DOI: 10.5433/1679-0359.2019v40n5Supl1p2247

Isolation and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* found in chilled chicken carcasses in the Federal District Region and surrounding areas

Isolamento e resistência antimicrobiana de *Campylobacter jejuni* e *Campylobacter coli* encontradas em carcaças de frango resfriadas na região do Distrito Federal e entorno

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Abstract

This study aimed to isolate *Campylobacter jejuni* and *Campylobacter coli* from chilled chicken carcasses marketed in the Federal District Region and surrounding areas, as well as to detect the occurrence of antimicrobial resistance and genes responsible for the same. A total of 105 chilled chicken carcasses were collected, of which 7 (6.67%) were positive for *C. jejuni* and 4 (3.81%) were positive for *C. coli*. These results were obtained using both the conventional microbiological isolation method and polymerase chain reaction assays. All of the positive strains were subjected to antimicrobial susceptibility testing for seven antimicrobials. The resistance incidences found in the *C. jejuni* strains were as follows: 71.43% for tetracycline and nalidixic acid, 42.86% for streptomycin and gentamicin, 57.14% for ciprofloxacin and erythromycin, and 28.57% for chloramphenicol. Among the *C. coli* strains, 100% were resistant to tetracycline and streptomycin, 75% were resistant to erythromycin, 50% were resistant to ciprofloxacin, gentamicin, and nalidixic acid, and no strains were resistant to chloramphenicol. While analyzing the presence of antimicrobial resistance genes in the isolated *C. jejuni* strains, the *aph3-1* (resistance to aminoglycosides), *aadE* (resistance to streptomycin), and *tet(O)* (resistance to tetracycline) genes were identified, with occurrence rates of 57.14%, 28.57%, and 42.86%, respectively, whereas in the *C. coli* strains, there was a 25% occurrence rate for both the *aph3-1* and *tet(O)* genes. The *aadE* gene was not found in the *C. coli* isolates. The results of this study demonstrated the presence of *C. jejuni* and *C. coli* in chilled chicken carcasses marketed in the Federal District Region and surrounding areas, as well as the antimicrobial resistance and the presence of resistance genes in these bacteria, which may pose threats to public health.

Key words: Antimicrobial resistance. *Campylobacter coli.* C*ampylobacter jejuni*. Chilled chicken carcass. Resistance gene.

Resumo

O objetivo deste trabalho foi isolar *Campylobacter jejuni* e *Campylobacter coli* de carcaças de frango resfriadas comercializadas no Distrito Federal e entorno, bem como detectar a ocorrência de resistência antimicrobiana e genes responsáveis pela resistência antimicrobiana. Foram coletadas um total de 105

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Received: Apr. 21, 2018 - Approved: June, 20, 2019

carcaças de frango resfriadas, das quais 7 (6,67%) foram positivas para *C. jejuni* e 4 (3,81%) para *C. coli*. Estes resultados foram obtidos usando tanto o método convencional de isolamento microbiológico quanto os ensaios de reação em cadeia da polymerase (PCR). Todas as cepas positivas foram submetidas ao teste de susceptibilidade antimicrobiana para sete antimicrobianos. As incidências de resistência encontradas nas cepas de *C. jejuni* foram as seguintes: 71,43% para tetraciclina e ácido nalidíxico, 42,86% para estreptomicina e gentamicina, 57,14% para ciprofloxacina e eritromicina e 28,57% para cloranfenicol. Entre as cepas de *C. coli*, 100% foram resistentes à tetraciclina e à estreptomicina, 75% eram resistentes à eritromicina, 50% eram resistentes à ciprofloxacina, gentamicina e ácido nalidíxico, e nenhuma cepa eram resistentes ao cloranfenicol. Ao analisar a presença de genes de resistência antimicrobiana nas cepas isoladas de *C. jejuni*, foram identificados os genes *aph3-1* (resistência a aminoglicosídeos), *aadE* (resistência à estreptomicina) e *tet (O)* (resistência à tetraciclina) genes foram identificados, com taxas de ocorrência de 57,14%, 28,57% e 42,86%, respectivamente, enquanto que nas cepas de *C. coli*, houve uma taxa de ocorrência de 25% para os genes *aph3-1* e *tet (O)*. O gene *aadE* não foi encontrado nos isolados de *C. coli*. Os resultados deste estudo demonstraram a presença de *C. jejuni* e *C. coli* em carcaças de frango resfriadas comercializadas na Região do Distrito Federal e entorno, além da resistência antimicrobiana e a presença de genes de resistência nestas cepas, que podem se tornar uma possível ameaça a saúde pública.
Palavras-chave: *Campylobacter coli. Campylobacter jejuni*. *C*arcaça de frango refrigerada. Gene de resistência. Resistência antimicrobiana.

