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Prevalence, strain identification and antimicrobial resistance of *Campylobacter* spp. isolated from slaughtered pig carcasses in Brazil

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ABSTRACT

The aim of this study was to investigate the rate of contamination, species identification and antimicrobial resistance of thermophilic *Campylobacter* spp. in pig carcasses during the slaughter process in a slaughterhouse in Brazil. Two hundred and fifty-nine samples were collected at 7 different stages of the slaughter process for *Campylobacter* determination by both qualitative and quantitative methods. Typical colonies were subjected to API Campy, real-time polymerase chain reaction (PCR) and antimicrobial resistance testing. *Campylobacter* was found in 18.9% of the carcasses and 3.5% of the samples. Dehairing was the slaughter stage with the highest *Campylobacter* contamination (55.6%). All *Campylobacter* strains were confirmed by real-time PCR and showed multi-drug resistance to cephalothin, nalidixic acid, norfloxacin, tetracycline and trimethoprim. None of the strains were resistant to amoxicillin/clavulanic acid, ampicillin and chloramphenicol. Despite the low occurrence of *Campylobacter* spp. in pig samples, the antimicrobial resistance of *Campylobacter* strains represents a considerable risk for the consumption of pork meat and confirms the need for continuous monitoring of *Campylobacter* in the pig production chain.

1. Introduction

In the food industry, *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* spp. represent the major potential causes of food-borne diseases in humans (Franchin, Aidoo, & Batista, 2005; Ghafir, China, Dierick, De Zutter, & Daube, 2007). Although *Salmonella* has been associated with most cases of food-borne gastroenteritis in past several years, in the last decade, *Campylobacter* has appeared as the leading cause of acute enteritis in humans in industrialized countries (Bardon, Kolar, Cekanova, Hejnar, & Koukalova, 2009; Ghafir et al., 2007; Guévremont, Nadeau, Sirois, & Quessy, 2006; Humphrey, O'Brien, & Madsen, 2007; Pezzotti et al., 2003; Suzuki & Yamamoto, 2009).

Although campylobacteriosis is not associated with high mortality rates and is generally self-limited, it has a significant impact on the economy and public health in industrialized countries (Josefsen, Lübeck, Hansen, & Hoorfar, 2004).

The occurrence of *Campylobacter* spp. in food appears to be fairly common in European countries (Ghafir, China, Dierick, Zutter, & Daube, 2008), and it is responsible for many cases of Guillain-Barré syndrome (GBS) (Blaser, Allos, & Lang, 1997; Caporale et al., 2006; Sinha, Prasad, Pradhan, Jain, & Jha, 2004; Tam, Rodrigues, & O'brien, 2003). In the United Kingdom, the annual estimated cost for the treatment of diseases caused by *Campylobacter* spp. was € 740 million in 2007 (Humphrey et al., 2007).

More recently, concern regarding the prevalence of campylobacteriosis has increased because of the frequent isolation of antimicrobial-resistant strains from humans and animals. Antimicrobial resistance develops from the massive use of antimicrobials in animals for preventive or therapeutic purposes as well as for increasing the growth rate in modern intensive animal production systems (Mayrhofer, Paulsen, Smulders, & Hilbert, 2004; Pezzotti et al., 2003; Rönner, Engvall, Andersson, & Kaijser, 2004).

Antimicrobial resistance of *Campylobacter* spp. strains isolated from animal products has been reported in different countries such as Italy (Pezzotti et al., 2003), Denmark (Alban, Nielsen, & Dahl, 2008), Korea (Shin & Lee, 2007), Sweden (Rönner et al., 2004), the United States (Olah, Doetkottb, Fakhra, & Loguea, 2006), the United Kingdom (Little, Richardson, Owen, Pinna, & Threlfall, 2008), Austria (Mayrhofer et al., 2004), Australia (Miflin,

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Templeton, & Blackall, 2007), Czech Republic (Bardon et al., 2009) and Canada (Guévremont et al., 2006; Inglis et al., 2005).

