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# Occurrence of *Campylobacter* in raw chicken and beef from retail outlets in São Paulo, Brazil

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## Abstract

The aim of this study was to evaluate the occurrence of *Campylobacter* in meat samples (120 chicken and 100 beef) and determine the antimicrobial susceptibility of the isolates. A total of 220 samples from retail outlets were purchased in the city of São Paulo, Brazil. *Campylobacter* detection was performed according to the International Organization for Standardization (ISO) method ISO-10272-1:2006. A PCR assay based on nucleotide sequence differences in the *lpxA* gene was used to distinguish between *C. jejuni* and *C. coli*. Antimicrobial resistance was determined by agar disc diffusion method. *Campylobacter* was isolated from 17 (7.7%) of 220 samples. Breast fillets exhibited the highest contamination rate (25%; 5/20), followed by wings (15%; 6/40), whole leg (15%; 3/20), drumstick (10%; 2/20), and drumette (5%; 1/20). All beef samples were negative for *Campylobacter*. The most prevalent species found was *C. coli*, followed by *C. jejuni*. The isolates were commonly resistant to nalidixic acid and ciprofloxacin. Data obtained confirm the need of monitoring and control of *Campylobacter* in poultry production chain.

## Practical applications

*Campylobacter* spp. cause foodborne illness in humans commonly through the consumption of contaminated poultry meat. Although Brazil is the world's largest poultry meat exporter, data regarding this pathogen are limited in our country. In the present study, chicken cuts purchased from retail stores in São Paulo may occasionally be contaminated with *Campylobacter*. This underline the importance of surveillance of foodborne pathogens in retail meats, and the data can contribute to risk analyses or control measures in the meat production chain.

## 1 | INTRODUCTION

*Campylobacter* is considered the most common bacterial cause of human gastroenteritis in the world. In many countries the organism is isolated 3–4 times more frequently from patients with foodborne disease than other bacterial enteropathogens such *Salmonella* or *Escherichia coli* (WHO, 2012). Since 2005, *Campylobacter* has been the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) (EFSA, 2016). In the United States in 2014, *Campylobacter* was the second most common cause of foodborne disease accounting for 31 of the 317 reported outbreaks (CDC, 2016). In Brazil, the cases of campylobacteriosis are under-diagnosed and under-reported, and there are few studies about molecular identification of *Campylobacter*.

*Campylobacter* infections result in an acute, self-limited gastrointestinal illness characterized by diarrhea, fever, and abdominal cramps (Allos, 2001). Almost all infected people recover without any specific treatment; however, antimicrobial therapy is necessary for patients at high risk for severe or long-lasting infections, such as immune-suppressed patients. A subset of patients develop sequelae such as reactive arthritis (ReA), Reiter's syndrome (RS), irritable bowel syndrome (IBS), Guillain–Barré syndrome (GBS), inflammatory bowel disease (IBD), Chron's disease (CD), and ulcerative colitis (UC) (Esan et al., 2017). Guillain–Barré syndrome is the most common and most severe acute paralytic neuropathy, with about 100,000 people developing the disorder every year worldwide (Willison, Jacobs, & van Doorn, 2016). Approximately 25–40% of GBS patients have evidence of *Campylobacter jejuni* infection 1–3 weeks prior to the illness (Nyati & Nyati, 2013).

The intestinal tract of food producing animals have been considered as one of the most important reservoirs for *Campylobacter* in the food supply. Human exposure can come through consumption of animal products, in particular raw or undercooked poultry meat

  

(Humphrey, O'Brien, & Madsen, 2007). *Campylobacter* may colonize poultry gastrointestinal tracts without deleterious effects upon the birds, and asymptomatic carriers freely spread the microorganisms during production and processing, resulting in further contamination of both live birds and processed carcasses (Skarp, Hänninen, & Rautelin, 2016). *Campylobacter* isolates from poultry resistant to quinolones and fluoroquinolones (nalidixic acid and ciprofloxacin), and to tetracycline have been observed (Ma et al., 2017; Woźniak-Biel et al., 2017). Both of these antimicrobial groups are often used in poultry industry, especially regarding enteric infections. Several factors contributing to the emergence of resistance have been hypothesized and include antimicrobial drug use in broiler breeder and broiler chickens and importation of poultry products.

