ANTIMICROBIAL SUSCEPTIBILITY OF CAMPYLOBACTER sp STRAINS ISOLATED FROM CALVES WITH AND WITHOUT DIARRHEA IN MINAS GERAIS STATE, BRAZIL

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ABSTRACT

The antimicrobial susceptibility of 25 Campylobacter sp strains isolated from calves with and without diarrhea - 7 C. coli, 16 C. fetus and 2 C. jejuni – was studied by the disk diffusion method. Eleven antimicrobial agents were tested: amikacin, ampicillin, kanamycin, chloramphenicol, erythromycin, gentamicin, neomycin, nitrofurantoin, penicillin G, tetracycline and sulfamethoxazole-trimethoprim. All Campylobacter sp strains were susceptible to amikacin, ampicillin, chloramphenicol, erythromycin, gentamicin, neomycin and nitrofurantoin. Three strains were moderately susceptible to kanamycin (2 C. coli and 1 C. fetus). All the strains were resistant to penicillin G. Two C. fetus strains were moderately susceptible to sulfamethoxazole-trimethoprim and 1 C. coli, 9 C. fetus and 2 C. jejuni strains were resistant. Two C. fetus strains were moderately susceptible to tetracycline and 3 C. coli, 2 C. fetus and 1 C. jejuni strains were resistant. Eleven strains showed multidrug resistance (2 C. coli, 8 C. fetus and 1 C. jejuni). There was no correlation between resistance of Campylobacter sp strains to antimicrobials and the occurrence of diarrhea in calves. The frequency of resistance and, most importantly, multidrug resistance found among Campylobacter sp strains isolated from calves in Minas Gerais, Brazil, were high and the patterns of resistance observed are related to the antimicrobials agents most largely used in cattle in Brazil.

Key words: Campylobacter sp, calf, diarrhea, antimicrobial agents, susceptibility

INTRODUCTION

Campylobacter jejuni and C. coli are some of the most frequent infectious agents of gastroenteritis in the world (8). In domestic animals they cause diarrhea, but they are also frequently isolated from asymptomatic animals (22,25). C. jejuni and C. coli are agents of great importance in patients with travelers’ diarrhea and infected with human immunodeficiency virus (HIV) and who, therefore, need antimicrobial therapy (8). Erythromycin, fluoroquinolones and tetracycline are the drugs of choice for treating Campylobacter sp diarrhea (31). C. fetus is divided in two subspecies: C. fetus subsp. venerealis, which colonize the bovine reproductive tract and causes abortion and subfertility in cattle, and C. fetus subsp. fetus, whose habitat is the bovine gastrointestinal tract and causes diarrhea (3,18) and sporadically abortion in cattle and sheep (33). In humans, C. fetus infections usually cause septicemia and extra-intestinal infections that require parenteral therapy (33). In these cases, gentamicin associated with ampicillin is the treatment of choice (13).

Domestic animals play an important role in the epidemiology of human intestinal infection caused by Campylobacter sp. Domestic and wild birds, pigs, cattle, dogs and cats may be considered important reservoirs of Campylobacter sp (8), constituting possible sources of infection for humans. Diarrhea outbreaks in humans caused by C. jejuni and C. coli are

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frequently associated with contaminated water and ingestion of unpasteurized milk. The consumption of raw chicken, pig and beef meat is also associated with sporadic cases of human diarrhea (8).

Antimicrobials are used in food animals as therapeutic agents and as growth promoters. The use of effective drugs has been essential to guarantee the high indices of productivity reached in the last decades, propitiating a reduction of mortality and morbidity and the maintenance of animal well-being. However, the indiscriminate use of antimicrobials can lead to the selection of resistant bacteria in food animals that could subsequently be transmitted to humans, a serious public health problem (23). Resistance to antimicrobials exists even before they were actually applied by humans, but this intrinsic form of resistance is not the major cause of concern about animal and human health. The great majority of the resistant microorganisms are the result of genetic changes, due to mutations or transference of genetic material, followed by a selection process, which happens mostly because an indiscriminate use of antimicrobials (23,29).