Although *Campylobacter* contamination in food of animal origin has been reported in several studies, most research has focused on poultry and poultry products (Mackiw, Popowski, & Szponar, 2008; Meremäe et al., 2010; Sallam, 2007) However, pigs are also considered potential reservoirs of *Campylobacter* spp. (Alban et al., 2008).

The contamination of pork meat may occur at the slaughterhouse itself by cross-contamination during slaughter procedures. Some recent studies have reported *Campylobacter* contamination in pork in European countries (Ghafir et al., 2008; Little et al., 2008). However, from an extensive review of the international scientific literature, no reports were found on the occurrence and antimicrobial resistance of *Campylobacter* in pig carcasses in Brazil. Because pork is one of the most widely consumed meats in the world, monitoring and obtaining information on *Campylobacter* contamination in different countries is highly relevant both for public health and to the international pork trade.

The aim of the present work was to investigate the prevalence of thermophilic *Campylobacter* spp. in pig carcasses during the slaughter process in Brazil and to identify the strains of *Campylobacter* spp. both phenotypically and genotypically. The resistance of the isolates to several antimicrobials was also investigated.

2. Materials and methods

2.1. Sample collection

Two hundred and fifty-nine samples were collected from 37 pig carcasses randomly selected along the slaughter line at a big pork processing plant in the city of Videira, Santa Catarina State, Brazil. Samples were taken from the same carcass at 7 different steps of the slaughter process: sticking/bleeding, dehairing, flaming, evisceration, washing, cooling and ham cutting.

At each slaughter step, an area of 100 cm^2 from 4 different sites of the carcass (medial neck, arm, loin and ham) was swabbed by thoroughly rubbing the surface of the site with sterile swabs in a total sample area of 400 cm^2 per carcass. Immediately after collection, swabs from 4 different carcass sites, composing one sample, were pooled together in plastic bags containing 400 mL of 0.1% sterile peptone water, for the determination of *Campylobacter* spp. *Campylobacter* determination was performed less than 30 min after sample collection.

Sampling methodology was performed according to the European Union recommendations for pig carcass sampling for microbiological analysis (European Union, 2001).

2.2. Detection and enumeration of Campylobacter spp.

Because it is known that the *Campylobacter* genus is sensitive to laboratory growing conditions, both qualitative and quantitative methods were used to determine the presence of *Campylobacter* spp. in samples. In this manner, the use of both techniques, i.e. preenrichment and direct isolation on agar, minimized recovery limitations inherent of each technique and allowed the recovery of injured cells and inhibited competing microorganisms, thereby providing reliable results.

For the detection of thermophilic *Campylobacter* spp. (qualitative method), 2 mL of the sample diluted in 400 mL of 0.1% sterile peptone water was transferred to 18 mL of Bolton broth (CM 983; Oxoid, Basingstoke, Hampshire, England) with selective supplement (SR 208E; Oxoid) and incubated at 36 ± 0.5 °C for 4 h, followed by further incubation at 42 ± 0.5 °C for 44 h under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N) (Franchin et al., 2005). A loop full of enrichment (Bolton broth) was streaked onto modified Bolton agar and incubated at 42 ± 0.5 °C in a microaerophilic atmosphere for 20–44 h. The modified Bolton agar was prepared as follows: dehydrated Bolton broth (CM 983; Oxoid) was suspended in water, added 1.5% agar–agar (HiMedia Laboratories, Mumbai, India) and 0.5 g/L ferrous sulphate (Carlo Erba Reagenti, Milan, Italy). After adjusting to pH 7.5 and sterilization by autoclaving at 121 °C for 15 min, the medium was cooled to 50 °C and added aseptically 200 ppm of 2,3,5-triphenyltetrazolium chloride solution (TTC) (Sigma, St. Louis, Missouri, USA) sterilized by membrane filtration and selective supplement (SR 183E; Oxoid) (Franchin et al., 2005; Line, 2001).

