistant strains detected by the MIC test ($n = 24$) and five full susceptible strains were addressed. The amplicons were purified using a QIAquick PCR purification kit (Qiagen, USA) and sequenced with an ABI 3730 DNA sequencer (Applied Biosystems) using the RPT/Fiocruz Sequencing Platform. Sequences were manually edited and compared with those in current databases using the BLAST suite of programs. Nucleotide alignments were generated using ClustalW in the Unipro UGENE software [27]. For L4 and L22, the sequences were aligned to the corresponding sequence of the parent strain NCTC 11,168 using the same software to identify specific mutations, and the *flaA-SVR* nucleotide allele was obtained in the database found at PubMLST database (http://pubmlst.org/organisms/campylobacter-jejunicoli) [28]. The dendrogram was generated with the CLC GenomicsWorkbench 23.0.2 (Qiagen, The Netherlands), using the UPGMA method with the Jukes and Cantor distance correction model and bootstrap values calculated in 1000 replicates.

Statistical analysis

The chi-square test was performed using Epi Info, version 6.0, software (Centers for Disease Control and Prevention, Atlanta, Ga.) to evaluate the differences in resistance levels to erythromycin, ciprofloxacin, and their combination in strains from different states. A significant level of 0.05 was considered statistically significant.

Results

Of the 80 *C. jejuni* isolates, 81.25% (65/80) were resistant to ciprofloxacin, 30% (24/80) were resistant to erythromycin, and 18.75% (15/80) were susceptible to both the antimicrobials. The resistance observed in the Paraná state to ciprofloxacin and erythromycin was 53.57% (15/28) and 7.14% (2/28), respectively; in the Santa Catarina state 92.31% (24/26) and 30.77% (8/26); and 100% (26/26) and 53.85% (14/26) in the Rio Grande do Sul state, respectively. Strains from Paraná were more sensitive to both antimicrobials than those from Santa Catarina and Rio Grande do Sul ($p_{\text{ciprofloxacin}} = 0.0000006$; $p_{\text{erythromycin}} = 0.00003$). MIC values for ciprofloxacin ranged from ≤ 0.125 to 64 µg/mL and for erythromycin

Table 1 Target genes/mutation, primer sequence, annealing temperature (°C), amplicon size, and reference of the primers used in this study

Gene	Primer sequence (5′-3′)	Sense	Annealing temperature	Size (pb)	Reference
cmeA	TTTGGATCCTTGATGGCTAAGGCAACTTTC	Forward	54°	771	[25]
	CTCCAATTTCTTAAGCTTCGCTACCAA	Reverse			
cmeB	GGTACAGATCCTGATCAAGCC	Forward		820	
	AGGAATAAGTGTTGCACGGAAATT	Reverse			
cmeC	GCTTGGATCCTTATCTTGGGAAAAA	Forward		624	
	TTTTTAAAGCTTTAAGGTAATTTTCTT	Reverse			
ermB	GGGCATTTAACGACGAAACTGG	Forward	55°	421	[13]
	CTGTGGTATGGCGGGTAAGT	Reverse			
gyrA	TTTTTAGCAAAGATTCTGAT	Forward	50°	265	[23]
	CAAAGCATCATAAACTGCAA	Reverse			
23sRNA	TTAGCTAATGTTGCCCGTACCG	Forward	59°	485	[24]
	TAGTAAAGGTCCACGGGGTCGC*	Reverse			
	AGTAAAGGTCCACGGGGTCTGG**	Reverse			
L4	TTATCCCTCTTTTGTAATAGATTCTAA	Forward	51°	614	[14]
	ATGAGTAAAGTAGTTGTTTTAAATGAT	Reverse			
L22	TTAGCTTTCCTTTTTCACTGTTGCTTT	Forward	55°	425	
	ATGAGTAAAGCATTAATTAAATTCATAAG	Reverse			
	TGAGAAGTTAAGTTTTGGAGAG	Reverse			
SVR-*flaA*	CTATGGATGAGCAATTWAAAAT	Forward	60°	402	[26]
	CAAGWCCTGTTCCWACTGAAG	Reverse			

*Mutation detection position 2074, **mutation detection position 207

0.5 to > 128 µg/mL. Most ciprofloxacin-resistant strains (55/80) showed MIC ≥ 16 µg/mL and almost all erythromycin-resistant strains (22/24) showed MIC > 128 µg/mL (Table 2).