Very little information has been published regarding the prevalence of *Campylobacter* spp. isolated from different types of meat from retail outlets in São Paulo city, Brazil. The main objective of this study was to evaluate *Campylobacter* occurrence in raw chicken and beef at retail level and determine antimicrobial susceptibility of the isolates.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

A total of 220 samples of raw chicken and fresh beef were purchased from five (A–E) retail outlets in the city of São Paulo over a 1-year period. The samples were packed by the industry or at the market, and only packages within expiration date were collected. Each package contained on average 500 g of meat. The chicken parts and number of samples were: wing (n = 40), drumstick (n = 20), whole leg (n = 20), drumette (n = 20), and breast fillets (n = 20). The beef cuts were: knuckle (n = 20), german trim striploin (n = 20), top side cap on (n = 20), eyeround skin on (n = 20), and rump tail on (n = 20). Samples were transported to the microbiology laboratory in isothermal boxes and immediately analyzed.

### 2.2 | Microbiology analysis

Before opening, packages of meat were submitted to external disinfection with 70% alcohol solution. The chicken cuts were aseptically weighed and rinsed with 100 ml buffered peptone water for 1 min. Regarding beef cuts, a 100 $cm^2$ area was sampled with sterile polyurethane sponge (Biotrace Int. Bioproducts & Nasco, Modesto, EUA) pre-moistened with 10 ml of sterile maximum recovery diluent (0.1% Peptone, 0.85% NaCl). Each sponge was put into sterile stomacher bag with 100 ml of maximum recovery diluent. The mixture was then homogenized for three minutes in a peristaltic homogenizer (Lab Blender 400, Seward, England) and the liquid was centrifuged at 1000 × g for 15 min at 5°C (Sorvall Instruments, RC-5B, Dupont, EUA).

*Campylobacter* detection was determined according to the International Organization for Standardization (ISO) method ISO-10272-1:2006. For this purpose, the pellet from beef cuts was suspended in 100 ml of Bolton broth and incubated under microaerophilic atmosphere ($O_2$ 5%, $CO_2$ 10%, $N_2$ 85%) for 4 hr at 37°C and then for 44 ± 4 at 41.5 ± 1°C. In case of the chicken cuts, 10 ml of the rinse broth was added to 90 ml of the Bolton broth and incubated under the same conditions. Subsequently, 10 μL of the culture was streaked onto mCCDA (modified Charcoal Cefoperazone Deoxycholate Agar) and Karmali agar with subsequent incubation for 4 hr at 37°C and then for 44 ± 4 at 41.5 ± 1°C under microaerophilic conditions. From each positive plate, three to five presumed *Campylobacter* colonies were cultured onto Columbia blood agar plates for further characterization. To confirm the genus *Campylobacter*, the isolates were submitted to microscopic examination, oxidase testing, microaerophilic growth at 25°C, and aerobic growth at 41.5°C. For species differentiation, the isolates were tested for catalase, susceptibility to nalidixic acid and cephalotin, hippurate and indoxyl acetate hydrolysis. The isolate collection was stored at −70°C in peptone broth containing 20% (vol/vol) glycerol.

### 2.3 | Genomic DNA extraction

*Campylobacter* isolates were inoculated onto Columbia blood agar plates and incubated for 48 hr at 41.5 ± 1°C under microaerophilic conditions. Bacteria were harvested and suspended in 1000 μL of sterile distilled water in a 1.5 ml microcentrifuge tube. Cells were collected by centrifugation at 5000 × g for 5 min at 4°C. Genomic DNA was extracted with DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's specifications. Extracted genomic DNA was stored at −20°C until use as a template for PCR amplification.

