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# ANTIMICROBIAL RESISTANCE IN *CAMPYLOBACTER* SPP ISOLATED FROM BROILER FLOCKS

**Suzete Lora Kuana[1]; Luciana Ruschel dos Santos[2]; Laura Beatriz Rodrigues[2]; Anderlise Borsoi[1]; Hamilton Luis do Souza Moraes[1]; Carlos Tadeu Pippi Salle[1]; Vladimir Pinheiro do Nascimento[1]***

[1]Programa de Pós Graduação em Ciências Veterinárias, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil; [2]Curso de Medicina Veterinária, Universidade de Passo Fundo, Passo Fundo, RS, Brasil

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## ABSTRACT

The aim of this study was to assess the antimicrobial susceptibility of 62 *Campylobacter* spp. strains obtained from broiler flocks using the agar diffusion method. The *Campylobacter* spp strains were isolated from 22 flocks aged between 3 and 5 weeks of life, isolated from cloacae swabs, stools and cecal droppings in the farm and from the carcass rinsing in the slaughterhouse. Campylobacter spp strains were tested on Mueller-Hilton (MH) agar (27 samples) and MH plus TTC agar (35 samples). The antimicrobial susceptibility test revealed a 62.5% resistance to at least one drug, especially to enrofloxacin (71%), neomycin (50%), lincomycin (50%), tetracycline (43%), penicillin (42%), ceftiofur (33%) amoxicillin (27%), spiramycin (20%), ampicillin (18%) and norfloxacin (14%), whereas a lower percentage of strains was resistant to erythromycin (10%) and doxycycline (10%). All strains were sensitive to gentamicin and lincomycin-spectinomycin and 80% of them to colistin. These results indicate that it is necessary to reduce the use of antimicrobials in veterinary and human medicine.

**Key words:** *Campylobacter* spp, antimicrobial resistance, broiler flocks

## INTRODUCTION

Thermophilic bacteria of the genus *Campylobacter* spp. are foodborne enteric pathogens, and *Campylobacter jejuni* is the most commonly reported cause of gastroenteritis in humans. The association between *Campylobacter* in poultry and human enteritis is due to the persistence of this agent in the rearing environment of broilers, which asymptomatically colonizes their intestine and eventually contaminates the carcasses (6). The development of antibiotic resistance in different countries, mainly among zoonotic microorganisms and the impact of foodborne diseases on consumers may be devastating to the food industry (11). The increase in the antimicrobial resistance of *Campylobacter* may lead to treatment failure in severely affected humans (12). After detecting resistance to fluoroquinolone, Unicomb *et al.* (12) recommended that this active ingredient should not be used in animals, once resistant *Campylobacter* may be transmitted to humans by contaminated foods. An increase in the number of ciprofloxacin-resistant *Campylobacter* strains isolated from pigs, associated with the veterinary use of enrofloxacin, was also reported (1). The strains were isolated from rectal swabs of pigs, from different regions, collected in the slaughterhouse. All ciprofloxacin-resistant strains were also resistant to erythromycin. A similar resistance to erythromycin (63%) was found in *C. jejuni* and *C. coli*, suggesting cross-resistance due to the use of tylosin, a drug that also belongs to the macrolide group of antibiotics. In the same study, a high resistance to gentamicin (50%) was found.

Nachamkin, Ung and Li (7) submitted *Campylobacter* strains to E test, and found an MIC equal to or greater than 32 µc/mL which indicates high resistance to fluoroquinolone, and pointed to the fact that this antimicrobial is often used to treat travelers'

*Corresponding Author. Mailing address: Universidade Federal do Rio Grande do Sul, UFRGS. Faculdade de Veterinária, Programa de Pós Graduação em Ciências Veterinárias (PPGCV-UFRGS). Av. Bento Gonçalves, 9090. Porto Alegre, RS, Brasil. CEP 91540000. Telefone: (51) 3316-6939; Fax: (51) 3316-7305. E-mail: vladimir@orion.ufrgs.br

diarrhea. Nonetheless, erythromycin resistance was low (<5%). Pedersen and Wederkopp (9) analyzed 10 quinolone resistant *Campylobacter* strains and obtained six different pulse-field gel electrophoresis (PFGE) patterns, whereas only four different patterns were observed in 12 quinolone susceptible strains. These authors considered the possible existence of new clones or evolution of these variants on the farm, which persisted through several flocks, even in the absence of selection pressure.