Antimicrobial susceptibility test represents one of the most important tasks of the clinical microbiology laboratory. It can be used as an epidemiological tool and for the definition of the proper treatment of infections, consequently preventing or creating strategies that minimize the dissemination of resistant bacterial strains, mainly multiresistant ones. With the observed increase in drug resistance, the study of antimicrobial susceptibility of any routinely isolated strain becomes important, mainly of Campylobacter sp strains, since studies on the susceptibility profile of Campylobacter sp strains isolated in Brazil are still rare.

The aim of this study was to determine the antimicrobial susceptibility of Campylobacter sp strains isolated from calves up to 60 days of age, with and without diarrhea, in Minas Gerais State, Brazil.

MATERIAL AND METHODS

A total of 25 Campylobacter sp strains were tested. The strains were isolated from calves up to 60 days of age, with and without diarrhea, from dairy farms in Minas Gerais State, Brazil, from May 1990 to February 1991 (18,19). The distribution of Campylobacter species and the clinical state of the calves are shown in Table 1.

The disk diffusion method was performed according to Woods and Washington (37). Campylobacter sp were grown in BHI agar (Difco, USA), at 37°C, under a microaerophilic condition (85% N₂, 10% CO₂, 5% O₂), for 48h. After incubation all strains were suspended in PBS pH 7.4 and density was adjusted to the 0.5 McFarland turbidity standard and inoculated on Mueller-Hinton agar plates (Difco, USA) supplemented with 5% of horse blood. Inoculated plates were incubated at 37°C under a microaerophilic atmosphere, for 48h. Disks of the following antimicrobial agents were used: amikacin 30 µg, ampicillin 10 µg, kanamycin 30 µg, chloramphenicol 30 µg, erythromycin 15 µg, gentamicin 10 µg, neomycin 30 µg, nitrofurantoin 300 µg, penicillin G 10 µg, trimethoprim-sulfamethoxazole 1.25/23.75 µg (sulfazotrim) and tetracycline 30 µg. All disks were supplied by Cecon (São Paulo – Brazil). After incubation, the inhibition zones were measured and the susceptibility patterns were determined (37).

Fisher and χ² tests were employed to study the correlation between resistant Campylobacter sp strains and the occurrence of diarrhea in animals (28).

RESULTS

All Campylobacter sp strains tested were susceptible to amikacin, ampicillin, chloramphenicol, erythromycin, gentamicin, neomycin and nitrofurantoin. Three strains (12%) were moderately susceptible to kanamycin (Table 2). Two strains (8%) were moderately susceptible and six (24%) strains were resistant to tetracycline. Twenty-two (100%) of the tested strains were resistant to penicillin G; 9 were from animals with diarrhea. Two strains (8%) were moderately susceptible and 12 (48%) were resistant (Table 2) to the combination sulfamethoxazole-trimetoprim.

Twelve strains (48%) showed multidrug resistance (Table 3), which meant that they were resistant to more than one antimicrobial group. Three C. coli strains, from 2 animals without and one with diarrhea; 8 C. fetus, 4 from animals with and 4 from animals without diarrhea, and one C. jejuni strain, isolated from an animal without diarrhea, showed multidrug resistance. There was no correlation between the resistance of Campylobacter sp strains to antimicrobials and the occurrence of diarrhea in calves.

DISCUSSION

Antimicrobial resistance of microorganisms is a real concern, especially in veterinary medicine, because of the interrelation

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<th>Table 1. Isolates of Campylobacter sp from calves up to 60 days of age, with and without diarrhea in Minas Gerais State, Brazil.</th>
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<td>Clinical State</td>
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1. All strains were isolated from calves in Minas Gerais State, Brazil, during the period from May 1990 to February 1991.
Sulfonamides and penicillin are used in calves to prevent infections and fed milk or milk replacer, which may contain antimicrobials, mainly tetracycline, with a consequent increase in antimicrobial use. On many dairy farms, one-day old calves are housed separately to control microbiota and prevent the dissemination of pathogens and disease. The husbandry adopted on several dairy farms, such as raising diarrheic animals; 4 – M – moderately susceptible; 5 – R – resistant; 6 – S – susceptible.

The results of the present study may reflect those problems associated with the veterinary use of antibiotics as the higher resistance patterns were found to antimicrobials largely employed for treating cattle infections or as growth promoters.

Some authors compared methods for antibiotic susceptibility testing of Campylobacter species and concluded that the disk diffusion method may constitute a simple, inexpensive, accurate and reliable antimicrobial testing method for Campylobacter strains.

