Pathogenic potential and genotypic diversity of Campylobacter jejuni: a neglected food-borne pathogen in Brazil

Miliane Rodrigues Frazão, Marta Inês Cazentini Medeiros, Sheila da Silva Duque and Juliana Pfrimer Falcão

Abstract

Purpose and methodology. Campylobacter jejuni is a major zoonotic pathogen that causes food-borne gastroenteritis worldwide. However, there are only a few studies available that have molecularly characterized C. jejuni strains isolated in Brazil. The aim of this study was to genotype 111 C. jejuni strains isolated from sick humans (43), monkey faeces (19), chicken faeces (14), chicken meat (33) and sewage (2) between 1996 and 2016 in Brazil using flaA-SVR (short variable region) sequencing and PFGE. Furthermore, the presence of 16 virulence genes was analysed by PCR.

Results. Using PFGE and flaA-SVR sequencing, the 111 C. jejuni strains studied were grouped into three and two clusters, respectively, and some strains of different origin presented a similarity of ≥80 %. In total, 35 flaA-SVR alleles were detected. Alleles gt65, gt69 and gt57 were the most prevalent, in contrast with those frequently described in the PubMLST database. All 111 C. jejuni strains contained the genes flaA, flaB, cadF, docA, cdtA, cdtB, cdtC, iamA, ciaA, sodB, dnaJ, pldA, racR and csrA. The wlaN gene was detected in 11 strains (9.9 %), and the virB11 in just one strain (0.9 %).

Conclusions. In conclusion, the pathogenic potential of the C. jejuni strains studied was highlighted by the high frequency of the majority of the virulence genes searched. The flaA-SVR sequencing and PFGE results showed that some of the strains studied presented a high genotypic similarity, suggesting potential for transmission between animal sources and humans in this country. Altogether, the results characterize further C. jejuni isolates from Brazil, an important producer and exporter of chicken meat.

INTRODUCTION

Campylobacter jejuni is a major zoonotic pathogen that causes food-borne gastroenteritis worldwide [1–3]. According to the European Food Safety Authority, in 2013 in 32 European countries, campylobacteriosis was the most commonly reported gastrointestinal bacterial pathogen in humans, with approximately 215 000 cases and a rate of 64.8 per 100 000 population [4]. In the USA, Campylobacter species are the most common cause of diarrhoea in humans, which affects over 2 million people annually [5].

Campylobacteriosis can be acquired by humans through consumption of undercooked or contaminated food, particularly undercooked chicken, contaminated water, unpasteurized milk and contact with some animals, such as wild birds, poultry, cattle, pigs and domesticated animals [6–10].

Campylobacter pathogenicity is associated with genes located on the chromosome and on the virulence plasmid pVir when present [11, 12]. Chromosomal genes such as cadF, dnaJ, docA and racR are responsible for adherence and colonization, and the flaA and flaB genes are necessary for motility. The cytotoxins are encoded by the cdtA, cdtB and cdtC genes. The iamA, ciaA and pldA genes are responsible for the invasion and survival in host cells, and the sodB and csrA genes provide protection against oxidative stress [6, 13, 14]. The wlaN gene was considered to be related to the Guillain–Barré syndrome [15]. The virB11 is a plasmidial gene located in the plasmid pVir, which encodes a type IV secretion system possibly involved in adherence [16, 17]. However, not all genes associated with virulence are currently known [12, 18].

Various molecular techniques, such as sequencing of the short variable region (SVR) of the flaA gene, PFGE and
multilocus sequence typing (MLST), among others, have been used successfully worldwide to study the epidemiology and genotypic diversity of Campylobacter spp. [19–29].

Clinical cases of campylobacteriosis have been underreported and underdiagnosed, and molecular studies of C. jejuni isolates are scarce in Brazil [30–34]. The majority of the studies performed in this country have investigated the occurrence and antimicrobial resistance profile of C. jejuni strains [35–41]. In this way, additional studies would help to assess the involvement of Campylobacter spp. in the causation of illness in humans and animals, and to determine the impact of its presence in non-clinical sources in Brazil.

In 2014, Brazil produced 12 691 000 tonnes of chicken meat and exported 4 099 000 tonnes. These values rank Brazil as the world’s largest poultry exporter since 2004 and the third largest producer of chicken meat, surpassed only by the USA and China [42]. Among the main importers are Saudi Arabia, South Africa, Jordan, Turkey and the European Union [43]. However, only a few studies have evaluated the occurrence of Campylobacter spp. isolated from chicken meat in Brazil [33, 36, 37, 40].