Introduction

Campylobacter jejuni and *Campylobacter coli* are presently considered as the leading causative agents of gastroenteritis in humans as well as the main source of poultry contamination (AARESTRUP; ENGBERG, 2001; FREDIANI-WOLF; STEPHAN, 2003; ISHIHARA et al., 2004; ENGLEN et al., 2007; HÄNNINEN; HANNULA, 2007; WASSENAAR et al., 2007). In 2011, it was estimated that there were more than 845,000 cases of campylobacteriosis in the United States alone (SCALLAN et al., 2011). In the European Union in 2015, campylobacteriosis was the most commonly reported zoonosis (EFSA, 2016); however, it is underreported in several countries, and its true incidence may be at least 10-fold higher than the actual documented cases (GIBREEL; TAYLOR, 2006). Despite the fact that campylobacteriosis is a short-lived, self-limiting, and mild infection, antibiotic treatments are required for the more severe cases.

Macrolides, particularly erythromycin and fluoroquinolones, are the drugs of choice for the treatment of these infections (AARESTRUP et al., 2005; HAN et al., 2007; KURINČIČ et al., 2007). According to Tafa et al. (2014) the antimicrobial resistance of this species of bacteria to the fluoroquinolones and macrolides has increased, mainly due to the approval of their use in food producing animals. Antimicrobials are used in animal husbandry as therapeutic agents, prophylactics, and growth promoters (FÀBREGA et al., 2008), and their indiscriminate use has inevitable side effects. The most troubling side effect is the spread of resistance genes and, consequently, of antimicrobial resistant microorganisms, mainly by horizontal gene transfer through mobile genetic elements, such as plasmids, transposons and integrons (ZHANG et al., 2006).

Brazil is presently the second largest chicken producer, and accounts for 37% of world exports and is the leader in exporting chicken products, which are imported by more than 150 countries (MAPA, 2018); however, there is, to date, no specific legislation for *Campylobacter* spp. detection, which highlights the need for further studies on the occurrence of this pathogen in Brazil and its potential risks to human health (CORTEZ et al., 2006). In Brazil, human cases of campylobacteriosis are underreported, whereas in other countries, such as Australia and the UK, cases of enteritis due to *Campylobacter* spp. are more

frequent than are those caused by *Salmonella* spp. or *Shigella* spp. (FOOD STANDARDS AGENCY, 2011; OZFOODNET, 2012). In a previous study conducted by our research group, Moura et al. (2013) detected *Campylobacter jejuni* in 19.56% of chilled chicken carcasses marketed in the Federal District Region and the surrounding areas, as well as the presence of antimicrobial resistance in these agents; however, the presence of antimicrobial resistance genes was not investigated. No reports exist regarding the presence of *C. coli* in this region.

Considering the aforementioned facts and the growing threat of *Campylobacter* spp. infections worldwide, incurred mainly via consumption of chicken meat or chicken by-products, this study aimed to investigate the occurrence of *C. jejuni* and *C. coli* in chilled chicken carcasses marketed in the Federal District Region and surrounding areas, to study the antimicrobial resistance of these bacteria, and to detect the antimicrobial resistance genes in these bacteria using polymerase chain reaction (PCR) assays.

Materials and Methods

Obtaining samples and bacterial isolation

In total, 105 samples of chilled chicken carcasses of various brands were acquired from the commercial establishments located throughout the Federal District Region and surrounding areas from March 2011 to June 2012. The carcasses were acquired while simulating an actual consumer purchase, with acquired samples comprising a whole chicken, with intact packaging, without any sign of damage, marked with the SIF (Serviço de Inspeção Federal [Federal Inspection Service]) stamp, chilled to an average temperature of 4 °C, and prior to the expiration date. The samples were transported in coolers to the Food Microbiology Laboratory of the College of Agronomy and Veterinary Medicine at the University of Brasilia for microbiological isolation.

