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Tese

Caracterização genômica e epidemiologia molecular de *Campylobacter* termofílicos isolados de produtos cárneos de frango comercializados no sul do Brasil

Simone de Fátima Rauber Würfel

Pelotas, 2018

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Tese apresentada ao Programa de Pós-Graduação em Veterinária da Faculdade de Veterinária da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área de concentração: Sanidade Animal).

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Resumo

WÜRFEL, Simone de Fátima Rauber. **Caracterização genômica e epidemiologia molecular de *Campylobacter* termofílicos isolados de produtos cárneos de frango comercializados no sul do Brasil.** 2018. 167f. Tese (Doutorado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2018.

Campylobacter spp. é o patógeno de origem alimentar considerado a causa bacteriana mais comum de gastroenterite humana, responsável por cerca de 400 a 500 milhões de casos anualmente em todo o mundo. Este patógeno é um habitante comum do intestino de frangos de corte e, frequentemente, causa doença em humanos por meio do consumo de carne de frango contaminada. O objetivo deste estudo foi analisar a epidemiologia molecular e caracterizar o genoma de *Campylobacter* termofílicos isolados de produtos cárneos de frango comercializados no sul do Brasil. A relação genética entre os isolados de *C. jejuni* e *C. coli* foi avaliada pela técnica de *pulsed-field gel electrophoresis* (PFGE), a resistência aos macrolídeos, ciprofloxacina e tetraciclinas foi avaliada pelo método de disco difusão, enquanto que a detecção de genes associados à virulência foi avaliada pela *polymerase chain reaction* (PCR). Dois isolados de *C. jejuni* foram submetidos ao sequenciamento genômico completo e análise comparativa baseada no genoma de referência *C. jejuni* NCTC 11168. Identificou-se grande diversidade genética entre os isolados de *C. jejuni* e *C. coli* avaliados, embora tenha-se observado recorrência de alguns clones. Foram observados níveis elevados de resistência às tetraciclinas e ciprofloxacina, enquanto que níveis menores de resistência aos macrolídeos e multirresistência foram identificados. Os isolados também portavam vários genes associados à virulência, mas nenhum gene específico foi relacionado com a resistência a antimicrobianos. A técnica de PFGE e a pesquisa de genes associados à virulência tiveram maior poder discriminatório, porém, a análise da resistência a antimicrobianos forneceu informações importantes sobre os isolados, melhorando a diferenciação entre eles. A primeira análise gênomica de dois *C. jejuni* isolados no Brasil revelou diferenças na *multilocus sequence typing* (MLST). Além disso, a análise comparativa de referência revelou diferenças na estrutura genômica (SNPs, rearranjos e inversões) em ambos os genomas, bem como a presença de alguns genes associados à virulência e de elementos genéticos móveis, como transposons, ilhas genômicas e sequências de profagos. Mecanismos de resistência a antimicrobianos e um novo megaplasmídeo de virulência também foram identificados. De modo geral, pode-se concluir que a presença de uma diversidade de isolados de *Campylobacter* resistentes a antimicrobianos e potencialmente virulentos contaminando produtos cárneos de frango no Brasil representa um risco potencial à saúde dos consumidores. Isso demonstra a necessidade de medidas de controle mais rigorosas para *Campylobacter* na cadeia de produção avícola do Brasil.

Palavras-chave: carne de frango; diversidade genética; resistência antimicrobiana; virulência; sequenciamento genômico

Abstract

WÜRFEL, Simone de Fátima Rauber. **Genomic characterization and molecular epidemiology of thermophilic *Campylobacter* isolated from poultry meat products marketed in Southern Brazil.** 2018. 167f. Thesis (Doctor degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2018.

Campylobacter spp. is the foodborne pathogen considered to be the most common bacterial cause of human gastroenteritis, and is responsible for as many as 400-500 million cases worldwide each year. This pathogen is a common inhabitant of the broilers gut and often causes human disease through consumption of contaminated poultry meat. The aim of this study was to analize the molecular epidemiology and characterize the thermophilic *Campylobacter* genome isolated from poultry meat products marketed in Southern Brazil. The genetic relationship among *C. jejuni* and *C. coli* isolates was evaluated by the pulsed-field gel electrophoresis (PFGE) technique, the resistance to macrolides, ciprofloxacin and tetracyclines was evaluated by the disk diffusion method, whereas the detection of virulence-associated genes was evaluated by the polymerase chain reaction (PCR). Two *C. jejuni* isolates were submitted to whole-genome sequence and comparative analysis based on the reference genome *C. jejuni* NCTC 11168. A high genetic diversity was identified among *C. jejuni* and *C. coli* isolates evaluated, although the recurrence of some clones has been observed. High levels of resistance to tetracyclines and ciprofloxacin were observed, while lower levels of resistance to macrolides and multidrug resistance were identified. The isolates also carryed several virulence-associated genes, but no specific gene was related to antimicrobial resistance. The PFGE technique and the virulence-associated genes research had greater discriminatory power, but the antimicrobial resistance analysis provided importante information about the isolates improving the differentiation among them. The first genomic analysis of two *C. jejuni* isolated in Brazil revelated differences in multilocus sequence typing (MLST). Moreover, the comparative reference analysis revealed differences in genomic structure (SNPs, rearrangements and inversions) in both genomes, as well as the presence of some virulence-associated genes and of mobile genetic elements such as transposons, genomic islands and prophages sequences. Mechanisms of antimicrobial resistance and a new virulence megaplasmid were also identified. In general, it can be concluded that the presence of a diversity of antimicrobial resistant and potentially virulent *Campylobacter* isolates contaminating the poultry meat products in Brazil represent a potential risk to health of consumers, since this country is the world's largest exporter of chicken meat. This demonstrates the need of more rigorous control measures for *Campylobacter* in poultry production chain from Brazil.

