

## Detection of *Campylobacter jejuni*, *Campylobacter coli*, and virulence genes in poultry products marketed in Northeastern Brazil

Detecção de *Campylobacter jejuni*, *Campylobacter coli* genes de virulência em produtos avícolas comercializados na região Nordeste do Brasil

Detección de *Campylobacter jejuni* y *Campylobacter coli* y genes de virulencia en productos avícolas comercializados en el Noreste de Brasil

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### Abstract

In this study, we evaluated the prevalence of *Campylobacter jejuni*, *Campylobacter coli*, and virulence genes in fresh, chilled, and frozen chicken carcasses with livers and gizzards sold in public markets and supermarkets. Of the 90 samples analyzed, *C. jejuni* was the most prevalent, with 28.8% of positive samples, whereas *C. coli* was positive in 15.6% of samples. In public market samples, *C. coli* had a higher prevalence than *C. jejuni*, with 16.7% positive samples detected, whereas in supermarket samples, *C. jejuni* was more prevalent (36.7% positivity). *C. jejuni* was detected in all forms of commercialized carcasses; however, there was a higher prevalence (43.3%) in chilled samples than *C. coli*, which was not detected in frozen samples but showed a higher prevalence (16.7%) in fresh samples. Both species were detected in different poultry products, with *C. jejuni* being more prevalent (53.3%) in liver samples. *C. coli* showed a higher prevalence in samples of meat pieces (10%). The presence of five virulence genes related to adherence (*Peb1*, *JlpA*, *CadF*, and *CapA*) and invasion (*CiaB*) was also observed in both species.

**Keywords:** *Campylobacter*; Broiler chickens; Microbiological control; PCR.

## Resumo

Neste estudo, avaliamos a prevalência de *Campylobacter jejuni*, *Campylobacter coli* e genes de virulência em carcaças de frangos frescos, resfriados e congelados com fígados e moelas comercializadas em mercados públicos e supermercados. Das 90 amostras analisadas, *C. jejuni* foi a mais prevalente, com 28,8% das amostras positivas, enquanto *C. coli* foi positiva em 15,6% das amostras. Nas amostras do mercado público, *C. coli* teve uma prevalência maior do que *C. jejuni*, com 16,7% de amostras positivas detectadas, enquanto nas amostras de supermercado, *C. jejuni* foi mais prevalente (36,7% de positividade). *C. jejuni* foi detectado em todas as formas de carcaças comercializadas; entretanto, houve prevalência maior (43,3%) nas amostras resfriadas do que *C. coli*, o que não foi detectado nas amostras congeladas, mas apresentou prevalência maior (16,7%) nas amostras frescas. Ambas as espécies foram detectadas em produtos avícolas diferentes, com *C. jejuni* sendo mais prevalente (53,3%) em amostras de fígado. *C. coli* apresentou maior prevalência em amostras de pedaços de carne (10%). A presença de cinco genes de virulência relacionados à adesão (*Peb1*, *JlpA*, *CadF* e *CapA*) e invasão (*CiaB*) também foi observada em ambas as espécies.

**Palavras-chave:** *Campylobacter*; Frangos de corte; Controle microbiológico; PCR.

## Resumen

En este estudio, evaluamos la prevalencia de *Campylobacter jejuni*, *Campylobacter coli* y genes de virulencia en canales de pollos frescos, refrigerados y congelados con hígados y mollejas vendidos en mercados públicos y supermercados. De las 90 muestras analizadas, *C. jejuni* fue la más prevalente, siendo el 28,8% de las muestras positivas, mientras que *C. coli* fue positiva en el 15,6% de las muestras. En muestras del mercado público, *C. coli* tuvo una mayor prevalencia que *C. jejuni*, con un 16,7% de muestras positivas detectadas, mientras que en muestras de supermercados, *C. jejuni* fue más prevalente (36,7% de positividad). *C. jejuni* se detectó en todas las formas de canales comercializadas; sin embargo, hubo una mayor prevalencia (43,3%) en muestras enfriadas que en *C. coli*, que no se detectó en muestras congeladas, pero mostró una mayor prevalencia (16,7%) en muestras frescas. Ambas especies se detectaron en diferentes productos avícolas, siendo *C. jejuni* más prevalente (53,3%) en las muestras de hígado. *C. coli* mostró mayor prevalencia en muestras de trozos de carne (10%). En ambas especies también se observó la presencia de cinco genes de virulencia relacionados con la adhesión (*Peb1*, *JlpA*, *CadF* y *CapA*) y la invasión (*CiaB*).