Therefore, the aims of this study were to assess the presence of certain virulence genes by PCR and to evaluate the genotypic diversity, using flaA-SVR sequencing and PFGE on C. jejuni strains isolated from humans, animals, chicken meat and sewage between 1996 and 2016 in Brazil, to better understand the pathogenic potential and genotypic diversity of strains of this species isolated in this country, an important chicken meat producer and exporter.

METHODS

Bacterial strains

A total of 111 C. jejuni strains were studied. Those strains were isolated from humans (43 strains), monkey faeces (19 strains), chicken faeces (14 strains), chicken meat (33 strains) and sewage (2 strains) from cities of Minas Gerais, São Paulo, Rio de Janeiro and Rio Grande do Sul states located in the southeast and southern regions of Brazil, between 1996 and 2016. Specifically, the strains isolated from monkeys were isolated from captive individuals of the species saimiri, rhesus and cynomolgus. Also, some strains were isolated from wild marmosets.

These strains were selected from the collections of the Campylobacter Reference Laboratories of the Oswaldo Cruz Institute of Rio de Janeiro (Fiocruz-RJ) and of the Adolfo Lutz Institute of Ribeirão Preto (IAL-RP) in Brazil. They were systematically chosen to represent isolates from sporadic cases, and from different years, from different clinical and non-clinical samples of the two collections of the above reference laboratories. Table S1 (available in the online Supplementary Material) summarizes the characteristics of the 111 C. jejuni strains used in this study.

Genus and species confirmation

The genomic DNA of the strains listed in Table S1 was extracted according to Campioni and Falcão [44], with a few modifications. Specifically, the strains were cultured at 42 °C on BBL Columbia Agar Base (Becton Dickinson), supplemented with charcoal (Neon) and FBP (0.5 % ferrous sulphate (Labsynth), 0.5 % sodium pyruvate (Vetec) and 0.5 % metabolisable sulphate (Labsynth) diluted in sterile water) under micro-aerobic conditions (10 % CO₂, 5 % O₂ and 85 % N₂), and the growing strains were placed directly in solution 1 (20 % sucrose, 50 mM Tris/HCl, pH 8.0, 50 mM EDTA) of the extraction protocol.

The purity of the genomic DNA was estimated as described by Sambrook and Russell [45], and its quantity was determined using NanoDrop 1000 (Thermo Fisher Scientific). Molecular confirmation of the genus and species was performed using specific regions of the genes 16S rRNA, ceuE and mapA, as described by Denis et al. [46].

Detection of virulence genes by PCR

The general PCR procedure was performed according to the method described by Falcão et al. [47]. The primers and PCR conditions used to detect virulence genes were described previously by Wassenaar and Newell [21] (for flaA), Müller et al. [48] (for flaA, docA and ianA), Konkel et al. [49] (for flaA), Hichey et al. [50] (for flaA), Datta et al. [14] (for cdtB, cdtC, virB11, dnal, pldA and racB), Rivera-Amill et al. [51] (for ciaB), Wassenaar et al. [52] (for wlaN), Biswas et al. [53] (for sodB) and Fields and Thompson [54] (for csrA). A template PCR without DNA was used as a negative control. C. jejuni strain ATCC 33291 was used as a positive control for all the genes searched. The PCR products were analysed by agarose gel electrophoresis and visualized by UV light after staining the gel with ethidium bromide (0.5 μg ml⁻¹).

flaA-SVR sequencing and analysis

Genomic DNA extraction of the 111 C. jejuni strains listed in Table S1 was performed as described above. All amplifications of the flaA gene were performed in a total volume of 50 μl, with 25 pmol 5’-ATG GGA TTT CG TAT TAA 3’ and 5’-CGT TAG TAA TCT TAA AAC ATT TCT 3’ primer pair described by Wassenaar and Newell [21] (Integrated DNA Technologies), 1× PCR buffer (Life Technologies), each deoxyribonucleotide at 0.2 mmol l⁻¹ (Life Technologies), 2 mmol l⁻¹ MgSO₄ (Life Technologies), 1 U Taq DNA Polymerase High Fidelity (Life Technologies) and 50 ng DNA template. A sample of the complete mix without DNA was used as a negative control. The PCR was carried out in a Mastercycler (Eppendorf). Amplicons of 1713 bp were detected by electrophoresis in 1.5 % agarose gel stained with ethidium bromide (0.5 μg ml⁻¹) and observed under UV light. Afterwards, the amplicons were purified for sequencing with a PureLink Quick PCR Purification kit (Life Technologies) according to the manufacturer’s recommendations.
To perform sequencing of the SVR of the flaA gene, the purified amplicons were resubmitted to amplification using the 5′-CTA TGG ATG AGC AAT T(ATT)A AAA T-3′ and 5′-CAA G(ATT)C CTG TTC C(ATT)A CTG AAG-3′ primer pair (Integrated DNA Technologies), as described by Meinersmann et al. [19]. Automated DNA sequencing was performed with ABI 3500xL (Life Technologies).