For the enumeration of thermophilic *Campylobacter* (quantitative method), 0.4 mL of the sample diluted in 400 mL of 0.1% sterile peptone water was streaked onto modified Bolton agar and incubated at 42 ± 0.5 °C for 24–48 h in a microaerophilic atmosphere. The results were expressed as colony forming units (CFU) per cm^2. Using the rule of three calculation, one colony grown on the modified Bolton agar was equivalent to 2.5 CFU/cm^2, because the agar was inoculated with 0.4 mL of the sample.

Oxidase-positive, red-coloured colonies that showed curved or spiral gram-negative rods on microscopy were presumptively identified as *Campylobacter* spp.

2.3. Biochemical and enzymatic methods for identification

The biochemical and enzymatic system API Campy (bioMérieux®, Marcy l'Etoile, France) was used for the identification of *Campylobacter* spp. strains following the manufacturer's recommendations.

2.4. Real-time PCR identification

For genotype confirmation, the DNA of the isolates identified by the biochemical enzymatic kit was extracted using the NewGen Prep and NewGen Preamp (Simbios Biotecnologia, Canoas, Rio Grande do Sul, Brazil), according to the manufacturer's protocol.

For the polymerase chain reaction, 2 mL of DNA extracted from the strains were added into a microtube and 28 mL of *Campylobacter* Master Mix Kit (Simbios Biotecnologia, Canoas, Rio Grande do Sul, Brazil) with Taq polymerase (Promega, Madison, Wisconsin, USA).

Real-time PCR was performed on the Step One Plus thermal cycler (Applied Biosystems) with the following amplification programme: an initial activation step of the enzyme at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, and 1 min of annealing and elongation at 60 °C.

As the positive control, a standard strain of *Campylobacter jejuni* subsp. *Jejuni* ATCC 33291 (Remel, Lenexa, Kansas, USA) was subjected to API Campy test and genetic identification by real-time PCR. For genetic identification, a blank test containing only the Master Mix Kit and Taq polymerase was adopted as a negative control.

2.5. Antibiotic susceptibility testing

Isolated cultures were analysed for antimicrobial resistance using the disc diffusion assay on Müller-Hinton agar plates (CM 0337B; Oxoid, Basingstoke, Hampshire, England) containing 5% (v/v) sheep blood (SUM 04; Newprov, Pinhais, Paraná, Brazil) incubated under microaerophilic conditions. The suspension was adjusted to match the 0.5 McFarland (Probac, Brazil) turbidity standard as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2005).

Antimicrobial susceptibility was performed using standard discs (Laborclin, Pinhais, Paraná, Brazil) containing ampicillin (10 μg), amoxicillin/clavulanic acid (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), gentamicin (10 μg), clindamycin (2 μg), norfloxacin (10 μg), cephalothin (30 μg), streptomycin (10 μg), tetracycline (30 μg) and trimethoprim (5 μg). The antibiotics used in this study were chosen because they are frequently used in the treatment of human infections (Rönner et al., 2004; Shin & Lee, 2007).

The inhibition zone diameter obtained for ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, gentamicin, clindamycin, norfloxacin, cephalotin and tetracycline were determined according to the criteria proposed for the genus *Campylobacter* by Huymans and Turnidge (1997). For amoxicillin/clavulanic acid, streptomycin and trimethoprim, the inhibition zone diameter was interpreted according to the breakpoints of the Clinical and Laboratory Standards Institute for sEnterobacteriaceae (CLSI, 2005; Han, Jang, Choo, Heu, & Ryu, 2007; Luangtongkum, Morishita, Al-Tayeb, Ison, & Zhang, 2007).

3. Results

The rates of isolation of *Campylobacter* spp. from the different pig slaughtering steps are shown in Table 1.

From a total of 259 samples, *Campylobacter* spp. was isolated in only 9 (3.5%), indicating a low prevalence. With regard to carcass contamination, of 37 carcasses, 7 (18.9%) were positive for *Campylobacter* spp. From those positive carcasses, 5 (13.5%) showed *Campylobacter* spp. contamination in only one collection step, while the other 2 carcasses (5.4%) were contaminated in more than one collection step (Table 1).