**Palabras clave:** *Campylobacter*; Pollos de engorde; Control microbiológico; PCR.

## 1. Introduction

Among the pathogens involved in foodborne outbreaks related to chicken meat, *Campylobacter* spp. are the most prevalent (Camino et al., 2017; Gourley et al., 2017). *Campylobacter* spp. infection, known as campylobacteriosis, is a worldwide zoonosis of serious public health concern, causing gastroenteritis in humans. Consumption of badly cooked poultry meat is one of the main risk factors associated with infection (Freitas and Noronha, 2007; Sharma et al., 2016).

Worldwide, *Campylobacter* spp. are among the main pathogens causing bacterial gastroenteritis (Li et al., 2018). They are gram-negative, microaerophilic bacteria with respiratory metabolism (Gorman and Adley, 2004; Wainwright et al., 2005). *Campylobacter jejuni* and *Campylobacter coli* have long been the most common species associated with cases of campylobacteriosis and other bacteremic infections (Iraola et al., 2014; Maziero and Oliveira, 2010). The *Campylobacter* infectious dose is approximately 500 colony-forming units/g, subject to the individual's physical condition or age (Granić et al., 2009). The infection is caused by virulence mechanisms involved in the production of toxins, motility flagella, adhesion, and epithelial invasion (Modi et al., 2015).

Worldwide, different health and food safety bodies, including the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO), have reported campylobacteriosis as the most prevalent foodborne disease, with rates higher than those reported for salmonellosis and shigellosis (Platts-Mills et al., 2014; WHO, 2013). Thus, a burden has been placed on both the food sector, owing to the need for monitoring and analyzing meat products to maintain the standard of food safety, and on public health, in terms of the costs of diagnosis and treatment (Naravaneni and Jamil, 2005).

In Brazil, research on *Campylobacter* is limited compared to developed and developing countries; however, over the years, studies conducted in different regions of Brazil have shown varying rates of *Campylobacter* prevalence, between 11% and 98% (Franchin et al., 2007; Kuana et al., 2008; Azeredo et al., 2010; Carvalho et al., 2013; Feistel et al., 2012; Silva,

2016). Although campylobacteriosis is classified as a foodborne disease and there is a specific information system for the investigation and notification of outbreaks and diseases, there is no specific Brazilian legislation for the control of *Campylobacter* (Brasil, 2010). This lack of control regarding the contamination of chicken meat by *Campylobacter* can compromise the food security of chicken meat considering that it is one of the most consumed meat products in Brazil and worldwide. In this study, we report the detection of *Campylobacter* spp. and virulence genes in chicken carcasses sold in different commercial establishments in a municipality in the northeast region of Brazil.

## 2. Methodology

### 2.1 Sample Location and Collection

Samples of broiler carcasses were acquired in two types of commercial establishments: three public markets and three supermarkets in Sanitary District III in Recife-PE. The carcasses marketed in the establishments originated from municipalities in one of the states in Northeast Brazil.

The carcasses acquired in the public markets did not have any inspection seal (municipal, state, or federal), whereas those acquired in supermarkets had a Federal Inspection Seal (SIF). Ten samples of carcasses with livers and gizzards sold in fresh, chilled, and frozen forms were obtained from two different commercial brands, totaling 90 samples.

Samples were transported in isothermal boxes (2–8°C) with recyclable ice to the Meat and Derived Products Inspection Laboratory (LICPD) of the Department of Veterinary Medicine (DMV) of the Federal Rural University of Pernambuco (UFRPE).

Fresh and chilled samples of carcasses with livers and gizzards were processed upon arrival at the laboratory, whereas frozen samples were kept under refrigerated temperature for 24 h to promote thawing.

### 2.2 Microbiological analysis of the samples

Samples of chicken carcasses with livers and gizzards were analyzed following the guidelines of the International Organization for Standardization (ISO 10272-1) for the isolation and identification of *Campylobacter* spp. (ISO 2006).