Therefore, the aim of this study was to assess the *in vitro* antimicrobial resistance of *Campylobacter* spp. strains isolated from broilers flocks, focusing on enrofloxacin, a fluoroquinolone approved for veterinary use in Brazil an erythromycin, is used in human clinical medicine, and on 14 other antimicrobials.

## MATERIALS AND METHODS

A total of 62 *Campylobacter* spp strains isolated from 22 flocks aged between 3 and 5 weeks of life were studied, among which 41 were collected on the farm (cloacae swabs, stools and cecal droppings) and 21 in the slaughterhouse (carcasses). *Campylobacter* spp strains were grown on Mueller-Hilton (MH) agar (27 strains) and MH plus TTC agar (35 strains). The *C. jejuni* ATCC 33291 reference strain and a *C. jejuni* IAL2247 field strain were grown on MH plus TTC agar. A suspension of the was cultures was prepared in saline solution (NaCl 0.85%), and turbidity was visually adjusted to the 0.5 McFarland standard ($1.5 \times 10^8$ cfu/mL). The absorbance of suspension was determined using a spectrophotometer at 630 nm in a flat-bottom microplate. The suspensions were surface plated, within 30 minutes after preparation, and the following antimicrobial disks were applied to the surface of the plates: amoxicillin (10 µg), ampicillin (10 µg), ceftiofur (30 µg), colistin (10 µg), doxycycline (10 µg), enrofloxacin (5 µg), spiramycin (100 ìg), streptomycin (10 µg), erythromycin (15 µg), gentamicin (10 µg), lincomycin-spectinomycin (9/100 µg), lincomycin (2 µg), norfloxacin (10 µg), neomycin (30 µg), penicillin (10 IU) and tetracycline (30 µg). The plates were incubated at 37ºC for 48 hours under microaerophilia (5% $O_2$, 10% $CO_2$, 85% $N_2$), when the inhibition zones around the disks were measured.

## RESULTS AND DISCUSSION

The results of antimicrobial resistance of *Campylobacter* spp. strain isolated from broiler flocks are shown in Table 1. There are no parameters for the susceptibility profile of Campylobacter spp. (2,5), thus the interpretation of results an inhibition zone diameters followed the National Committee for Clinical Laboratory recommendations (8) for microorganisms requiring 5% $CO_2$ for growth. The following bacteria were considered: *Enterococcus* for penicillin, amoxicillin and ampicillin, *Streptococcus* for tetracyclines and erythromycin, and *Enterobacteriacea*e for enrofloxacin. For gentamicin, streptomycin, neomycin and norfloxacin, the interpretations were based on inhibition zone diameter tables for aerobic microorganisms provided by disc suppliers for antibiogram analysis in veterinary and human medicine. The activity of the other antimicrobials (colistin, spiramycin, ceftiofur, lincomycin-spectinomycin, lincomycin) was determined following the recommendation of suppliers for aerobic agents, due to the lack of specific parameters for poultry isolates.

