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Original Article

Antimicrobial Resistance in Campylobacter jejuni Isolated from Brazilian Poultry Slaughterhouses

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INTRODUCTION

Campylobacteriosis is one of the most common foodborne diseases in the world. It is considered the most frequently reported foodborne illness in the European Union (EU) and one of the most important in the United States (US) (EFSA & ECDC, 2018; CDC, 2019a; WHO, 2019). Poultry is known to be the major reservoir and an important source for pathogen transmission to humans (Kaakoush *et al.*, 2015). *Campylobacteriosis* is most often associated with the consumption of raw and undercooked poultry or the cross-contamination of other foods by these items (CDC, 2019a). Although Brazil is a leading supplier of the world's poultry meat (ABPA, 2018), Brazil's official data does not report *Campylobacter* infections.

Resistance in foodborne pathogens presents the potential for their transmission to humans through the food chain (Wang *et al.,* 2013). *Campylobacteriosis* is generally a self-limiting disease. However, in some patients, *Campylobacter* infection can result in a systemic disease requiring the use of antimicrobials (CDC, 2019b). Erythromycin is considered the first-line treatment, but fluoroquinolones are also frequently used due to their broad-spectrum activity against enteric pathogens (Engberg *et al.,* 2001). Recently, however, multidrug-resistant *Campylobacter* strains have been detected in poultry and several other sources around the world (Szczepanska *et al.,* 2017; Du *et al.,* 2018; Montgomery *et al.,* 2018).

In the EU, *Campylobacter* isolated from human and poultry sources have shown high to extremely high resistance to ciprofloxacin and tetracycline (EFSA & ECDC 2018), and both substances have been widely used in Brazilian poultry production in recent decades (Machinski Júnior *et al.,* 2005). Ciprofloxacin resistance in *Campylobacter* strains is usually related to the Tre-86-Ile mutation in the quinolone resistance-determining region (QRDR) of the *gyrA* gene, which results in the replacement of the amino acid threonine by isoleucine (Frasao *et al.,* 2015a). Resistance to tetracycline is usually related to the presence of the *tetO* gene (Pratt & Korolik, 2005). Our aim was to assess the minimum inhibitory concentrations (MICs) for *Campylobacter jejuni* strains and determine their molecular resistance profiles to tetracycline and ciprofloxacin.

MATERIALS AND METHODS

Bacterial strains and growth conditions

A total of 54 *C. jejuni* strains were selected for this study (Table 1). Isolates were obtained from broiler carcass samples collected between 2011 and 2012 from different Brazilian poultry slaughterhouses according to criteria described by the International Organization for Standardization (ISO 10272-1:2017). The bacterial isolates were stored

Table 1 – *Campylobacter jejuni* strains: identification, source of isolation, phenotypic resistance profiles and molecular resistance profile.

Identification	Source of isolation	Phenotypic resistance profile		Molecular resistance profile			
		CLSI breakpoints [a]	EUCAST breakpoints	tetracycline (*tetO*)[b]	ciprofloxacin (mutation in *gyrA*)[c]		
					Ter-86-Ile	Val-149-Ile	silent mutations
1	cooled carcass	CIP, TET, NAL	CIP, TET	-	+	+	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
2	cooled carcass	*	*	NA	NA	NA	
3	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro
4	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	+	NA	NA	
5	carcass after washing	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	+	+	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
6	cooled carcass	CIP, TET, NAL, ERY	CIP, ERY, NAL	NA	NA	NA	NA
7	carcass before scalding	CIP, TET, NAL, ERY	CIP, ERY, NAL	NA	NA	NA	NA
8	frozen carcass (60 days)	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	NA	NA	NA
9	carcass after plucking	NAL	TET	-	NA	NA	NA
10	cooled carcass	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	+	+	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
11	cooled carcass	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	+	+	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
12	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro
13	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
14	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	-	NA	NA	NA
15	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
16	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
17	cooled carcass	CIP, TET, NAL	CIP, TET, NAL	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
18	cooled carcass	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	+	+	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
19	cooled carcass	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	-	NA	NA	NA
20	carcass after chiller	CIP, TET, NAL	CIP, TET, NAL	-	NA	NA	NA
21	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
22	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro
23	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
24	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
25	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
26	cloacal swab	CIP, TET, NAL	CIP, TET, NAL	-	NA	NA	NA
27	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	NA	NA	NA
28	chicken cuts	CIP, NAL	CIP, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro, Gli-110-Gli
29	chicken cuts	CIP, NAL	CIP, NAL	NA	NA	NA	NA