The methodology provides for the enrichment of 25 g of sample in Bolton broth under microaerophilic conditions, with oven incubation at 37–42°C for 24–48 h. In the present study, a 1:10 dilution was adopted, and from the 25 g portion (pieces and skin of the neck, breast, wing, cloacal region, livers, and gizzards), 10 g of sample was weighed, placed in a sterile stomacher bag, and 90 mL of Bolton Broth was added to the selective enrichment medium containing 5% (v/v) blood from lysed and defibrinated blood from the equine and selective supplement.

The stomacher bags, containing prepared samples and the selective enrichment medium, underwent a pre-enrichment stage under microaerophilic conditions at 37°C for 4 h ± 1 h and an enrichment stage under the same conditions at 42°C for 48 h. After the enrichment period, each sample was homogenized and isolated on plates of modified charcoal cefoperazone agar deoxycholate (m-CCDA) and Campy Cefex, both supplemented with cefoperazone and amphotericin B. Samples were subsequently incubated under the same temperature conditions and period mentioned above.

Subsequently, colonies suspected of being *Campylobacter*, based on colony morphology for each medium used, were selected and replicated for new cultures on Columbia blood agar (CBA) supplemented with defibrinated sheep blood, following the same incubation pattern.

Colonies obtained from the plates were stored and frozen at -20°C in Eppendorf microtubes containing ultrapure water for subsequent DNA extraction and confirmation of *Campylobacter* spp. by conventional PCR, while some were frozen under the same temperature conditions in microtubes containing 1 mL of brain-heart infusion broth (BHI) with 20% glycerol (v/v).

All analyses were conducted in parallel with control strains of *C. jejuni* (ATCC 29428) and *C. coli* (CCAMP 1068).

### 2.3 DNA extraction

DNA was extracted from colonies obtained by cultivation and from the 15 mL aliquots of the enrichment broth, with the extraction process carried out in parallel with the microbiological analysis of the samples.

Genomic DNA was extracted from isolates via thermal extraction. Tubes were placed in a dry bath at 90°C for 15 min, then in a freezer for 15 min, followed by centrifugation for 5 min at 14 000 × g. The supernatants were stored in a freezer at -20°C and used as target DNA for PCR analysis.

The extraction process from the enrichment broth was performed using the commercial Wizzard® Genomic DNA Purification kit (Promega®) according to the manufacturer's protocol. Samples were stored in a freezer at -20°C until PCR analysis.

### 2.4 Molecular identification of *Campylobacter* spp.

Specific primers for *C. jejuni* and *C. coli* were used to confirm species from the isolates and enrichment broth (Table 1). PCR followed the methodology by Casaril (2010). Subsequently, 6 µL of the amplified DNA products were transferred, to which 0.5 µL of Bluegreen® and 1 µL of 1.5% or 2% agarose buffer were added, along with a 100 bp Ladder® molecular weight marker, and submitted to electrophoresis, under ultraviolet light and photo-documented by the gel documentation system, following conditions established by the laboratory protocols for carrying out the tests.

**Table 1.** Primers used in PCR reactions to detect *C. jejuni* and *C. coli*.

Primers <sup>a</sup>	Sequence (5' - 3')	Product	Reference
<i>mapA</i> -F	AGTCCTGGTGGTTTGAAGC	202 pb	Casaril, 2010
<i>mapA</i> -R	CCGCATTAAAATTCACATCG		
<i>CeuE</i> -F	ATGAAAAAATCTTTAGTTTTTGGCA	889pb	Casaril, 2010
<i>CeuE</i> -R	ATTTTATTATTTGTAGCAGCG		

<sup>a</sup>F= Forward, R= Reverse.  
Source: Authors.

### 2.5 Molecular detection of virulence genes

The detection of virulence genes was performed according to the methodologies recommended by the authors, using the following primer sequences: *Peb1* (5'-GCAGAAGGTAACTTGAGTCTATT-3') and (5'-TTATAAACCCATTTTTTCGCTA A-3') (Pei et al., 1993); *JlpA* (5'-CACAGGGAATCGACAGCATAGA-3') and (5'-ACGCTCCGCCATTAACATA-3') (Veras et al., 2016); *CadF* (5'-TTGAAGGTAATTTAGATATG-3') and (5'-CTAATACCTAAAGTTGAAAC-3'); *CiaB* (5'-TCATGCGGTGGCATTAGAATGGG-3') and (5'-AGGTCTAACTTCATCAACCCTTTGCCA-3') (Konkel et al., 1999); and *CapA* (5'-GGATCCATGGGTGTAAATGTTTCGTTTC-3') and (5'-GTCGACTTACCAAAGATAATTAAC TGAGC-3') (Ashgar et al., 2007).

