Pesq. Vet. Bras. 35(7):637-642, julho 2015
DOI: 110.1590/S0100-736X2015000700006

Resistance to β-lactam and tetracycline in *Campylobacter* spp. isolated from broiler slaughterhouses in southern Brazil[1]

Yuli M. Sierra-Arguello[2]*, Rafaela B. Morgan[2], Gustavo Perdoncini[2], Leonardo M. Lima[2], Marcos José P. Gomes[3] and Vladimir Pinheiro do Nascimento[2]

ABSTRACT.- Sierra-Arguello Y.M., Morgan R.B., Perdoncini G., Lima L.M., Gomes M.J.P. & Nascimento V.P. 2015. **Resistance to β-lactam and tetracycline in *Campylobacter* spp. isolated from broiler slaughterhouses in southern Brazil.** *Pesquisa Veterinária Brasileira 35(7):637-642.* Centro de Diagnóstico e Pesquisa em Patologia Aviária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 8824, Porto Alegre, RS 91540-000, Brazil. E-mail: yuli_melisasierra@yahoo.com

The study was carried out to screen and analyze the genetic characteristics of antimicrobial resistance in *Campylobacter* spp. from poultry sources. A total of 141 strains of *Campylobacter* isolated from samples of broilers of slaughterhouses in southern Brazil was identified by phenotypic and genotypic methods. *Campylobacter* isolates were evaluated for its antimicrobial susceptibility and the presence of resistance genes. The strains were investigated for antimicrobial susceptibility against two agents (ampicillin and tetracycline) by disk diffusion method. PCR assay was used to confirm the specie and the presence of ampicillin (bla_{OXA-61}), tetracycline *tet*(O), and the energy-dependent multi-drug efflux pump (*cme*B) genes. *Campylobacter jejuni* was the most ubiquitous; its presence was determined in 140 samples out of 141 (99.3%), whereas *Campylobacter coli* was found only in one of the contaminated samples (0.70%). The results obtained showed 65% and 35.5% of *Campylobacter* isolates resistant to β-lactams and tetracyclines, respectively. The *cme*B gene responsible for multidrug resistance was detected in 26 isolates out 141 strains (18.5%). Moreover, 36 out of 141 *Campylobacter* strains (25.6%) were found to be resistant to at least two different antimicrobia resistance markers (β-lactams and tetracyclines).

INDEX TERMS: *Campylobacter*, tetracycline, β-lactam, efflux pump, resistance genes, PCR.

RESUMO.- [**Resistência a β-lactâmicos e tetraciclina em *Campylobacter* spp. isolados de matadouros-frigoríficos de aves no sul do Brasil.**] O presente estudo foi realizado para examinar e analisar as características genéticas de resistência antimicrobiana de *Campylobacter* spp. a partir de fontes avícolas. Um total de 141 amostras de *Campylobacter* isolados em matadouros-frigoríficos de aves do estado do Rio Grande do Sul, Brasil, foi identificado por métodos fenotípicos e genotípicos. Foi analisada a susceptibilidade antimicrobiana e a presença de genes de resistência. As cepas foram testadas para detectar sensibilidade frente a dois antimicrobianos (ampicilina e tetraciclina) pelo método de difusão em disco. A seguir, usando a reação em cadeia da polimerase foi confirmada a espécie e a presença dos genes de resistência à ampicilina (bla_{OXA-61}) e tetraciclina *tet*(O), assim como a detecção da bomba de efluxo (*cme*B). *Campylobacter jejuni* foi a espécie mais isolada, sua presença foi determinada em 140 amostras (99,3%), e *Campylobacter coli* foi encontrada em uma única amostra (0,70%). Os resultados obtidos mostraram 65% e 35,5% de *Campylobacter* isolados resistentes a β-lactâmicos e tetraciclinas, respectivamente. O gene *cme*B responsável pela resistência a múltiplos antimicrobianos foi detectado em 26 amostras (18,5%). Neste contexto, 36 das 141 amostras (25,6%) foram consideradas resistentes a dois grupos diferentes de antimicrobianos (β-lactâmicos e tetraciclinas).

TERMOS DE INDEXAÇÃO: *Campylobacter*, tetraciclinas, β-lactâmicos, bomba de efluxo, genes de resistência, PCR.

[1] Received on May 8, 2015.
Accepted for publication on July 21, 2015.

[2] Centro de Diagnóstico e Pesquisa em Patologia Aviária (CDPA), Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 8824, Porto Alegre, RS 91540-000, Brazil. *Corresponding author: yuli_melisasierra@yahoo.com

[3] Faculdade de Veterinária, UFRGS, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil.

