

ANTIMICROBIAL SUSCEPTIBILITY OF 51 *CAMPYLOBACTER* STRAINS ISOLATED FROM DIARRHEIC AND DIARRHEA-FREE DOGS*

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ABSTRACT

The minimum inhibitory concentrations (MICs) were determined by agar dilution for six antimicrobial agents (cephalexin, erythromycin, gentamicin, enrofloxacin, chloramphenicol, and oxytetracycline) using 51 *Campylobacter* strains (29 *Campylobacter jejuni* subsp. *jejuni*, 14 *Campylobacter jejuni* subsp. *doylei*, 5 *Campylobacter upsaliensis*, 2 *Campylobacter coli*, and 1 *Campylobacter lari*) isolated from 29 diarrheic and 22 diarrhea-free dogs. Percentages corresponding to 50% and 90% strain inhibition (MIC 50% and MIC 90%) were determined. The results show that erythromycin, enrofloxacin, and gentamicin can be recommended for the treatment of campylobacteriosis in dogs; this opens the possibility of their therapeutic use in human medicine.

KEY WORDS: *Campylobacter*, dogs, minimum inhibitory concentration, antimicrobials, public health.

RESUMO

SUSCEPTIBILIDADE ANTIMICROBIANA DE 51 AMOSTRAS DE *CAMPYLOBACTER* ISOLADAS DE CÃES COM E SEM DIARRÉIA. As concentrações inibitórias mínimas de seis antimicrobianos (cefalexina, eritromicina, gentamicina, enrofloxacina, cloranfenicol e oxitetraciclina) para 51 estirpes de *Campylobacter* (29 de *Campylobacter jejuni* subsp. *jejuni*, 14 de *Campylobacter jejuni* subsp. *doylei*, 5 de *Campylobacter upsaliensis*, 2 de *Campylobacter coli* e 1 de *Campylobacter lari*), isoladas de 29 cães com diarreia e 22 cães sem diarreia foram determinadas pelo método de diluição em ágar. Foram determinadas as percentagens correspondentes à inibição de 50% e 90% das estirpes isoladas (CIM 50% e CIM 90%). De acordo com os resultados, a eritromicina, a enrofloxacina e a gentamicina podem ser recomendadas como drogas de eleição para o tratamento da campilobacteriose em cães, ampliando também as possibilidades terapêuticas para o uso em medicina humana.

PALAVRAS-CHAVE: *Campylobacter*, cães, concentração inibitória mínima, antimicrobianos, saúde pública.

INTRODUCTION

Over the past few decades, *Campylobacter* species have emerged as the common cause of enteritis in humans and animals. Enteric campylobacteriosis has been considered as a highly prevalent worldwide zoonosis in developed countries (NACHAMKIN, 1997)

Dogs are contaminated by oral-fecal contact, and by manifesting gastroenteritis or acting as healthy carriers, represent a potential infection source to humans (BLASER et al., 1978; BRUCE & FLEMING, 1983; GOOSSENS et al., 1991). Antimicrobial therapy for dogs

with clinical campylobacteriosis reduces the duration and severity of the disease, decreases microorganism shedding time, minimizing the risk of human exposure to the bacteria (BOOSINGER & DILLON, 1992).

Antimicrobial resistance studies of *Campylobacter* strains isolated from dogs are rare. Most studies deal with strains isolated from humans and animals used for human consumption.

Erythromycin and chloramphenicol, the most common antibiotics used to treat dogs have sometimes failed to eliminate the organism from the stool (MONFORT et al., 1990).

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The aim of this study was to evaluate minimum inhibition concentrations (MICs) of six antimicrobial agents (cephalexin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, and oxytetracycline) on *Campylobacter* strains isolated from both diarrheic and diarrhea-free dogs.

MATERIALS AND METHODS

Fifty-one *Campylobacter* strains were isolated from dogs: 29 *Campylobacter jejuni* subsp. *jejuni*, 14 *Campylobacter jejuni* subsp. *doylei*, 5 *Campylobacter upsaliensis*, 2 *Campylobacter coli*, and 1 *Campylobacter lari*.

Serial dilutions in solid medium, according to the National Committee for Clinical Laboratory Standards recommendations (NCCLS, 1998), were used to study the susceptibility of these strains to antimicrobial agents and to determine each agent's MIC. The antibiotics cephalexin, erythromycin, gentamicin, enrofloxacin, chloramphenicol, and oxytetracycline were submitted to the following procedures: each one was first diluted in 1,000 µg/mL saline, then new dilutions were made on Petri dishes with thioglycolate agar supplemented with 20% bovine blood, corresponding to the following concentrations per plate: 0.06 µg/mL, 0.125 µg/mL, 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, and 128 µg/mL. The final volume of each plate was 20 mL. Bacterial colonies were suspended in saline to 0.5 MacFarland standard turbidity (1.5×10^8 CFU/µL) and inoculated in culture medium using a 3-mm replicator. *Escherichia coli*, ATCC 25922, inoculated in one of the plates was used as a control. The plates were kept at 35° C under microaerophilic conditions; readings were made 48 hours later.

From these results, MICs corresponding to 50% and 90% inhibition of *Campylobacter* (MIC₅₀ and MIC₉₀) samples were determined.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results of the different antimicrobial concentrations.