### 2.4 | Differentiation of campylobacter spp

A PCR assay based on nucleotide sequence differences in the *lpxA* gene was used to distinguish between *C. jejuni* and *C. coli*, as previously described (Klena et al., 2004). Forward primers for *C. jejuni* (lpxAC. jejuni: 5′-ACA ACT TGG TGA CGA TGT TGT A-3′) and for *C. coli* (lpxAC. coli: 5′-AGA CAA ATA AGA GAG AAT CAG-3′) were used in combination with unique reverse primer lpxARKK2m (5′-CAA TCA TGD GCD ATA TGA SAA TAH GCC AT-3′). For PCR reaction, GoTaq Green Master Mix (Promega Corporation, Madison, EUA) was used with 10 pmol/μL of forward primer and 10 pmol/μL of reverse primer, 50 ng of genomic DNA mixed in a 25 μL reaction volume. The reaction mixtures were placed into an epMastercycler S thermocycler (Eppendorf, Hamburg, Germany). The cycling conditions used were initial denaturation of 94°C for 4 min, 35 cycles with 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension time of 5 min. The PCR products were detected by electrophoresis by using 2% agarose gels. The bands were visualized under UV light after staining with ethidium bromide (1 μg/mL, Pharmacia) and the images were captured on an EDAS120 system (Eastman Kodak, New York, NY).

### 2.5 | Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles of the isolates were determined by agar disc diffusion according to the specifications given in the document M100-S25 of the Clinical and Laboratory Standards Institute (CLSI, 2015). Eight antimicrobial discs (Oxoid, Basingstoke, United Kingdom) were used: cephalothin (KF; 30 μg), chloramphenicol (CHL;

**TABLE 1** Occurrence of *Campylobacter* spp. in raw chicken purchased at retail

| | No. of samples | No. of positive (%) | No. of *C. jejuni* (%) | No. of *C. coli* (%) |
|---|---|---|---|---|
| Wing | 40 | 6 (15.0) | 3 (7.5) | 3 (7.5) |
| Drumette | 20 | 1 (5.0) | 0 | 1 (5.0) |
| Breast fillet | 20 | 5 (25.0) | 1 (5.0) | 4 (20.0) |
| Drumstick[a] | 20 | 2 (10.0) | 1 (5.0) | 2 (10.0) |
| Whole leg | 20 | 3 (15.0) | 0 | 3 (15.0) |
| Total | 120 | 17 (14.2) | 5 (4.2) | 13 (10.8) |

[a]Cross-contamination of *C. jejuni* and *C. coli* in one sample.

30 μg), ciprofloxacin (CIP; 5 μg), erythromycin (ERY; 10 μg), gentamicin (GEN; 120 μg), nalidixic acid (NAL; 30 μg), streptomycin (STR; 10 μg), and tetracycline (TET; 30 μg). *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560 were used for quality control. Isolates were defined as multi-resistant if resistant to three or more antimicrobial classes.

### 2.6 | Statistical analysis

The frequency of *Campylobacter* isolates from different meat samples were compared by chi-square test ($\chi^2$) using 95% confidence interval and the Stata Data Analysis and Statistical Software, version 12.0 (College Station, TX). A $p$ value of $< .05$ was considered significant.

## 3 | RESULTS

*Campylobacter* was isolated from 17 (7.7%) of 220 samples analyzed in this study. Among the chicken meat samples (n = 120), *Campylobacter* was found most frequently in breast fillets (5/20), followed by wings (6/40) and whole leg (3/20), drumstick (2/20), and drumette (1/20) (Table 1). The occurrence of *Campylobacter* in the various parts of the chicken varied in the range 5–25%, which differed significantly ($p < .05$). All beef samples were negative for *Campylobacter*.

