Antimicrobial susceptibility profile of *Campylobacter* spp. isolated from different animal species in Minas Gerais

Perfil de suscetibilidade a antimicrobianos de amostras Campylobacter *spp isoladas de diferentes espécies animais em Minas Gerais*

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Abstract

Susceptibility pattern of 45 *Campylobacter* spp. isolates – 16 *C. jejuni*, eight *C. coli,* and 21 *C. fetus* isolated from different animal species in Brazil – to twelve antimicrobial agents was determined. All *Campylobacter* spp. isolates were susceptible to gentamicin, sulfadiazine, and sulfamethoxazole. *C. jejuni* and *C. coli* were also sensitive to chloramphenicol, whereas all *C. fetus* strains were susceptible to kanamycin. Cefoperazone showed the highest percentage of resistance among *C. jejuni* (68.75%), followed by nalidixic acid (31.25%), ampicillin (37.50%), tetracycline (37.50%), erythromycin (12.50%), and kanamycin (6.25%). Likewise, cefoperazone exhibited the highest percentage of resistance among *C. coli* (75.00%), followed by nalidixic acid (50.00%), tetracycline (50.00%), erythromycin (37.50%), ampicillin (12.50%), and kanamycin (12.50%). Among *C. fetus* strains, nalidixic acid showed the highest resistance rate (85.71%), followed by cefoperazone (71.43%), tetracycline (42.86%), ampicillin (19.05%), chloramphenicol (9.52%), and erythromycin (4.76%). Therefore, it was found that the majority of *Campylobacter* spp. isolated from animals was sensitive to gentamycin, chloramphenicol, kanamacyn, and sulfonamides; however, a high proportion of the strains showed reduced susceptibility to nalidixic acid, ampicillin, cefoperazone, and tetracycline. Moreover, *C. coli* and *C. fetus* isolates showed a high percentage of multidrug resistant strains.

Keywords: Antimicrobial drug resistance. *Campylobacter* spp.. Brazil. Cattle. Pigs. Broilers. Marmosets. Dogs.

Resumo

O padrão de sensibilidade de 45 amostras de *Campylobacter* spp, incluindo 16 amostras de *C. jejuni*, 8 de *C. coli* e 21 *C. fetus*, isoladas de diferentes espécies de animais do Brasil, foi determinado para doze antimicrobianos. Todas as amostras de *Campylobacter* spp foram sensíveis à gentamicina, sulfadiazina e sulfametoxazol. *C. jejuni* e *C. coli* foram também sensíveis ao cloranfenicol, enquanto todas as amostras de *C. fetus* foram sensíveis à canamicina. Cefoperazona mostrou o maior percentual de resistência entre *C. jejuni* (68,75%), seguido pelo ácido nalidíxico (31,25%), ampicilina (37,50%), tetraciclina (37,50%), eritromicina (12,50%) e canamicina (6,25%). Similarmente, cefoperazona também exibiu o maior percentual de resistência entre as amostras de *C. coli* (75,00%), seguido pelo ácido nalidíxico (50,00%), tetraciclina (50,00%), eritromicina (37,50%), ampicilina (12,50%) e canamicina (12,50%). Entre os isolados de *C. fetus*, ácido nalidíxico apresentou maior taxa de resistência (85,71%), seguido de cefoperazona (71,43%), tetraciclina (42,86%), ampicilina (19,05%), cloranfenicol (9,52%) e eritromicina (4,76%). Assim, os nossos resultados mostraram que a maioria das amostras de *Campylobacter* spp isolados de animais foram sensíveis à gentamicina, cloranfenicol, canamicina e sulfonamidas. No entanto, uma proporção elevada das amostras apresentou susceptibilidade reduzida ao ácido nalidíxico, ampicilina, cefoperazona e tetraciclina. Além disso, *C. coli* e *C. fetus* mostraram uma alta porcentagem de amostras resistentes a múltiplas drogas.

Palavras-chave: Resistência a antimicrobianos. *Campylobacter* spp.. Brasil. Bovinos. Suínos. Frangos. Saguis. Cães.

Introduction

Campylobacter spp. are Gram-negative, non-spore forming, microaerophilic, curved or spiral rods responsible for important diseases in human and animals (DEBRUYNE et al., 2008) *C. jejuni* and *C. coli* are some of the major culture-confirmed bacteria associated with

human gastroenteritis in the United States (CDC, 2013), and *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* are important livestock pathogens, besides being human pathogens (ALVES et al., 2011).

