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Research Note

Antimicrobial Resistance of *Campylobacter jejuni* Isolated from Chicken Carcasses in the Federal District, Brazil

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ABSTRACT

The aim of the present study was to perform microbiological isolation of *Campylobacter jejuni* from chilled chicken carcasses marketed in the Federal District of Brazil and to subject the strains to an antibiogram. A total of 92 samples from chilled chicken carcasses were acquired, 18 of which (19.56%) tested positive for *C. jejuni*. A total of 16 strains were tested for susceptibility to eight antimicrobial drugs. All 16 strains were resistant to ciprofloxacin, 15 strains to nalidixic acid, streptomycin, tetracycline, and gentamycin, 14 strains to amoxicillin, 11 strains to erythromycin, and 6 strains to chloramphenicol. The present study is the first to report on the presence of *C. jejuni* in chilled chicken carcasses marketed in the Federal District region of Brazil. These results may indicate flaws in certain steps of this food processing and highlight a possible public health problem due to the high level of resistance exhibited by the isolated strains.

Campylobacteriosis is a zoonosis that causes diarrhea in humans. It is frequently diagnosed in developed countries, and one of its major causal agents is *Campylobacter jejuni (4, 10, 19)*. Brazilian aviculture has exhibited high growth rates over the last 3 decades; as such, Brazil has become the third largest producer of chicken meat worldwide and the leader in exports. According to the Ministry of Agriculture *(13)*, Brazilian meat production is expected to supply 44.5% of the international market by 2020, and chicken meat will account for 48.1% of the global exports. Although Brazil is the world's largest chicken meat exporter, and the internal and external markets are increasingly demanding the hygienic safety of foodstuffs, there is not yet any specific legislation for the control of *Campylobacter* in animal-derived foods in Brazil.

Antibiotic treatment is usually prescribed for patients with severe, long-lasting, or recurrent diarrhea, and macrolides and fluoroquinolones are the drugs of choice *(2)*. However, such drugs have also been empirically employed in patients with gastroenteritis without etiological diagnosis.

The aims of the present study were to investigate the occurrence of *C. jejuni* in chilled chicken carcasses in the Federal District region of Brazil and in Brasília, the capital, and to analyze the resistance profiles of the isolated *C. jejuni* strains to antimicrobials.

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MATERIALS AND METHODS

A total of 92 samples from chilled chicken carcasses of various brands marketed in the Federal District region and Brasília were purchased at various commercial establishments from March to December 2009.

The method used to isolate *C. jejuni* was previously described by Taremi et al. *(19)*, and a positive-control strain of *C. jejuni* was acquired from the Instituto Adolf Lutz (IAL/SP, Brazil) to verify the microbiological isolation. Each sample (25 g) was weighed and homogenized in a mixture containing 225 ml of Campylobacter Enrichment Broth Base (Preston Enrichment Broth Base, M899, HiMedia, Mumbai, India), 2.25 ml of Campylobacter Selective Supplement IV (Preston Selective Supplement, FD042, HiMedia), and 12 ml of 5% sheep blood using a stomacher (Mayo International, Milan, Italy) for 1 min. The homogenate was incubated at 42°C for 48 h in an anaerobic jar (Permution, Curitiba, Brazil) with a microaerophilic atmosphere generator (BD BBL CampyPak Plus Microaerophilic System Envelopes with Palladium Catalyst, BD, Franklin Lakes, NJ). An approximately 0.1-ml aliquot of preincubated Preston broth was streaked using a platinum wire on the surface of a plate containing Campylobacter Agar Base (M994, HiMedia) supplemented with Campylobacter Supplement I (Blaser-Wang Selective Medium, FD006, HiMedia) and 5% sheep blood to obtain isolated colonies. The plates were incubated at 42°C for 48 hours under microaerophilic conditions. The presumptive identification of *Campylobacter* was determined with Gram staining, verification of catalase and oxidase production, and hippurate hydrolysis.