The lack of correlation between the occurrence of diarrhea in calves and resistance to antimicrobials among the Campylobacter sp strains in the present study might be associated to the reduced number of strains tested. However, as animals are usually raised under similar conditions, it just can reflect that most animals are submitted to the same risk factors for the appearance of antimicrobial resistance.

Diarrhea outbreaks caused by C. jejuni and C. coli are frequently associated to unpasteurized milk and raw chicken, pork and beef meat ingestion, what emphasizes even more the importance of studies on susceptibility of Campylobacter sp strains isolated from animals to the antibiotics of current use in human medicine or to the drugs of choice for treating Campylobacter infections. In the present study, all the isolated strains of Campylobacter sp. were susceptible to erythromycin, the drug of first choice for the treatment of human enteric infection caused by C. jejuni and C. coli. Some studies have already demonstrated the development of resistance of C. jejuni and C. coli strains to erythromycin, although Gaudreau and Gilbert (14), in Canada, did not find resistant C. jejuni strains to this antimicrobial. In general, strains isolated from swine present higher rates of resistance to erythromycin, because macrolides are largely used to control respiratory diseases in this species. Aarestrup et al. (1) reported that the wide use of tylosin in Denmark was probably responsible for the high rates of resistance of C. coli and C. jejuni strains to macrolides in swine. Although many products containing macrolides have been approved for treating cattle with respiratory, hoof and mastitis problems in Brazil, their use does not seem to influence the appearance of resistance in Campylobacter sp strains, probably because of their restricted use in cattle production due to their high costs.

In the present study, all the tested strains were susceptible to the drugs of choice for the treatment of systemic infection caused by C. fetus, gentamicin and ampicillin. Tremblay et al.

1 – All strains were isolated from calves in Minas Gerais State, Brazil, during the period from May 1990 to February 1991. 2 – Number of strains submitted to the same risk factors for the appearance of antimicrobial resistance. 3 – Number of strains isolated from animals to the development of resistance of Campylobacter sp strains to erythromycin, although Gaudreau and Gilbert (14), in Canada, did not find resistant C. jejuni strains to this antimicrobial. In general, strains isolated from swine present higher rates of resistance to erythromycin, because macrolides are largely used to control respiratory diseases in this species. Aarestrup et al. (1) reported that the wide use of tylosin in Denmark was probably responsible for the high rates of resistance of C. coli and C. jejuni strains to macrolides in swine. Although many products containing macrolides have been approved for treating cattle with respiratory, hoof and mastitis problems in Brazil, their use does not seem to influence the appearance of resistance in Campylobacter sp strains, probably because of their restricted use in cattle production due to their high costs.

In the present study, all the tested strains were susceptible to the drugs of choice for the treatment of systemic infection caused by C. fetus, gentamicin and ampicillin. Tremblay et al.
Milk and eggs may cause public health issues in Brazil because of the risks that residues of this drug in meat pose. Campylobacter jejuni demonstrated a very low resistant rate of strain isolates, which were reported by Sáenz (27) in Spain. Lariviére et al. (20) demonstrated a very low resistant rate of Campylobacter sp strains to chloramphenicol, varying from 0.6% to 10%. However, despite the good activity of chloramphenicol against Campylobacter sp, its use in food animals was forbidden in Brazil because of the risks that residues of this drug in meat, milk and eggs may cause to public health (6).

Similar results as those found in the present study, showing a lack of resistance to chloramphenicol among Campylobacter sp isolates, were reported by Sáenz et al. (27) in Spain. Lariviére et al. (20) demonstrated a very low resistant rate of Campylobacter sp strains to chloramphenicol, varying from 0.6% to 10%. However, despite the good activity of chloramphenicol against Campylobacter sp, its use in food animals was forbidden in Brazil because of the risks that residues of this drug in meat, milk and eggs may cause to public health (6).