Three to five colonies from the 17 chicken samples, totalizing 75 isolates, were subjected to phenotypic tests and to PCR assay for identification of *Campylobacter* spp. The aim was to analyze if more than one species would be present in the same chicken sample. Amplicons of 331 and 391 bp for *C. jejuni* and *C. coli*, respectively, were generated by PCR assay (Figure 1). The most prevalent species found was *C. coli*, corresponding to 66 isolates belonging to 13 chicken samples. While *C. jejuni* was found in nine isolates from five chicken samples. Regarding chicken cuts, *C. coli* was seen in four breast fillets, three wings, three whole leg, one drumette, and one drumstick. Furthermore, *C. jejuni* was found in three wings, one breast fillet, and one drumstick. The simultaneous presence of *C. jejuni* and *C. coli* was observed in just one drumstick sample (Table 1).

One isolate from each positive chicken sample was subjected to antimicrobial susceptibility test, totalizing 18 *Campylobacter* isolates. Among them, four (22.2%) isolates were susceptible to all antimicrobial agents and fourteen (77.8%) isolates were resistant at least one antimicrobial agent. Resistance to nalidixic acid (77.8%), ciprofloxacin (72.2%), tetracycline (16.7%), and streptomycin (16.7%) were the most common resistance profiles found (Table 2). Only two isolates were multi-drug resistant showing resistance to chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, and streptomycin.

## 4 | DISCUSSION

*Campylobacter* is one of the most common causes of diarrheal illness worldwide and particularly associated with raw and undercooked poultry meat or by cross-contamination of other foods by these items. Broiler have been regarded as one of the main reservoirs of *Campylobacter* and the colonization level in broiler ceca can reach $10^9$ cfu/g (Stern et al., 2008). Fecal contamination of carcasses is difficult to avoid during the chicken slaughtering process and is most likely responsible for the high prevalence of *Campylobacter* in chicken meat (Elvers, Morris, Newell, & Allen, 2011).

The prevalence of *Campylobacter* is variable according to the countries or geographic regions. In European countries, the proportion of contaminated broiler carcasses collected at slaughterhouses after chilling varied from 4.9% in Estonia to 100.0% in Luxembourg (EFSA, 2010). In China, *Campylobacter* prevalence was accessed along a broiler production chain from farm to retail and prevalence in fresh broiler carcass samples obtained from supermarkets was 31.3% (40/128) (Ma, Wang, Shen, Zhang, & Wu, 2014). The average prevalence of *Campylobacter* in fresh broiler meat commercialized in the United States from 2005 to 2011 was 41% (Williams & Oyarzabal, 2012).

A previous study in Brazil have described high prevalence of *Campylobacter* in broiler carcasses during processing (Franchin, Ogliari, & Batista, 2007). At Brazilian retail level there are also few studies conducted and *Campylobacter* was isolated in 10.8% (21/194) of the refrigerated broiler carcasses in São Paulo city (Carvalho et al., 2014). In the Federal District region and Brasilia, *Campylobacter* was found in 19.6% (18/92) of chilled chicken carcasses purchased at various commercial establishments (Moura et al., 2013). Although *Campylobacter* has been detected in broiler carcasses, the prevalence of *Campylobacter* in chicken cuts at retail in Brazil is poorly known.

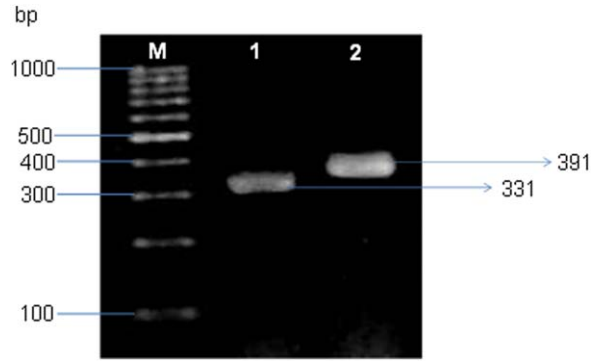


**FIGURE 1** Specie-specific PCR amplicons of *Campylobacter* after electrophoresis in 2% agarose gel. M: 100 bp DNA ladder. 1: *Campylobacter jejuni* amplicon with 331 bp. 2: *C. coli* amplicon with 391 bp