**Table 1.** Antimicrobial resistance of *Campylobacter* spp. strains isolated from broiler flocks

| Antimicrobial drug | Number of strains | Diameter (mm) | S% | I% | R% |
|---|---|---|---|---|---|
| Amoxicillin | 15 | 0-32 | 73 | 0 | 27 |
| Ampicillin | 12 | 14-44 | 82 | 0 | 18 |
| Ceftiofur | 9 | 0-24 | 34 | 33 | 33 |
| Colistin | 20 | 9-32 | 80 | 20 | 0 |
| Doxycycline | 10 | 12-30 | 90 | 0 | 10 |
| Enrofloxacin | 17 | 0-30 | 17 | 12 | 71 |
| Erythromycin | 10 | 11-30 | 90 | 0 | 10 |
| Spiramycin | 10 | 13-30 | 60 | 20 | 20 |
| Estreptomicina | 14 | 10-30 | 86 | 0 | 4 |
| Gentamicin | 11 | 23-30 | 100 | 0 | 0 |
| Lincomycin-Spectinomycin | 5 | 31 | 100 | 0 | 0 |
| Lincomycin | 12 | 0-30 | 17 | 33 | 50 |
| Neomycin | 6 | 0-28 | 50 | 0 | 50 |
| Norfloxacin | 14 | 0-38 | 72 | 14 | 14 |
| Penicilin | 12 | 0-26 | 58 | 0 | 42 |
| Tetracycline | 14 | 0-40 | 57 | 0 | 43 |

S= sensitive I= intermediate R= resistant.

The *C. jejuni* ATCC 33291 reference strain was resistant to ampicillin and ceftiofur while *C. jejuni* IAL 2247 strain was susceptible to colistin and lincomycin. It was not possible to measure the activity of the other antimicrobials and the TTC control disc, since there was no growth around the discs. The use of MH agar supplemented with 5% sheep blood (3,5) has been recommended in the literature. MH agar diffusion test compared to agar dilution test was considered to be reliable, easy-to-perform and inexpensive in monitoring the antimicrobial resistance of *C. jejuni* strains (2).

*Campylobacter* spp. strains evaluated in this study were resistant to at least one antimicrobial (62.5%). The resistance to enrofloxacin (71%), neomycin (50%), tetracycline (43%), lincomycin (50%), penicillin (42%), ceftiofur (33%) amoxicillin (27%), spiramycin (20%), ampicillin (18%) and norfloxacin (14%) was considered high, whereas the resistance to erythromycin and to doxycycline was only 10%. The strains were sensitive to gentamicin (100%), lincomycin-spectinomycin (100%) and

colistin (80%). There was high resistance to enrofloxacin and, despite the paucity of previous data on fluoroquinolone susceptibility, this result indicated the persistence of its effects in the environment (9), since enrofloxacin was not used on the poultry farm during this study.

In this study, only a small number of strains were resistant to erythromycin, which has been the antimicrobial of choice in Brazil for many cases that require treatment in human medicine (4,5,10). Spiramycin resistance was twice as high. Considerable resistance, to neomycin was detected, possibly due to its sporadic use in the prophylactic treatment of enteroparasitosis in chicken. The unexpected resistance to some unused antimicrobials, such as tetracycline and lincomycin, needs to be further investigated. With regard to penicillin, the beta-lactamase produced by *Campylobacter* may have contributed towards resistance (2).

## RESUMO

### Resistência antimicrobiana em *Campylobacter* spp isoladas de frangos de corte

O objetivo deste estudo foi verificar a susceptibilidade antimicrobiana de 62 amostras de *Campylobacter* spp. em amostras isoladas de 22 lotes de frango de corte, pelo método de difusão em Agar. As amostras de *Campylobacter* spp foram isoladas de frangos com idade entre 3 e 5 semanas, isoladas a partir de swabs cloacais, fezes e descarga cecal obtidos nas granjas e de rinsagem de carcaças no abatedouro. Das 62 amostras avaliadas, 27 foram testadas em ágar MH e 35 no ágar MH com TTC. O perfil de susceptibilidade antimicrobiana apresentou 62,5% de resistência para, no mínimo, uma droga, sobretudo para enrofloxacina (71%), neomicina (50%), lincomicina (50%), tetraciclina (43%), penicilina (42%), ceftiofur (33%) amoxicilina (27%), espiramicina (20%), ampicilina (18%) e norfloxacina (14%), enquanto uma percentagem menor foi observada frente eritromicina (10%) e doxiciclina (10%). Todas as amostras foram sensíveis a gentamicina e linco-espectinomicina e 80% à colistina. Pelo exposto, faz-se necessária a redução do uso dos antimicrobianos na medicina veterinária e em humanos.

**Palavras chave:** *Campylobacter* spp, resistência antimicrobiana, frangos de corte

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