Attention to *Campylobacter* infections has increased since they were recognized in 1970's as important human pathogens and public health issue, especially *C. jejuni* and, to a lesser extent, *C. coli.* (OLSON et al., 2008; CDC, 2013) Although the majority of the human cases of *Campylobacter* spp. gastroenteritis are related to self-limited diarrhea, severe sequels as reactive arthritis and Guillain-Barré syndrome may sporadically occur (OLSON et al., 2008). Humans are infected by *C. jejuni* or *C. coli* mainly through direct contact with carrier animals or consumption of contaminated food and water, poorly processed poultry meat and non-pasteurized milk (OLSON et al., 2008). *C. fetus* is a rare human pathogen frequently involved in systemic and extra-intestinal disease in patients with preexisting illnesses (WOO et al., 2002). In contrast to the secondary role in human infections, *C. fetus* is an important cause of abortion and infertility in cattle, being responsible for great economic losses (ALVES et al., 2011). Moreover, bacteria of the genus *Campylobacter* can be isolated from several domestic and wildlife animal species, as commensal microorganisms or causing a large number of non-reproductive diseases such as enteritis and mastitis (DEBRUYNE et al., 2008).

Antimicrobial treatment of human campylobacteriosis is usually not needed; however, for severe or long-lasting cases and for systemic infections it becomes mandatory (ADVICE FOR TRAVELERS, 2012). Erythromycin and azithromycin are the drugs of choice to treat *C. jejuni* and *C. coli* gastrointestinal infections in humans, but fluoroquinolones and tetracyclines are also recommended (ADVICE FOR TRAVELERS, 2012; DEKATE et al., 2013). The optimal treatment regimen for human *C. fetus* infection has not been determined, but gentamicin and imipenem are suggested as empirical therapy of choice and ampicilin, chloramphenicol, and fluoroquinolones could also be considered (TREMBLAY et al., 2003).

The resistance of *Campylobacter* spp. isolates, from both humans and animals, to different classes of antimicrobial agents such as quinolones, macrolides, aminoglycosides, tetracyclines, β-lactams, and sulfonamides has been widely reported (VAN DEN BOGAARD; STOBBERINGH, 2000; CHUMA et al., 2001; AVRAIN et al., 2003; TREMBLAY et al., 2003; MIRANDA; LAGE, 2007; ABAY et al., 2014;

DI GIANNATALE et al., 2014). Nonetheless, mainly the resistance to quinolones and macrolides has been considered an emerging public health hazard, due to its fast increase in the last decades (GIBREEL; TAYLOR, 2006; FITZGERALD et al., 2008; GARCIA-MIGURA et al., 2014). The high incidence of antibiotic resistance among *Campylobacter* spp. has led to an intensification of the discussion over the rational use of antimicrobial agents, especially in veterinary medicine, since their massive use, mainly as feed additives, has been related to the increase of drug resistance in *Campylobacter* spp. (JUNTUNEN et al., 2011; GARCIA-MIGURA et al., 2014; USUI et al., 2014).

Thus, the aim of the present study was to determine the antimicrobial susceptibility profile of 45 *Campylobacter* spp. isolates from different animal species in the State of Minas Gerais, Brazil.

Materials and Methods

Bacterial strains and culture conditions

A total of 45 *Campylobacter* spp. were used – 16 *C. jejuni,* eight *C. coli,* and 21 *C. fetus* strains from the collection of Laboratório de Bacteriologia Aplicada, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG) and Departamento de Microbiologia, Instituto de Ciências Biológicas, UFMG, isolated between 1977 and 2000 (LEITE, 1977; CARVALHO, 1992; LAGE, 1992; LAGE et al., 1992; STYNEN et al., 2003). *C. jejuni* strains were isolated from feces or rectal swabs of dogs (n = 5), marmosets (n = 5), pigs (n = 3), broilers (n = 2), and calf (n = 1). *C. coli* strains were isolated from feces or rectal swabs of pigs (n = 6), broiler (n = 1), and calf (n = 1). *C. fetus* strains were isolated from feces of calves (n = 14) or from genital tract (n = 7) of adult cattle. All strains were isolated from animals from Minas Gerais, Brazil, and identified through routine laboratory methods (DEBRUYNE et al., 2008).

Campylobacter spp. isolates were cultured in brain heart infusion (BHI) agar (Difco, USA) supplemented with 10% of defibrinated horse blood and incubated for 48 h at 37°C under microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2) (DEBRUYNE; GEVERS; VANDAMME, 2008).