INTRODUCTION

Campylobacter is recognized as the leading causes of bacterial foodborne diarrheal disease throughout the worldwide (Park 2002, Silva et al. 2011). Campylobacteriosis is estimated to cause about 1.3 million infections, 13,000 hospitalizations and 120 deaths each year in the United States (CDC 2013). It is also the most commonly reported antecedent infection in the development of Guillain Barré syndrome (GBS) and Miller Fisher syndrome (MFS) (Godschalk et al. 2004, Hardy et al. 2011, Van den Berg et al. 2014). A risk factor for human disease is the consumption of contaminated poultry products (Conlan et al. 2007, Ellström et al. 2014). Transmission to man usually results in sporadic infection, and is often associated with improper handling or cooking of food (Moore et al. 2005). *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* are considered to be zoonotic pathogens, antimicrobial resistance among isolates in the animal reservoir has serious implications for the treatment in humans (Moore et al. 2006, EFSA 2011). The majority of cases of clinical *Campylobacter* spp. enteritis are generally mild or self-limiting disease not to require of antimicrobial chemotherapy (Moore et al. 2005). However, antimicrobial therapy may be used in a subset of patients with severe and prolonged systemic complications or to control infection (Avrain et al. 2003, Janssen et al. 2008). Currently, macrolides and fluoroquinolones are the antimicrobial agents of choice when therapeutic intervention is warranted (Engberg et al. 2001, Moore et al. 2005). Bacterial populations can respond to the threat of an antimicrobial agent by evolving some type of resistance mechanism(s) (Rowe-Magnus et al. 2002, Luangtongkum et al. 2009). These resistant bacteria may be transferred to humans either through the food supply or by direct contact with animals (Khachatourians 1998, Angulo et al. 2004). Ampicillin and tetracycline have activity against *Campylobacter*, but in general, are not recommended for the treatment of *Campylobacter* infections (Blaser 1995, Dasti et al. 2007). However, the increment of resistant strains to commonly used antimicrobials in clinical practices makes it necessary to consider alternatives therapies as well as the search of easy and reliable methods to study antimicrobial susceptibility (Luangtongkum et al. 2009). The aim of the present study was to determine the occurrence of *Campylobacter* spp. strains carrying resistance genes (tetracycline and β-lactam) and the energy-dependent multi-drug efflux pump through phenotypic and molecular analyses in poultry sources from slaughterhouses in Rio Grande do Sul state, Brazil.

MATERIALS AND METHODS

Sample collection. Since January 2012 to December 2013, a total of 141 isolates from: carcasses through slaughter line (n=115); water collected from the chiller tank (n=18); and from swabs (cloacal and boxes of transport) (n=8) were obtained from broiler slaughterhouses of Rio Grande do Sul state, Brazil.

Antimicrobial susceptibility screening. Isolation was performed in accordance with the International Standards Organization guidelines (ISO 10272-1:2006). *Campylobacter* isolates were analyzed for antimicrobial resistance using the agar disk diffusion method. The suspension was adjusted to match the 0.5 McFarland turbidity standards as recommended by the Clinical and Laboratory Standards Institute (CLSI 2010). Isolated cultures were analyzed for antimicrobial resistance using the disk diffusion assay on Mueller-Hinton agar plates (CM0337 Oxoid®, containing 5% sheep blood) incubated under microaerophilic conditions using a gas tank with a mixture (10% CO_2, 2% H_2, 5% O_2, and 85% N_2) for 48 hr at 41.5°C. Sheep blood agar plates were inoculated, and disks (Oxoid®) including tetracycline (30 µg), and ampicillin (10 µg) were added. Plates were incubated as described above. In view of lack of interpretative CLSI criteria for *Campylobacter* strains, the criteria used for the *Enterobacteriaceae* family were employed as breakpoints for *Campylobacter* resistance (CLSI 2012). *C. jejuni* ATCC 33560 strain was used as control throughout the testing period.

DNA extraction. Genomic DNA was extracted using an adapted protocol described by Borsoi et al. (2009). Stored *Campylobacter* isolates were cultured on 5% sheep blood agar plates and incubated at 41.5ºC for 48 hrs in microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2). One milliliter of bacterial culture was centrifuged at 12.000 r.p.m. for 2 min (5415C Microcentrifuge, Eppendorf, Hamburg, Germany) and the supernatant was discarded. The pellet was suspended in 800 µL of sterile distilled water and the resulting mixture was centrifuged at 12.000 r.p.m. for 2 min. The pellet was again suspended in 200 µL of sterile distilled water. The sample was placed on a thermal block (Multi-Block Heater, Baxter, USA) at 95ºC for 10 min. The mixture was centrifuged as describe above and the supernatants were transferred into fresh Eppendorf tubes to serve as a DNA template for subsequent processing.

Multiplex-PCR assay. The isolates were confirmed by Multiplex-PCR based detection of 16S rRNA, *ceu*E and *map*A genes (Denis et al. 1999).