The PCR consisted of a final volume of 12.5 µL, and all thermal profiles of each reaction had an initial stage of 15' at 95°C and a final extension stage of 10' at 72°C. Strains of *C. jejuni* (ATCC 29428) and *C. coli* (CCAMP 1068) were used as positive controls; for the negative control, a compound from all the constituents of the reaction mix was used without the

addition of DNA. The reactions of the amplified products were visualized following the same protocol used to confirm the species.

### 3. Results and Discussion

#### 3.1 Detection of *C. jejuni* and *C. coli* in chicken carcasses marketed according to the type of establishment

All results presented were from samples from Sanitary District III in Recife-PE.

Results of the analyses using PCR to detect *C. jejuni* and *C. coli* in chicken carcasses sold in different types of commercial establishments are shown in Tables 2 and 3, respectively.

**Table 2.** Detection of *C. jejuni* and *C. coli* in chicken carcasses according to the type of establishment.

Type of Establishment	Number of samples	<i>C. jejuni</i>	<i>C. coli</i>
Market	30	4 (13,3%)	5 (16,7%)
Supermarket	60	22 (36,7%)	9 (15,0%)
Total	90	26 (28,8%)	14 (15,6%)

Source: Authors.

The present study showed a higher prevalence of *C. jejuni* than *C. coli* in chicken carcasses sold in commercial establishments. Various worldwide studies on the prevalence of *Campylobacter* in poultry products have indicated that *C. jejuni* is the main species involved in cases of campylobacteriosis in humans, with detection rates of up to 90% (EFSA, 2019). In turn, *C. coli* is the second most prevalent species, with detection rates between 5% and 10% (CDC 2018).

**Table 3.** Use of PCR to detect *C. jejuni* and *C. coli* in chicken carcasses from public markets and supermarkets.

Establishment	Number of samples	<i>C. jejuni</i>	<i>C.coli</i>
Market 1	10	0	1 (10,0%)
Market 2	10	4 (40,0%)	3 (30,0%)
Market 3	10	0	1 (10,0%)
Total	30		
Supermarket 1	24	8 (33,3%)	5 (20,8%)
Supermarket 2	24	8 (33,3%)	1 (4,2%)
Supermarket 3	12	5 (41,7%)	3 (25,0%)
Total	60		

Source: Authors.

Regarding the presence of the two species based on the commercial establishment, *C. coli* was more prevalent than *C. jejuni* in samples from public markets. A large number of studies have shown a higher prevalence of *C. jejuni* than *C. coli* in chicken carcasses (EFSA, 2019); however, it is important to mention that, depending on the material sampled, the origin of the samples, and storage conditions, the rates of detection can vary considerably (Melo et al., 2013).

*C. coli* is more susceptible to refrigeration or freezing storage conditions than *C. jejuni* (Suzuky and Yamamoto, 2009; Wei et al., 2016); therefore, it is suggested that the higher positivity of *C. coli* in this study was associated with the sale of

carcasses at room temperature.

The higher occurrence of *C. coli* concerning to *C. jejuni* in public markets can be associated with two factors: origin and health status of the lots, and temperature of the commercialization process. According to Wegener (2010), in some regions of cities, products from bird flocks with lower sanitary status than those from flocks sold in supermarkets are commonly supplied; the public establishments in this study were in such peripheral regions. Regarding the temperature factor, in harvesting places, the sale of carcasses at room temperature was predominant, a factor favoring the growth of *C. coli*, whereas even *C. jejuni* growing at room temperature had better resistance to the storage conditions employed (CDC, 2014).

In supermarket samples, *C. jejuni* had higher detection rates than *C. coli*. *C. jejuni* has been reported to be the most prevalent species (EFSA, 2019; Kudirkienė et al., 2013; Praakle-Amin et al., 2007), consistent with the results in this study. It is worth mentioning that even though there is a difference between the percentages of positivity between species, one of the most important factors that imply high prevalence rates is the contamination of chicken carcasses by *Campylobacter*.