Table 1 – *Campylobacter jejuni* strains: identification, source of isolation, phenotypic resistance profiles and molecular resistance profile.

Identification	Source of isolation	Phenotypic resistance profile		Molecular resistance profile			
		CLSI breakpoints [a]	EUCAST breakpoints	tetracycline (*tetO*)[b]	ciprofloxacin (mutation in *gyrA*)[c]		
					Ter-86-Ile	Val-149-Ile	silent mutations
30	chicken cuts	CIP, NAL	CIP, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro, Gli-110-Gli
31	chicken cuts	CIP, NAL	CIP, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro, Gli-110-Gli
32	chicken cuts	CIP, NAL	CIP, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Gli-110-Gli
33	cooled carcass	CIP, ERY, NAL	CIP, ERY, NAL	NA	NA	NA	NA
34	frozen carcass (60 days)	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
35	chicken cuts	CIP, TET, NAL	CIP, TET	-	NA	NA	NA
36	chicken cuts	CIP, TET, NAL, ERY	CIP, TET, NAL, ERY	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
37	carcass after evisceration	CIP, TET, NAL	CIP, TET	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
38	cloacal swab	TET, NAL	TET, NAL	-	NA	NA	NA
39	carcass after plucking	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
40	chiller water	CIP, TET, NAL	CIP, TET, NAL	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
41	cloacal swab	CIP, TET, NAL	CIP, TET, NAL	+	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
42	pre chiller water	CIP, ERY, NAL	CIP, ERY, NAL	NA	NA	NA	NA
43	carcass after evisceration	CIP, NAL	CIP, ERY, NAL	NA	NA	NA	NA
44	carcass after chiller	CIP, ERY, NAL	CIP, TET, NAL, ERY	-	NA	NA	NA
45	carcass after evisceration	CIP, ERY, NAL	CIP, NAL, ERY	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
46	cooled carcass	CIP, ERY	CIP, ERY	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
47	chicken cuts	CIP, ERY, NAL	CIP, ERY, NAL	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
48	chicken cuts	CIP, ERY, NAL	CIP, TET, NAL, ERY	-	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val
49	chicken cuts	CIP, NAL	CIP	NA	NA	NA	NA
50	cooled carcass	CIP, NAL	CIP, NAL	NA	NA	NA	NA
51	chicken cuts	CIP, NAL	CIP, ERY	NA	+	-	His-81-His, Ser-119-Ser, Ala-120-Ala, Ser-157-Ser, Val-161-Val, Pro-186-Pro, Gli-110-Gli
52	carcass after plucking	CIP, NAL	CIP, ERY, NAL	NA	NA	NA	NA
53	carcass after plucking	CIP	CIP	NA	NA	NA	NA
54	chiller water	CIP, ERY, NAL	CIP, ERY, NAL	NA	NA	NA	NA

Antimicrobial agents: ciprofloxacin (CIP), erythromycin (ERY), nalidixic acid (NAL), tetracycline (TET).

[a] Intermediate strains were also classified as non-susceptible.

[b] Molecular characterization performed only if MIC ≥ 2 mg/L. Other strains are identified as "Not Applicable" (NA).

[c] Molecular characterization performed only if MIC ≥ 4 mg/L. Other strains are identified as "Not Applicable" (NA).

at –80 °C in ultra-high temperature-processed milk and were reactivated on blood base agar (Oxoid, Hampshire UK) supplemented with 5% defibrinated sheep blood. The plates were incubated within a jar at 42 ± 1 °C under microaerobic conditions.