Of 9 isolates of *Campylobacter* from the samples, 5 (55.6%) were found after dehairing, 2 (22.2%) after sticking/bleeding, 1 (11.1%) after flaming and 1 (11.1%) after the cooling process. Seven isolates were detected by the qualitative method and 2, by the quantitative method. Of the *Campylobacter* spp. isolated using the quantitative method, one was found in the dehairing step, showing a viable count of 2.5 CFU/cm^2 and the other one—isolated from the cooling process—showed a count of 150 CFU/cm^2. All the other samples submitted to quantitative determination showed the number of viable cells to be lower than 2.5 CFU/cm^2.

In accordance with the biochemical and enzymatic identification of the isolates by API Campy, of the 9 cultures, 8 (88.9%) were identified as belonging to the genus *Campylobacter* and 1 (11.1%) as belonging to the genus *Arcobacter* (Table 2).

The strains were subjected to real-time PCR to confirm the identification of *Campylobacter* by API Campy. All the 9 cultures exhibited high correlativity with the chromosomal DNA of the genus *Campylobacter*, including the strain identified as *Arcobacter* according to the API Campy kit.

With regard to the sensitivity to antibiotics, all strains were resistant to at least 5 (41.7%) of the antimicrobial agents tested—cephalothin, nalidixic acid, norfloxacin, tetracycline and trimethoprim. The antimicrobial resistance results are presented in Table 3.

Table 1
Prevalence of *Campylobacter* sp. in a total of 259 samples collected from different steps of pig slaughtering process.

Collection step	Number of isolates	Frequency (%)
Sticking/bleeding	2	0.8%
Dehairing	5	1.9%
Flaming	1	0.4%
Evisceration	0	0%
Washing	0	0%
Cooling	1	0.4%
Ham cutting	0	0%
Total	9	3.5%

Table 2
Results for *Campylobacter* identification by API Campy in samples collected from pig carcasses.

Sample identification	Collection step[a]	*Campylobacter* specie	Probability (%)[b]
1	D	*Campylobacter jejuni Doylei*	98.2%
2	D	*Campylobacter jejuni Jejuni*	96.4%
3	S/B	*Campylobacter coli*	99.9%
4	D	*Arcobacter cryaerophilus*	99.0%
5	D	*C. coli*	99.2%
6	D	*Campylobacter jejuni Jejuni*	98.6%
7	F	*C. coli*	99.6%
8	C	*Campylobacter jejuni Doylei*	97.9%
9	S/B	*Campylobacter jejuni Jejuni*	97.5%

[a] D, dehairing, S/B, sticking/bleeding, F, flaming, C, cooling.
[b] Probability given by the API Campy system.

4. Discussion

The results showed that the occurrence of *Campylobacter* spp. in pig samples collected at the slaughterhouse was low, especially when compared with samples collected from poultry, turkey and sheep, but showed similar results as samples originating from cattle (Inglis et al., 2005; Mayhofer et al., 2004). Studies have noted *Campylobacter* spp. contamination at levels ranging from 28% to 72% in chicken carcasses and at 0–3.3% in bovine carcasses (Ghafir et al., 2007; Ono & Yamamoto, 1999).

The prevalence of *Campylobacter* spp. in samples evaluated in this study was 3.5%, similar to the frequency of 0–5% found in studies in Japan, Austria and United Kingdom for swab samples (Ono & Yamamoto, 1999, Mayrhofer et al., 2004, Little et al., 2008). The prevalence in pig carcasses was 18.9%, which was also similar to that reported by Ghafir et al. (2008), i.e. 17%, from 1997 to 1999 in Belgium.

Pig slaughter is an open process with many opportunities for the contamination of the carcass with pathogenic bacteria; it does not contain any step that could be considered completely free of hazard. Thus, contamination needs to be controlled by safety procedures (Borch, Nesbakken, & Christensen, 1996). Furthermore, *Campylobacter* spp. are known to be more frequently found in pig carcasses than in cattle or sheep carcasses (Franco, 1998).