All ciprofloxacin-resistant strains (65/80) had a mutation at codon 86 (Thr-86-Ile) in the QRDR of *gyrA*, whereas the sensitive strains had no substitution. Regarding the erythromycin-resistant strains, 62.5% (15/24) had a mutation in both the A2074C and A2075G positions of *23S rRNA*, and 37.5% (9/24) had only the A2075G mutation. None of the sensitive strains harbored the A2074C or A2075G mutations. Six strains had amino acid substitutions in three codons (I65V, A103V, and S109A), and seven had two substitutions (I65V/S109A) in the L22 ribosomal protein. Seven strains harbored T177S substitutions in the L4 protein, and no strain had L4 and L22 substitutions concurrently. The *ermB* gene was not detected in the present study and all strains (80/80) harbored the *cmeABC* operon.

SVR-*flaA* typing revealed 12 different alleles in the 29 investigated strains. Allele 287 was the most predominant, covering 31.03% (9/29) of the isolates, followed by alleles 975 and 54 with four isolates each (4/29). The five SVR-*flaA* alleles detected in the five susceptible strains differed from those detected in resistant strains. Table 3 lists *flaA* alleles, antimicrobial resistances, and molecular markers associated with the 29 sequenced strains (Fig. 1). Two clusters were identified with a similarity greater than 84.11%. Only the Cj89 strain was grouped alone in one cluster and the other 28 strains evaluated were grouped in another cluster with a similarity ≥ 94.70%.

Discussion

High incidence and levels of resistance to both antimicrobials tested were detected in the *C. jejuni* strains tested in this study. The presence of these resistant strains represents an additional risk to public health, as infections may be more difficult to treat in cases where antimicrobial therapy is required. Strains from Paraná were more sensitive to both antimicrobials than those from Santa Catarina and Rio Grande do Sul. This may be related to the level of awareness of antimicrobial use by chicken producers, biosecurity in poultry farms leading to lower antimicrobial use, or even aspects related to differences in *Campylobacter* strains that circulate in each state. Several studies have detected fluoroquinolone-resistant strains isolated from chicken carcasses in Brazil and worldwide, with MIC exceeding 128 µg/mL [5, 29]. In our study, 81.25% (65/80) of the strains were resistant to ciprofloxacin with MIC reaching 64 µg/mL. Studies show that a single mutation at position Thr-86-Ile in the QRDR of *gyrA* is considered the main mechanism of resistance to fluoroquinolones and leads to the replacement of the amino acid threonine for isoleucine [5, 30, 31]. All resistant strains in our study harbored this mutation, which was not observed in the susceptible strains. This mutation confers a fitness benefit to *Campylobacter* in chickens by reducing the supercoiling activity of *gyrA*, which may help in the emergence, maintenance, and spread of fluoroquinolone-resistant isolates in poultry farms [32] and can overcome the colonization of susceptible strains [33]. Previous results obtained by our research group [29–31, 34] suggested the substitution of susceptible strains with resistant strains over time in the poultry production chain of Rio de Janeiro State, located in the southeast region of Brazil. The results obtained in the present study, including strains isolated between 2017 and 2018 from three other states, suggest that the replacement of susceptible strains with fluoroquinolone-resistant strains is widespread.

In our study, high levels (MIC ≥ 64 µg/mL) of erythromycin resistance were detected in 30% (24/80) of the strains. In previous studies in Brazil, erythromycin resistance in *Campylobacter* spp. isolated from poultry, ranged from 0 to 42.60% [35–38]. All resistant strains had the mutations A2074C/A2075G simultaneously or only A2075G. These mutations are considered to be the main mechanisms involved in the high levels of resistance to macrolides and do not represent any advantage for bacterial cells. Studies comparing colonization capacity demonstrated that strains without mutations supplanted mutant strains, indicating that

Table 2 Distribution of the minimal inhibitory concentration values for 80 *C. jejuni* isolates from broiler carcasses