Phenotypic characterization of antimicrobial resistance

As described by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2013b), a broth microdilution test was performed to determine the MIC for six clinically relevant antibiotics (Sigma-Aldrich): chloramphenicol (0.25–128 mg/L), ciprofloxacin (0.007–16 mg/L), erythromycin (0.064–128 mg/L), gentamicin (0.064–32 mg/L), nalidixic acid (1–256 mg/L), and tetracycline (0.064–64 mg/L). The strains were classified as susceptible or non-susceptible (including intermediate strains) according to the breakpoints described in the CLSI standards (CLSI, 2013a; El-Adawy *et al.,* 2015). The strains were also classified as wild type or non-wild type (nWT) based on their epidemiological MIC cut-off (ECOFFs), which were determined according to the EUCAST guidelines available at the time of data analysis (January, 2019) (EUCAST, 2019). A *C. jejuni* reference strain (ATCC 33560) was selected to ensure the validity of the tests. Strains that were resistant to three or more classes of antimicrobials were classified as multidrug resistant (MDR) strains (Schwarz *et al.*, 2010). The multiple antibiotic resistance (MAR) index was determined as previously described (Krumperman, 1983).

Molecular characterization of antimicrobial resistance

Thermal extraction of DNA was performed as described (Sambrook & Russel, 2012). The strains with tetracycline MICs ≥2 mg/L were selected for PCR detection of the *tet*O gene. The primers were designed by Bacon *et al.* (2000). All PCR reactions (25 µL) contained 10X PCR buffer, 2.5 mM dNTPs, 10 pmol primer, 2 mM $MgCl_2$, 5 U Taq DNA polymerase, and 2 µL template DNA. The cycling program consisted of 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min. The amplified products (559 bp) were separated by electrophoresis in a 1% agarose gel stained with ethidium bromide, which was photographed under UV illumination. A total of 31 strains with ciprofloxacin MICs ≥4 mg/L were selected to characterize their molecular resistance. First, the QRDR in the *gyrA* gene was detected by PCR with primers designed by Price *et al.* (2005). All PCR reactions (25 µL) contained 10X PCR buffer, 1 mM dNTPs, 10 pmol primer, 2 mM $MgCl_2$, 1 U Taq DNA polymerase, and 5 µL template DNA. The cycling program consisted of 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The amplified products (454 bp) were separated by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and photographed under UV illumination. All reactions were repeated three times. A PCR control containing the PCR mixture without the addition of the template DNA was included with all reactions.

The amplified products of *gyrA* were then sequenced in triplicate in an automated sequencer (ABI-PRISM 3500® Genetic Analyzer; Applied Biosystems) with 50 cm capillaries and polymer POP7 (Applied Biosystems). The sequences obtained in the chromatograms were processed using Chromas Lite (Technelysium) and Geneious (Biomatters) software. To confirm the presence of the mutation, the sequence of strain *C. jejuni* (L04566.1), obtained from GenBank, was used as a ciprofloxacin-sensitive strain standard.

Statistical analysis

The data were subjected to a descriptive statistical analysis using PASW Statistics software. The kappa index (Landis & Koch, 1977) was determined to evaluate the concordance between the classifications based on the CLSI breakpoints and ECOFF values.

RESULTS

The phenotypic antimicrobial resistance profiles and MIC results are described in Tables 1 and 2. Only one strain was susceptible to all substances and all strains were clinically susceptible to gentamicin and chloramphenicol, regardless of the breakpoint (CLSI or EUCAST) evaluated. Resistance for tetracycline and erythromycin was higher when EUCAST parameters were applied. 46.3% (25/54) of the strains were classified as non-susceptible and 51.8% (28/54) as nWT for tetracycline, and 42.6% (23/54) of the strains were classified as non-susceptible and 48.1% (26/54) as nWT for erythromycin. Resistance to ciprofloxacin was equal for both parameters, and 94.4% (51/54) of the strains were classified as non-susceptible or nWT. Regarding resistance for nalidixic acid, 94.4% (51/54) of the strains were non-susceptible according to the CLSI breakpoints and 83.3% (45/54) were nWT according to EUCAST breakpoint. CLSI also classifies the strains as "intermediate", which were considered as non-susceptible in the present study (Borges *et al.*, 2019) due to their uncertain therapeutic effect *in vivo* (CLSI, 2013b).