In this study, 55.5% of *Campylobacter* spp. were found after dehairing, 22.2% after sticking/bleeding, 11.1% after flaming and 11.1% after the carcass cooling process.

The contamination observed after sticking/bleeding is probably due to cross-contamination by microorganisms from the intestinal

Table 3
Percentages of antimicrobial-resistant *Campylobacter* strains isolated from pig carcasses.

Antibiotic	Resistance (%)	Resistant *Campylobacter* species
Ampicillin	0	
Amoxicillin/clavulanic acid	0	
Chloramphenicol	0	
Streptomycin	33.3	*C. jejuni Jejuni, C. coli*
Gentamicin	44.5	*C. jejuni Jejuni, C. coli*
Ciprofloxacin	77.8	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*
Clindamycin	77.8	*C. jejuni Jejuni, C. coli*
Cephalothin	100	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*
Nalidixic acid	100	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*
Norfloxacin	100	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*
Tetracycline	100	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*
Trimethoprim	100	*C. jejuni Jejuni, C. jejuni Doylei, C. coli*

flora of the pigs, since different degrees of contamination by *Campylobacter* spp. have been recorded in the faeces of these animals by previous researchers (Adesiyun & Krishnan, 1995; Modolo, Margato, Gottschalk, Lopes, & De, 1999, Ono & Yamamoto, 1999).

Among the slaughtering steps sampled, dehairing of carcasses showed the highest contamination (55.6%). This could occur as a result of low water renewal or bad temperature control of the scalding tank. Another important factor for the occurrence of *Campylobacter* spp. in carcasses in the dehairing step was related to the type of the material used on the fingers of the dehairing machine. As these fingers were made of rubber and attached to the machine, they could not be easily removed for cleaning and disinfection, which could lead to the accumulation of residual material and bacterial growth.

Low *Campylobacter* spp. counts in pig carcass in different slaughter steps found in the current study was also found by Gill and Bryant (1993) which recorded *Campylobacter* counts ranging from 30 to 70 CFU/cm^2 in samples collected after carcass dehairing and from 1 to 6 CFU/cm^2 in samples obtained after the scorching and toilette of the carcasses. In their study, Ghafir et al. (2008) observed *Campylobacter* counts ranging from <1 CFU/cm^2 to >249 CFU/cm^2 in samples obtained from pig carcasses collected immediately after slaughter.

Although the thermophilic species of *Campylobacter* are recognized as emergent human pathogens, the epidemiological studies for these microorganisms are recent, and there are yet no legal standards established for the presence of *Campylobacter* spp. in food in Brazil and other countries. However, since the presence of this pathogen has a great impact on public health and on meat trading, it is likely that the microbiological standards for *Campylobacter* spp. in meat will be defined similar to those for pathogens such as *Salmonella* spp. and *Listeria monocytogenes*, which require the absence of pathogens in 25-g samples.

In the present work, 33.3% of the isolates were biochemically and enzymatically identified as *Campylobacter coli, 33.3% as Campylobacter jejuni* subsp. *Jejuni*, 22,2% as *Campylobacter jejuni* subsp. *Doylei and 11,1% as Arcobacter cryaerophilus*, 11.1%. However, PCR confirmed all the isolates to be *Campylobacter* strains, since all samples showed high compatibility with the primer used and rapid replication of their DNA in real-time PCR identification.

The real efficiency of enzymatic and biochemical tests for the identification of microorganisms is being challenged due to observations of failure when these tests are compared with genetic tests. Moran, Scates, and Madden (2009) reported the misidentification of 41 strains phenotyped as *C. coli,* but genotyped as *Arcobacter butzleri*. The same researchers found that a temperature of 25 °C under microaerophylic conditions proved to be a suitable cultivation technique for the confirmation of *Campylobacter* spp., since *Campylobacter* spp. do not grow well at this temperature, whereas *Arcobacter* spp. do grow.