Antimicrobial	State	MIC(µg/mL)											Susceptible isolates (%)	Resistant isolates (%)	Total
		≤0.125	0.25	0.5	1	2	4	8	16	32	64	>128			
Ciprofloxacin	Paraná	12	0	1	0	0	0	1	10	4	0	0	13 (46.42)	15 (53.58)	28 (100)
	Santa Catarina	2	0	0	0	0	0	6	7	11	0	0	2 (7.70)	24 (92.30)	26 (100)
	Rio Grande do Sul	0	0	0	0	0	0	3	9	13	1	0	0 (0)	26 (100)	26 (100)
Erythromycin	Paraná	0	0	4	7	13	0	2	0	0	0	2	26 (92.85)	2 (7.15)	28 (100)
	Santa Catarina	0	0	2	5	9	2	0	0	0	0	8	18 (69.23)	8 (30.77)	26 (100)
	Rio Grande do Sul	0	0	1	2	8	1	0	0	0	2	12	12 (46.15)	14 (53.85)	26 (100)

Table 3 Strains, state, year of isolation, minimal inhibitory concentration (MIC) for ciprofloxacin (CIP) and erythromycin (ERY), molecular mechanisms associated with antimicrobial resistance and *flaA* alleles of 29 *Campylobacter jejuni* strains isolated from Broiler Carcasses in southern, Brazil

Strain	State	Year of isolation	MIC CIP	Thr- 86-Ile	MIC ERY	23S A2074C	23S A2075G	*ermB*	L4	L22	*cmeABC*	*flaA* Allele
Cj80	SC	2018	16	Positive	> 128	Negative	Positive	Negative	WT	WT	Positive	5
Cj81	SC	2018	16	Positive	> 128	Positive	Positive	Negative	WT	WT	Positive	5
Cj89*	PR	2018	≤ 0.125	Negative	2	Negative	Negative	Negative	WT	WT	Positive	16
Cj86	PR	2018	16	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	34
Cj88	PR	2018	16	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	34
Cj23	RS	2017	16	Positive	> 128	Negative	Positive	Negative	T177S	WT	Positive	34
Cj43*	SC	2017	≤ 0.125	Negative	0.5	Negative	Negative	Negative	NT	NT	Positive	36
Cj7*	PR	2017	≤ 0.125	Negative	1	Negative	Negative	Negative	NT	NT	Positive	45
Cj32	RS	2017	32	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	54
Cj33	RS	2017	32	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	54
Cj50	RS	2018	32	Positive	> 128	Positive	Positive	Negative	WT	I65V/S109A	Positive	54
Cj90	RS	2018	32	Positive	> 128	Negative	Positive	Negative	WT	I65V/S109A	Positive	54
Cj93	RS	2018	8	Positive	> 128	Positive	Positive	Negative	WT	WT	Positive	243
Cj10	SC	2017	8	Positive	> 128	Positive	Positive	Negative	WT	WT	Positive	287
Cj26	RS	2017	32	Positive	> 128	Negative	Positive	Negative	WT	WT	Positive	287
Cj31	SC	2017	16	Positive	> 128	Positive	Positive	Negative	WT	I65V/S109A	Positive	287
Cj53	SC	2018	8	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	287
Cj54	RS	2018	16	Positive	> 128	Positive	Positive	Negative	WT	I65V/ A103V/ S109A	Positive	287
Cj55	RS	2018	32	Positive	> 128	Positive	Positive	Negative	WT	I65V/ A103V/ S109A	Positive	287
Cj57	RS	2018	32	Positive	> 128	Negative	Positive	Negative	WT	I65V/ A103V/ S109A	Positive	287
Cj59	RS	2018	16	Positive	> 128	Positive	Positive	Negative	WT	I65V/ A103V/ S109A	Positive	287
Cj77	RS	2018	8	Positive	64	Positive	Positive	Negative	WT	I65V/ S109A	Positive	287
Cj12*	PR	2017	≤ 0.125	Negative	2	Negative	Negative	Negative	NT	NT	Positive	496
Cj25	SC	2017	32	Positive	> 128	Negative	Positive	Negative	WT	I65V/ A103V/ S109A	Positive	652
Cj30	SC	2017	32	Positive	> 128	Negative	Positive	Negative	WT	I65V/ S109A	Positive	975
Cj37	SC	2017	32	Positive	> 128	Negative	Positive	Negative	WT	I65V/ S109A	Positive	975
Cj58	RS	2018	16	Positive	64	Negative	Positive	Negative	WT	I65V/ S109A	Positive	975
Cj87	RS	2018	32	Positive	> 128	Positive	Positive	Negative	T177S	WT	Positive	975
Cj72*	PR	2018	≤ 0.125	Negative	1	Negative	Negative	Negative	NT	NT	Positive	1123