The molecular antimicrobial resistance profiles are described in Table 1. Only 42.8% (12/28) of tetracycline non-susceptible strains presented the gene *tetO*. All strains tested for the presence of mutations in the QRDR fragment of the *gyr*A gene showed a threonine to isoleucine (Thr-86-Ile) mutation at codon 86 and 16,1% (5/31) of them presented a second mutation at codon 149 (Val-149-Ile). The silent mutations His-85-His, Ser-119-Ser, Ala-120-Ala, and Val-161-Val were observed in all the analyzed strains, while 22.6% (7/31) and 12.9% (4/31) also contained the silent mutations Pro-186-Pro and Gly-110-Gly, respectively.

DISCUSSION

Antimicrobial resistance is a complex challenge and a major problem for global public health. Each year, about 25,000 patients in the EU and 23,000 in the US die from infections caused by multiresistant bacteria, with annual treatment costs of approximately 1.5 billion euros and 20 billion dollars, respectively (WHO, 2014). The Brazilian government does not have an integrated program for monitoring antimicrobial resistance in the primary human and production animal pathogens such as *Salmonella* spp. and *C. jejuni*, making the adoption of new measures to control and restrict the use of antimicrobials difficult (Borges *et al.,* 2019). In addition, unlike European countries, Brazil has no specific legislation mandating the analysis of *campylobacteriosis*. Therefore, studies addressing antimicrobial resistance are essential for characterizing the circulating *C. jejuni* strains in the Brazilian poultry production chain.

Although similar, the results based on the ECOFF values showed a great number of nWT strains (non-susceptible). Considering that MIC determinations depend on breakpoints and that MIC results affect clini-cal decisions and official data reports (Kassim *et al.*, 2016), changes in the breakpoint parameters can result in significant changes in the final MIC. The breakpoints are set by three international agencies: the U.S. Food and Drug Administration Center for Drug Evaluation and Research (USDA-CDER), the CLSI, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Humphries *et al.,* 2019). The guidelines proposed by CLSI are some of the most used worldwide and are based on the drugs' properties and mechanisms of resistance (Kassim *et al.,* 2016), whereas EUCAST bases its breakpoints on the drugs' properties and ECOFFs (EUCAST, 2019). We compared the results for both interpretative criteria through kappa analysis. It showed perfect agreement for ciprofloxacin, gentamicin and chloramphenicol, almost perfect agreement for tetracycline (κ = 0.889) and erythromycin (κ = 0.888) and fair agreement for nalidixic acid (κ = 0.400). Comparisons among studies is challenging due to the wide variation in interpretative techniques and parameters. The agreement seen between the EUCAST and CLSI guidelines not only provides important information about antimicrobial susceptibility, it indicates that international data on *C. jejuni* resistance could be compared, thus allowing the establishment of more specific control measures for this species in the poultry production chain.

The use of chloramphenicol in production animals has been banned in Brazil since 2003 (MAPA, 2003) and the use of gentamicin in poultry production is restricted (Giacomelli *et al.,* 2014). These are probably the reasons for the absence of non-susceptible strains in our study.

Our results indicate that almost 50% of the strains were resistant to erythromycin, which is higher than the previously published data (Bolinger & Kathariou, 2017; Szczepanska *et al.,* 2017). These results are a public health concern, because this agent is the treatment of choice for *Campylobacteriosis* (Engberg *et al.,* 2001). These high rates may be associated with the wide use of this drug in animal production up until 2012, when erythromycin was banned as a food additive in Brazil (MAPA, 2012). Higher erythromycin resistance rates can also be related to the several mechanisms by which *Campylobacter* acquires resistance to these antimicrobial agents (Bolinger & Kathariou, 2017).