The dendrogram was generated using the software package BioNumerics 7.0 (Applied Maths), using the UPGMA (unweighted pair group method with arithmetic mean) method with the Jukes–Cantor distance correction model. Bootstrap values (1000 samples) were used to estimate the robustness of the phylogenetic analysis. The flaA-SVR nucleotide allele nomenclature was obtained on the Oxford database at http://pubmlst.org/campylobacter.

**PFGE typing and analysis**

The bacterial strains were grown as described above. The genomic DNAs of all isolates were prepared in agarose blocks using the CDC Pulsenet protocol for C. jejuni [20]. The plugs were digested with 40 U Smal (Life Technologies) at 25 °C for 2 h.

Macro-restriction fragments were resolved by counter-clamped homogeneous electric field electrophoresis in a CHEF-DR III apparatus (Bio-Rad Laboratories) as described by Gomes et al. [29]. The pulse times were increased from 6.8 to 35.4 s over 19 h, as described by Ribot et al. [20].

Salmonella serotype Braenderup H9812 strain, digested with 40 U XbaI at 37 °C for 2 h, was used as a reference for the molecular mass standard and was included three times on each gel to allow comparison of the fingerprinting over several gels. The gels were stained with ethidium bromide (0.5 µg ml⁻¹) for 30 min and destained in distilled water for 80 min. The restriction fragments were viewed under UV light.

Relatedness among the PFGE profiles was analysed using the software package BioNumerics 7.0 (Applied Maths). Only bands that represented fragments between 20.5 and 1135 kb in size were included in the analysis. A similarity dendrogram was constructed by the UPGMA method using the Dice similarity coefficient and a position tolerance of 1.5 %.

**Discrimination index**

The discriminatory index of flaA-SVR sequencing and PFGE was assessed by Simpson’s diversity index as described by Hunter and Gaston [55].

**RESULTS**

The results described below characterized 111 C. jejuni strains isolated from diverse clinical and non-clinical sources that represent the diversity of two important collections of Campylobacter Reference Laboratories in Brazil.

**Genus and species confirmation**

All the strains analysed in this study were confirmed as belonging to the species C. jejuni by the amplification of 16S rRNA and mapA genes.

**Detection of virulence genes by PCR**

Sixteen genes were analysed by PCR, and all 111 C. jejuni strains studied carried the genes flaA, flhA, cadF, docA, cdA, cdtB, cdtC, iamA, ciaB, sodB, dmal, pldA, racR and csaA. The wlaN gene was detected in 11 (9.9 %) strains, and the virB11 gene was found in only one (0.9 %) strain.

**flaA-SVR sequencing**

The dendrogram generated by flaA-SVR sequencing revealed 35 SVR types and grouped the 111 C. jejuni strains into two clusters, designated SVR-A and SVR-B, with a similarity of more than 83.4 % (Fig. 1).

Cluster SVR-A was composed of 13 (11.7 %) strains with ≥89.2 % similarity, which were isolated from humans (n=7), monkey and chicken faeces (n=5) and sewage (n=1) between 2004 and 2016 in São Paulo, Minas Gerais and Rio de Janeiro states (Fig. 1).

Cluster SVR-B comprised 98 (82.3 %) strains exhibiting a similarity of more than 91.2 %, which were isolated from humans (n=36), monkey and chicken faeces (n=28), chicken meat (n=33), and sewage (n=1) between 1996 and 2015 in São Paulo, Minas Gerais, Rio de Janeiro and Rio Grande do Sul states (Fig. 1).