*Sensitive strains to both antimicrobials. *NT*, non-tested; *WT*, wild type; *PR*, Paraná; *SC*, Santa Catarina; *RS*, Rio Grande do Sul

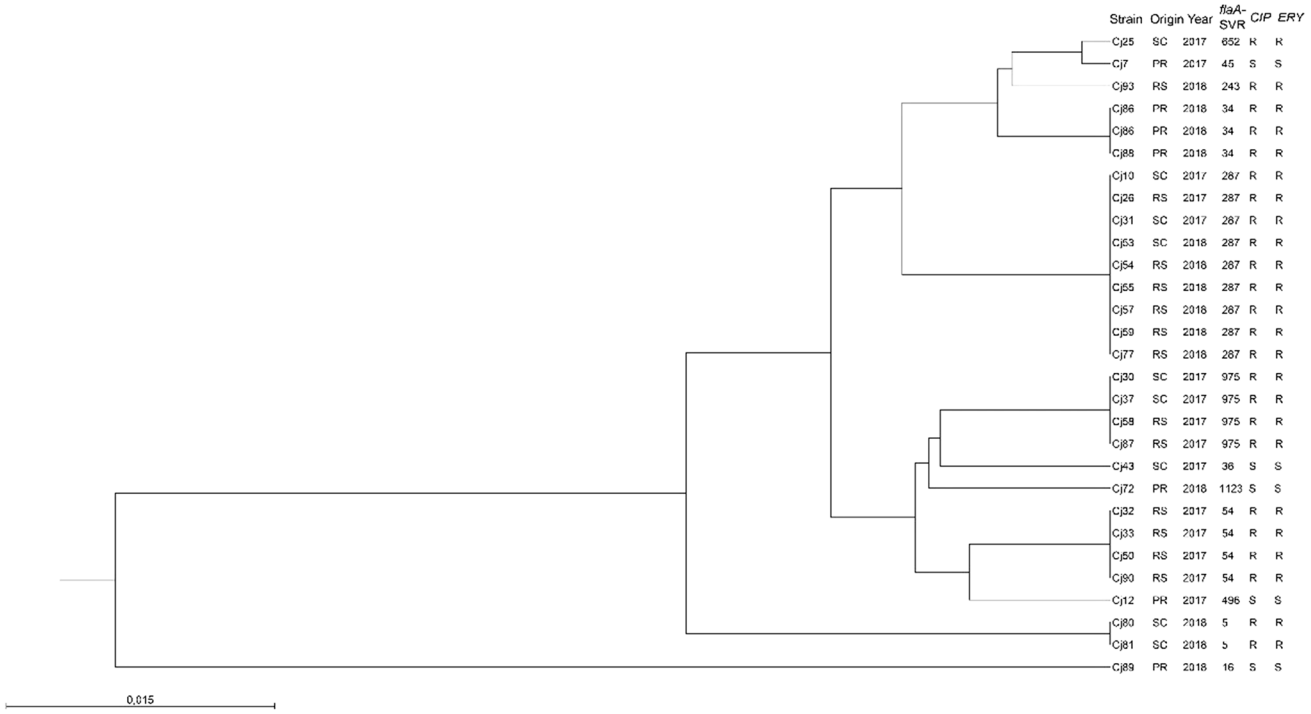

Fig. 1 Phylogenetic tree based on 29 SVR-*flaA* gene sequences of *C. jejuni* isolates from chicken carcasses in southern Brazil. The dendrogram was inferred by using the UPGMA method with Jukes and Cantor distance correction model and bootstrap values calculated in 1000 replicates. "PR" Paraná, "SC" Santa Catarina, "RS" Rio Grande do Sul, "CIP" Ciprofloxacin, "ERY" Erythromycin, "R" resistant strain, and "S" a susceptible strain

in the absence of macrolides, these mutations decrease cell fitness [39]. *ErmB* encodes a ribosomal RNA methylase capable of methylating the macrolide-binding site and has also been linked to high levels of resistance in *Campylobacter* [13]. Since its first detection in China [13], reports of *ermB*-positive *Campylobacter* isolate have occurred in several countries [40–43]. Although one report was published in Latin America [44], this gene has not been detected in *Campylobacter* isolates from Brazil [36, 45, 46].