The disk diffusion method was selected to test susceptibility to antimicrobials, following the methods described by Taremi et al. *(19)* and the National Committee for Clinical Laboratory Standards

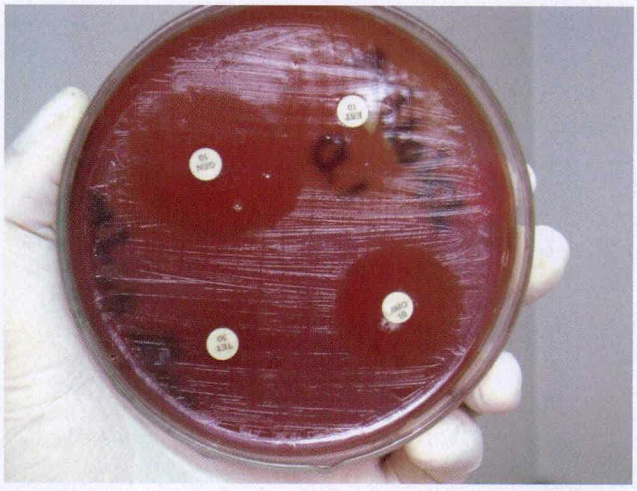

FIGURE 1. *Results of antimicrobial susceptibility test of strains of* C. jejuni *isolated from chicken carcasses in Federal District, Brazil, using disc diffusion in a plate with 5% sheep blood. Gentamicin, upper left (sensitive); streptomycin, upper right (sensitive); tetracycline, bottom left (resistant); amoxicillin, bottom right (sensitive).*

(11). Approximately 25 ml of Mueller-Hinton agar (M1084, HiMedia) was supplemented with 5% sheep blood, dispensed into petri dishes, and incubated at 37°C for 24 hours to confirm sterility. The Newprov antibiotic discs used contained nalidixic acid (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), tetracycline (15 μg), gentamycin (10 μg), amoxicillin (30 μg), chloramphenicol (30 μg), and streptomycin (30 μg).

RESULTS AND DISCUSSION

From the 92 samples of chicken carcasses analyzed in this study, 18 (19.56%) strains of *C. jejuni* were isolated. Because two strains did not remain culturable for the test of susceptibility to antimicrobials, a total of 16 strains of *C. jejuni* were subjected to an antibiogram. All 16 strains (100%) exhibited resistance to ciprofloxacin; 15 strains (93.75%) were concomitantly resistant to nalidixic acid, streptomycin, tetracycline, and gentamycin; 14 strains (87.5%) were resistant to amoxicillin; 11 strains (68.75%) were resistant to erythromycin; and the lowest rate of resistance was to chloramphenicol, which was exhibited by 6 strains (37.5%), as shown in Figure 1. The present study is the first report on the isolation of *C. jejuni* from chicken carcasses in the Federal District region. Our results regarding the isolation of *C. jejuni* are similar to those reported in different animal-derived foods by several authors *(3, 8, 9, 16, 19, 21)*.

C. jejuni is seldom investigated in Brazil. Reiter et al. *(14)* analyzed 120 poultry carcasses at a slaughterhouse in Blumenau (Santa Catarina, southern Brazil) and found *Campylobacter* spp. in 52 samples (43%) using the indirect detection method with MiniVIDAS equipment (bioMérieux, Inc., Marcy l'Etoile, France). These authors did not identify the species, nor did they investigate the resistance to antimicrobials. Dias et al. *(1)* isolated 19 strains (38%) of *C. jejuni* from 50 samples of poultry carcasses marketed in Belo Horizonte (Minas Gerais, southeastern Brazil) and found 100% resistance to amikacin, chloramphenicol, erythromycin, gentamycin, kanamycin, and neomycin, 95% resistance to tetracycline, and 90% resistance to ampicillin; however, none of the strains were resistant to trimethoprim-sulfamethoxazole. In a study conducted in Belém (Pará, northern Brazil), Freitas and Noronha *(5)* isolated 12 strains (87.5%) of *Campylobacter* spp. from 14 samples of chicken carcasses and giblets acquired from slaughterhouses, farmers' markets, and supermarkets. These authors did not differentiate the *Campylobacter* species, nor did they perform an analysis of the antimicrobial resistance.