According to flaA-SVR sequencing, 35 flaA-SVR alleles were detected. The flaA-SVR allele most frequently detected was gt57, with 19 (17.1 %) strains isolated from humans (n=3), monkey faeces (n=4) and chicken meat (n=12) between 2003 and 2009 in São Paulo, Minas Gerais and Rio de Janeiro states. The next most frequently detected flaA-SVR alleles were allele gt45, with 13 (11.7 %) strains isolated from humans (n=6) and chicken meat (n=7) between 1996 and 2010 in São Paulo, Minas Gerais and Rio de Janeiro, and allele gt49, with 13 (11.7 %) strains isolated from humans (n=8), monkey and chicken faeces (n=4) and chicken meat (n=1) between 1997 and 2014 in São Paulo, Minas Gerais and Rio de Janeiro states. Next most frequently detected was allele gt21, which was composed of nine (8.1 %) strains isolated from human (n=1), chicken faeces (n=3) and chicken meat (n=5) between 2004 and 2011 in Minas Gerais and Rio de Janeiro states. Alleles gt51, gt100, gt230 and gt287 comprised five (4.5 %) strains; allele gt551 comprised four (3.6 %) strains; allele gt53 comprised three (2.7 %) strains; and alleles gt14, gt190, gt274, gt595 and gt855 each comprised two (1.8 %) strains. The following 20 alleles were represented by only one strain: gt2, gt5, gt9, gt11, gt22, gt23, gt24, gt36, gt54, gt149, gt153, gt162, gt198, gt239, gt260, gt975, gt1336, gt1379, gt1440 and gt1651.

**PFGE**

The dendrogram constructed with the PFGE profiles obtained is shown in Fig. 2. PFGE revealed 64 different
Fig. 1. Dendrogram representing the genetic relationship among the 111 C. jejuni strains based on flaA-SVR sequencing and analysis using the Jukes–Cantor distance method and UPGMA algorithm. Phylogenetic analysis was performed using BioNumerics 7.0. Bootstrap (1000 samples) are shown behind the nodes, and the similarity values are presented in front. SP, São Paulo; MG, Minas Gerais;
types and grouped the 111 C. jejuni strains into three main clusters, designated PFGE-A, PFGE-B and PFGE-C. The similarity of the three clusters was above 47.7% (Fig. 2).

Cluster PFGE-A comprised 48 (43.2%) strains exhibiting a similarity of more than 50.4% isolated from humans, monkey and chicken faeces, chicken meat and sewage between 1996 and 2016 in São Paulo, Minas Gerais and Rio de Janeiro states. PFGE-A was subdivided into 10 sub-clusters composed of strains with ≥80% similarity, designated PFGE-A1 to PFGE-A10. Specifically, PFGE-A1, PFGE-A4, PFGE-A5, PFGE-A6, PFGE-A7 and PFGE-A8 comprised strains from different sources. Cluster PFGE-A1 included two strains exhibiting ≥82.4% similarity isolated from human and chicken faeces in 2003 and 2016 in São Paulo and Rio de Janeiro states. Cluster PFGE-A4 included two indistinguishable strains isolated from monkey faeces and sewage in 1999 in Rio de Janeiro state. Cluster PFGE-A5 included six strains exhibiting ≥89.8% similarity isolated from humans (n=2) and monkey and chicken faeces (n=4) between 1999 and 2013 in Minas Gerais, São Paulo and Rio de Janeiro states. Cluster PFGE-A6 was composed of seven strains with ≥93.3% similarity isolated from humans (n=7) and monkey faeces (n=1) in São Paulo and Rio de Janeiro states between 1997 and 2014. Cluster PFGE-A7 included three strains exhibiting ≥94.1% similarity isolated from human and chicken meat (n=2) in 2003 and 2008 in Minas Gerais and São Paulo states. Cluster PFGE-A8 included eight strains with more than 80.8% similarity isolated from humans (n=2) and monkey faeces (n=6) in Rio de Janeiro State between 1996 and 2013 (Fig. 2).