Keywords: poultry meat; genetic diversity; antimicrobial resistance; virulence; genomic sequencing

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1 Introdução

Bactérias do gênero *Campylobacter* pertencem à família *Campylobacteriaceae* (VANDAMME; DE LEY, 1991; FRANCO; LANDGRAF, 1996). São bastonetes Gram-negativos em forma de “S”, “asa de gaivota” ou espiral, com dimensões que variam de 0,2 a 0,8 µm de largura por 0,5 a 5,0 µm de comprimento (HOLT et al., 1994; KEENER et al., 2004). Possuem um único flagelo polar posicionado em uma ou ambas as extremidades da célula (VANDAMME; DE LEY, 1991; HOLT et al., 1994; KEENER et al., 2004), o qual é responsável pelo movimento característico de “saca-rolha” ou “vaivém” (FRANCO; LANDGRAF, 1996).

As condições necessárias para crescimento de *Campylobacter* spp. são incomuns e as diferencia de outras bactérias patogênicas de origem alimentar (CHLEBICZ; ŚLIŻEWSKA, 2018). Essas bactérias possuem metabolismo do tipo respiratório e são tipicamente microaerofílicas (VANDAMME; DE LEY, 1991; HOLT et al., 1994). A concentração ideal de O_2 para sua multiplicação é 5%, a qual é inibida em concentrações menores que 3% e maiores que 15%. Além disso, são capnofílicas, sendo necessária uma concentração de cerca de 10% de CO_2 para sua multiplicação (FRANCO; LANDGRAF, 1996). Em condições laboratoriais, requer uma atmosfera especial que geralmente consiste em 5% de O_2, 10% de CO_2 e 85% de N_2 (KEENER et al., 2004). No entanto, algumas estirpes podem se multiplicar em condições aeróbias ou anaeróbias (VANDAMME; DE LEY, 1991).

Campylobacter spp. também requer temperaturas restritas para multiplicação, que variam de 30 a 46ºC (CHLEBICZ; ŚLIŻEWSKA, 2018). As espécies denominadas termofílicas se multiplicam em temperaturas entre 37 e 42ºC, sendo 42ºC a temperatura ótima, porém são incapazes de se multiplicar abaixo de 30ºC (KEENER et al., 2004). O gênero *Campylobacter* é composto, atualmente, por 32 espécies e 13 subespécies (CHLEBICZ; ŚLIŻEWSKA, 2018). Dentre elas, o grupo termofílico contempla a maioria das espécies de *Campylobacter* associadas a infeções em humanos, sendo *C. jejuni* e *C. coli* as espécies mais frequentemente isoladas de alimentos e responsáveis pela maioria dos casos de campilobacteriose humana (SILVA et al., 2018). Cerca de 95% das infecções são associadas à *C. jejuni* enquanto que 5% são atribuídas a *C. coli* (CHLEBICZ; ŚLIŻEWSKA, 2018).

Campylobacter spp. estão amplamente distribuídos na natureza e uma grande variedade de animais, inclusive naqueles utilizados na produção de alimentos, como bovinos, ovinos, suínos e aves, podem ser hospedeiros assintomáticos desse patógeno (SILVA et al., 2018). A maioria das infecções por *Campylobacter* spp. está relacionada à ingestão de carne de frango crua ou malcozida, bem como à contaminação cruzada para alimentos que serão consumidos *in natura*, uma vez que esses micro-organismos são comumente encontrados no intestino de frangos de corte e podem ser transferidos para a carcaça no momento do abate (CDC, 2017a). De acordo com a *European Food Safety Authority* (EFSA), a manipulação, o preparo e o consumo da carne de frango podem ser responsáveis por 20-30% dos casos de campilobacteriose humana (EFSA, 2018), enquanto que 50-80% dos casos podem ser atribuídos ao frango como reservatório (HEREDIA; GARCÍA, 2018).

Apesar de *Campylobacter* spp. serem normalmente incapazes de se multiplicar fora de um hospedeiro animal, podem sobreviver por várias semanas em alimentos, principalmente quando armazenados sob refrigeração (CHLEBICZ; ŚLIŻEWSKA, 2018). Estima-se que aproximadamente 400-800 células de *Campylobacter* spp. sejam suficientes para causar uma infecção em humanos, pois o patógeno encontra no intestino humano um nicho microaerofílico ideal para colonização (USHANOV, 2018).

A campilobacteriose geralmente ocorre como eventos esporádicos, sendo rara a ocorrência de surtos (CDC, 2017a). O período de incubação da doença normalmente é de 1 a 7 dias antes do desenvolvimento dos sintomas, mas pode variar de acordo com a dose infecciosa ingerida (CHLEBICZ; ŚLIŻEWSKA, 2018). Pessoas acometidas podem apresentar sintomas como diarreia sanguinolenta, dor abdominal, febre, dor de cabeça e náusea, que normalmente desaparecem dentro de 2 a 5 dias, mas podem durar até 10 dias (EFSA; ECDC, 2018). Além disso, os sintomas podem variar de acordo com o indivíduo (SILVA et al., 2018) e algumas pessoas infectadas não apresentam sintomas (CDC, 2017a). A doença normalmente é autolimitada e a maioria dos pacientes se recupera sem auxílio de tratamento; entretanto, o tratamento antimicrobiano é necessário em infecções graves ou prolongadas (CDC, 2017a; SHEN et al., 2018).

Uma série de complicações gastrointestinais tem sido associada à infecção por *Campylobacter* spp., como doenças esofágicas, diarreia sanguinolenta intestinal, distúrbios gastrointestinais funcionais, doença celíaca e câncer de cólon (HEREDIA; GARCÍA, 2018). Além disso, pacientes com neoplasias malignas ou submetidos à terapia com corticosteroides correm risco de bacteremia (USHANOV, 2018). Em alguns casos, *Campylobacter* spp. podem desencadear reações inflamatórias autoimunes do sistema nervoso central, coração e articulações, resultando em doença prolongada e debilitante, como mielite transversa, artrite reativa e síndrome de Guillain-Barré (GBS) (EFSA; ECDC, 2018), uma condição neurológica de paralisia ascendente que pode comprometer a musculatura respiratória e levar à morte (USHANOV, 2018).