### 3.2 Detection of *C. jejuni* and *C. coli* in fresh, chilled, and frozen chicken carcass samples

The results of the analyses using the PCR technique to detect *C. jejuni* and *C. coli* in fresh, chilled, and frozen chicken carcass samples and types of chicken carcass samples (pieces), livers, and gizzards are presented in Tables 4 and 5.

**Table 4.** Results of the detection of *C. jejuni* and *C. coli* in fresh, frozen, and frozen chicken carcasses.

Samples	Number	<i>C. jejuni</i>	<i>C. coli</i>
Fresh	30	4 (13,3%)	5 (16,7%)
Cold	30	13 (43,3%)	4 (13,3%)
Frozen	30	9 (30,0%)	0
Total	90		

Source: Authors.

The profile of the occurrence of *C. jejuni* in different forms of the samples showed the highest detection rate in chilled carcasses, followed by frozen carcasses, with the lowest rate in fresh carcasses, thus, demonstrating the viability of *C. jejuni* under three different commercialization processes. According to Sampers et al. (2010) and CDC (2014), the storage temperature of the carcasses reduces the final counts of *Campylobacter* in chicken meat, which may explain the decrease in detection rates between the chilled and frozen carcasses as the treatment applied in frozen carcasses is more intense.

By comparing the detection rates of *C. jejuni* between samples subjected to heat treatment (cooling and freezing) and carcasses kept at room temperature, a lower detection rate was found in fresh samples, even though the latter were in favorable conditions for the multiplication of the bacteria. Lee et al. (1998) pointed to the ability of *Campylobacter* to replicate at 4°C and room temperature, which justified the higher detection rate of *C. jejuni* in chilled samples.

According to Birk et al. (2004), chicken meat is composed of protective compounds such as peptides and lipids, which prolong the viability of *C. jejuni* during storage. The use of cooling further enhances this characteristic, which may explain the higher percentage of detection obtained for chilled carcasses in this study.

There was a reduction in the detection rate of *C. jejuni* in frozen carcasses when compared to that of chilled carcasses. According to ISO (2006), *Campylobacter* spp. are highly sensitive to freezing; however, owing to their ability to assume a viable but non-cultivable form (VNC), they can remain in food as a sign of favorable conditions. As the enrichment broth was

used in this study in processing the samples to recover cells, it is believed that the cells of the microorganisms present in the frozen carcasses were greatly stimulated by the conditions provided, favoring their detection.

It is important to note that the higher or lower detection rate in frozen carcasses may be associated with the origin and health status of chicken batches. In Denmark, Wegener (2010) found that chicken flocks positive for *Campylobacter* were generally used in the production of frozen birds, one of the forms of control of *Campylobacter* used in the country. In Brazil, unlike what occurs in other countries, the control of *Campylobacter* is not carried out, showing that birds of positive flocks can reach the consumer market through the three different forms of commercialization.

The type of slaughter used can be a compromising factor concerning greater or lesser contamination of carcasses. In this study, less contamination with *C. jejuni* was found in fresh carcasses than in chilled and frozen samples. This could be explained by the manual slaughter used in public markets to obtain fresh carcasses. In addition, a smaller number of birds are slaughtered in public markets than that for chilled and frozen carcasses that pass through the slaughter platform, thus, potentially favoring contamination by *Campylobacter* for the time that the carcasses spend in the slaughter line (Wei et al., 2016).

The profile of the occurrence of *C. coli* in the three commercialization processes occurred in a descending manner, with a detection rate of 16.7% in fresh samples. There was a percentage reduction to 13.3% in positive samples of carcasses subjected to cooling, with no detection in carcasses where freezing was employed, showing the inherent sensitivity of *C. coli* to the thermal stress generated by the use of conservation methods. Corroborating with observations similar to the present study, Maziero and Oliveira (2010) evaluated the same quantity of frozen samples and did not obtain positivity for *C. coli*.

Abd El-Aziz and Abd-Allah (2017) obtained superior results when assessing the presence of *C. coli* in fresh carcasses, with 87.5% of the samples positive. In their study, Igwaran and Okoh (2020) evaluated the presence of different species of *Campylobacter* in different types of chilled meat at retail and slaughterhouses, including chicken meat, and obtained a prevalence rate of 22.08% for *C. coli*.