Cluster PFGE-B was composed of 25 (22.5%) strains that exhibited a similarity above 52.3% isolated from humans, chicken faeces and chicken meat in São Paulo, Minas Gerais, Rio de Janeiro and Rio Grande do Sul between 1996 and 2015. PFGE-B was subdivided into four sub-clusters composed of strains with ≥80% similarity, designated PFGE-B1 to PFGE-B4. Specifically PFGE-B1, PFGE-B2 and PFGE-B3 comprised strains from different sources. Cluster PFGE-B1 included 10 strains with more than 85.7% similarity isolated from humans (n=4), chicken faeces (n=1) and chicken meat (n=5) in Rio de Janeiro and Rio Grande do Sul states between 1996 and 2015. Cluster PFGE-B2 was composed of four strains with ≥94.1% similarity isolated from human (n=1) and chicken faeces (n=3) in Minas Gerais and São Paulo states in 2004 and 2006. Cluster PFGE-B3 contained seven strains that exhibited a similarity of more than 82.4% isolated from human (n=1) and chicken meat (n=6) in Minas Gerais and São Paulo states between 2004 and 2009 (Fig. 2).

Cluster PFGE-C comprised 38 (34.2%) strains with a similarity above 59.9% isolated from humans, monkey and chicken faeces, chicken meat, and sewage between 1996 and 2011 in Minas Gerais, São Paulo and Rio de Janeiro states. Cluster PFGE-C was subdivided into six sub-clusters composed of strains with ≥80% similarity, designated PFGE-C1 to PFGE-C6. Specifically PFGE-C1, PFGE-C2, PFGE-C3 and PFGE-C6 comprised strains from different sources. Cluster PFGE-C1 included 14 strains isolated from humans (n=4), monkey and chicken faeces (n=2) and chicken meat (n=8) between 1997 and 2009 in Minas Gerais, São Paulo and Rio de Janeiro states with a similarity above 81.5%. Cluster PFGE-C3 was composed of three strains isolated from humans (n=2) and monkey faeces (n=1) in 1996 and 2009 in São Paulo and Rio de Janeiro states with 85.7% similarity. Cluster PFGE-C6 was composed of five strains isolated from humans (n=1) and monkey faeces (n=4) in 2003 and 2009 in São Paulo and Rio de Janeiro states with 91.9% similarity (Fig. 2).

Discrimination index

The discrimination index (DI) was 0.932 and 0.980 by flaA-SVR sequencing and PFGE, respectively.

DISCUSSION

C. jejuni is a major food-borne pathogen that causes gastroenteritis worldwide [1, 4, 5]. However, this pathogen has been underdiagnosed and understudied in Brazil, and molecular studies that have characterized the genotypic diversity of C. jejuni strains are scarce in this country [31, 33, 34]. The majority of the Brazilian studies investigated the occurrence and antibiotic resistance profile of C. jejuni strains [35, 37, 38, 40, 41].

In this way, we investigated and characterized representative strains of C. jejuni isolated from diverse sources. Also, strains isolated from asymptomatic chickens and monkeys were chosen because these two animals have been reported as important C. jejuni carriers in other studies performed in this country [32, 33, 36]. Furthermore, we decided to analyse sewage isolates since the environment can be an important source of contamination for humans, animals and food.

The present study used flaA-SVR sequencing and PFGE to type 111 C. jejuni strains isolated from humans, monkey and chicken faeces, chicken meat and sewage between 1996 and 2016 from various cities in São Paulo, Minas Gerais, Rio de Janeiro and Rio Grande do Sul states, which are located in the southeast and southern regions of Brazil. Moreover, the presence of 16 virulence genes was
Sorted by the UPGMA method. SP, MG, RS, RJ, RS.

Fig. 2. Dendrogram representing the genetic relationship among the 111 *C. jejuni* strains based on PFGE Smal fingerprints. Similarity (%) between the patterns was calculated using the Dice index and is represented by the numbers beside the nodes. The data were sorted by the UPGMA method. SP, São Paulo; MG, Minas Gerais; RJ, Rio de Janeiro; RS, Rio Grande do Sul. Red, strains isolated from humans; blue, strains isolated from animals; yellow, strains isolated from chicken meat; green, strains isolated from sewage.
investigated by PCR to verify the pathogenic potential of those strains.

All 111 C. jejuni strains in this study contained the majority of the virulence genes analysed. All strains contained the genes flaA, fliA, cadF, docA, cdtA, cdtB, cdtC, iamA, ciaB, sodB, dnaJ, pldA, racR and csrA. The wlaN gene was detected in 11 (9.9 %) strains while the virB11 gene was found in only one (0.9 %) strain. Thus, the high frequency of the majority of the 16 virulence genes searched highlighted the pathogenic potential of the C. jejuni strains studied in this work.