Estima-se que ocorra um caso de GBS a cada 1000 casos de campilobacteriose, e que 40% dos casos de GBS nos Estados Unidos da América (EUA) estejam associados a uma infecção prévia por *Campylobacter* spp. (CDC, 2017a). De acordo com o CDC (2017a), cerca de 3.000 a 6.000 pessoas desenvolvem GBS anualmente nos EUA. Além disso, aproximadamente 1% dos pacientes com campilobacteriose desenvolvem a síndrome de Reiter, artropatia aguda ou crônica e artrite reativa (USHANOV, 2018). No Brasil, não existem estudos epidemiológicos avaliando a frequência de casos de GBS em decorrência de campilobacteriose (SILVA et al., 2018). Entretanto, um estudo realizado no nordeste do país encontrou evidência sorológica de infecção recente por *C. jejuni* em um terço dos pacientes acometidos pela GBS (DOURADO et al., 2003).

Campylobacter spp. são patógenos de origem alimentar considerados a causa bacteriana mais comum de gastroenterite humana (SHEN et al., 2018; WHO, 2018), responsável por cerca de 400 a 500 milhões de casos anualmente em todo o mundo (HEREDIA; GARCÍA, 2018), e a incidência das infeções por *Campylobacter* spp. tem aumentado constantemente (CHLEBICZ; ŚLIŻEWSKA, 2018). Nos últimos anos, o número de casos de campilobacteriose aumentou drasticamente na América do Norte, Europa e Austrália, e a doença tornou-se endêmica em alguns países africanos, asiáticos e do Oriente Médio (HEREDIA; GARCÍA, 2018). Na União Europeia (UE), a campilobacteriose tem sido a doença transmitida por alimentos mais frequentemente relatada desde 2005, com cerca de 200.000 casos humanos anualmente (EFSA; ECDC, 2017). No entanto, estima-se que o número real de casos anuais seja em torno

de nove milhões e que as perdas econômicas ultrapassem €2,4 milhões por ano (EFSA, 2018).

Nos EUA, *Campylobacter* spp. estão entre os micro-organismos mais envolvidos em doenças diarreicas e acredita-se que acometam mais de 1,3 milhão de pessoas anualmente (CDC, 2017a). De acordo com o *Foodborne Diseases Active Surveillance Network* (FoodNet) dos EUA, *Campylobacter* spp. foram responsáveis pela maior incidência de infecções transmitidas por alimentos (19,2 para cada 100.000 habitantes) em 2017, com um aumento de 10% em relação ao período 2014-2016 (MARDER et al., 2018). Entretanto, a maioria dos casos não são diagnosticados ou não são notificados (CDC, 2017a). No Brasil, assim como na maioria dos países em desenvolvimento, existem poucos dados epidemiológicos disponíveis sobre a campilobacteriose humana e o diagnóstico laboratorial de doenças entéricas não é rotineiramente realizado (WHO, 2013; SILVA et al., 2018). Nos últimos 15 anos, apenas 37 casos de campilobacteriose transmitida por alimentos foram relatados no Brasil. Essa baixa incidência pode ser consequência da ineficácia dos programas de vigilância do governo, que resulta em subnotificação e dificuldade de estimar o número real de casos (SILVA et al., 2018).

De acordo com o relatório anual de 2017 da Associação Brasileira de Proteína Animal (ABPA), o Brasil é o maior exportador mundial de carne de frango e segundo maior produtor, e a região sul do país produz e exporta mais de 50% da carne de frango brasileira (ABPA, 2017). Apesar disso, não existem padrões legais para *Campylobacter* spp. em alimentos no Brasil e os estudos relacionados a esses micro-organismos são escassos em comparação com outros patógenos transmitidos por alimentos (SILVA et al., 2018). Segundo o *National Antimicrobial Resistance Monitoring System for Enteric Bacteria* (NARMS) dos EUA, 33% da carne de frango *in natura* vendida no varejo estava contaminada com *Campylobacter* spp. em 2014 (CDC, 2017a). Além disso, os resultados do monitoramento de *Campylobacter* spp. em alimentos realizado na EU em 2016, demonstraram que esses patógenos estavam presentes em 36,7% da carne de frango *in natura* (EFSA; ECDC, 2017). No Brasil, altos níveis de isolamento de *C. jejuni* e *C. coli* em frangos de corte e produtos cárneos de frango foram reportados em diferentes regiões do país (MAZIERO; DE OLIVEIRA, 2010; MEDEIROS, 2011; VAZ et al., 2011; ALVES et al., 2012; GONÇALVES et al., 2012; MEDEIROS et al., 2012; PERDONCINI, 2012), o que demonstra a importância do controle desses patógenos no Brasil (SILVA et al., 2018).

Embora o número de casos de infecções invasivas por *Campylobacter* spp. seja normalmente baixo, pessoas imunossuprimidas necessitam de tratamento terapêutico (CDC, 2017a), o que é preocupante, pois a eficácia de tais tratamentos é atualmente comprometida pelo aumento da resistência de *C. jejuni* e *C. coli* aos principais antimicrobianos utilizados para tratamento da campilobacteriose humana, ou seja, macrolídeos, fluorquinolonas e tetraciclinas (BOLTON, 2015). A resistência a antimicrobianos de *Campylobacter* spp. provenientes de alimentos de origem animal tornou-se uma grande preocupação de saúde pública, existindo uma clara correlação entre o uso de antimicrobianos na produção animal e cepas resistentes em humanos (WIECZOREK; OSEK, 2013), o que pode resultar em falhas no tratamento terapêutico (EFSA; ECDC, 2018). Segundo a Organização Mundial de Saúde (WHO, 2017), *Campylobacter* spp. estão entre os "patógenos prioritários" que representam maior ameaça à saúde humana, para os quais novos antimicrobianos são necessários urgentemente. Por conseguinte, o monitoramento da resistência a antimicrobianos é essencial para a identificação de padrões de resistência emergentes ou específicos, fornecendo dados relevantes para avaliações de risco e intervenções direcionadas (EFSA; ECDC, 2018).

Estudos demonstraram que infecções por cepas de *Campylobacter* spp. resistentes a antimicrobianos estão associadas a um risco aumentado de doença invasiva ou morte em comparação às infecções por cepas suscetíveis, sugerindo que a resistência pode estar associada ao aumento da virulência (TRAVERS; BARZA, 2002; ENGBERG et al., 2004; HELMS et al., 2005; MØLBAK, 2005). Os mecanismos subjacentes podem ser a co-seleção de características de virulência, supra-regulação da virulência ou melhor adequação das cepas resistentes (BARZA, 2002; MØLBAK, 2005; TRAVERS). No entanto, a patogênese da infecção por *Campylobacter* spp. é complexa e pouco compreendida (KOOLMAN et al., 2015) e os mecanismos exatos pelos quais *C. jejuni* ou *C. coli* causam infecção são desconhecidos (WHO, 2013).