Genotypic antimicrobial resistance. The confirmed *C. jejuni* isolates were screened for the presence of three genes: tetracycline (*tet*O), β-lactam (bla_{OXA-61}), and the energy-dependent multi-drug efflux pump (*cme*B). Primers, PCR conditions and lengths of products generated in this study are listed (Table 1). The PCR conditions were adapted (Pratt & Korolik 2005, Obeng et al. 2012). All PCR amplifications were performed in a mixture (25 µL) consisting of 5 µL of 10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 0.25 µL (5U/µL) of Taq thermostable DNA polymerase (Invitrogen®), 2 µmol 1^{-1} of $MgCl_2$ (25 mM), 2 µL dNTPs (dATP, dCTP, dGTP and dTTP, each at 2.5 mM), 2 µL extracted template DNA and 0.5 µL (10 pmole 1^{-1}) of each primer. Sterile Milli-Q water was added q.s.p 25 µL. All amplification reactions are performed in thermal cycler (Peltier Thermal Cycler Biocycler-MJ96+/MJ96G). The cycles were performed as described in Table 1. For visualization of PCR products, 10 µL aliquots were subjected to electrophoresis in a 2% agarose gel (Invitrogen®) in Tris-Acetated EDTA (TAE) buffer. DNA bands were stained with ethidium bromide for 2h at 100V, viewed under Ultraviolet (UV) transilluminator (ATTO®) and photographed (Fig. 1). The size of the PCR amplicons was compared to the 100 bp DNA ladder (Invitrogen®).

Statistical analysis. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) v18 (IBM). Discrete variables were expressed as percentages, and proportions were compared using the Chi-square test with the significance level defined at P value <0.05.

RESULTS AND DISCUSSION

All the isolates were confirmed by Multiplex-PCR based detection of 16SrRNA, *ceu*E and *map*A genes. The most ubiquitous of the thermotolerant *Campylobacter* spp. was

Table 1. List of primers and PCR conditions used in this study

Target gene	Primers	Sequence (5'→3')	PCR conditions	Product (bp)	Reference
16S rRNA	MD16S*F*[a]	ATCTAATGGCTTAACCATTAAAC	95°C/10 min, 35 cycles: 95°C/30s, , 59°C/90 s 72°C/1 min, and 72°C/10 min.	857 for *Campylobacter* genus identification 589 for *C. jejuni* species identification 462 for *C. coli* species identification	Linton et al. 1997 Denis et al. 1999 Denis et al. 1999
	MD16S*R*[b]	GGACGGTAACTAGTTTAGTATT			
*map*A	MDmapA*F*[a]	CTATTTTATTTTTGAGTGCTTGTG			
	MDmapARb	GCTTTATTTGCCATTTGTTTTATTA			
*ceu*E	col3*F*[a]	AATTGAAAATTGCTCCAACTATG			
	MDcol2*R*[b]	TGATTTTATTATTTGTAGCAGCG			
*tet*O	tetO*F*[a]	GCGTTTTGTTTATGTGCG	94°C/5 min, 30 cycles: 94°C/30 s, 54°C/30 s, 72°C/1 min, and 72°C/7 min.	559	Pratt & Korolik et al. 2005
	tetO*R*[b]	ATGGACAACCCGACAGAAG			
bla_{OXA-61}	$bla_{OXA-61}F$[a]	AGAGTATAATACAAGCG		372	Obeng et al. 2012
	$bla_{OXA-61}R$[b]	TAGTGAGTTGTCAAGCC			
*cme*B	cmeB*F*[a]	TCCTAGCAGCACAATATG		241	
	cmeB*R*[b]	AGCTTCGATAGCTGCATC			

C. jejuni. It was found in 140 of the contaminated samples (99.3%), whereas *C. coli* was identified in the remaining sample (0.7%). The PCR-amplified products of *Campylobacter* species and three resistance associated genes in agarose gel are summarized in Figure 1. The most prevalent species in this study was *C. jejuni* detected in 99.3% of positive findings, similar to what has been observed by other researchers (Garin et al. 2012, Hungaro et al. 2015).

An initial screening was performed to identify the antimicrobial susceptibility of 141 *Campylobacter* strains against ampicillin and tetracycline by using the disk diffusion test. Resistance of *Campylobacter* isolates among this method to ampicillin and tetracycline was detected in 65% and 35.5%, respectively (Fig. 2). The resistance we found to β-lactam among both our *Campylobacter* spp. isolates was at the lower range of that reported for amoxicillin *(*87.5%) in the prior Brazilian study (De Moura et al. 2013). Expression of a penicillinase-type of β-lactamase in *Campylobacter* confers resistance to amoxicilin, ampicillin and ticarcilin (Iovine 2013). This enzyme provided resistance to penicillins but not to cefotaxime and imipenem (Alfredson & Korolik 2005). The level of resistance to *Campylobacter* spp. to tetracycline by phenotypic method was similar to that reported in other studies in Brazil (Kuana et al. 2008). However, in contrast high presence of resistance (93.75%) was found by De Moura et al. (2013).