Consistent with our work, virulence genes with high frequency were found in C. jejuni strains isolated in other countries [14, 53, 57, 58]. In a study by Datta et al. [14], performed with 111 C. jejuni strains isolated from human and cattle faeces in Canada. Koolman and colleagues [58] assessed the presence of various virulence genes in 17 C. jejuni strains isolated from poultry and clinical sources in Ireland and found that all of the strains contained the genes flaA, fliA, cadF, dnaJ, ciaB, sodB, cdtB and cdtC in 92 C. jejuni strains isolated from human faeces and poultry carcasses in Poland. The study performed by Biswas et al. [53] detected a high rate of genes dnaJ, pldA, ciaB, docA, sodB, racR, iamA and cadF in 102 C. jejuni strains isolated from human and cattle faeces in Canada. Koolman and colleagues [58] assessed the presence of various virulence genes in 17 C. jejuni strains isolated from poultry and clinical sources in Ireland and found that all of the strains contained the genes flaA, fliA, cadF, dnaJ, ciaB, sodB, cdtB and cdtC. The virB11 gene was detected in five (29 %) strains, and the wlaN gene was detected in only two (11 %) strains.

In the study of Melo and colleagues [33], performed in Brazil with 55 C. jejuni strains isolated from chicken carcasses, seven virulence genes were investigated. The genes flaA, pldA, cadF and ciaB and the CDT complex were detected in 41 (74.5 %), 35 (63.6 %), 37 (67.3 %), 37 (67.3 %) and 36 (65.5 %) strains, respectively. Quetz et al. [59] assessed the presence of the genes ciaB, dnaJ, racR, flaA, pldA and cdtAB in 60 C. jejuni strains isolated from human diarrhoeal faeces in northeastern Brazil. The genes ciaB, racR and cdtABC were found in 57 (95 %) strains, and the genes dnaJ, flaA and pldA were found in 52 (86.7 %), 48 (80 %) and 27 (45 %) strains, respectively. The aforementioned Brazilian studies showed lower rates for the virulence genes analysed compared with the data obtained in the present study.

In this work, the genotypic diversity of the 111 C. jejuni strains studied was assessed by flaA-SVR sequencing and PFGE typing. Using flaA-SVR sequencing, the 111 C. jejuni strains studied were grouped into two major clusters designated SVR-A and SVR-B, exhibiting a similarity of more than 83.4 %. The most frequently detected flaA-SVR alleles were gt57, gt45 and gt49, with 19, 13 and 13 strains, respectively. Some strains isolated from different sources, years and sites were indistinguishable and belonged to the same flaA-SVR allele, suggesting the potential for transmission between clinical and non-clinical sources (Fig. 1).

In some countries, the alleles most frequently observed were different from those obtained in our work. Sails et al. [60] studied 47 C. jejuni strains isolated from humans in the USA, and the most prevalent alleles found were gt3 and gt6 with eight and seven strains, respectively. In Canada, the most frequently found allele in 81 C. jejuni strains isolated from humans, bovines and chickens was gt36 [61]. Wassenaar et al. [62], using flaA-SVR sequencing, investigated the genotypic diversity of 293 C. jejuni strains isolated from humans and poultry in the Basque country (Spain), Norway and Iceland and observed that alleles gt36, gt32 and gt34 were the most prevalent, with 25, 20 and 19 strains, respectively. Giacomelli et al. [63] studied 30 C. jejuni strains isolated from broilers in northern Italy, and allele gt36 was found to be the most frequent. A study performed in Portugal by Duarte et al. [27] evaluated 89 C. jejuni strains isolated from humans, food and animals between 2009 and 2012, and revealed that allele gt34 was the most frequently detected, comprising 10 strains.

However, the above-mentioned studies detected, in some strains, the same alleles that were detected in the present work. For instance, the studies performed in Italy, the USA, Canada, Portugal, Spain, Norway and Iceland [27, 60–63] found alleles gt2, gt5, gt9, gt11, gt22, gt23, gt36, gt239 and gt260, which were each found in just one strain in our work.

According to the literature, this is the first study performed in Brazil to use flaA-SVR sequencing to type C. jejuni strains isolated from humans, monkey and chicken faeces, chicken meat and sewage. One study from Brazil typed C. jejuni strains isolated from humans and animals by PCR-restriction fragment length polymorphism (RFLP) of the flaA gene using three different restriction endonucleases [31]. Therefore, our work provides new data on the genotypic diversity of C. jejuni strains typed by flaA-SVR sequencing isolated in Brazil from different sources over a period of 20 years.