Acredita-se que sistemas multifatoriais complexos relacionados à motilidade e quimiotaxia (envolvendo os genes *docB*, *docC*, *flaA*, *flaB*, *fliA*, *fliM*, *fliY* e *rpoN*), adesão (envolvendo os genes *cadF*, *cj0588* e *pldA*), produção de toxinas (envolvendo os genes *cdtA*, *cdtB* e *cdtC*), invasão celular (envolvendo os genes *ciaB*, *flhA* e *flhB*), lipo-oligossacarídeos (LOS) (envolvendo os genes *wlaN* e *cgtB*), cápsula (envolvendo o gene *kpsM*), resposta ao estresse (envolvendo os genes *ahpC*, *cj1000*, *clpP*, *dnaJ*, *docA*, *katA*, *luxS*, *racR* e *sodB*) e sistema de secreção do tipo 6 (T6SS; envolvendo o

gene *hcp*) são primordiais para o sucesso da multiplicação de *Campylobacter* spp. em frangos de corte, sobrevivência durante as etapas de processamento de alimentos e virulência em humanos (KRUTKIEWICZ; KLIMUSKZO, 2010; HARRISON et al., 2014; BOLTON, 2015; GHUNAIM et al., 2015; KOOLMAN et al., 2016).

A ampla diversidade genética entre as cepas de *Campylobacter* spp. pode contribuir para as diferenças nas respostas fenotípicas do patógeno (SILVA et al., 2018). Apesar desse gênero bacteriano possuir um genoma pequeno (1.6 a 2.0 Mb), apresenta extensa variação genética devido a mecanismos intragenômicos e troca genética entre linhagens, uma vez que é naturalmente competente e pode captar DNA exógeno (YOUNG et al., 2007). Há uma alta taxa de recombinação devido à transferência gênica horizontal, que resulta no aumento da diversidade alélica nos genomas de *Campylobacter* spp. e graus variados de ruptura da estrutura populacional total clonal, de tal forma que a introdução de um grande número de polimorfismos pode gerar novos fenótipos (SHEPPARD; MAIDEN, 2015). A transferência horizontal contribui muito para a evolução das espécies bacterianas e os genes adquiridos horizontalmente representam uma grande proporção de genomas bacterianos. Aproximadamente 75% dos genes em cada genoma foram adquiridos por transferência horizontal durante a evolução. Eles são disseminados por elementos genéticos móveis, como plasmídeos, transposons, bacteriófagos ou ilhas genômicas (JUHAS, 2015).

A sequência genômica completa da cepa *C. jejuni* NCTC 11168 revelou a presença de vários genes codificadores de proteínas com potencial de virulência e propriedades de sobrevivência, além de sequências hipervariáveis encontradas em regiões que codificam proteínas envolvidas na biossíntese ou modificação de estruturas de carboidratos acessíveis à superfície (PARKHILL et al. 2000). Essas variações estruturais surgem de mecanismos como mutações pontuais, duplicação e deleção de genes, *frameshift* e variação de fase (YOUNG et al. 2007). No entanto, a compreensão limitada dos mecanismos genéticos, fisiológicos e de virulência de *Campylobacter* spp. dificulta seu controle eficaz na cadeia alimentar, bem como o desenvolvimento de estratégias de prevenção de doenças (PARKHILL et al. 2000). Recentes avanços científicos, como o sequenciamento do genoma, vêm oferecendo novas abordagens, levando a uma melhor compreensão da patogênese desse intrigante patógeno (WHO, 2013).

2 Objetivos

2.1 Objetivo geral

Estudar a epidemiologia molecular e caracterizar o genoma de *C.* jejuni e *C. coli* isolados de produtos cárneos de frango comercializados no sul do Brasil.

2.2 Objetivos específicos

1) Avaliar a relação genética entre os isolados de *C. jejuni* e *C. coli*;
2) Investigar a resistência a antimicrobianos dos isolados de *C. jejuni* e *C. coli*;
3) Investigar a presença de genes associados à virulência nos isolados de *C. jejuni* e *C. coli*;
4) Avaliar a relação entre resistência a antimicrobianos e potencial de virulência nos isolados de *C. jejuni* e *C. coli*;
5) Realizar o sequenciamento e a análise genômica de dois isolados de *C. jejuni*, comparando com uma cepa de referência.

3 Artigos

3.1 Artigo 1

Genetic diversity of *Campylobacter* species isolated from poultry meat products at retail market in Southern Brazil

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GENETIC DIVERSITY OF *Campylobacter* SPECIES

MICROBIOLOGY AND FOOD SAFETY

Genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry meat products sold on the retail market in Southern Brazil

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ABSTRACT

Campylobacter is regarded as the most common bacterial cause of gastroenteritis throughout the world and most cases of human campylobacteriosis can be traced back to the consumption of poultry meat. In Brazil, few studies evaluated the genetic relatedness among *Campylobacter* isolates. The aim of this research was to evaluate the genetic diversity of *Campylobacter* spp. isolated from poultry meat products sold on the retail market in Southern Brazil. The presumptive identification of *Campylobacter* was performed using traditional microbiological analysis, followed by molecular confirmation by PCR. The genetic diversity of isolates was analyzed by pulsed-field gel electrophoresis (PFGE). *Campylobacter* spp. was isolated from 91.7% (33/36) of the samples, totaling 48 isolates. *Campylobacter jejuni* was the most prevalent species isolated (90.8%). PFGE data revealed 26 pulsotypes and 18 PFGE patterns composed of only one isolate. *Campylobacter* isolates exhibited high genetic diversity; however, some clones were recurrent in the poultry meat products sold on the retail market. As the south region of Brazil is an important producer and exporter of chicken meat, our results highlight the need to control this pathogen in the food chain in this area of the world to reduce the risks of exposing consumers to campylobacteriosis.