Microbiological isolation was performed according to the method of Kudirkienė et al. (2011), in which 10 g of each sample was weighed and placed in a sterile bag for homogenization, 90 ml of Bolton enrichment broth (Bolton Broth, Oxoid, CM0983) containing Bolton Broth Selective Supplement (Oxoid, SR183E) and 5% defibrinated horse blood was added, and the samples were incubated at 42 °C for 48 h in anaerobic jars (Permution®) in a microaerophilic gas generator (BD BBL™ CampyPak™ Plus Microaerophilic System Envelopes with a Palladium Catalyst). After incubation, a 0.1-ml aliquot of each sample was spread on the surface of a Petri dish containing a blood-free *Campylobacter* selective agar base (mCCDA, Oxoid, CM 0739) with the CCDA Selective Supplement (Oxoid, SR155E), which was incubated at 37 ºC for 48 h under microaerophilic conditions. Spiral or curved gram-negative colonies that were oxidase and catalase positive were classified as *Campylobacter*. A sodium hippurate hydrolysis test was performed for biochemical differentiation of *C. jejuni* and *C. coli*, with colonies that were positive for this test being classified as *C. jejuni*.

Identification of the species C. jejuni and C. coli using PCR

The *Campylobacter* species in the 105 samples were identified using PCR assays. A 1-ml aliquot was removed from the Bolton Enrichment Broth sample after incubation at 42 °C for 48 h under microaerophilic conditions, and the total DNA was extracted from it following the method described by Sambrook and Russel (2006), using phenol:chloroform:isomyl alcohol at a ratio of 25:24:1. PCR was performed using the method described by Linton et al. (1997) for identifying *C. jejuni* and *C. coli.* The gene *hip(O)* was amplified to identify *C. jejuni*, using primers HIP400 F 5′-GAAGAGGGTTTGGGTGGTG-3′ and HIP1134 R 5′- AGCTAGCTTCGCATAATAACTTG-3′,

which generated a 735-bp product. To identify *C. coli*, a fragment of the *aspA* gene was amplified using primers CC18 F 5′-GGTATGATTTCTACAAAGCGAG-3′ and CC519 R 5′-ATAAAAGACTATCGTCGCGTG-3′, which generated a 500-bp product. The reactions were performed in a final volume of 25 µl containing 2.5 mM $MgCl_2$, 2 µl of 2 mM dNTPs, 1 µl of 10 pmol of each primer, 2.0 U of Taq DNA polymerase (Invitrogen), 2.5 µl of 10× PCR buffer, and 1 µl of DNA template. Twenty-five amplification cycles were performed, with denaturation at 94 ºC for 1 min, annealing at 66 ºC (*C. jejuni*) or 60 ºC (*C. coli*) for 1 min, and extension at 72 ºC for 1 min.

Phenotypic profiling and identification of antimicrobial resistance genes

The *Campylobacter* spp. strains isolated using conventional microbiological methods were subjected to antimicrobial susceptibility testing following the method described by Taremi et al. (2006). The technique used was disc diffusion in Mueller-Hinton agar (HiMedia, M1084), supplemented with 5% sheep blood. The plates were incubated at 42 ºC for 24 h under microaerophilic conditions. The following antimicrobials (Newprov®) were tested: tetracycline (15 µg), streptomycin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), and chloramphenicol (30 µg). The test readings were performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