Moreover, one of the factors related to the erroneous identification by the API Campy system is the difficulty in reading the results presented by this test. On (1996) stated that although the API Campy system comprises multiple tests for strain identification, it does not include all the clinically relevant species of Campylobacteriaceae.

Huymans and Turnidge (1997) found 100% correlations between the results of API Campy and the conventional phenotypic and biochemical method for *Campylobacter* spp. identification for *C. jejuni*. However, only 66.6% of *Campylobacter lari* and 74% of *C. coli* were correctly identified by both systems. The same authors observed misidentification of *C. coli* and *C. lari* as *Arcobacter cryaerophilus* by API Campy.

Regardless of the strain misidentification described for API Campy in a few studies, Kuana, Dos Santos, Rodrigues, and Do Nascimento (2009); Reina, Ros, and Serra (1995) and still consider this identification system as the best option for *Campylobacter* species when more efficient systems are not available.

Although *Campylobacter coli* is considered a commensal species of the intestinal tract of pigs (Quinn, Markey, Carter, Donnelly, & Leonard, 2002), *Campylobacter jejuni* represented 55.5% of the *Campylobacter* species identified in pig carcasses in this study. Workman, Mathison, and Lavoie (2008) consider pig products an important source of *C. coli* as all isolates from pigs tested in their work were identified as belonging to this species. Identical results were obtained by Shin and Lee (2007). However, several studies have reported a higher prevalence of *Campylobacter jejuni* than *C. coli* in farm animals (Açik & Çetinkaya, 2006; Bae et al., 2005; Zweifel, Zychowska, & Stephan, 2004;).

The introduction of antimicrobial agents in human and animal therapy has had a great impact on population growth and food production. The first agents were introduced during the 1930s, and resistance to these drugs gradually emerged with their worldwide use. However, in the last decade, the isolation of antimicrobial-resistant microorganisms from farm animals has increased greatly (EFSA, 2008).

Because *Campylobacter* spp. is classified as an emerging human pathogen, studies regarding the prevention and control of this pathogen in the poultry and pig production chains are recent. Therefore, the criteria for susceptibility to antibiotics and other parameters for the control of *Campylobacter* spp. have not been internationally standardised.

Studies related to the sensitivity to antibiotics of *Campylobacter* spp. in different countries show different degrees of resistance to the same drug (Alban et al., 2008; Armi, Senok, Ismaeel, Al-Mahmeed, & Botta, 2007; Gebreyes, Thakur, & Morrow, 2005; Guévremont et al., 2006, Han et al., 2007; Little et al., 2008, Moran et al., 2009).

In this study, all *Campylobacter* spp. isolates showed resistance to at least 5 of the antibiotics tested, indicating multi-drug resistance.

Multi-drug resistant strains obtained from pork samples have been reported by Gebreyes et al. (2005) and (Guévremont et al., 2006). Resistance of *Campylobacter* spp. isolated from other animal species has also been reported. Little et al. (2008) observed antibiotic resistance of *Campylobacter* spp. obtained from cattle and pigs; (Laungtongkum et al., 2007) verified resistance in strains from poultry; Inglis et al. (2005), from cattle; and Mayrhofer et al. (2004), from cattle, poultry and pigs.

In the present work, all isolates were sensitive to chloramphenicol, amoxicillin/clavulanic acid, and ampicillin. Sensitivity to chloramphenicol by all *Campylobacter* isolates was also observed by Sáenz et al. (2000) and Guévremont et al. (2006) in pigs, whereas Bardon et al. (2009) and Miflin et al. (2007) found similar resistance patterns for *Campylobacter* spp. obtained from humans and chickens, respectively.

High resistance to ampicillin for *Campylobacter* spp. was recorded by Little et al. (2008) (57.3%) and Sáenz et al. (2000) (65.7%) in samples from pigs and by Han et al. (2007) (43.1%) and Miflin et al. (2007) (40.8%) in samples from chicken. Contrasting results were obtained in this study, where none of the strains showed resistance to ampicillin.