Key words: *Campylobacter*; food safety; genetic diversity; poultry meat; pulsed-field gel electrophoresis

INTRODUCTION

Campylobacter is regarded as the most common bacterial cause of human gastroenteritis worldwide (Zendehbad et al., 2015). In the European Union, campylobacteriosis is the most frequently reported foodborne disease, with around 200,000 human cases annually. However, the actual number of cases each year is estimated to be around nine million, placing a major economic and disease burden on society (EFSA, 2014). In the United States of America (USA), *Campylobacter* is one of the microorganisms most common in diarrheal diseases, which is believed to affect over 1.3 million people every year (CDC, 2017a). In Brazil, only 37 foodborne campylobacteriosis cases were reported during the last 15 years. The low incidence of campylobacteriosis in Brazil might be a consequence of ineffectiveness of government surveillance programs, which lead to under-reporting and difficulty to estimate the actual number of cases (Silva et al., 2018).

Campylobacteriosis is generally self-limiting, and almost all people infected recover without any specific treatment. However, antimicrobial therapy is necessary for patients who are deemed to be at a high risk of severe or long-lasting infections, such as immune-suppressed patients (Wieczorek et al., 2015; CDC, 2017a). Among the post-infectious consequences, the Guillain-Barré syndrome stands out. Guillain-Barré syndrome is an acute inflammatory demyelinating disease that affects the peripheral nervous system resulting in flaccid paralysis, which can compromise the respiratory muscles and cause death (Hadden and Gregson, 2001).

Campylobacter jejuni and *C. coli* species are the most important threats to public health and are responsible for a large number of cases of foodborne infection in humans (EFSA, 2011). The majority of cases of human campylobacteriosis are associated with the consumption of raw or undercooked poultry meat or caused by the cross-contamination with foods consumed uncooked (EFSA, 2011; CDC, 2017a). Birds, especially poultry, are regarded as the primary reservoir for *C. jejuni*, and can be asymptomatic carriers (O'Leary et al., 2011; Wieczorek et

al., 2015). Since these microorganisms are commonly found in the intestinal tract of poultry, they can contaminate meat during the slaughter process (Wieczorek et al., 2015), making it difficult to remove them from the poultry processing plant due to high levels of contamination and constant reintroduction (Keener et al., 2004). These microorganisms can also be easily spread via cross-contamination inside the poultry processing plant (Keener et al., 2004). Control measures and hygienic practices in the poultry industry are essential for decreasing the incidence of initial carcass contamination; however, they are insufficient to eradicate *Campylobacter* from the final product (Silva et al., 2016). The most effective strategy to reduce rates of human campylobacteriosis is to control the pathogen in a significant source such as poultry meat (Prachantasena et al., 2016).

Despite the significance of *Campylobacter* as an agent of foodborne disease, there is little information available about *Campylobacter* contamination levels in commercially sold poultry meat in Brazil (Gritti et al., 2011). Furthermore, legal standards have yet to be established by which the presence or counts of *Campylobacter* in foods are monitored or controlled (Hungaro et al., 2015). Research in this field is necessary in Brazil because it is the world's largest exporter of chicken meat and the second largest producer, surpassed only by the USA. Moreover, according to the 2017 annual report of Brazilian Association of Animal Protein (ABPA, 2017), the south region of Brazil produces and exports more than 50% of Brazilian chicken meat.

The methods of molecular typing represent excellent tools to help prevent human campylobacteriosis, since they provide opportunities to understand the epidemiology and population genetics, as well as to understand the effectiveness of prevention and monitoring practices for the control of foodborne pathogens (Eberle and Kiess, 2012). Furthermore, they play a major role in elucidating the genetic diversity of *Campylobacter* and the transmission routes of these microorganisms in the food chain (Wassenaar and Newell, 2000). Among them,

pulsed-field gel electrophoresis (**PFGE**) is a highly discriminatory method that has been widely used for the molecular characterization of *Campylobacter* spp. (Ribot et al., 2001; Eberle and Kiess, 2012; Kovačić et al., 2015; Wieczorek et al., 2015; Gomes et al., 2016). PFGE is among PulseNet's main subtyping tools (CDC, 2017b) and was currently employed in the investigation of an outbreak of *C. jejuni* in Colorado, USA (Burakoff et al., 2018). It has been used successfully to identify the genetic variability of *C. jejuni* and *C. coli* (Ge et al., 2006; O'Leary et al., 2011; Alves et al., 2012; Perez-Boto et al., 2012; Abay et al., 2014; Kovačić et al., 2015; Wieczorek et al., 2015). In Brazil, few studies evaluated the genetic relationship among *Campylobacter* isolates. The aim of this study was to evaluate the genetic diversity of *C. jejuni* and *C. coli* isolated from poultry meat products in Southern Brazil.

MATERIAL AND METHODS

Sampling

Thirty-six samples were collected from the retail market in Southern Brazil. For each collection, samples of carcass, cuts (breast, drumstick, thigh, drumette), and poultry liver from the same batch of two brands (A and B) were obtained in a supermarket at intervals of 60 d, totaling three sampling events. All samples were obtained in the original package (from manufacturer) and transported to the laboratory in isothermal containers with ice. The manufacturers are not related from a commercial perspective, are located 400 km away from each other, and do not have broiler flock suppliers in common. Manufacturer A provides their products only for Brazilian retail, while manufacturer B exports chicken meat to 28 countries located in the Middle East, Africa, Far East, Eastern Europe, and Central America.

Isolation and Phenotypic Identification

The isolation and phenotypic identification of *Campylobacter* were performed in accordance with the International Organization for Standardization (ISO 10272-1:2006), with adaptations. From each sample, two methods of sampling were performed. First, a representative 10 g portion (a mix of skin and meat) was aseptically weighed and added to 90 ml of Bolton broth (CM0983; Oxoid, Basingstoke, Hampshire, UK) containing a selective supplement (SR0208; Oxoid) and potassium clavulanate (Sigma-Aldrich, St. Louis, Missouri) to a final concentration of 2 $mg.l^{-1}$ (Moran et al., 2011). After, the rest of the sample was rinsed with 150 ml of buffered peptone water (**BPW**; Oxoid), and one aliquot (10 ml of this broth) was added to the Bolton broth supplemented according to the description above. The samples were then incubated at 37 °C for 4 h and then 42 °C for 24 h under microaerophilic conditions (5% O_2, 10% CO_2, and balance N_2; White Martins, Danbury, Connecticut).