Antimicrobial susceptibility testing

Minimum Inhibitory Concentration (MIC) was determined according to the Clinical and Laboratory Standards Institute (CLSI) VET01-A4 manual

recommendations (CLSI, 2013a) for ampicillin (Life Technologies, Carlsbad, CA, USA), cefoperazone (Pfizer, New York, NY, USA), chloramphenicol (Life Technologies, Carlsbad, CA, USA), erythromycin (Gerbrás, Anápolis, GO, Brazil), gentamicin (Inlab, São Paulo, SP, Brazil), kanamycin (Inlab, São Paulo, SP, Brazil), nalidixic acid (Inlab, São Paulo, SP, Brazil), penicillin G (Inlab, São Paulo, SP, Brazil), streptomycin (Merck, Darmstadt, HE, Germany), sulfadiazine (Inlab, São Paulo, SP, Brazil), sulfamethoxazole (Sigma-Aldrich, St. Louis, MO, USA), and tetracycline (Inlab, São Paulo, SP, Brazil) in a dilution ranging from 0.0625 to 256 μg/mL. Briefly, Mueller-Hinton agar (Difco, Sparks, MD, USA) plates supplemented with 5% defibrinated horse blood plus the antimicrobial were inoculated with bacterial suspensions adjusted to turbidity equivalent to a 0.5 McFarland standard and incubated for 48h at 37ºC under microaerophilic conditions.

Determination of the MIC was performed in duplicate and all antibiotics were tested with the reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *C. jejuni* NCTC 11351 to ensure that the results were within acceptable limits of quality control for susceptibility testing (CLSI, 2013b). Furthermore, two Mueller-Hinton agar plates supplemented with 5% defibrinated horse blood, without antibiotics, were employed at the beginning of the antibiotic plate sequence, and two at the end of this sequence, as growth controls.

MIC breakpoints were set in accordance to the CLSI for *C. jejuni / C. coli* or *Enterobacteriaceae* (Table 1) (CHEN et al., 2010; CLSI, 2010, 2012, 2013b). Based on these criteria the strains were classified as resistant or sensitive to antimicrobials. However, the breakpoints to penicillin G and streptomycin were not defined by CLSI, and therefore the percentage of resistant strains could not be determined.

Table 1 – Minimal Inhibitory Concentration (MIC) breakpoints to determine antimicrobial susceptibility of *Campylobacter* spp. isolates

Antibiotic	MIC breakpoint			Reference	
	S[a]	I[b]	R[c]	Document	Strain
Nalidixic acid	≤ 8	-	> 16	CA-SFM[d]	*C. jejuni / C. coli*
Ampicillin	≤ 8	16	≥ 32	CLSI[e] – VET01-S2	*Enterobacteriaceae*
Kanamycin	≤ 16	32	≥ 64	CLSI – M100-S22	*Enterobacteriaceae*
Cefoperazone	≤ 16	32	≥ 64	CLSI – M100-S22	*Enterobacteriaceae*
Chloramphenicol	≤ 8	16	≥ 32	CLSI – VET01-S2	*Enterobacteriaceae*
Erythromycin	≤ 8	16	≥ 32	CLSI – M45-A2	*C. jejuni / C. coli*
Gentamycin	≤ 2	4	≥ 8	CLSI – VET01-S2	*Enterobacteriaceae*
Sulfadiazine	≤ 256	-	≥ 512	CLSI – VET01-S2	*Enterobacteriaceae*
Sulfamethoxazole	≤ 256	-	≥ 512	CLSI – VET01-S2	*Enterobacteriaceae*
Tetracycline	≤ 4	8	≥ 16	CLSI – M45-A2	*C. jejuni /C. coli*

[a] Susceptible; [b] Intermediate; [c] Resistant; [d] Antibiogram Committee of the French Society for Microbiology; [e] Clinical and Laboratory Standards Institute

Multidrug resistance was defined as resistance to three or more antimicrobial classes. The antimicrobial classes were as follows: (i) quinolones (nalidixic acid); (ii) macrolides (erythromycin); (iii) tetracycline (tetracycline); (iv) β-lactams (penicillin G, cefoperazone, and ampicillin); (v) aminoglycosides (gentamicin, streptomycin, and kanamycin); (vi) sulfonamides (sulfamethoxazole and sulfadiazine); and (vii) phenicols (chloramphenicol). Strains resistant to three or more antimicrobial groups were considered multidrug resistant. (MAGIORAKOS et al., 2012)