In the present study, the isolation method for *C. jejuni* described by Taremi et al. *(19)* was applied successfully, and it allowed the isolation of pure *C. jejuni* colonies from chilled chicken carcasses. *C. jejuni* was detected in chilled chicken carcasses purchased at commercial establishments located in the Federal District region, and our data corroborate the hypothesis that poultry carcasses might represent an important vector of transmission of *C. jejuni* to humans. The presence of this bacterial species might indicate flaws in some step in the hygienic industrial practices, including the processing, transportation, or sale of poultry meat *(3, 5, 14)*. The results of the present study emphasize the need to improve the hygienic-sanitary control measures during the processing of poultry meat. In a study by Taremi et al. *(19)* to determine the prevalence and antimicrobial resistance of *Campylobacter* sp. from retail raw chicken and beef, the resistance to ciprofloxacin was 69.4%, and the resistance rates to the antimicrobial agents nalidixic acid, streptomycin, tetracycline, and gentamycin were 75, 4.2, 45.8, and 4%, respectively. The resistance to amoxicillin was 11.1%, and the chloramphenicol resistance was 2.8%; no resistance to erythromycin was observed. In Korea, Han et al. *(7)* examined the resistance to antimicrobials in strains of *C. jejuni* isolated from poultry carcasses and observed 100% resistance to tetracycline, nalidixic acid, ciprofloxacin, and ampicillin and 43.1% resistance to amoxicillin. Although these results are similar to those obtained in the present study, the strains isolated by Han et al. *(7)* were sensitive to erythromycin and gentamycin.

In Spain, Sánchez et al. *(17)* examined the resistance to antimicrobials in strains of *C. jejuni* isolated from the stools of hospitalized patients with diarrhea. These authors observed 2.3% resistance to erythromycin, 28.5% to ciprofloxacin, and 36.8% to nalidixic acid, all of which are similar to the rates measured in the present study. Rodrigo et al. *(15)* analyzed *C. jejuni* in poultry carcasses at six health departments in Trinidad and Tobago. An antibiogram was performed on 319 *C. jejuni* isolates, and the results indicated 86.6% resistance to ciprofloxacin, 5.4% to gentamycin, 30% to streptomycin, and 26.8% to enrofloxacin. These results are similar to those of the present study with regard to the high rates of ciprofloxacin resistance; in our study, all of the strains analyzed exhibited 100% resistance to ciprofloxacin.

The increasing prevalence of *Campylobacter* strains that are resistant to quinolones as a function of their indiscriminate use might threaten the future efficacy of such drugs. The resistance of *C. jejuni* strains to various drugs

represents a worldwide public health problem *(7, 18, 19)* and is a source of constant concern for the World Health Organization *(20)*. However, researchers unanimously assert that the origin of the antimicrobial resistance of *C. jejuni* strains isolated from both poultry meat and human patients is still unknown.

The low rate of resistance to chloramphenicol observed in the present study might be due to Normative Instruction No. 9 of the Ministry of Agriculture, Livestock, and Supply *(12)* from 27 June 2003, which forbids the use of chloramphenicol in Brazilian veterinary medicine.

The present study isolated from chicken carcasses a considerable number of *C. jejuni* specimens that were resistant to the main antimicrobials used in medical care. However, comparisons among the results obtained from various studies on *C. jejuni* are difficult due to differences in the sample collection procedures and the use of several different methods for isolation and analysis of susceptibility to antimicrobials. According to Ge et al. *(6)*, antimicrobial resistance might vary among the multiple species of *Campylobacter* and, thus, hinder attempts to standardize this test. *C. jejuni* was detected in poultry carcasses marketed in the Federal District region of Brazil. The results of the present study might contribute to the establishment of measures for the prevention and control of contamination with these bacterial species and serve as a warning to the Brazilian health authorities regarding the indiscriminate use of antimicrobials, which might threaten the efficacy of such drugs in the treatment of campylobacteriosis. Finally, further studies must be conducted to investigate the origin of the high level of resistance observed in the isolated strains.

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