After this period, they were streaked onto modified Charcoal Cefoperazone Deoxycholate agar (**mCCDA**, CM0739; Oxoid) with CCDA selective supplement (SR0155; Oxoid) and Preston agar (*Campylobacter* agar base CM0689 and Preston *Campylobacter* selective supplement SR0017; Oxoid) with 5% (vol/vol) lysed horse blood. They were then incubated at 42 °C under microaerophilic conditions for 48 h. Two presumptive colonies of *Campylobacter* from each plate were analyzed under the microscope by Gram staining for morphological identification. Afterwards, they were inoculated on blood agar base no. 2 (CM0271; Oxoid) with 5% (vol/vol) lysed horse blood and Columbia blood agar base (CM0331; Oxoid) to carry out the phenotypic tests of catalase and oxidase production, and hydrolysis of indoxyl acetate and sodium hippurate. The reference strain *C. jejuni* ATCC 33291 was used as the control.

Genotypic Identification

All isolates identified as *Campylobacter* were subjected to PCR for genus confirmation and multiplex PCR (**mPCR**) for species differentiation. The DNA of each isolate was extracted and purified using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin) according to the manufacturer's protocol. The PCR genus confirmation was performed by amplifying a sequence of 287 bp of the 16S rRNA gene of *C. jejuni*, *C. coli* and *C. lari* using the primers and conditions previously described (Josefsen et al., 2004). To differentiate between the species, mPCR protocol (Maćkiw et al., 2012) was used, yielding a product of 773 bp to *C. jejuni* and 364 bp to *C. coli*. The agarose gel was stained with GelRed (Biotium, Fremont, CA) and the amplification products were visualized using a transilluminator L-Pix Touch (Loccus Biotecnologia, São Paulo, Brazil).

As a positive control, the reference strains *C. jejuni* ATCC 33291, *C. coli* ATCC 33559, and *C. lari* ATCC 35221 were used. *Salmonella* Typhimurium ATCC 14028 was used as a negative control for molecular analysis. Moreover, a mixture without addition of DNA was incorporated as a negative control reaction.

Genetic Diversity

Isolates of *Campylobacter* were subjected to PFGE to compare DNA profiles according to the standardized protocol used in the PulseNet program of the Center for Disease Control and Prevention (CDC, 2017b), with minor changes (Ribot et al., 2001). Briefly, colonies were suspended in 0.85% NaCl (wt/vol) and adjusted to an absorbance of 0.680 at a wavelength of 600 nm using a spectrophotometer. DNA in agarose plugs was prepared with a 200 µl aliquot of adjusted cell suspensions, 0.2 mg of proteinase K (New England Biolabs Inc., Ipswich, Massachusetts), 1% (wt/vol) agarose gel PFGE certified (Bio-Rad Laboratories, Hercules, California) with 0.5 X Tris-Borate buffer (0.045 M Tris-borate, 0.001 M EDTA). The lysis of cells in plugs was performed with cell lysis buffer (50 mM Tris:EDTA [pH 8.0], 1% sarcosine

(wt/vol), 0.1 mg of proteinase K/ml). Subsequently, *Campylobacter* DNA cleavage was achieved by restriction with 40 U of *Sma*I enzyme (New England Biolabs) at 25 °C for 2 h. DNA of *Salmonella enterica* serotype Braenderup strain H9812 (ATCC BAA-664) was used to determine fragment size after digestion with restriction enzyme *Xba*I (New England Biolabs). The macrorestriction products were separated in 1% (wt/vol) agarose gel PFGE certified (Bio-Rad Laboratories) with 0.5 X Tris-Borate buffer for 19 h at 6 V/cm, using CHEF-DR II system (Bio-Rad Laboratories) with initial and final switch times of 6.8 s and 35.4 s respectively. After the electrophoresis run was completed, agarose gel was stained with ethidium bromide (1 µg/ml) and visualized using a transilluminator L-Pix Touch (Loccus Biotecnologia). The band profiles obtained were analyzed using BioNumerics software package version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). A dendrogram was generated by cluster analysis using the unweighted pair group method with arithmetic averages (**UPGMA**). The similarity between PFGE patterns was expressed as a Dice coefficient (position tolerance 1%). To assess the genetic diversity of *Campylobacter* populations, the Simpson's index of diversity (**SID**) was determined according to Hunter (1990). Isolates sharing the number and position of DNA fragments were considered to belong to the same PFGE profile. The isolates that exhibited PFGE patterns that were indistinguishable from the primary enzyme were also digested with the *Kpn*I restriction enzyme (New England Biolabs), recommended as secondary enzyme according to the PulseNet protocol (CDC, 2017b).

Statistic Analysis

A statistical ANOVA using Fishers Least Significant Difference (**LSD**) test was performed to evaluate the isolation results. A P value < 0.05 was deemed to be significant.

RESULTS

Campylobacter was isolated from 91.7% (33/36) of the samples tested, of which 48% were from Brand A and 52% from Brand B. We did not observe a significant difference in the prevalence of *Campylobacter* spp. between brands ($P > 0.05$). Among the positive samples, *Campylobacter* were detected in 66.7% of the carcasses, 100% of the breasts, drumsticks, thighs, drumettes, and 83.3% of the livers analyzed. A total of 48 isolates were obtained, of which 87.5% were *C. jejuni* and 12.5% were *C. coli*. *Campylobacter jejuni* was significantly more frequent than *C. coli* ($P < 0.05$). Moreover, two samples contained the species *C. jejuni* and *C. coli* simultaneously, which were identified through mPCR.

The genetic relationship among isolates of *Campylobacter* analyzed by PFGE using *Sma*I restriction enzyme was represented by a dendrogram (Figure 1). The isolates that exhibited an identical genotypic profile when analyzed by *Sma*I restriction enzyme but differed by analysis with *Kpn*I restriction enzyme are presented in Figure 2. The genotypic profiles obtained from digestion with *Sma*I and *Kpn*I restriction enzymes were designated as S (S1-S24) and K (K1-K26) respectively. All isolates that were genetically identical (100% similarity) with both enzymes were considered as a pulsotype. The P13 and P14 pulsotypes and the P24 and P25 pulsotypes revealed indistinguishable genotypic profiles following restriction with enzyme *Sma*I (Figure 1) but differed when digested with the *Kpn*I restriction enzyme (Figure 2); as such, they were classified as different pulsotypes (Table 1).