We also observed a high level of resistance to tetracycline. Tetracycline resistance in *Campylobacter* has been previously reported in strains isolated from animal products (Abdi-Hachesoo *et al.,* 2014; Giacomelli *et al.,* 2014; Hungaro *et al.,* 2015; Sierra-Arguello *et al.,* 2015). Over the past decade, the tetracycline compound class has been used in farm animal production as a growth promoter and for the treatment of diseases. The high resistance levels suggest that the overuse of tetracycline may have selected resistant strains. The majority of tetracycline resistance determinants confer increased resistance to the other compounds from the same class, though it is also possible that the use of oxitetracycline and doxycycline has also caused tetracycline resistance (Fairchild *et al.,* 2005). A high level of tetracycline resistance in *Campylobacter* is usually associated with the presence of the *tetO* gene. This gene encodes the TetO protein, which protects ribosomes from the inhibitory effects

of tetracycline (Connel *et al.*, 2003). A total of 28 isolates had tetracycline MICs ≥2 mg/L, and 42.8% of them carried the gene. Reports from Brazil have shown lower frequencies of this gene than in other countries (Sierra-Arguello *et al.,* 2015; Du *et al.,* 2018). This gene can be present in conjugative plasmids containing resistance genes for other antimicrobials that continue to undergo selective pressure. The *tetO* gene can also be found as a chromosomal element. In this case, the stability of the chromosomal location ensures the gene replicates from generation to generation, even in the absence of the drug (Avrain *et al.,* 2004; Crespo *et al.,* 2012).

Fluoroquinolones are considered the second-line treatment against *Campylobacter* infection in humans (Engberg *et al.,* 2001). *Campylobacter* strains showed high resistance to fluoroquinolones, with the CLSI breakpoints and ECOFF values indicating that 90.7% and 81.5% of the strains, respectively, were resistant to both ciprofloxacin and nalidixic acid. These high fluoroquinolone resistance rates have been previously described in Brazilian poultry sources (Sierra-Arguello *et al.,* 2016) and are likely due to the large use of this antimicrobial class in poultry production (Iovine & Blaser, 2004). Although ciprofloxacin is more commonly used in humans, it is structurally related to enrofloxacin (WHO, 2011), which has been widely used in poultry production (Garcia-Migura *et al.,* 2014), and previous studies have demonstrated that resistance for ciprofloxacin and enrofloxacin is developed through the same mechanisms (Frasao *et al.,* 2015b). *Campylobacter* resistance to fluoroquinolones is usually related to a mutation in the QRDR region of the *gyrA* gene (Frasao *et al.,* 2015b). This gene codes for the 'A' subunit of the enzyme DNA gyrase and confers a decreased susceptibility to fluoroquinolones (Wilson *et al.,* 2000). All strains tested for the presence of mutations in the QRDR fragment of the *gyr*A gene showed a threonine to isoleucine (Thr-86-Ile) mutation at codon 86 (Table 1). This result confirms that this substitution is always related to high fluoroquinolone MICs. A second mutation at codon 149 (Val-149-Ile) was observed in 19.3% of the strains. As these amino acids belong to the same class, the replacement may not lead to any significant conformational modifications of the protein. Consequently, its function probably remains unmodified (Taylor, 1986). Other mutations associated with an intermediate level of resistance to quinolones such as Asp-90-Asn and Ala-70-Thr (Iovine, 2013) were not encountered in this study. Mutation in QRDR of gene *gyrA* is the main resistance mechanism to fluoroquinolones. However, chromosomal efflux pumps, especially those codified by *cmeA, cmeB* and *cmeC* genes, are important factors to antimicrobial in *Campylobacter* species (Wieczorek & Osek, 2013; Nascimento *et al.*, 2019). Previous studies have demonstrated that almost all strains of *Campylobacter jejuni* isolated in Brazil presented these three genes (Nascimento *et al.*, 2019).

Since 2000, several Latin American countries are part of the Pan American Health Organization Network for Monitoring/Surveillance of Antibiotic Resistance. However, very few of them are conducting surveillance for *Campylobacter* species. In this context,

Table 2 – Minimum inhibitory concentration (MIC) results: non susceptible strains (CLSI breakpoints) and non-wildtype (ECOFF values).