All of the isolated *C. jejuni* and *C. coli* strains were screened for the presence of the tet(O) (tetracycline), *aph*-3 (aminoglycosides), and *aadE* (streptomycin) genes using PCR. Table 1 presents the primer sequences used to detect these antimicrobial resistance genes. The method used to detect the aph-3 and *aadE* genes was described by Obeng et al. (2012). The reactions were performed in a final volume of 25 µl containing 4.0 mM $MgCl_2$, 2 µl of 2 mM dNTPs, 1 µl of 10 pmol of each primer, 2.0 U of Taq DNA polymerase (Invitrogen), 2.5 µl of 10× PCR buffer, and 1 µl of DNA template. The following PRC protocol was used: initial denaturation at 95 °C for 4 min, followed by 30 amplification cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 4 min. The method described by Pratt and Korolik (2005) was used to detect the *tet(O)* gene. The reactions were performed in a final volume of 25 µl, using the same concentrations of reagents used to amplify the *aph-3* and *aadE* genes, with the exception of the MgCl2 concentration, which was 3.5 mM. The following PRC protocol was used: initial denaturation at 95 °C for 2 min, followed by 35 amplification cycles of denaturation at 95 °C for 2 min, annealing at 52 °C for 30 s, and extension temperature at 72 °C for 1 min, with a final extension at 72 °C for 2 min.

Table 1. Primer sequences used to detect antimicrobial resistance genes.

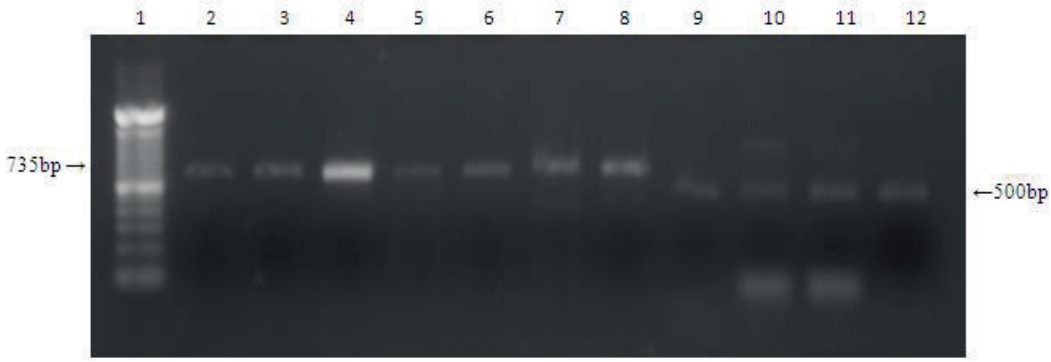
Primer	5′-3′ Sequence	Product (bp)	Reference
aadE F	GAACAGGATGAACGTATTCG		
aadE R	GCATATGTGCTATCCAGG	837	Obeng et al. (2012)
*aph*A-3 F	TGCGTAAAAGATACGGAAG		
*aph*A-3 R	CAATCAGGCTTGATCCCC	701	Obeng et al. (2012)
tet(O) F	GGCGTTTTGTTTATGTGCG		
tet(O) R	ATGGACAACCCGACAGAAGC	559	Pratt and Korolik (2005)

Results and Discussion

Identification of the C. jejuni and C. coli species by microbiological isolation and PCR

Analysis of the 105 chilled chicken samples conducted using the microbiological isolation method revealed that 11 (11.55%) samples tested positive for *Campylobacter* spp., 7 (63.64%) of which were identified as *C. jejuni* and 4 (36.36%) as *C. coli*. PCR analysis of all 105 samples was performed to further identify the *Campylobacter* species isolated. The results obtained using PCR matched the results obtained using the microbiological isolation method. Figure 1 depicts the results of the PCR assay for the presence of the *hip(O)* gene, which is specific for *C. jejuni,* and those for the presence of the *aspA* gene, which is specific for *C. coli*, which yielded 735-bp and 500-bp products, respectively.

Figure 1. Results of the PCR assay for the presence of the *hip(O)* gene which is specific for *C. jejuni,* and those of the PCR assay for the presence of the *aspA* gene, which is specific for *C. Coli* 1) 100 bp DNA Ladder (Invitrogen®); 2-8) 735 bp product, result of gene amplification *hip(O)*; 9-12) 500 bp product, result of gene amplification *aspA*.