**TABLE 2** Antimicrobial resistance profile of *Campylobacter* isolates from raw chicken

| Market | Chicken cut (n) | Identified species (n) | Resistance profile (n) |
|---|---|---|---|
| Market A (n = 43) | Wing (13) | *C. jejuni* (1)<br>*C. coli* (3) | Susceptible<br>CIP-NAL (3) |
| | Drumette (2) | – | – |
| | Breast fillet (18) | *C. coli* (3) | Susceptible (2)<br>CIP-NAL-TET (1) |
| | Drumstick[a] (3) | *C. jejuni* (1)<br>*C. coli* (1) | CIP-NAL<br>CHL-ERY-GEN-NAL-STR |
| | Whole leg (7) | *C. coli* (1) | CHL-CIP-ERY-NAL-STR |
| Market B (n = 15) | Wing (4) | *C. jejuni* (1) | CIP-NAL-TET |
| | Drumette (1) | *C. coli* (1) | CIP-NAL |
| | Breast fillet (1) | *C. coli* (1) | CIP-NAL |
| | Drumstick (8) | – | – |
| | Whole leg (1) | *C. coli* (1) | CIP-NAL-STR |
| Market C (n = 20) | Wing (5) | – | – |
| | Drumette (9) | – | – |
| | Breast fillet (1) | *C. jejuni* (1) | CIP-NAL-TET |
| | Drumstick (3) | – | – |
| | Whole leg (2) | – | – |
| Market D (n = 15) | Wing (5) | *C. jejuni* (1) | Susceptible |
| | Drumette (2) | – | – |
| | Breast fillet (0) | – | – |
| | Drumstick (2) | *C. coli* (1) | CIP-NAL |
| | Whole leg (6) | *C. coli* (1) | CIP-NAL |
| Market E (n = 27) | Wing (13) | – | – |
| | Drumette (6) | – | – |
| | Breast fillet (0) | – | – |
| | Drumstick (4) | – | – |
| | Whole leg (4) | – | – |

[a]Cross-contamination of *C. jejuni* and *C. coli* in one sample.
CIP = ciprofloxacin (5 μg); NAL = nalidixic acid (30 μg); TET = tetracycline (30 μg); CHL = chloramphenicol (30 μg); ERY = erythromycin (10 μg); GEN = gentamicina (120 μg); STR = streptomycin (10 μg) according to CLSI (2015).

Our study reports that 17 of 120 chicken parts (14.2%) purchased in supermarkets were positive for *Campylobacter*, being most frequently found in breast fillets (25%), followed by wings (15%) and whole leg (15%), drumstick (10%), and drumette (5%). The highest occurrence of *Campylobacter* among breast fillets is probably because these areas are exposed to the feces-contaminated litter on the grow-out facility floors as the birds dust themselves and settle onto their breasts while resting. In addition, the breast presents a larger surface area and the structure of the skin follicles facilitates the permanence of *Campylobacter* in this region.

Among five sampled markets (A–E), four contained positive samples for *Campylobacter* (Table 2). Samples from market B had highest prevalence (26.7%), followed by those from market A (23.3%). The market E showed no *Campylobacter* contamination in chicken samples, probably because the meat came from *Campylobacter*-negative lots and due to good processing practices during slaughtering. Horizontal transmission of *Campylobacter* in the farm environment represents the usual rote of transmission. Once colonized, chickens remain colonized until they are slaughtered and contamination of raw meat has been shown to occur in the slaughterhouse.