Ampicillin is not recommended for the treatment of *Campylobacter* infections. However, the increasing resistance to fluoroquinolones, tetracycline and erythromycin of *C. coli* and *C. jejuni* strains, might compromise the effectiveness of treatment (Aarestrup & Engberg 2001, Engberg et al. 2001, Gibreel & Taylor 2006, Alfredson & Korolik 2007, Silva et al. 2011). Recently, it was proposed that an oral β-lactam may provide an alternative therapy, although epidemiological studies have shown β-lactam resistance to be commonly observed in *Campylobacter* spp. (Griggs et al. 2009, Gormley et al. 2010). β-lactam antimicrobials, which inhibit bacterial cell wall biosynthesis, have been the most commercially available antimicrobials in the market because of their high specificity and potent killing effect (Zeng et al. 2014). Several major β-lactam resistance mechanisms have been characterized, including the production of β-lactamases (Lachance et al. 1991, Griggs et al. 2009). A majority of *C. jejuni* and *C. coli* isolates are able to produce

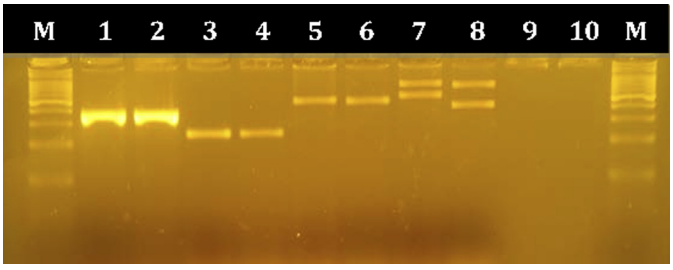

Fig.1. Agarose gel electrophoresis of PCR products from *C. jejuni*. Lanes: M, 100 bp marker; 1-2, bla_{OXA-61} (372 bp); 3-4, *cme*B (241bp); 5, *tet*O (559 bp); 7, *map*A (589 bp); 8, *ceuE* (462 bp); 9-10, Reaction control.

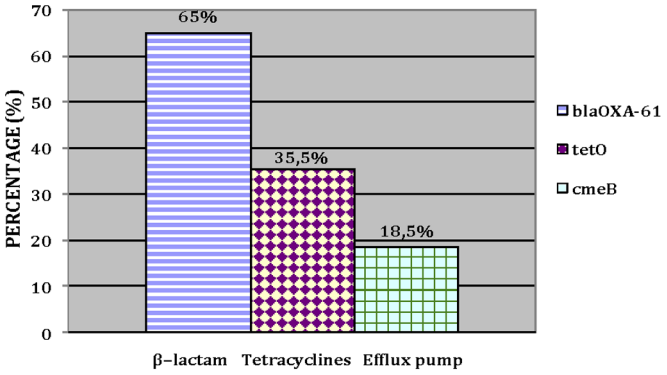

Fig.2. The occurrence of *Campylobacter* spp. strains carrying resistance genes (tetracycline and β-lactam) and the energy-dependent multi-drug efflux pump.

β-lactamases, which inactivate the β-lactam molecule by hydrolysis the structural lactam ring (Tajada et al. 1996, Wieczorek & Osek 2013). In *Campylobacter*, ampicillin resistance and the associated β-lactamase production are chromosomally encoded (Alfredson & Korolik 2005). A single nucleotide mutation (G→T transversion) upstream of bla_{OXA-61} was identified in the ampicillin-resistant derivative of *C. jejuni* NCTC 11168 (Zeng et al. 2014). The results of phenotypic and genetic analyses of antimicrobial susceptibility were fully concordant. We showed that ampicillin resistance by disk diffusion in campylobacters isolated was associated with the presence of the bla_{OXA-61} gene carried on the chromosome. Griggs et al. (2009) reported that 91% of the ampicillin-resistant isolates carried the bla_{OXA-61} gene.

This study found a high level of resistance to β -lactam gene in 65% (91/141) of isolates. Our results are similar to those described by Obeng et al. (2012), who observed resistance of 59-65.4% for *C. jejuni*. In Brazilian studies, Hungaro et al. (2015) was found 100% resistant isolates to ampicillin.