The present results regarding the occurrence of *C. jejuni* in chilled chicken carcasses marketed in the Federal District Region were similar to those obtained in a previous study conducted by our research group. In the previous study, Moura et al. (2013) detected *Campylobacter* spp. in 19.56% of 92 chilled chickens marketed in the Federal District Region of Brazil using microbiological methods, with all of the strains identified as *C. jejuni*; however, in this previous study, the presence of *C. coli* was not investigated; thus, to our knowledge, this is the first study to identify the presence off *C. coli* in 36.36% of the samples for this region. Results similar to those of this study were reported by Frediani-Wolf and Stephan (2003), who detected *C. jejuni* in the neck skin of 24.4% of chickens collected in a slaughterhouse in Switzerland, and by Cortez et al. (2006), who isolated *Campylobacter* spp. from 5.2% of fecal, feather, and water samples collected in several areas of a chicken slaughterhouse located in São Paulo, Brazil, with 4.9% of the strains being identified as *C. jejuni* and 0.35% as *C. coli.*

Nevertheless, the results differing from those obtained in our study were reported by some authors. Fernández and Pisón (1996) detected strains of *Campylobacter* spp. in 92.9% of the samples isolated from frozen chicken livers in Chile, of which 78.6% were identified as *C. coli* and 21.4% as *C. jejuni*. Wei et al. (2016) found a similar result that demonstrated a high prevalence of *Campylobacter* in the retail poultry meat in South Korea, with an isolation rate of 80.1% and the most prevalent species was *C. jejuni* with 66.7%, followed by *C. coli* with 12.9%. in 64.7% of samples isolated from the chicken carcasses and by-products in Japan, identifying 85.5% of the positive samples as *C. jejuni* and 24.5% as *C. coli*.

The differences between the results may be due to distinct variable count, such as the geographical location, the study time and year, management practices, and isolation techniques used, among others (KASHOMA et al., 2015).

The occurrence rate of *C. jejuni* (63.64%) was higher than that of *C. coli* (36.36%). Similar results were reported by Kang et al. (2006), who observed an occurrence rate of 36.3% for *C. jejuni* and 26.4% for *C. coli* in the isolates obtained from chicken carcasses in Korea; by Obeng et al. (2012), who found an occurrence rate of 30.4% for *C. jejuni* and 14.5% for *C. coli* in isolates obtained from the rectal swabs of chickens in Australia; and by Han et al. (2007), who found a 37.7% occurrence rate for *C. jejuni* and a 35.5% for *C. coli* in the isolates obtained from chicken carcasses in Korea. Chicken meat and chicken by-products are the major food sources contaminated by *C. jejuni*, although some studies have reported that the occurrence rate of *C. coli* in pork is greater than that in poultry (HARVEY et al., 1999; SÁENZ et al., 2000; PEZZOTTI et al., 2003). The microbiological and molecular diagnostic methods were effective in detecting the occurrence of these two microorganisms in this study.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility tests of *C. jejuni* and *C. coli* strains isolated in this study are presented in Table 2. Among the *C. jejuni* strains isolated, resistance to tetracycline and nalidixic acid (71.43%) were the most common forms of antimicrobial resistance detected, followed by resistance to ciprofloxacin and erythromycin (57.14%). Three of the *C. jejuni* strains isolated (42.86%) were resistant to streptomycin and gentamicin, and only two *C. jejuni* strains (28.57%) were resistant to chloramphenicol. Among the *C. coli* strains, resistance to tetracycline and streptomycin were the most frequently detected forms of resistance, with all four strains (100%) resistant, followed by erythromycin resistance (75%). Two strains (50%) were resistant to ciprofloxacin, gentamicin, and nalidixic acid, and no resistance to chloramphenicol was observed. Five strains (71.43%) of *C. jejuni* and all four strains (100%) of *C. coli* were multi-drug resistant, that is, being resistant to three or more antimicrobials.

Table 2. Results of the antimicrobial susceptibility tests of *Campylobacter jejuni* and *Campylobacter coli* strains isolated in this study, which were performed using the disc diffusion method.

Antimicrobial	*C. jejuni* (*n* = 7)			*C. coli* (*n* = 4)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
TET	14.29	14.29	71.43	0	0	100
STR	28.57	28.57	42.86	0	0	100
CIP	28.57	14.29	57.14	25	25	50
ERY	28.57	14.29	57.14	25	0	75
GEN	42.86	14.29	42.86	50	0	50
NAL. AC.	28.57	0	71.43	0	50	50
CHL	28.57	42.86	28.57	50	50	0

TET = tetracycline, STR = streptomycin, CIP = ciprofloxacin, ERY = erythromycin, GEN = gentamicin, NAL AC,. = nalidixic acid, CHL = chloramphenicol; Resistant profile: S = susceptible, I = intermediate, R = resistant.