**Table 5.** Results of the detection of *C. jejuni* and *C. coli* in carcasses of chicken, liver, and gizzards.

Samples	Number	<i>C. jejuni</i>	<i>C. coli</i>
Pieces	30	11 (36,7%)	3 (10%)
Liver	30	16 (53,3%)	1 (3,3%)
Gizzard	30	10 (33,3%)	1 (3,3%)
Total	90		

Source: Authors.

The prevalence of *C. jejuni* in the samples of meat pieces was 36.7%, the second-highest percentage among the different types of meat (beef, pork, and sheep). Most of the studies that searched for *Campylobacter* spp. in chicken carcasses, whether whole or in pieces of carcasses, use the rinsing methodology or cut pieces. According to Hansson et al. (2014), the presence of skin in the samples greatly influences the achievement of positive results. Similarly, Sampers et al. (2010) observed a high incidence of bacteria when analyzing meat preparations with skin. The exposed data corroborated the prevalence found in this study, as the fragments of carcasses collected to compose the samples of meat pieces were collected with skin.

Among the different samples, *C. jejuni* occurred in more than half of the liver samples, followed by considerable percentages for the samples of meat pieces and gizzards. Detection rates for *C. jejuni* and *C. coli* species in chicken liver vary

from 10% to 90% (Chaloner et al., 2014; Firlieyanti et al., 2016; Whyte et al., 2006).

In gizzard samples, the detection rate of *C. jejuni* was 33.3% positive. Bouffleur (2009) obtained a prevalence of 44.4% positive samples, a higher percentage than the present study. A higher prevalence was also verified by Trassi (2012), who analyzed samples of gizzards from slaughterhouses, obtaining 50.0% positivity. A lower percentage of *C. jejuni* than that in this study was verified by Chaves et al. (2010), who analyzed the same quantity of gizzards and obtained only 3.3% of positive samples for *C. jejuni*. The same percentage (3.3%) found for *C. jejuni* by the author was also found for *C. coli*.

Gutting has been identified as a critical point for contamination in the slaughter of carcasses and chicken products (liver and gizzards) (Rosenquist, 2006). Once contaminated, the products pass through the slaughter flow and are sent to the market. Research also points to the long-term survival of *Campylobacter* for long periods in retailed chicken products (Birk et al., 2006; El-Shibiny et al., 2009; Solow et al., 2003). This explains the reported outbreaks associated with the consumption of this food type (Glashower et al., 2017; Hanson et al., 2014; Scott et al., 2015; Tompkins et al., 2013).

The prevalence of *C. coli* in the three different products was considerably lower than that in *C. jejuni*, with detection rates of 10.0% for lump samples and 3.3% for livers and gizzards. The detection of the highest prevalence of the species in samples of meat pieces further confirmed that the presence of skin in the samples favored the detection of the bacteria. The lower percentage of positivity could also be associated with the low population of microorganisms in different samples and their fastidious nature (Vandamme, 2000), slowing the growth of *Campylobacter*, which suffers the action of competing microbiota. Thus, it is suggested that the growth of competing microbiota is different from that of *Campylobacter* and that when using detection techniques, in this case, PCR the amount of target DNA of the species in question does not present detectable levels in the reaction.

### 3.3 Detection of virulence genes in positive samples for *C. jejuni* and *C. coli*

The results obtained from the analyses using the PCR technique to detect the virulence genes *Peb1*, *JlpA*, *CadF*, *CiaB*, and *CapA* of the 26 positive samples for *C. jejuni* and 14 for *C. coli* are described in Table 6.

**Table 6.** Detection of virulence genes in positive samples for *C. jejuni* and *C. coli*.

Virulence genes	<i>C. jejuni</i>	<i>C. coli</i>
<i>Peb1</i>	13 (43,2%)	7 (50%)
<i>JlpA</i>	18 (48,6%)	6 (42,8%)
<i>CadF</i>	27 (72,9%)	7 (50%)
<i>CiaB</i>	26 (70,2%)	10 (71,4%)
<i>CapA</i>	8 (21,6%)	4 (28,5%)

Source: Authors.