Analysis of the PFGE data for the 48 isolates yielded a total of 26 pulsotypes (isolates with 100% similarity). The Simpson's index of diversity for *Sma*I-*Kpn*I restriction enzymes was high (0.95), which indicated a high level of genetic diversity among *Campylobacter* isolates. The use of a second enzyme increased the discriminatory power of PFGE technique. Among the 26 PFGE patterns, 9 (34.6%) were shared by two or more *Campylobacter* isolates and 17 (65.4%) PFGE patterns were composed of only one isolate (Table 1), which also

indicates a diversified population of *Campylobacter* in the products analyzed. It is important to highlight that these 26 pulsotypes were obtained from 33 positive samples.

The isolates were divided into two major groups (CJ and CC) according to the species, clearly demonstrating that *C. jejuni* and *C. coli* are genetically highly diverse, with an average similarity of 28.9% (Figure 1). Moreover, the *C. jejuni* isolates were grouped into two clusters (CJ-A and CJ-B) and shared 40.4% similarity. Table 1 shows the distribution of PFGE patterns according to the sample. The predominant PFGE pattern was the P8 pulsotype composed of seven isolates, followed by the P2 pulsotype (6 isolates). P2, P5, P8, P13, P16 and P19 pulsotypes included isolates from different samples of the same batch and brand that were manufactured on the same date. P8, P17, P18 and P23 pulsotypes included isolates from different samples of the same batch, of the same brand, and manufactured on different dates. Among the studied population, the P8 pulsotype stands out for including *Campylobacter* isolated throughout the sampling period. Moreover, the P18 pulsotype included isolates from different samples, brands, and manufacturing dates (Table 1).

In our study, two carcass samples were contaminated with *C. jejuni* and *C. coli* concomitantly. The carcass sample manufactured by Brand A on 6/27/13 contained *C. jejuni* (P2 and P17 pulsotypes) and *C. coli* (P23 pulsotype) isolates (Table 1). Similarly, the carcass sample manufactured by Brand A on 9/18/13 was contaminated with *C. jejuni* (P6 pulsotype) and *C. coli* (P23 pulsotype).

DISCUSSION

There was a high occurrence of *Campylobacter* in the samples evaluated. Studies in Brazil have described detection rates of this pathogen in poultry meat products sold for human consumption between 0 and 99% (Aquino et al., 2002; Freitas and Noronha, 2007; Kuana et

al., 2008; De Carvalho et al., 2010; Gritti et al., 2011; Alves et al., 2012; Gonçalves et al., 2012; Medeiros et al., 2012; De Carvalho et al., 2013; De Moura et al., 2013; Campos et al., 2015; Silva et al., 2016). According to Ugarte-Ruiz et al. (2013), the *Campylobacter* isolation can vary depending on the protocol used and the effectiveness of isolation, and the methods used to detect these microorganisms can also vary according to the level of bacterial contamination in the sample.

The higher prevalence of *C. jejuni* than *C. coli* in our study can be attributed to the role of chicken as primary reservoir of this species, which are normally found in high levels in the chicken's intestines during the slaughter process. These microorganisms can spread throughout the poultry processing plant by means of intestinal rupture and, consequently, contaminate the final product (Keener et al., 2004). Previous studies also indicate a higher prevalence of *C. jejuni* than *C. coli* in carcasses or poultry meat products (De Carvalho et al., 2010, 2013; Hungaro et al., 2015; Kuana et al., 2008; Silva et al., 2016; Zendehbad et al., 2015; Zhao et al., 2010). However, these species were isolated in the same proportion in a study carried out by Aquino et al. (2002) in Brazil, whereas Campos et al. (2015) reported a higher prevalence of *C. coli* than *C. jejuni* in poultry meat and organs sold in Rio de Janeiro state.

Genotyping of *Campylobacter* exploits differences in the DNA sequences among different strains, and plays an essential role in epidemiological studies (Ge et al., 2006). In this study, we employed PFGE to determine the genetic relatedness among 48 *Campylobacter* isolates from poultry meat products (carcass, cuts, and liver) sold in Southern Brazil.

The amount of unmatched PFGE patterns found in this study reflected the broad genetic diversity of isolates analyzed and, consequently, the large variety of molecular patterns present in poultry meat products sold in retail stores in Southern Brazil. O'Leary et al. (2011) also identified PFGE patterns composed of only one isolate in *C. jejuni* and *C. coli* isolated from

retail poultry meat in Ireland (27.4% and 31.5%, respectively), and Ge et al. (2006) in 36% of *C. jejuni* isolates analyzed.

Pulsotypes that included isolates from different samples of the same batch and brand that were manufactured on the same date can be result of cross-contamination during the slaughter process or the occurrence of clones contaminating the broiler flocks. According to Prachantasena et al. (2016), genetically identical isolates are often found in broiler flocks, slaughter line equipment, and the final product. *Campylobacter* can be expected to contaminate meat during the slaughter process as a result of fecal contamination, and the process operations that have been considered in risk assessment are evisceration, scalding, defeathering, washing, and chilling (FAO, 2003). Ugarte-Ruiz et al. (2013) also pointed out the significant role the slaughterhouse plays in the process of cross-contamination among broiler flocks, which can lead to contamination of carcasses from negative or not fully colonized flocks.

Pulsotypes that included isolates from different samples of the same batch, of the same brand, and manufactured on different dates, can suggest the recurrence of *Campylobacter* strains in the retail market given that the samples were obtained over a period of 6 months with intervals of 60 d between each collection. Other studies have demonstrated evidence of recurrent *Campylobacter* strains in the meat supply (Ge et al., 2006; Zhao et al., 2010; O'Leary et al., 2011). Zhao et al. (2010) identified by PFGE several *Campylobacter* clones repeatedly contaminating the same retail meat product in the USA over a six-year period. In Brazil, Gomes et al. (2016) found high genetic similarity among some *C. coli* isolated from various sources over the 16-year study period.