Resistance to tetracycline was most commonly found among the strains of *C. jejuni* and *C. coli* isolated in this study, with an incidence of 71.43% and 100%, respectively. Similar values were observed in other studies, such as that of Han et al. (2007), who found that 99.1% of the *C. jejuni* samples isolated from chicken meat in Korea were tetracycline resistant, and that of Ishihara et al. (2004), who found that 87.9% of the *C. coli* strains isolated from the production animals in Japan were resistant to this antimicrobial. Jamali et al. (2015) hypothesized that the widespread use of tetracycline for the treatment, prevention, and control of poultry diseases during recent decades may have led to an increased level of resistance to this drug in *Campylobacter* spp. strains.

The level of resistance to nalidixic acid and ciprofloxacin observed in *C. jejuni* in this study was 71.43% and 57.14%, respectively, and that to both of these antimicrobials in *C. coli* was 50%. An increasing level of resistance to quinolones in *Campylobacter* spp. has been reported in several countries (ISHIHARA et al., 2004; HÄNNINEN; HANNULA, 2007; SON et al., 2007). Wassenar and Newell (2000) and Rodrigo et al. (2007) cited studies linking an increased resistance to these drugs to the use of quinolones in farmed chickens during their 1st week of life, administered to reduce the prevalence of vaccination-related issues, and during their 3rd and 4th weeks of life, administered to treat and prevent the respiratory diseases.

Erythromycin resistance was discovered in 57.14% of the *C. jejuni* and 75% of the *C. coli* strains isolated. According to Aarestrup and Engberg (2001) and Iovine (2013), erythromycin is preferred for the treatment of campylobacteriosis in humans. In samples of animal products, the level of resistance to this drug is generally higher in *C. coli* strains, particularly in the strains isolated from pigs, than in *C. jejuni* strains (ENGBERG et al., 2001). The high level of resistance to erythromycin in *C. coli* strains observed in the present study, as do the results of Pezzotti et al. (2003), who detected erythromycin resistance in 25% and 12.5% of *C. coli* and *C. jejuni* strains, respectively, isolated from chicken carcasses and in 60% and 16.7% of *C. coli* and *C. jejuni* strains, respectively, isolated from pig carcasses in northeastern Italy. On May 17, 2012, The Ministry of Agriculture, Livestock and Food supply of Brazil banned the use of erythromycin as an additive in the poultry farming feed, coinciding with the realization period of this research; wherein the collects were realized to June 2012, it was possible to detect the resistance of this class of Antimicrobials.

The results of research on the resistance to aminoglycosides in *Campylobacter* spp. has been widely documented, generally indicating low levels of resistance, as demonstrated by Rodrigo et al. (2007), who observed 30% and 5,4% rate of resistance to streptomycin and gentamicin, respectively, in *Campylobacter* spp. samples isolated from chickens in Trinidad and Tobago and by Marinou et al. (2012), who found a 14.3% rate of resistance to gentamicin in *C. coli* strains isolated from the fecal swabs of chickens in Greece. These phenomena presumably occurred due to the minimal use of these antibiotics in animal husbandry (GIACOMELLI et al., 2014). The results of this study regarding the resistance of *Campylobacter* spp. to streptomycin and gentamicin, both of the aminoglycoside class, were similar to those obtained by Moura et al. (2013), who reported a high level of resistance (93.75%) to both of these antimicrobials in *C. jejuni* strains isolated from chilled chickens collected from the same region as that considered in the present study,

The low level of resistance to chloramphenicol, which was observed in only two of the *C. jejuni* strains (28.57%) and not in any of the *C. coli* strains isolated in this study, may be explained by the use of the active ingredients of this antimicrobial being banned in animal feed in Brazil via Instruction No. 9 of the 27th of June 2003 of the Ministry of

Agriculture, Livestock and Supply due to reports of aplastic anemia occurring in humans (BRASIL, 2003). Similar results were reported by Moura et al. (2013), in which 37.5% of *C. jejuni* strains were resistant to this antimicrobial.