An increase in tetracycline resistance in *C. jejuni* and *C. coli* strains has been observed in recent years (Luangtongkum et al. 2008). The *tet*O gene was detected in 35.5% (50/141) of isolates. Previous reports have indicated that *tet*O is present in 40% of *C. jejuni* isolates from chicken carcasses (Hungaro et al. 2015), and of *C. jejuni* from chickens 19.2–40.7% (Obeng et al. 2012). Tetracyclines have been suggested as an alternative choice in the treatment of clinical campylobacterosis, but in practice are rarely used (Moore et al. 2006). The *tet*(O) gene in *Campylobacter* encodes the Tet(O) protein that protects the ribosome from the inhibitory effect of tetracycline (Connell et al. 2003). Its primary antimicrobial effect takes place by direct steric hindrance by binding to the A site in the 30S subunit, thus hindering the movement of transfer RNA and inhibits peptide elongation (Wieczorek & Osek 2013). Tet(O) is coded by a gene located on plasmids of different sizes and in some strains chromosomally (Lee et al. 1994, Gibreel et al. 2004, Dasti et al. 2007). However, studies provide evidence that tetracycline–resistance *Campylobacter* have been recovered from organic and other production systems in which no antimicrobial have been used, which indicates that prior or current use may not be a defining attribution to resistance (Piddock et al. 2000, Luangtongkum et al. 2008, Cox et al. 2009).

In *Campylobacter* is described a multidrug efflux system (CmeABC), belonging to the Resistance Nodulation Division (RND) family of transporters that conferring intrinsic resistance to various antimicrobials and toxic compounds (Pumbwe & Piddock 2002, Lin et al. 2002, Guo et al. 2010). This pump is widely distributed in *Campylobacter*, including *C. coli*, and is constitutively expressed (Payot et al. 2002). Lin et al. (2003) showed that this efflux pump is essential for the growth and survival of *C. jejuni* in chicken intestinal extracts, and their report indicates that, through mediating resistance to bile salts in the intestinal tract, CmeABC allows *C. jejuni* to colonize chickens successfully. It is also possible that, in some species, efflux pumps that export antimicrobial agents also export virulence determinants, such as adhesins, toxins or other proteins that are important for the colonization and infection of human and animal cells (Piddock 2006). The role of efflux pumps in biofilm formation, persistence, resistance to antimicrobials and biocides was largely hypothetical; until some recent studies have discovered that expression of efflux pumps contribute to antimicrobial resistance by biofilms (Zhang & Mah 2008, Andersen et al. 2015). The higher expression of efflux pumps offers physiological advantage in the form of expelling toxic wastes from the densely populated biofilms and in the process efflux antimicrobial compounds, which is manifested as enhanced antimicrobial resistances of biofilms (Andersen et al. 2015). In addition, adaptation to the environment, such as quorum sensing and biofilm formation can also contribute to bacterial persistence (Martins et al. 2013). In this study, twenty six isolates (18.5%) presents the gene that encode a multidrug efflux pump. The CmeABC efflux pump may also contribute to β-lactam resistance (Iovine 2013). Gibreel et al. (2007) reported that inactivation of the *cme*B gene in the resistant isolates examined led to a 16 to 128-fold decrease in tetracycline minimum inhibitory concentration (MIC), resulting in the complete restoration of tetracycline susceptibility. Studies also suggested that when both CmeABC and *tet*O are functional, the impact on tetracycline resistance is synergistic (Lin et al. 2002, Pumbwe & Piddock 2002).

The obtained data revealed that 7.1% *Campylobacter* strains possess three resistance determinant (n=10), and 43 isolates (30.5%) presents two *r*esistance-associated genes. A percentage 35.5% of isolates presents one markers (n = 50). No resistance markers were found in 38 isolates (27%). Moreover, 36 out of the 141 *Campylobacter* strains (25.6%) were found to be resistant to at least two different antimicrobial resistance groups (bla_{OXA-61} and *tet*O). The distribution of resistance markers among *Campylobacter* spp. tested according to the source of isolation are summarized in Table 2. This study has found a high genetic diversity of *Campylobacter* spp. isolated from broiler slaughterhouses. This observation suggests that such genotypes may be particularly adapted to survive cleaning and disinfection stress in poultry slaughterhouses and may contaminate the carcasses during processing (Peyrat et al. 2008). Additionally, our results showed that *Campylobacter* may easily contaminate poultry carcasses at slaughter process.