None of the beef samples was contaminated with *Campylobacter*. The prevalence of this microorganism in beef is generally low. In the Fargo metropolitan area of North Dakota (United States), none of the 133 beef samples was positive for this pathogen (Kegode, Doetkott, Khaitsa, & Wesley, 2008). In Italy, *Campylobacter* was detected in 1.3% of the beef samples (Pezzotti et al., 2003). Similar results were observed in a Belgium study that reported no positive result for *Campylobacter* in minced meat from beef and veal, and only a very small number of beef carcasses (3.3%) and cutting meat (5%) were contaminated with *Campylobacter* (Ghafir, China, Dierick, De Zutter, & Daube, 2007). A total of 770 samples of retail raw meat were examined for the presence of *Campylobacter* in Korea and 1.2% of the beef samples were positive for this microorganism (Hong et al., 2007). The lower levels of *Campylobacter* in beef may be due to a lower incidence of this organism in cattle than in poultry, mainly due to the lower body temperature of bovines when compared to poultry.

In this study, chicken meat samples from retail presented most often *C. coli* (72.2%) followed by *C. jejuni* (27.8%). Similar results were found elsewhere. (Pezzotti et al., 2003) described in Italy that 55.6% of the broiler samples harbored *C. coli* and 44.4% *C. jejuni*. In Korea, most chicken meat samples were contaminated with *C. coli* (62.9%), being *C. jejuni* detected in 51.9% of the samples (Hong et al., 2007). A study conducted with 678 *Campylobacter* isolates from broilers using PCR assay was able to identify almost the same percentage of isolates as *C. jejuni* (49.2%) and *C. coli* (47.5%) (Manfreda, De Cesare, Bondioli, Stern, & Franchini, 2006). The species identification by PCR assay is more sensitive than biochemical tests, but care must be taken to avoid false positives arising from DNA contamination, as well as false negatives caused by inhibitory substances in food or enrichment broths. The current study also shows that *C. jejuni* and *C. coli* were detected simultaneously in one drumstick sample from supermarket A (Table 2). This could probably due to the cross-contamination from different chicken meats in the slaughterhouse or from different meats in the supermarket.

Whereas patients with campylobacteriosis usually recover without antimicrobial treatment, for a group of patients (those with severe disease or those at high risk for serious complications) antimicrobial agents are recommended. Macrolides and fluoroquinolones have been the recommended agents for treatment of severe campylobacteriosis, but resistance to these agents, particularly fluoroquinolones such as ciprofloxacin, is common, limiting treatment options (Aarestrup & Engberg, 2001). The World Health Organization (WHO, 2017) catalogue 12 families of antibiotic-resistant "priority pathogens" that pose the greatest threat of human health, they include *Campylobacter* spp. fluoroquinolone-resistant. This list was drawn up in a bid to guide and promote research and development of new antibiotics.

Among the *Campylobacter* isolates obtained in our study, fourteen (77.8%) were resistant to at least one of the seven antimicrobial tested. The isolates showed resistance to quinolone/fluoroquinolones,

macrolides, aminoglycosides and/or tetracycline (Table 2). Resistance to nalidixic acid (77.8%) and ciprofloxacin (72.2%) were the most frequently found in our study. Surveillance of antimicrobial resistance has identified important levels of resistance to fluoroquinolones among *Campylobacter* isolates around the world. In Korea, a study reported that 95.9% of *Campylobacter* from retail raw meats were resistant to ciprofloxacin (Hong et al., 2007). Resistance to fluoroquinolones was also common in the United States although in lower rates. Zhao et al. (2010) showed that 17% of the isolates obtained from retail broiler meat were resistant to ciprofloxacin and 19% of the isolates to nalidixic acid. High level of resistance to fluoroquinolones, such as enrofloxacin, appears to be associated with the use of these drugs to treat poultry. The frequent resistance observed to ciprofloxacin highlights the importance of adopting restrictive measures to control the use of the antimicrobial agents in food producing animals.

## 5 | CONCLUSIONS

In summary, our results demonstrate that chicken purchased from retail outlets in São Paulo city, may occasionally be contaminated with *C. jejuni* and *C. coli*, being a potential source of infection to consumers. The presence of antimicrobial-resistant isolates underline the additional risk that *Campylobacter* can represent to human health. The data presented here contribute to risk assessment and highlight the need of monitoring and control of this pathogen in poultry production chain.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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