We detected the I65V, A103V, and S109A in L22 amino acid substitutions, and only the T177S substitution in the L4 ribosomal protein, as previously reported [14, 47, 48]. Mutations in L4 and L22 have been associated with a lower level of resistance to macrolides (erythromycin MIC = 32 µg/mL) in the absence of mutations in *23S rRNA* genes [39]. In our study, it was difficult to estimate the real contribution of these substitutions to the level of resistance because the erythromycin-resistant strains had other mutations at positions 2074 and/or 2075 in the *23S rRNA*, and low levels of resistance were not detected.

In addition to mutations in sites of antimicrobial action, such as *gyrA* and domain V of the 23S *rRNA*, the presence of efflux pumps in *Campylobacter* spp. is recognized as a secondary mechanism of resistance to ciprofloxacin and erythromycin and has been considered a factor that may potentiate resistance to these classes [49]. We detected genes related to the *CmeABC* efflux pump in 100% (80/80) of the strains, similar to the results obtained in other studies [50, 51]. The high prevalence of these genes is probably due to the known participation of efflux pumps in bacterial cell metabolism, mediation of resistance to bile salts in the intestinal tract, uptake of essential nutrients and ions, and excretion of bacterial metabolism products and toxic substances, which play a fundamental role in colonization by *Campylobacter* [49, 52].

Campylobacter is characterized by its high genetic variability, and the formation of new combinations of genetic alleles can be accelerated because this bacterium is naturally competent for DNA uptake and transformation [53]. Despite the wide use of MLST and PFGE, *flaA* typing is a commonly-employed technique offering discriminatory power, high reproducibility even if performed in different laboratories, and the possibility of comparison with strains from other countries deposited in the databases [18, 54]. However, SVR-*flaA* typing is a single-locus analysis method that does not assess the entire genome. Twelve different alleles of *C. jejuni* were identified. Although many strains clustered into the same allele, such as those belonging to the *SVR-flaA* alleles 287, 975, and 54, few differences in mutations and antimicrobial resistance levels to ciprofloxacin were

observed. Frequent intra-and interspecies genetic mutations in *C. jejuni* result in different molecular variants, as determined by SVR-*flaA* typing [55]. The *fla*A allele type 287 was the most common, occurring in 31.03% (9/29) of the strains, although it was not detected in the Parana state. Other studies [56, 57] have detected this allele in samples from chickens and cases of human campylobacteriosis, reinforcing the role of chickens as a source of *Campylobacter*. Wieczorek et al. (2019) [55] found that this SVR-*flaA* type was the most common among numerous multidrug-resistant profiles, including resistance to ciprofloxacin in *Campylobacter* isolates from poultry chains. However, it was not clear whether there was a correlation between *flaA-SVR* 287 genotype and antimicrobial resistance. Other *flaA* allele types detected in this study, such as 34 and 45, have also been associated with human cases and chicken meat [57–59]. Additional research is necessary to investigate the connection between antimicrobial resistance and genotypes. Apart from the strains clustered into alleles 287, 975, and 54, we observed variability among alleles in the three different states. Since evolution is a dynamic process, monitoring the genotypes of *Campylobacter* is vital, as several anthropogenic factors such as intensive animal production and antibiotic use can act as selective pressures, changing epidemiological chains and how this microorganism interacts with its hosts.

Conclusion

A high incidence and level of resistance to ciprofloxacin and erythromycin and related point mutations were detected in *C. jejuni*. These results are of public health concern when antibiotic therapy is required for human campylobacteriosis caused by poultry strains. We also detected variability in the SVR-*fla*A alleles among the resistant strains, corroborating the high diversity reported in several *Campylobacter* studies. Owing to the high plasticity and genetic diversity of *Campylobacter jejun*i, whole-genome sequencing should be performed on strains circulating in the Brazilian poultry industry to investigate the possible relationship between certain genotypes and antimicrobial resistance.

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Declarations

Conflict of interest The authors declare no competing interests.

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