Antimicrobial resistance in both medicine and agriculture is documented by the World Health Organization (WHO), along with other various national authorities as a major emerging problem of public health importance, as well as consequences for animal treatment (Moore et al. 2006, Silva et al. 2011). Bacteria have developed multiple ways of becoming resistant to antimicrobials; in most cases bacteria are exposed to these substances, but have found a way to evade or resist the antimicrobial agent (Lin et al. 2003, Griggs et al. 2009, Wieczorek & Osek 2013). Further research in understanding the antimicrobial resistance mechanisms will facilitate the selection of antimicrobials for

Table 2. Distribution of resistance markers among *Campylobacter* spp. tested according to the source of isolation

Sources	Percentage (number) of strains		
	Found positive by PCR for:		
	bla_{OXA-61}	*tet*O	*cme*B
Cloacal swab (n=5)	60 (3/5)	60 (3/5)	40 (2/5)
Swab of broiler transportation cage (n=3)	66.6 (2/3)	0	33.3 (1/3)
Broiler carcasses through slaughter process(n=115)			
Scalding (n=4)	25 (1/4)	50 (2/4)	0
Defeathering (n=10)	80 (8/10)	30 (3/10)	40 (4/10)
Evisceration (n=5)	60 (3/5)	40 (2/5)	60 (3/5)
Spray-washing (n=7)	57.1 (4/7)	57.1 (4/7)	28.5 (2/7)
Cooling (n=89)	66.3(59/89)	30.33 (27/89)	11.2 (10/89)
Chiller tank processing water (n=18)	61.11(11/18)	50 (9/18)	22.2 (4/18)
Total	65 (91/141)	35.5 (50/141)	18.5 (26/141)

clinical treatment and the formulation of diagnostic media for various *Campylobacter* spp. To our knowledge, this is the first study to determine the frequency of the bla_{OXA-61}, *tet*O and *cme*B genes in campylobacters from poultry origin of Rio Grande do Sul state, Brazil. Our results also emphasize the need for a surveillance and monitoring system and risk analyzes for the prevalence and resistances of *Campylobacter* in poultry and other food animals.

REFERENCES

Aarestrup F.M. & Engberg J. 2001. Antimicrobial resistance of thermophilic *Campylobacter*. Vet. Res. 32:311-321.

Alfredson D.A. & Korolik V. 2007. Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli.* FEMS Microbiol. Lett. 277:123-132.

Alfredson D. & Korolik V. 2005. Isolation and expression of a novel molecular class D β-lactamase, OXA-61, from *Campylobacter jejuni*. Antimicrob. Agents Chemother. 49:2515-2518.

Andersen J.L., He G.X., Kakarla P., KC R., Kumar S., Lakra W.S., Mukherjee M.M., Ranaweera I., Shrestha U., Tran T. & Varela M.F. 2015. Multidrug efflux pumps from *Enterobacteriaceae*, *Vibrio cholerae* and *Staphylococcus aureus* bacterial food pathogens. Int. J. Environ. Res. Public Health 12:1487-1547.

Angulo F.J., Nunnery J.A. & Bair H.D. 2004. Antimicrobial resistance in zoonotic enteric pathogens. Rev. Sci. Tech. OIE 23:485-496.

Avrain L., Humbert F., L'Hospitalier R., Sanders P., Vernozy-Rozand C. & Kempf I. 2003. Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. Vet. Microbiol. 96:267-276.

Blaser M.J. 1995. *Campylobacter* and related species, p.1948-1956. In: Mandell G.L., Bennett J.E. & Dolin R. (Eds), Principles and Practice of Infectious Diseases. 4th ed. Churchill Livingstone, New York, NY.

Borsoi A., Santin E., Santos L.R., Salle C.T.P., Moraes H.L.S. & Nascimento V.P. 2009. Inoculation of newly hatched broiler chicks with two Brazilian isolates of *Salmonella* Heidelberg strains with different virulence gene profiles, antimicrobial resistance, and pulsed field gel electrophoresis patterns to intestinal changes evaluation. Poult. Sci. 88:750-758.

CDC 2013. Incidence and trends of infection with pathogens transmitted commonly through food. Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996-2012. Morbidity and Mortality Weekly Report 19:283-287.

CLSI 2010. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria: approved guideline. 2nd ed. Clinical and Laboratory Standards Institute (M45eA2), Wayne, PA.

CLSI 2012. Performance Standards for Antimicrobial Susceptibility Testing. 22nd Informational Supplement. Clinical and Laboratory Standards Institute (M100eS22), Wayne, PA.

Conlan A.J., Coward C., Grant A.J., Maskell D.J. & Gog J.R. 2007. *Campylobacter jejuni* colonization and transmission in broiler chickens: a modelling perspective. J. R. Soc. Interface 4:819-829.

Connell S., Tracz D., Neirhaus K.H. & Taylor D.E. 2003. Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrob. Agents Chemother. 47:3675-3681.

Cox N.A., Richardson L.J., Buhr R.J. & Fedorka-Cray P.J. 2009. *Campylobacter* species occurrence within internal organs and tissues of commercial caged Leghorn laying hens. Poultry Sci. 88:2449-2456.

Dasti J.I., Gross U., Pohl S., Lugert R., Weig M. & Schmidt-Ott R. 2007. Role of the plasmid-encoded *tet*(O) gene in tetracycline-resistant clinical isolates of *Campylobacter jejuni* and *Campylobacter coli.* J. Med. Microbiol. 56:833-837.