In this study, the PFGE methodology revealed 64 different PFGE types and grouped the 111 C. jejuni strains into three clusters, designated PFGE-A, PFGE-B and PFGE-C, with a genotypic diversity over 47.7 %. However, these three clusters were divided into 20 different sub-clusters that contained 99 strains with a similarity of ≥80 % (Fig. 2). The analysis of these sub-clusters showed a high similarity of some strains isolated from humans, monkey and chicken faeces, chicken meat and sewage from different years and sites of isolation, a fact previously observed by flaA-SVR sequencing data. Additionally, the PFGE results suggest possible in Brazil contamination between clinical and non-clinical sources (PFGE-A4, PFGE-A7, PFGE-B1, PFGE-B3, PFGE-C1 and PFGE-C2) and between humans and animals (PFGE-A1, PFGE-A5, PFGE-A6, PFGE-A8, PFGE-B2, PFGE-C3 and PFGE-C6) (Fig. 2).
In contrast, some studies performed in other countries using PFGE revealed a high genotypic diversity among *C. jejuni* strains. For instance, Oporto et al. [64] typed 87 *C. jejuni* strains isolated from animals in the Basque country (Spain) between 2003 and 2006, and revealed 55 PFGE types, with a genotypic diversity of more than 30%, and only 10 strains presented a similarity of more than 80%. Schweitzer et al. [65] demonstrated a high genetic diversity among 60 *C. jejuni* strains isolated from animals in Hungary, of which 40 yielded a unique Smal profile and a similarity of $\geq 50\%$. In a study conducted by Clark and colleagues [66] in Canada, 152 *C. jejuni* strains isolated from animals showed 103 PFGE types, of which 82 were of only one strain. According to Abay et al. [67], 115 different PFGE profiles were obtained from 174 *C. jejuni* strains isolated from humans and chicken in Turkey, with the strains showing a genotypic diversity of $\geq 27.1\%$.

In Brazil, a few studies have genotyped *C. jejuni* strains by PFGE. A study performed in southern Brazil by Silva and colleagues [34] detected a high diversity of 61 *C. jejuni* strains isolated from poultry products, poultry faeces and humans faeces. In that work, the dendrogram was generated with a tolerance of 3% and the *C. jejuni* strains showed a genotypic diversity of more than 30%. Moreover, the human strains showed no relatedness to the poultry strains. Thus, the present work provides additional information on *C. jejuni* strains typed by PFGE and isolated from humans, monkey and chicken faeces, chicken meat and sewage in different states of Brazil, over a 20-year period country.

Regarding DI, flaA-SVR sequencing and PFGE yielded DI values of 0.932 and 0.980, respectively. Therefore, in our work, PFGE was found to be more suitable to discriminate the *C. jejuni* strains studied. Similar to our results, Sails et al. [60] typed 47 *C. jejuni* strains isolated from humans using MLST, flaA-SVR sequencing and PFGE and observed that the discriminatory power of PFGE was the best among the techniques used. Oporto et al. [64] used flaA PCR-RFLP, MLST and PFGE to type 71 *C. jejuni* strains isolated from humans, and found that PFGE was the most discriminatory method. The same result was observed by Behringer and colleagues [68], who typed 49 *C. jejuni* strains isolated from live and retail broiler chickens USA, and observed that PFGE had a better discriminatory power in comparison with flaA PCR-RFLP, MLST and repetitive-element palindromic PCR.

Although we found that PFGE was more efficient than flaA-SVR sequencing in differentiating *C. jejuni* strains, both assays gave the same epidemiological information, confirming the suitability of the flaA-SVR technique in complementing PFGE data.

In conclusion, the pathogenic potential of the *C. jejuni* strains studied was observed by the prevalence of the majority of the virulence genes searched. Taken together, the results obtained by flaA-SVR sequencing and PFGE, showed that some of the strains studied presented a high genotypic similarity among, suggesting the potential for transmission between clinical and non-clinical sources, and between human and animal sources, over the course of 20 years in different states of the southeast and southern regions in Brazil. This potential source of contamination is of concern, as this country has been the world’s largest exporter and the third largest producer of chicken meat since 2004. Furthermore, our results further characterize this important foodborne pathogen, which has been underdiagnosed and understudied in Brazil.

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### Conflicts of interest
The authors declare that there are no conflicts of interest.

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