The high levels of multidrug resistance (resistance to three or more antimicrobials), observed in five strains (71.43%) of *C. jejuni* and in all four strains (100%) of *C. coli* isolated in this study, constitute a public health concern, considering that according to Rodrigo et al. (2007), the microorganisms inhabiting the gastrointestinal tract can exchange genetic information, particularly gram-negative bacteria, broadening the resistance spectrum of microorganisms. More studies should be conducted to assess the origins of antimicrobial resistance.

Detection of antibiotic resistance genes in C. jejuni and C. coli strains

The results of our study on the presence of genes responsible for resistance to aminoglycosides (aph3-1), streptomycin (*aadE*), and tetracycline (*tet(O)*), evaluated via PCR assays, in the *C. jejuni* and *C. coli* strains are presented in Table 3. A 701-bp fragment of the *aph3-1* gene was amplified in four (isolated 57.14%) strains of *C. jejuni* and one (25%) strain of *C. coli*; this gene is known to confer resistance to aminoglycosides. An 837-bp fragment of the *aadE* gene, which was amplified in two (28.57%) strains of *C. jejuni*, confers resistance to streptomycin. A 559-bp fragment of the *tet(O)* gene was amplified in three (42.86%) strains of *C. jejuni* and one (25%) strain of *C. coli*, and this gene confers resistance to tetracycline (Figure 2).

Table 3. Results of the presence of genes responsible for resistance to aminoglycosides (*aph3-1*), streptomycin (*aadE*), and tetracycline (*tet(O)*), which were evaluated via PCR assays, in the *C. jejuni* and *C. coli* strains.

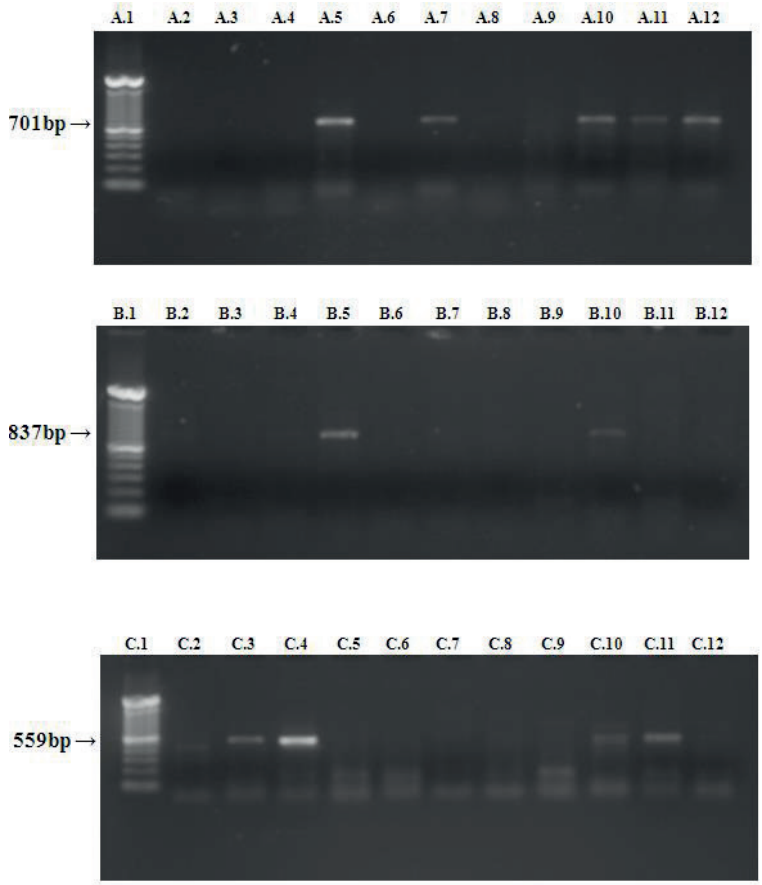
Species (*n*)	Gene	Positive strains (%)
C. jejuni (07)	*aph*-3-1	04 (57.14%)
	aadE	02 (28.57%)
	tet(O)	03 (25%)
C. coli (04)	*aph*-3-1	01 (25%)
	aadE	0
	tet(O)	01 (25%)