Results

The MIC range, MIC50 and MIC90 found for the 45 *Campylobacter* spp. studied strains, as well as the number of resistant strains to each antimicrobial agent tested are shown in figure 1.
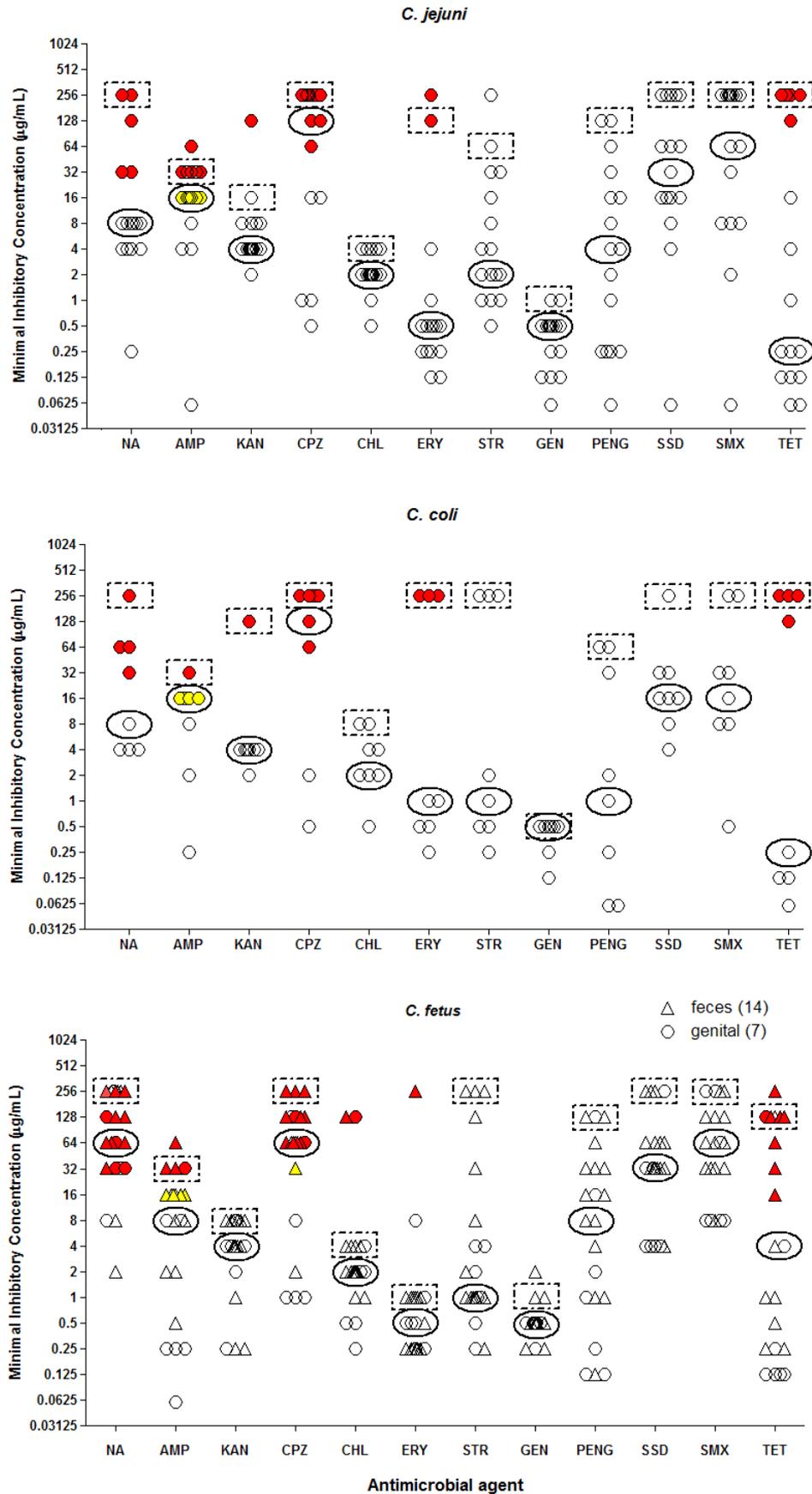
Figure 1 – Scatter plot of the Minimal Inhibitory Concentration (MIC) by the agar dilution method to nalidixic acid (N=A), ampicillin (AMP), kanamycin (KAN), cefoperazone (CPZ), chloramphenicol (CHL), erythromycin (ERY), streptomycin (STR), gentamycin (GEN), penicillin G (PENG), sulfadiazine (SSD), sulfamethoxazole (SMX), and tetracyclin (TET) of *C. jejuni* (n = 16), *C. coli* (n = 8), and *C. fetus* (n = 21) strains isolated from animals in the state of Minas Gerais, Brazil. Resistant strains are shown in red symbols and intermediate susceptibility profile in yellow symbols. The ellipses indicate the MIC_{50} for each antimicrobial agent, while dotted rectangles indicate the MIC_{90}