On the other hand, resistance to gentamicin (44.5%) was high compared to the results obtained by other researchers such as Gebreyes et al. (2005), Guévremont et al. (2006), Little et al. (2008), Pezzotti et al. (2003), Sáenz et al. (2000), Shin and Lee (2007) and who recorded levels of resistance ranging from 0% to 22.2%.

All the isolates were resistant to cephalothin, nalidixic acid, norfloxacin, tetracycline and trimethoprim. Sáenz et al. (2000) also observed 100% resistance to cephalothin and nalidixic acid for

Campylobacter spp. isolated in Spain and 94.4% tetracycline resistance. Kang et al. (2006) found high resistance to nalidixic acid (91%) for *Campylobacter* spp. from chicken products.

Bardon et al. (2009) observed greater resistance of *C. jejuni* isolated from poultry than that from human faeces from intestinal disease patients. The only exception was for tetracycline, for which higher resistance was noted in human strains. The same authors also found the greatest resistance of *Campylobacter* strains to quinolones among various antibiotics. Among *Campylobacter* strains isolated from chicken and human samples, 72% and 55% were resistant to ciprofloxacin, respectively. These results were similar to those obtained in the current study, in which 77.8% of the isolates were resistant to ciprofloxacin.

On the other hand, Miflin et al. (2007) found sensitivity to ciprofloxacin for all the 152 strains of *Campylobacter* spp. isolated from chicken in Australia, whereas Inglis et al. (2005) confirmed ciprofloxacin resistance in only 0.3% of isolates from cattle in Canada.

Because of the high use of ciprofloxacin in human antimicrobial therapy, the assessment of *Campylobacter* spp. resistance to this antibiotic is particularly relevant to pathogen monitoring programs (EFSA, 2008).

In 2006, data reported by the European Food Safety Authority (EFSA) demonstrated a resistance rate of 57% to ciprofloxacin for *Campylobacter* spp. in Italy and 53% in Germany. The lowest rate of resistance to quinolones for *Campylobacter* spp. isolated from chicken in European Union has been observed in the Scandinavian countries. In Denmark, the resistance to quinolones was 7%, and in Sweden, no resistance to quinolones was found in *C. jejuni* isolated from broilers. These low antimicrobial resistance rates observed in the Nordic countries were mainly due to the implementation of national monitoring for pathogens in food of animal origin and the banning of growth promoters in farm animal production (EFSA, 2008).

Of the 12 antibiotics tested in this study, 7 were not currently used for growth promotion or prophylactic therapy in pig production, whereas 5 were used for any of the mentioned purposes. Among the 3 antibiotics for which 100% of the strains showed sensitivity (amoxicillin/clavulanic acid, ampicillin and chloramphenicol), 2 were not used in pig production; among the remaining 9 antibiotics for which the strains showed some level of resistance (cephalothin, ciprofloxacin, clindamycin, streptomycin, gentamicin, nalidixic acid, norfloxacin, tetracycline and trimethoprim), 5 were not used in the pig production process. These results did not allow the firm establishment of a relationship between the recurrent use of antibiotics in pig production and antimicrobial resistance. However, *Campylobacter* antimicrobial resistance can be related to some specific genes, and the dissemination of these genes of microorganisms from one animal species to another and to humans is possible. Antimicrobial resistance observed in the present work could therefore probably be related to the antibiotics used in broiler production, since in Brazil, animal production farmers generally raise both broilers and pigs in the same land area (Aarestrup & Wegener, 1999; Guévremont et al., 2006).

Despite the low number of viable cells and the low frequency of *Campylobacter* spp. in pig samples, the risk of contamination by *Campylobacter* spp. in pork meat should not be disregarded as it has been classified as an emerging human pathogen. Given the relevance of the genus *Campylobacter* in human gastroenteritis, its occurrence in farm animals and the fact that all *Campylobacter* strains showed multi-drug resistance, a continuous monitoring of the prevalence and the antimicrobial resistance of *Campylobacter* spp. in pig production and in the pork meat product chain is essential to the implementation of effective policies for controlling and preventing contamination and infection by this pathogen.

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