The presence of the *aph-3* gene in *Campylobacter* spp. strains was previously reported by Frye et al. (2011), who demonstrated their presence in 9.7% of *C. coli* strains isolated from fecal swabs of pigs in the United States. Obeng et al. (2012) investigated the occurrence of this gene in *C. coli* strains isolated from pigs and in *C. jejuni* and *C. coli* strains isolated from poultry fecal samples. The results revealed the occurrence of the *aph-3* gene in 0.9% of *C. coli* strains isolated from pigs and the absence of this gene in *C. jejuni* and *C. coli* strains isolated from fecal samples of poultry in Australia. According to Iovine (2013), the *aph-3* gene, which is normally present in plasmids, encodes phosphotransferase, an enzyme involved in the resistance to aminoglycosides that can be found associated with the *aadE* gene, which confers resistance to streptomycin via an adenyl transferase.

Pinto-Alphandary et al. (1990) reported the occurrence of the *aadE* gene in 67% of *C. coli* strains that were isolated from humans and animals in France and Spain. The same authors also reported 100% occurrence of this gene in *C. jejuni* strains isolated from humans in Thailand. Obeng et al. (2012) investigated the occurrence of the *aadE*

gene in *C. coli* and *C. jejuni* strains isolated from poultry and pigs in Australia but did not obtain positive results. The resistance of *Campylobacter* spp. strains to aminoglycosides is generally not monitored because this class is not preferred for treating campylobacteriosis in humans; however, according to Thibodeau et al. (2013), such monitoring is important due to the possibility of these microorganisms acting as a reservoir of resistance genes that could be transferred to other microorganisms. It is known that erythromycin resistance can also occur due to modification of this antimicrobial through the activity of phosphotransferase, but this mechanism has been demonstrated only in *Staphylococcus* spp. (PAYOT et al., 2006).

Figure 2. Results of the PCR assay for the presence of the resistance genes *aph-3* (aminoglycosides), *aadE* (streptomycin), and *tet(O)* (tetracycline) in *C. coli* and *C. jejuni* strains. A.1) 100 bp DNA Ladder (Invitrogen®); A.5, A.10, A.11, A.12) 701 bp fragment of the *aph3-1* gene, detected in *C. jejuni*; A.7) 701 bp fragment of the *aph-3* gene, detected in *C. coli*; B.1) 100 bp DNA Ladder (Invitrogen®); B.5, B.10) 837 bp fragment the *aadE* gene, detected in *C. jejuni*; C.1) 100 bp DNA Ladder (Invitrogen®); C.3, C.10, C.11) 559 bp fragment of the *tet (O)* gene, detected in *C. jejuni*; C.4) 559 bp fragment of the *tet(O)* gene, detected in *C. coli*.



Similar to the results of the present study, the presence of the *tet(O)* gene in *Campylobacter* spp. strains has been reported by other authors, such as Lee et al. (1994), who investigated its occurrence in *C. jejuni* and *C. coli* strains isolated from chickens in Taiwan and found them in 98% of the samples, of which 87% were located in the bacterial plasmids and 11% in the bacterial chromosome. According to Luangtongkum et al. (2009), the *tet(O)* gene may be present in the bacterial chromosome or, more

commonly, may be present in bacterial plasmids. The main mechanism underlying the resistance to tetracycline in gram-negative bacteria, according to Iovine (2013), involves the binding of the *tet(O*) protein to the tetracycline-binding site on the ribosome, protecting it from the activity of this antimicrobial.

Conclusion

The high level of resistance to certain drugs and the occurrence of multi-drug resistance observed in the *C. jejuni* and *C. coli* isolates in this study might be associated with the indiscriminate use of these agents in animal husbandry. These results are of great concern because the antimicrobial resistant *Campylobacter* strains can be easily transmitted to humans via animal products, particularly chicken products. The resistance genes can be passed to other bacteria present in the same environment as the *Campylobacter* strains, which may limit the efficacy of treatments for more severe infections.

Coordinated actions are needed to reduce the level of contamination of this pathogen in the food chain, such as the proper management of animal production and the implementation of quality control programs, such as Good Practices in Manufacturing, Hazard Analysis and Critical Points of Control, during each process of the production chain.

Acknowledgment

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)

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