All *Campylobacter* spp. strains tested were susceptible to gentamicin, sulfadizine, and sulfamethoxazole (Figure 1). *C. jejuni* strains were also 100% sensitive to chloramphenicol. Cefoperazone was the antimicrobial that presented the lowest activity, against *C. jejuni*, with 68.75% (11/16) of resistant strains, followed by nalidixic acid [31.25% (5/16)], ampicillin [37.50% (6/16)], tetracycline [37.50% (6/16)], erythromycin [12.50% (2/16)], and kanamycin [6.25% (1/16)] (Figure 1). Moreover, *C. jejuni* strains also exhibited an intermediate susceptibility profile to ampicillin [37.50% (6/16)].

As observed in *C. jejuni,* all tested *C. coli* strains exhibited sensitivity to chloramphenicol. The antibiotic with the lowest activity against *C. coli* was cefoperazone, with 75.00% (6/8) of resistant strains, followed by nalidixic acid [50.00% (4/8)], tetracycline [50.00% (4/8)], erythromycin [37.50% (3/8)],

ampicillin [12.50% (1/8)], and kanamycin [12.50% (1/8)]. Furthermore, 50.00% (4/8) of *C. coli* strains also showed an intermediate susceptibility profile to ampicillin.

Among *C. fetus* strains, the highest percentage of resistance was observed to nalidixic acid [85.71% (18/21)], followed by cefoperazone [71.43% (15/21)], tetracycline [42.86% (9/21%)], ampicillin [19.05% (4/21)], chloramphenicol [9.52% (2/21)], and erythromycin [4.76% (1/21)]. Intermediate susceptibility patterns to ampicillin and cefoperazone were observed in 28.57% (6/21) and 4.76% (1/21) of *C. fetus* strains, respectively. All *C. fetus* strains were susceptible to kanamycin.

The susceptibility profile of *Campylobacter* spp. strains tested for ten antimicrobials with established breakpoints is shown in table 2.

Table 2 – Antimicrobial susceptibility profile of *Campylobacter* spp. strains isolated from animals in the state of Minas Gerais, Brazil

Species	NA	AMP	KAN	CPZ	CHL	ERY	GEN	SSD	SMX	TET	N[d]	Profile[e]
C. jejuni	R[a]	I[b]	S[c]	R	S	S	S	S	S	R	1	1
C. jejuni	S	R	S	R	S	R	S	S	S	R	1	2
C. jejuni	R	S	S	R	S	R	S	S	S	S	1	3
C. jejuni	S	R	S	R	S	S	S	S	S	S	3	4
C. jejuni	S	I	S	R	S	S	S	S	S	R	2	5
C. jejuni	S	I	S	R	S	S	S	S	S	S	2	6
C. jejuni	R	S	S	S	S	S	S	S	S	S	1	7
C. jejuni	S	R	S	R	S	S	S	S	S	R	1	8
C. jejuni	S	I	S	S	S	S	S	S	S	S	1	9
C. jejuni	R	S	R	S	S	S	S	S	S	S	1	10
C. jejuni	S	R	S	S	S	S	S	S	S	S	1	11
C. jejuni	R	S	S	S	S	S	S	S	S	R	1	12
C. coli	S	I	S	R	S	R	S	S	S	R	1	1
C. coli	R	R	R	R	S	R	S	S	S	R	1	2
C. coli	R	S	S	R	S	S	S	S	S	R	1	3
C. coli	S	I	S	R	S	S	S	S	S	R	1	4
C. coli	R	S	S	R	S	S	S	S	S	S	1	5
C. coli	S	S	S	S	S	S	S	S	S	S	1	6
C. coli	R	I	S	S	S	S	S	S	S	S	1	7
C. coli	S	I	S	R	S	R	S	S	S	S	1	8
C. fetus	R	S	S	R	S	S	S	S	S	R	3	1
C. fetus	R	I	S	R	S	S	S	S	S	R	2	2
C. fetus	R	R	S	R	S	S	S	S	S	R	2	3
C. fetus	R	I	S	R	R	S	S	S	S	R	1	4
C. fetus	S	I	S	R	S	R	S	S	S	R	1	5
C. fetus	R	R	S	R	R	S	S	S	S	S	1	6
C. fetus	R	S	S	S	S	S	S	S	S	S	4	7
C. fetus	R	S	S	R	S	S	S	S	S	S	2	8
C. fetus	R	I	S	R	S	S	S	S	S	S	2	9
C. fetus	R	R	S	I	S	S	S	S	S	S	1	10
C. fetus	S	R	S	R	S	S	S	S	R	S	1	11
C. fetus	S	S	S	S	S	S	S	S	S	S	1	12