De Moura H.M., Silva P.R., da Silva P.H.C., Souza N.R., Racanicci A.M.C. & Santana A.P. 2013. Antimicrobial resistance of *Campylobacter jejuni* isolated from chicken carcasses in the Federal District, Brazil. J. Food Prot. 76:691-693.

Denis M., Soumet C., Rivoal K., Ermel G., Blivet D., Salvat G. & Colin P. 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett. Appl. Microbiol. 29:406-410.

Ellström P., Hansson I., Söderström C., Olsson Engvall E. & Rautelin H. 2014. A prospective follow-up study on transmission of *Campylobacter* from poultry to abattoir workers. Foodborne Pathog. Dis. 11:684-688.

Engberg J., Aarestrup F.M., Taylor D.E., Gerner-Smidt P. & Nachamkin I. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: Resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. 7:24-34.

EFSA 2011. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J. 9:2090-2468.

Garin B., Gouali M., Wouafo M., Perchec A.-M., Thu P.M., Ravaonindrina N., Urbès F., Gay M., Diawara A., Leclercq A., Rocourt J. & Pouillot R. 2012. Prevalence, quantification and antimicrobial resistance of *Campylobacter* spp. on chicken neck-skins at points of slaughter in 5 major cities located on 4 continents. Int. J. Food Microbiol. 157:102-107.

Gibreel A. & Taylor D.E. 2006. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. 58:243-255.

Gibreel A., Tracz D.M., Nonaka L., Ngo T.M., Connell S.R. & Taylor D.E. 2004. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to *tet*(O)-mediated tetracycline resistance. Antimicrob. Agents Chemother. 48:3442-3450.

Gibreel A., Wetsch N.M. & Taylor D.E. 2007. Contribution of the CmeABC efflux pump to macrolide and tetracycline resistance in *Campylobacter jejuni*. Antimicrob. Agents Chemother. 51:3212-3216.

Godschalk P.C., Heikema A.P., Gilbert M., Komagamine T., Ang C.W., Glerum J., Brochu D., Li J., Yuki N., Jacobs B.C., van Belkum A. & Endtz H.P. 2004. The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barré syndrome. J. Clin. Invest. 114:1659-1665.

Gormley F.J., Strachan N.J., Reay K., MacKenzie F.M., Ogden I.D., Dallas J.F. & Forbes K.J. 2010. Antimicrobial resistance profiles of *Campylobacter* from humans, retail chicken meat, and cattle feces. Foodborne Pathog. Dis. 7:1129-1131.

Griggs D.J., Peake L., Johnson M.M., Ghori S., Mott A. & Piddock L.J. 2009. β-Lactamase-mediated β-Lactam resistance in *Campylobacter* species: Prevalence of Cj0299 (bla_{OXA-61}) and evidence for a novel β-Lactamase in *C. jejuni*. Antimicrob. Agents Chemother. 53:3357-3364.

Guo B., Lin J., Reynolds D.L. & Zhang Q. 2010. Contribution of the multidrug efflux transporter CmeABC to antibiotic resistance in different *Campylobacter* species. Foodborne Path. Dis. 7:77-83.

Hardy C.G., Lackey L.G., Cannon J., Price L.B. & Silbergeld E.K. 2011. Prevalence of potentially neuropathic *Campylobacter jejuni* strains on commercial broiler chicken products. Int. J. Food Microbiol. 145:395-399.

Hungaro H.M., Mendonça R.C.S., Rosa V.O., Badaró A.C.L., Moreira M.A.S. & Chaves J.B.P. 2015. Low contamination of *Campylobacter* spp. on chicken carcasses in Minas Gerais state, Brazil: Molecular characterization and antimicrobial resistance. Food Control 51:15-22.

Iovine N.M. 2013. Resistance mechanisms in *Campylobacter jejuni.* Virulence 4:230-240.

ISO 2006. International Standard Organization 10272-1:2006 describes a horizontal method for the detection of *Campylobacter* spp.

Janssen R., Krogfelt K.A., Cawthraw S.A., van Pelt W., Wagenaar J.A. & Owen R.J. 2008. Host-pathogen interactions in *Campylobacter* infections: the host perspective. Clin. Microbiol. Rev. 21:505-518.

Khachatourians G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can. Med. Assoc. J. 159:1129-1136.

Kuana S.L., Santos L.R., Rodrigues L.B., Borsoi A., Moraes H.L.S., Salle C.T.P. & Nascimento V.P. 2008. Ocorrência de *Campylobacter* em lotes de frangos de corte e nas carcaças correspondentes. Ciênc. Anim. Bras. 9:480-486.

Lachance N., Gaudreau C., Lamothe F. & Lariviere L.A. 1991. Role of the β-lactamase of *Campylobacter jejuni* in resistance to β-lactam agents. Antimicrob. Agents Chemother. 35:813-818.