The virulence genes studied are associated with the virulence of *Campylobacter* spp. and encode proteins involved in adhesion, invasion, and colonization. The presence of each of these genes suggested a biological and potentially pathogenic action involved in *Campylobacter* infection, and the mechanism by which they cause diseases in humans is multifactorial (Chukwu et al., 2019; Silva et al., 2011).

In the molecular analysis of the presence of five virulence genes for *C. jejuni* and *C. coli*, *CiaB* and *CadF* genes showed remarkable detection rates. These genes are associated with the invasion and colonization of host intestinal cells (Cróinín et al., 2012; Wiczorek et al., 2012). Different studies have reported the absence of the *CiaB* gene when analyzing

samples of chicken meat, at retail positive for *C. jejuni* and *C. coli* (Igwaran and Okoh, 2020), to 100% prevalence in stool samples from children with diarrhea positive for *C. jejuni* (Ghorbanalizadgan et al., 2014). For the *CadF* gene, there are reports of 100.0% prevalence of the gene for both species (Biswas et al., 2011; Ghunaim et al., 2015; Koolman et al., 2015).

Another gene detected was the *Peb1* gene, which encodes the protein *Peb1*, an adhesin located in the periplasm, whose function is related to adhesion to host cells (Pei et al., 1998). The prevalence was 43.2% and 50.0% in *C. jejuni* and *C. coli* samples, respectively. Kim et al. (2019) reported detection rates of the gene in chicken meat samples at retail positive for *C. jejuni* (93.3%) but did not include *C. coli* in the study.

The prevalence of the *JlpA* gene in *C. jejuni* and *C. coli* samples was similar, with 48.6% and 42.8% positivity, respectively. There are reports of detection of the gene with the detection range between 43.1% and 96.1% (Biswas et al., 2011; Koolman et al., 2015; Veras et al., 2016); however, these studies were performed only with samples positive for *C. jejuni*. In the present study, the *JlpA* gene was detected in both *C. jejuni* and *C. coli*, with similar percentages, noting that most studies researched the expression of this and other genes only in the species *C. jejuni*. The *JlpA* gene is responsible for the secretion of lipoproteins that act as adhesins, linking microorganisms to epithelial cells (Jin et al., 2001).

The lowest detection rate was for *CapA*, with 21.6% and 28.5% for *C. jejuni* and *C. coli*, respectively. This gene also encodes proteins related to adhesion to epithelial cells and acts as a cell colonization factor (Ashgar et al., 2007).

### 3.4 Description of virulence profiles for *C. jejuni* and *C. coli* species

Description of virulence profiles of the 26 *C. jejuni* and 14 *C. coli* samples are presented in Table 7.

**Table 7.** Virulence profiles concerning the genes detected in the species *C. jejuni* and *C. coli*.

Virulence profile	<i>C. jejuni</i>	<i>C. coli</i>
P-1: <i>Peb1</i> , <i>JlpA</i> , <i>CadF</i> , <i>CiaB</i> , <i>CapA</i>	0 (0%)	1 (7,1%)
P-2: <i>Peb1</i> , <i>JlpA</i> , <i>CadF</i> , <i>CiaB</i>	4 (15,3%)	2 (14,2%)
P-3: <i>Peb1</i> , <i>JlpA</i> , <i>CadF</i>	0 (0%)	1 (7,1%)
P-4: <i>Peb1</i> , <i>JlpA</i> , <i>CiaB</i>	3 (11,55%)	0 (0%)
P-5: <i>Peb1</i> , <i>CadF</i> , <i>CiaB</i>	1 (3,8%)	1 (7,1%)
P-6: <i>Peb1</i> , <i>CadF</i>	2 (7,6%)	1 (7,1%)
P-7: <i>Peb1</i> , <i>CiaB</i>	1 (3,8%)	1 (7,1%)
P-8: <i>Peb1</i> , <i>CapA</i>	0 (0%)	0 (0%)
P-9: <i>Peb1</i>	0 (0%)	1 (7,1%)
P-10: <i>JlpA</i> , <i>CadF</i> , <i>CiaB</i> , <i>CapA</i>	1 (3,8%)	1 (7,1%)
P-11: <i>JlpA</i> , <i>CadF</i>	1 (3,8%)	0 (0%)
P-12: <i>JlpA</i> , <i>CadF</i> , <i>CiaB</i>	0 (0%)	0 (0%)
P-13: <i>JlpA</i> , <i>CiaB</i> , <i>CapA</i>	0 (0%)	2 (14,2%)
P-14: <i>JlpA</i> , <i>CapA</i>	0 (0%)	1 (7,1%)