Vanhoof et al. (35) and Butzler and Skirrow (7) observed that aminoglycosides, chloramphenicol and tetracyclines have a good activity on isolates of C. jejuni from domestic animals. However, the occurrence of strains resistant to tetracycline has been reported by several authors (2,14,27,34,35). Aarestrup et al. (1), in a study performed in Denmark, observed that the resistance to tetracycline is more common in strains isolated from humans than from domestic animals. In Brazil, some studies (9,17,24) show the occurrence of strains resistant to tetracycline. However, Aquino et al. (4) did not find any Campylobacter sp strain isolated from men resistant to this antibiotic, but they reported resistance in strains isolated from swine. In the present study, 8% of the tested strains were moderately susceptible and 24% were resistant to this antibiotic, what is a remarkable finding, because tetracycline is one of the drugs of choice for treatment of C. jejuni and C. coli enteric infections. This may reflect the large use of tetracycline in cattle production in Brazil (30).

Vargas et al. (36) determined the susceptibility of 21 Brazilian isolates of C. fetus subsp. venerealis to the antimicrobial agents that are usually used for therapeutic and semen treatment. Resistance was only found to lincomycin (42.86%), enrofloxacin (4.76%) and nalidixic acid (100%), evidencing susceptibility of C. fetus subsp. venerealis strains to antimicrobials usually used for clinical and semen treatment. In the present study, from the 16 C. fetus strains tested, 12.5% were resistant to tetracycline, 56.25% were resistant to sulfazotrim and all of them were resistant to penicillin, what is a highly concerning finding, since some authors point out penicillin and streptomycin as the most used drugs to control C. fetus infections (12,16).

In the last years, multiresistant Campylobacter sp strains have been reported (4,21,26) and their frequencies are of concern for human and animal health authorities. In Brazil, Aquino et al. (4) reported C. coli and C. jejuni isolates from human and animal sources that were resistant to sulphonamide, norfloxacin, erythromycin, ciprofloxacin, ampicillin and tetracycline. Although the multiresistance patterns were different, the high frequency of multiresistant Campylobacter sp strains (48%), found in the present study, suggests that strict norms to the use of antimicrobials in veterinary medicine should be adopted in Brazil. The cases of multiresistance found were probably related to an indiscriminate and incorrect use of antimicrobials frequently observed in the production of cattle. Animals and food from animal origin play an important role in the transmission of campilobacteriosis to humans. So the findings of multi resistant Campylobacter sp strains from domestic animals stressed the importance of epidemiological surveillance and preventive actions that may avoid the dissemination of these strains over animal and human populations (23).

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RESUMO

Susceptibilidade a antimicrobianos de amostras de Campylobacter sp isoladas de bezerros com e sem diarréia, no estado de Minas Gerais, Brasil

Foi estudado o perfil de susceptibilidade aos antimicrobianos de 25 amostras de Campylobacter sp isoladas de bezerros com e sem diarréia (7 C. coli, 16 C. fetus e 2 C. jejuni). Foram testados pelo método de difusão 11 agentes antimicrobianos: amicacina, ampicilina, canamicina, cloranfenico, eritromicina, gentamicina, ...
neomicina, nitrofurantoína, penicilina G, tetraciclina e sulfametoxazole-trimetoprim. Todas as amostras de *Campylobacter* sp foram susceptíveis a amicacina, ampicilina, cloranfenicol, eritromicina, gentamicina, neomicina e nitrofurantoína. Três amostras foram moderadamente sensíveis à canamicina (2 *C. coli* e 1 *C. fetus*). Todas as amostras foram resistentes à penicilina G. Duas amostras de *C. fetus* foram moderadamente sensíveis a sulfametoxazole-trimetoprim e 1 *C. coli*, 9 *C. fetus* e 2 *C. jejuni* foram resistentes. Duas amostras de *C. fetus* foram moderadamente sensíveis à tetraciclina e 3 de *C. coli*, 2 de *C. fetus* e 1 de *C. jejuni* foram resistentes. Onze amostras apresentaram multirresistência (2 *C. coli*, 8 *C. fetus* e 1 *C. jejuni*).

Não houve correlação entre resistência de amostras de *Campylobacter* sp aos antimicrobianos e a ocorrência de diarréia nos bezerros. A frequência de resistência e, principalmente, a multirresistência encontradas nas amostras *Campylobacter* sp isoladas de bezerros em Minas Gerais, Brasil, foram altas. O perfil de resistência de amostras de *Campylobacter* sp está relacionado aos agentes antimicrobianos mais utilizados em bovinos no Brasil.

**Palavras chave:** *Campylobacter* sp, bezerro, diarréia, antimicrobianos, sensibilidade

**REFERENCES**