Different results were observed for each drug, which were also considered in other investigations. The variability can be used as an important marker in epidemiological research (ABRAHANS et al., 1990)

The strains isolated from both diarrheic and diarrhea-free dogs were highly resistant to cephalexin (MIC₅₀ > 128 µg/mL for both). This is due to the high resistance of these bacteria to cephalosporins and β-lactamic in general (ABRAHANS et al., 1990; TAJADA et al.,

1996). Cephalosporins are useful in selective media for primary bacterial isolation; however, studies report 2-25% susceptibility of these bacteria to cephalexin (ABRAHANS et al., 1990; MODOLO et al., 1991). It is therefore important that cephalosporin selective media are not exclusively used for the primary isolation of this bacterium; the concomitant use of filtration techniques is recommended (MODOLO, 2000). Chloramphenicol showed higher MIC values in diarrheic dogs (2-32 µg/mL) than diarrhea-free (2-8 µg/mL). Considering the chloramphenicol cut-off point of 8 µg/mL for *Campylobacter*, it is significant that 27.4% of the diarrheic strains were susceptible to the antibiotic. In contrast, all diarrhea-free strains were susceptible to the drug. There is no solid explanation for this discrepancy, since feces were not collected from diarrheic dogs after receiving medication. Variations in sensitivity patterns to chloramphenicol occur in the *Campylobacter* genus (ALEKSANDROVA et al., 1990). However, many studies have reported high sensitivity to the drug when considering strains isolated from humans (SHANKER & SORRELL, 1983) and dogs (FOX et al., 1984; MODOLO et al., 1991).

Chloramphenicol was effective in the treatment of dogs with campylobacteriosis, but failed in the elimination of chronic fecal excretion (DAVIES et al., 1984; MONFORT et al., 1990). In contrast, due to good liposolubility that can enhance body tissue penetration, it can be recommended for treating extra-intestinal infections in dogs, as long as the animals show no *Campylobacter* fecal excretion.

Enrofloxacin showed very similar MIC variations in diarrheic (1-4 µg/mL) and diarrhea-free dogs (1-8 µg/mL). It was active against isolated *Campylobacter* strains demonstrated by the low concentrations, at which 90% of strains were susceptible (2-4 µg/mL, diarrheic dogs and 4-8 µg/mL, diarrhea-free dogs). *Campylobacter* is frequently sensitive to quinolones; however, an increased resistance to these drugs is seen, probably due to genetic mutations interfering with bacterial DNA girase (GREIG et al., 2003). Selective pressure caused by the indiscriminate use of these drugs in aviculture is a contributory factor. Considering the existence of cross resistance between quinolones, the occurrence of nalidixic acid resistant *Campylobacter jejuni* may interfere with tests for this bacterium classification.

Erythromycin was the most effective against *Campylobacter*, with total strain sensitivity from both groups at very low concentrations (lower or equal to 0.06 µg/mL). This is corroborated by several authors (FOX et al., 1984; REINA et al., 1984; SKIRROW, 1994; GANDREAU & GILBERT, 1998). Erythromycin is effective in treating dogs, but was not able to eliminate the passive-carrier status of some treated dogs (MONFORT et al., 1990; BOOSINGER & DILLON, 1992; BURNENS et al., 1992).

Table 1 - Antimicrobial drug concentration variations and minimum inhibitory concentrations (MIC) capable of inhibiting 50% and 90% of the 29 thermophilic *Campylobacter* strains isolated from diarrheic dogs.

Drug	Concentration Variation	MIC 50%	MIC 90%
Cephalexin	2->128	4-8	>128
Chloramphenicol	2-32	8-16	16-32
Enrofloxacin	1-4	1	2-4
Erythromycin	0.06-0.125	0.05-0.125	< 0.06
Gentamicin	0.5-2	0.06-0.125	1-2
Oxytetracycline	16-64	16-32	32-64

Table 2 - Antimicrobial drug concentration variations and minimum inhibitory concentrations (MIC) capable of inhibiting 50% and 90% of the 29 thermophilic *Campylobacter* strains isolated from diarrhea-free dogs.

Drug	Concentration Variation	MIC 50%	MIC 90%
Cephalexin	2->128	>128	>128
Chloramphenicol	2-8	4-8	4-8
Enrofloxacin	1-8	0.5-1	4-8
Erythromycin	<0.06	-	-
Gentamicin	0.5-4	2	2-4
Oxytetracycline	16-64	16-32	32-64

Gentamicin showed similar MIC variations for both groups; all strains were sensitive (MIC \leq 4 $\mu\text{g}/\text{mL}$). *Campylobacter* is frequently sensitive to gentamicin (FOX et al., 1984; YOSHIMURA et al., 1984; MODOLO et al., 1991; LI et al., 1998). This was the second most effective antibiotic against *Campylobacter*. However, it should only be used in the absence of severe dehydration or renal failure, as it is nephrotoxic (GREENE & WATSON, 1998).

Oxytetracycline showed the same MIC variation for both groups (16-64 $\mu\text{g}/\text{mL}$). This shows that these bacteria are usually resistant to tetracycline and its derivatives. Sensitivity levels from 78 to 500 $\mu\text{g}/\text{mL}$ have already been reported (MOORE et al., 1996). Resistance to this drug by strains from dogs and humans may range from 15 to 55.7% (MODOLO et al., 1991; GANDREAU & GILBERT, 1998).

The great variability in this antibiotic's efficacy is probably due to its worldwide use in cattle, both at therapeutic or low doses; this would increase selective pressure on bacteria. Clinical assays, however, have shown the therapeutic efficacy of tetracycline in treating dogs with campylobacteriosis and a decrease in re-excretion rate (ABRAHANS et al., 1990; BURNENS et al., 1992).

In view of the heterogeneity in the results reported in the literature in comparison to data in this study, we recommend that *Campylobacter* antimicrobial susceptibility tests be performed for therapeutic purposes. Our results indicate erythromycin, enrofloxacin, and gentamicin as drugs suitable for the treatment of canine campylobacteriosis. This also opens up therapeutic possibilities for these drugs in human medicine.

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