[a] Resistant [b] Intermediate; [c] Susceptible; [d] Number of strains; [e] Profile highlighted in bold italics showed resistance to three or more antimicrobial groups and were considered multidrug resistant

Three [14.29% (3/21)] *C. jejuni* strains were multidrug resistant: two isolated from pigs and one from poultry. Among the observed multidrug-resistant *C. coli* strains [37.50% (3/8)], two were isolated from pigs and one from calf. *C. fetus* strains exhibited the highest percentage of multidrug-resistant strains [47.62% (10/21)]. Among *C. fetus* isolates from calf feces the percentage of multidrug resistant strains was 57.14% (8/14), whereas among the *C. fetus* isolated from genital tract 28.57% (2/7) were considered multidrug-resistant.

Discussion

The emergence of antibiotic resistance in *Campylobacter* spp. has been associated with the use of antimicrobials in veterinary medicine (THRELFALL et al., 2000; GARCIA-MIGURA et al., 2014). Therefore, the present study investigated the susceptibility profile of *C. jejuni, C. coli,* and *C. fetus* isolated from different animal species in Minas Gerais, Brazil, to twelve antimicrobial agents, and observed that cefoperazone, nalidixic acid, and tetracycline were the antibiotics that exhibited the lowest activity against all *Campylobacter* spp. tested, whereas the highest rate of susceptible strains found was to chloramphenicol, gentamycin, sulfadiazine, and sulfamethoxazole.

Beta-lactam, including ampicillin and cefoperazone, was the antimicrobial class that showed the highest level of resistance among all *Campylobacter* spp. strains tested (Table 2). Since *C. jejuni* and *C. coli* spp. are universally resistant to cephalosporins (FITZGERALD et al., 2008) as well as cefoperazone, and this drug is regularly used in *Campylobacter* selective media (LAGE et al., 1992) to inhibit other bacteria, the excessive number of resistant strains to this antimicrobial was not an unexpected result; on the contrary, it confirms media with cefoperazone as a good choice for primary isolation of *C. jejuni* and *C. coli*. Furthermore, the high rate of resistance to cefoperazone also observed among *C. fetus* strains could be explained considering that all *C. fetus* strains isolated from feces samples were cultivated in medium containing this drug (LAGE et al., 1992), which is a surprising result, since *C. fetus* is not intrinsically resistant to cefoperazone. Reduced susceptibility, with a large number of resistant or intermediate patterns, especially amongst *C. jejuni* isolates, was also observed to ampicillin. Ampicillin resistance is of special clinical interest since this drug may be used for the treatment of severe human campylobacteriosis infections, particularly in children (GALLAY et al., 2007). *C. jejuni*

and *C. coli* isolated from animals, mainly poultry, were broadly reported to be resistant to ampicillin (GALLAY et al., 2007; LEHTOPOLKU et al., 2010; ADZITEY et al., 2012; CHOKBOONMONGKOL et al., 2013; GIACOMELLI et al., 2014). Nevertheless, resistance to ampicillin among *C. fetus* strains has not been described, even for *C. fetus* isolated in the same region as the strains tested in the present study (Minas Gerais State, Brazil) (TREMBLAY et al., 2003; VARGAS et al., 2005; GALLAY et al., 2007; MIRANDA; LAGE, 2007). These differences are probably due to differences in the methodology used or in the origin of the strains, since a low-level agreement was observed for ampicillin in the comparison of agar dilution and agar disk diffusion methods (LUANGTONGKUM et al., 2007). Although there is no established breakpoint for penicillin G to *Campylobacter* spp., the wide range of MIC values observed as well as the high MIC50 and MIC90 values indicate a tendency to resistance to penicillin G among the tested *C. jejuni, C. coli,* and *C. fetus* strains (Figure 1). Moreover, a large number of those *Campylobacter* spp. strains were also resistant to other beta-lactams (cefoperazone and ampicillin), which strongly supports this trend of resistance to penicillin G, as the production of beta-lactamases is the dominant mechanism of resistance to beta-lactam drugs and they are widely reported in *Campylobacter* spp. (WIECZOREK; OSEK, 2013).