P-15: <i>CadF, CiaB</i>	1 (3,8%)	2 (14,2%)
P-16: <i>CadF, CiaB, CapA</i>	0 (0%)	0 (0%)
P-17: <i>CadF</i>	2 (7,6%)	0 (0%)
P-18: <i>CiaB, CapA</i>	1 (3,8%)	1 (7,1%)
P-19: <i>CiaB</i>	2 (7,6%)	0 (0%)
P-20: Absence of all genes	2 (7,6%)	2 (14,2%)

Source: Authors.

From gene detection, 20 virulence profiles were constructed for each species. The constructed profiles contained everything from the presence of the five genes to the absence of all of them.

The P-1 profile (*Peb1, JlpA, CadF, CiaB, CapA*) is noteworthy for grouping the five genes, although it was detected only in *C. coli* samples and was absent in *C. jejuni* samples. This profile showed a variation in the expression of virulence factors between *C. jejuni* and *C. coli*. It is worth mentioning that there is no description in the literature on the detection of the gene profiles described in this study, which is important to study because, as these genes are associated with the ability of the cells to adhere to and colonize the host (Silva et al., 2011).

The P-2 profile (*Peb1, JlpA, CadF, CiaB*) was the second most prevalent, and unlike the P-1 profile, it occurred in both species, with the presence of four of the studied genes being verified. The presence of these genes gives species the ability to adhere and colonize host cells (Veras et al., 2016).

There was variation in other profiles for their detection for both species. However, it is worth mentioning the presence of the profiles P-5 (*Peb1, CadF, CiaB*), P-6, P-7 (*Peb1, CiaB*), P-10 (*JlpA, CadF, CiaB, CapA*), P-15 (*CadF, CiaB*), and P-18 (*CiaB, CapA*), all of which, even with different detection rates between species, were detected in both *C. jejuni* and *C. coli* samples. Additionally, in all profiles, there was the presence of the *CiaB* gene and in four of them the presence of the *CadF* gene, thus signaling that their expression by the species is a mechanism that gives it marked virulence.

The absence of the five genes was represented by the P-20 profile expressed for both species. Even in the absence of the detected genes, it was not possible to state that the species are not virulent, considering the diversity of other virulence genes that exist and that may confer an even greater capacity than those of the present study.

In recent years, *Campylobacter* spp. has been recognized an emerging pathogen and indicator of gastroenteritis worldwide. The main risks associated with infections by this pathogen are contaminated chicken carcasses in slaughterhouses, post-slaughter treatments, temperature control, and hygiene management during processing or storage (Rozynek et al., 2005; Stern et al., 2001). The combination of these factors associated with the presence of *Campylobacter* spp. with different virulence profiles detected warns of the potential risk for humans in the appearance of cases and/or outbreaks of *Campylobacter* infections if appropriate measures are not implemented.

#### 4. Conclusion

*C. jejuni* and *C. coli* occur in different poultry products (carcasses, livers, and gizzards) sold in different establishments in northeastern Brazil, with *C. jejuni* being the most prevalent species.

The study revealed that even with the use of cooling and freezing in food, it was possible to recover *Campylobacter*, demonstrating that the methods act as a limiter, but do not eliminate the bacteria from the product.

The PCR assay proved to be a suitable method for detecting virulence gene species.

The presence of virulence genes played a role in the ability of *C. jejuni* and *C. coli* to remain viable in food.

There should be continued surveillance for the presence of these pathogens and their genes associated with poultry products. Control measures must be established from the field to the industry. The use of agents such as bacteriophages and probiotics is a promising alternative in the biocontrol of foodborne pathogens such as *Campylobacter* at the field level. In terms of industry, strengthening the monitoring of handling measures and practices during processing continues to be essential to reduce the contamination of poultry products and the probable risk of infection. Additionally, awareness-raising measures must be taken on the risks associated with the consumption of undercooked meat and on the care that the consumer must take in handling these food products to avoid possible cross-contamination, to preserve their food safety.

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