Lee C., Tai C., Lin S. & Chen Y. 1994. Occurrence of plasmids and tetracycline resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from whole market chickens and clinical samples. Int. J. Food Microbiol. 24:161-170.

Lin J., Michel L.O. & Zhang Q. 2002. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob. Agents Chemother. 46:2124-2131.

Lin J., Sahin O., Michel L.O. & Zhang Q. 2003. Critical role of multi-drug efflux pump CmeABC in bile resistance and *in vivo* colonisation of *Campylobacter jejuni*. Infect. Immun. 71:4250-4259.

Luangtongkum T., Jeon B., Han J., Plummer P., Logue C.M. & Zhang Q. 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol. 4:189-200.

Luangtongkum T., Morishita Y., Martin L., Choi I., Sahin O. & Zhang Q. 2008. Prevalence of tetracycline-resistant *Campylobacter* in organic broilers during a production cycle. Avian Dis. 52:487-490.

Martins M., McCusker M.P., Viveiros M., Couto I., Fanning S., Pagès J.M. & Amaral L. 2013. A simple method for assessment of MDR bacteria for over-expressed efflux pumps. Open Microbiol. J. 7:72-82.

Moore J.E., Barton M.D., Blair I.S., Corcoran D., Dooley J.S., Fanning S., Kempt I., Lastovica A.J., Lowery C.J., Matsuda M., McDowell D.A., McMahon A., Millar B.C., Rao R.J., Rooney P.J., Seal B.S., Snelling W.J. & Tolba O. 2006. The epidemiology of antibiotic resistance in *Campylobacter*. Microbes Infect. 8:1955-1966.

Moore J.E., Corcoran D., Dooley J.S., Fanning S., Lucey B., Matsuda M., McDowell D.A., Mégraud F., Millar B.C., O'Mahony R., O'Riordan L., O'Rourke M., Rao J.R., Rooney P.J., Sails A. & Whyte P. 2005. *Campylobacter*. Vet. Res. 36:351-382.

Obeng A.S., Rickard H., Sexton M., Pang Y., Peng H. & Barton M. 2012. Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. J. Appl. Microbiol. 113:294-307.

Park S.F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int. J. Food Microbiol. 74:177-188.

Payot S., Cloeckaert A. & Chaslus-Dancla E. 2002. Selection and characterization of fluoroquinolone-resistant mutants of *Campylobacter jejuni* using enrofloxacin. Microb. Drug Resist. 8:335-343.

Peyrat M.B., Soumet C., Maris P., Sanders P. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. Int. J. Food Microbiol. 124:188-194.

Piddock L.J. 2006. Multidrug-resistance efflux pumps? not just for resistance. Nat. Rev. Microbiol. 4:629-636.

Piddock L.J.V., Ricci V., Stanley K. & Jones K. 2000. Activity of antibiotics used in human medicine for *Campylobacter jejuni* isolated from farm animals and their environment in Lancashire, UK. J. Antimicrob. Chemother. 46:303-306.

Pratt A. & Korolik V. 2005. Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates. J. Antimicrob. Chemother. 55:452-460.

Pumbwe L. & Piddock L.J. 2002. Identification and molecular characterization of CmeB, a *Campylobacter jejuni* multidrug efflux pump. FEMS Microbiol. Lett. 206:185-189.

Rowe-Magnus D.A., Guerout A.M. & Mazel D. 2002. Bacterial resistance evolution by recruitment of super-integron gene cassettes. Mol. Microbiol. 43:1657-1669.

Silva J., Leite D., Fernandes M., Mena C., Gibbs P.A. & Teixeira P. 2011. *Campylobacter* spp. as a foodborne pathogen: a review. Frontiers Microbiol. 2:200.

Tajada P., Gomez-Garces J.-L., Alós J.-I., Balas D. & Cogollos R. 1996. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* to 12 β-lactam agents and combinations with β-lactamase inhibitors. Antimicrob. Agents Chemother. 40:1924-1925.

Van den Berg B., Walgaard C., Drenthen J., Fokke C., Jacobs B.C. & van Doorn P.A. 2014. Guillain-Barré syndrome: pathogenesis, diagnosis, treatment and prognosis. Nat. Rev. Neurol. 10:469-482.

Wieczorek K. & Osek J. 2013. Antimicrobial resistance mechanisms among *Campylobacter*. BioMed Res. 2013:1-12.

Zeng X., Brown S., Gillespie B. & Lin J. 2014. A single nucleotide in the promoter region modulates the expression of the β-lactamase OXA-61 in *Campylobacter jejuni*. J. Antimicrob. Chemother. 69:1215-1223.

Zhang L. & Mah T.F. 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J. Bacteriol. 190:4447-4452.