Nalidixic acid, together with beta-lactam drugs, was the drug with the highest rates of resistance among the studied *Campylobacter* spp. strains, especially *C. fetus* strains, which are naturally resistant to nalidixic acid (Figure 1) (BURNENS et al., 1993). This nalidixic acid resistance may indicate resistance to other quinolones such as ciprofloxacin, although it was not assessed in the present study, since for *C. jejuni* and *C. coli* nalidixic acid resistance correlates well with resistance to other quinolone drugs (KINANA et al., 2006). Resistance to quinolones amongst *Campylobacter* spp. is a global concern and has increased and spread very quickly (FITZGERALD et al., 2008; GARCIA-MIGURA et al., 2014). Administration of quinolones, especially its subtherapeutic use in food animals, has been implicated as the force behind increase in resistance to quinolones in *Campylobacter* spp. isolates from humans and animals (JUNTUNEN et al., 2011; GARCIA-MIGURA et al., 2014; USUI et al., 2014). In Brazil, fluoroquinolones were used as growth-promoter, although this not allowed anymore, which could explain the great incidence of nalidixic acid resistant *C. jejuni* and

C. coli strains observed in the present study (PAMVET-PR, 2005; SILVA; HOLLENBACH, 2010; SILVA et al., 2013). Moreover, this link is corroborated by poultry and pigs being, respectively, the main hosts of *C. jejuni* and *C. coli,* and quinolones being commonly use as feed additive in poultry and pork industries (PAMVET-PR, 2005; SILVA; HOLLENBACH, 2010).

Tetracycline was one of the three classes of antimicrobial drugs presenting the lowest activity against the tested *Campylobacter* spp. strains, with *C. fetus* strains showing the highest rate of resistance to it. This high level of resistance to tetracycline is probably a direct reflex of its intense use in veterinary medicine, especially in cattle (FDA, 2010). Indeed, cattle and sheep are the main hosts of *C. fetus,* which are found in the intestines of heathy animals and abundantly in their genital tract (LASTOVICA; ALLOS, 2008). The increase in resistance rates to tetracyclines of *Campylobacter* spp. is worrisome and has been constantly described, mainly in animal isolates (GALLAY et al., 2007; SAHIN et al., 2008; CHEN et al., 2010; LEHTOPOLKU et al., 2010; ADZITEY et al., 2012; CHOKBOONMONGKOL et al., 2013; DI GIANNATALE et al., 2014). Resistance to tetracycline has a clinical importance, since tetracycline is considered a second-line treatment for human *Campylobacter* spp. infections because of its low cost and toxicity (AARESTRUP et al., 2008; FITZGERALD et al., 2008). Furthermore, even though cattle are not considered the main sources of human campylobacteriosis, a recent study has demonstrated that their potential as source of infection in humans is underestimated (JONAS et al., 2015).

The obtained results showed a higher frequency of erythromycin resistance among *C. coli* strains, whereas it was less frequent among the tested *C. jejuni* and *C. fetus* strains. These findings are of public health importance as erythromycin is the first choice to treat human gastroenteritis caused by *Campylobacter* spp. (AARESTRUP et al., 2008; FITZGERALD et al., 2008). The higher incidence of erythromycin resistance among *C. coli* compared to other *Campylobacter* species may be due to the fact that the resistant *C. coli* strains were isolated from pigs, which are the only food animals for which erythromycin is allowed to be used as feed additive in Brazil (SILVA et al., 2013). Moreover, a higher frequency of erythromycin resistance has been reported worldwide among *C. coli* strains than *C. jejuni* strains (GIBREEL; TAYLOR, 2006; GALLAY et al., 2007; CHEN et al., 2010; LEHTOPOLKU et al., 2010).

For the tested aminoglycoside drugs, the findings were diverse. None of the tested *Campylobacter* spp. strains was resistant to gentamycin and only one *C. coli* was resistant to kanamycin (Figure 1). Susceptibility to gentamycin has been frequently described (LUANGTONGKUM et al., 2007; MIRANDA; LAGE, 2007; LEHTOPOLKU et al., 2010; ADZITEY et al., 2012; CHOKBOONMONGKOL et al., 2013; DI GIANNATALE et al., 2014; GIACOMELLI et al., 2014), being an important result, since gentamycin is commonly used in adherence and invasion assay (KONKEL; JOENS, 1989). Interestingly, the kanamycin susceptibility pattern observed in this study was also demonstrated to *Campylobacter* spp. strains also isolated in animals from Minas Gerais State, Brazil (MIRANDA; LAGE, 2007). Kanamycin is not an antibiotic usually included in susceptibility tests, but, differently from the observed to *Campylobacter* spp. from Minas Gerais, Luangtongkum *et al.* (LUANGTONGKUM et al., 2007) observed 36.38% of resistance among *Campylobacter* spp. isolated from poultry. Although there is no breakpoint to streptomycin for either *C. jejuni / C. coli* or *Enterobacteriaceae*, the wide range of MIC values reported chiefly among *C. jejuni* and *C. fetus* strains (Figure 1) suggests that some strains may be resistant to this drug. However, it is important to consider that among the tested *C. fetus* strains the isolates from feces exhibited a wider MIC range (0.25 to 256 µg/mL) than strains isolated from genital tract (0.25 to 4 µg/mL), indicating that feces isolates had a greater tendency to streptomycin resistance. These results endorse the indication of streptomycin for treatment of bovine genital campylobacteriosis (LAGE; LEITE, 2000).