Antimicrobial agent[a]	Minimum inhibitory concentration (MIC) - n (%)[b]															
	≤0.007	0.016	0.031	0.062	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
CHL				6 (11.1)	23 (42.6)	22 (40.7)	2 (3.7)	1 (1.8)	0	0	0	0	0			
CIP	2 (3.7)	1 (1.8)	0	0	0	0	0	0	5 (9.2)	16 (29.6)	20 (37)	10 (18.5)				
ERY				15 (27.7)	5 (9.2)	4 (7.4)	2 (3.7)	1 (1.8)	1 (1.8)	0	1 (1.8)	2 (3.7)	4 (7.4)	9 (16.6)	10 (18.5)	
GEN				16 (29.6)	22 (40.7)	13 (24)	3 (5.5)	0	0	0	0	0	0	0	0	
NAL								2 (3.7)	0	0	1 (1.8)	6 (11.1)	26 (48.1)	19 (35.9)	0	0
TET					22 (40.7)	2 (3.7)	1 (1.8)	1 (1.8)	2 (3.7)	1 (1.8)	3 (5.5)	7 (13)	9 (16.6)	6 (11.1)		

[a]Chloramphenicol (CHL), ciprofloxacin (CIP), erytromycin (ERY), gentamycin (GEN), nalidixic acid (NAL), tetracycline (TET).

[b] MIC breakpoints, according to CLSI guidelines, also include "intermediate" strains, which are considered non-susceptible.

Continuous lines indicate CLSI breakpoints.

Dotted lines indicate ECOFF values (EUCAST breakpoints).

Shaded areas indicate the tested concentrations.

data of *Campylobacter* resistance are mainly published by academic research groups (Fernández & Pérez-Pérez, 2016). Available data shows that antimicrobial resistance in *Campylobacter jejuni* varies among Latin American countries. The higher rates are described for fluorquinolones in several countries besides Brazil, including Ecuador, Argentina and Peru (Pollett *et al.*, 2012; Zbrum *et al.*, 2015; Fernández & Pérez-Pérez, 2016; Vinueza-Burgos *et al.*, 2017). Resistance to aminoglycosides is usually lower among these countries (Zbrum *et al.*, 2015; Vinueza-Burgos *et al.*, 2017; Toledo *et al.*, 2018). Resistance rates for erythromycin and tetracycline is variable according to the country (Pollett *et al.*, 2012; Zbrum *et al.*, 2015; Vinueza-Burgos *et al.*, 2017).

The individual maximum and minimum multiple-antibiotic resistance (MAR) indices for the isolates were 0.7 and 0.2, respectively, with an average index of 0.5, regardless of the interpretative criteria used. According to Proroga *et al.* (2011), the MAR index is a good risk assessment tool and can be applied to differentiate low- (MAR < 0.2) and high-risk (MAR > 0.2) regions where antibiotics are overused. Our results (overall MAR = 0.5) may indicate high antibiotic usage and high selective pressure in the poultry chain, but the practical significance of this finding may be lost, because antibiotic use is widespread in developing countries, including Brazil (Davis & Brown, 2016).

Based on the CLSI results, 13% (7/54) of the strains were multidrug-resistant (MDR), whereas 16.7% (9/54) of the strains were classified as MDR using the ECOFF values. Emerging resistance to the antimicrobial agents of choice for treating *Campylobacter* infections is becoming a serious threat to public health (Du *et al.,* 2018). The frequency of MDR strains found in this study is similar to that in previous reports from Brazil (Sierra-Arguello *et al.,* 2015). Given such results, Brazilian authorities should consider establishing an integrated surveillance network for antibiotic resistance in *Campylobacter*.

Considering that poultry is the major source of human *Campylobacter* spp. infection and that antimicrobial-resistant strains can be easily transmitted to humans via the food chain, our results show the need for the implementation of prudent antimicrobial-use policies in the Brazilian food production chain.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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