All of the *C. jejuni* and *C. coli* strains tested and most of the *C. fetus* strains were susceptible to chloramphenicol (Figure 1). This high susceptibility to chloramphenicol may be due to the prohibition of the use of this antibiotic in veterinary medicine in Brazil because of the risk of residues of this drug in meat, milk, and eggs (BRASIL, 1998). Hence, chloramphenicol as well as kanamycin and gentamycin could be suggested as options to treat human *Campylobacter* infections in Brazil. Likewise, the sulfonamides tested, sulfadizine and sulfamethoxazole, could be recommended to treat infections by *Campylobacter* spp., since they also demonstrated very good activity against *C. jejuni, C. coli,* and *C. fetus* strains isolated in Minas Gerais. As sulfonamides are not indicated for treatment of human or animal campylobacteriosis, they are usually not included in the antimicrobial susceptibility test for *Campylobacter*

spp., preventing any comparison with the present results. However, Italian *C. jejuni* and *C. coli* showed high rate of resistance to combination sulfamethoxazole plus trimethoprim (GIACOMELLI et al., 2014).

Besides the high rates of resistance to individual antimicrobial agents, the tested *Campylobacter* spp. strains from animal origin, mainly *C. coli* and *C. fetus*, also exhibited a high frequency of multidrug resistance. Considering that *C. jejuni* and *C. coli* are naturally resistant to cefoperazone, the erythromycin-tetracycline and nalidixic acid-tetracycline resistance patterns were the most frequent ones amongst the tested *C. jejuni* and *C. coli*. Quinolone-tetracycline concomitant resistance has also been described as one of the most prevalent in *C. jejuni* and *C. coli* strains from broilers, cattle, ducks, humans, and pigs in China, France, Finland, Italy, Malaysia, and Thailand (HAKANEN et al., 2003; GALLAY et al., 2007; CHEN et al., 2010; ADZITEY et al., 2012; CHOKBOONMONGKOL et al., 2013; DI GIANNATALE et al., 2014; GIACOMELLI et al., 2014). The tested *C. fetus* strains showed cefoperazone-tetracycline as the most common multidrug-resistance profile, taking into account that this species is naturally resistant to nalidixic acid. The present results were different from those observed by Miranda and Lage (2007) and Vargas et al. (2005) for Brazilian *C. fetus* isolates, which exhibited a low prevalence of multidrug resistant strains, with absence or low observation of resistance to tetracycline. These differences could be explained, considering that the multiple drug resistance observed was higher in *C. fetus* isolates from feces (57.14%) than from genital tract (28.57%), and that

all *C. fetus* strains tested by Vargas et al. were from genital origin. Besides, differences in the methodology used or in the origin of the strains could also contribute to the differences detected. Efflux system conferring resistance to several classes of antimicrobials such as tetracyclines, macrolides, fluoroquinolones, and aminoglycosides has been widely described in *Campylobacter* spp. (GIBREEL; TAYLOR, 2006; FITZGERALD et al., 2008), and could be responsible for the multidrug resistance patterns observed.

In conclusion, it was found that the majority of *C. jejuni, C. coli,* and *C. fetus* strains isolated from different animal species in Brazil were sensitive to gentamycin, chloramphenicol, and sulfonamides. However, a high proportion of strains showed reduced susceptibility to nalidixic acid, ampicillin, cefoperazone, and tetracycline, antimicrobial agents commonly used for the treatment of human campylobacteriosis. Moreover, the high percentage of multidrug resistant strains observed among *C. coli* and *C. fetus* animal isolates confirms the need for continuous monitoring of *Campylobacter* spp. resistance in the pig and cattle production chains.

Acknowledgments

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