Given the significant distance between manufacturers, which did not share providers of broiler flocks, it is believe that P18 pulsotype is widely distributed, since it included isolates from different samples, brands, and manufacturing dates. Similar observations were also made by Wieczorek et al. (2015), who identified the same PFGE profiles in *Campylobacter* isolated

from poultry caeca and carcasses collected in different regions of Poland. Kovačić et al. (2015), in Croatia, found identical PFGE profiles in *Campylobacter* isolated from poultry and humans over different periods of time and in different places consecutively for two year. Studies have suggested that flies play a linking role in the spread and epidemiology of *Campylobacter* infections by transmitting the microorganism from fecal sources to poultry and the environment (Bahrndorff et al., 2013; Royden et al., 2016). Wild birds also help to spread campylobacteriosis to humans and other bird species (Kwon et al., 2017).

In our study, two samples were contaminated with *C. jejuni* and *C. coli* concomitantly. Ge et al. (2006) reported similar results. They found multiple PFGE patterns within the same species of *Campylobacter*, different PFGE patterns for *C. jejuni* isolates in the same sample, and one chicken meat sample containing two *C. jejuni* and one *C. coli* PFGE patterns. It is well demonstrated that poultry carcasses frequently have the same genotype as the one present in the origin flock (Duffy et al., 2015; Prachantasena et al., 2016; Silva et al., 2016). The occurrence of clones within the broiler flocks is common due to the horizontal transmission of endemic strains among the poultry (Silva et al., 2016). However, monitoring broiler flocks has shown that some are infected with more than one species or genotype of *Campylobacter*, suggesting various infection sources (Alves et al., 2012; Perez-Boto et al., 2012; El-Adawy et al., 2013; Duffy et al., 2015; Kovačić et al., 2015; Prachantasena et al., 2016; Silva et al., 2016). Duffy et al. (2015) found up to six different genotypes of *Campylobacter* in the caecal samples taken from one chicken. Thus, it is believed that the two carcass samples evaluated may have been contaminated from a broiler flock infected with more than one species and genotype of *Campylobacter*, or could have been contaminated by more than one source during the slaughter process.

The high genetic diversity of *Campylobacter* isolates identified in our study was also reported in previous studies (Ge et al., 2006; Zhao et al., 2010; El-Adawy et al., 2013; Abay et

al., 2014; Kovačić et al., 2015; Wieczorek et al., 2015); however, it remains poorly understood. According to Wilson et al. (2009), the genetic diversity is influenced by the genetic instability of *Campylobacter*, since it is naturally competent and demonstrates a high rate of DNA recombination. They demonstrated that recombination is the primary mechanism driving molecular change, facilitating gene flow within the *Campylobacter* species, as well as importing genes from other sources. Furthermore, the potential of the pathogen to adapt rapidly to changes in selection pressure was highlighted.

Ge et al. (2006) found evidence of genetic instability in the *Campylobacter* isolates evaluated, which can affect the PFGE results (Wassenaar and Newell, 2000; Wieczorek et al., 2015) and hinder the interpretation of their molecular epidemiology (Perez-Boto et al., 2012). However, although the PFGE method has a few limitations as high cost and time consuming (Eberle and Kiess, 2012; CDC, 2017b), it is highly discriminatory and robust (Ribot et al., 2001; Kovačić et al., 2015). Moreover, the use more than one restriction enzyme increase the discriminatory power of PFGE technique (Kovačić et al., 2015; Zhao et al., 2010).

CONCLUSION

The results of this study provide important insights into the occurrence of *Campylobacter* in poultry meat products sold in Southern Brazil. *Campylobacter* isolates exhibited high genetic diversity; however, some clones were recurrent in the retail market. The south region of Brazil is an important producer and exporter of chicken meat. As such, it is of great importance to control this pathogen in the food chain to reduce the risks of exposing consumers to campylobacteriosis.

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Table 1. Genotypic profile of *Campylobacter* isolated from poultry meat products sold on the retail market in Southern Brazil

Sampling	Manufacture date	Brand	Positive sample	*Campylobacter* species	Genotypic profile		Pulsotype
					*Sma*I	*Kpn*I	
I	6/21/13	B	carcass	*C. coli*	S24	K26	P26
		B	liver	*C. jejuni*	S9	K9	P9
				C. jejuni	S4	K4	P4
	6/26/13	B	breast	*C. jejuni*	S20	K21	P21
				C. jejuni	S8	K8	P8
		B	drumstick	*C. jejuni*	S19	K20	P20
				C. jejuni	S18	K19	P19
		B	thigh	*C. jejuni*	S18	K19	P19
		B	drumette	*C. jejuni*	S18	K19	P19
	6/27/13	A	carcass	*C. jejuni*	S2	K2	P2
				C. jejuni	S16	K17	P17
				C. coli	S22	K23	P23
		A	breast	*C. jejuni*	S1	K1	P1
				C. jejuni	S2	K2	P2
		A	drumstick	*C. jejuni*	S2	K2	P2
		A	thigh	*C. jejuni*	S2	K2	P2
		A	drumette	*C. jejuni*	S2	K2	P2
		A	liver	*C. jejuni*	S2	K2	P2
II	9/16/13	A	breast	*C. jejuni*	S17	K18	P18
		B	thigh	*C. jejuni*	S8	K8	P8
		B	drumette	*C. jejuni*	S8	K8	P8
	9/17/13	B	liver	*C. jejuni*	S8	K8	P8
	9/18/13	A	carcass	*C. jejuni*	S6	K6	P6
				C. coli	S22	K23	P23
		A	drumstick	*C. jejuni*	S15	K16	P16
		A	thigh	*C. jejuni*	S15	K16	P16
		A	drumette	*C. jejuni*	S15	K16	P16
				C. jejuni	S16	K17	P17
		A	liver	*C. coli*	S23	K24	P24
				C. coli	S23	K25	P25
		B	breast	*C. jejuni*	S10	K10	P10
		B	drumstick	*C. jejuni*	S7	K7	P7

				C. jejuni	S8	K8	P8
				C. jejuni	S11	K11	P11
III	12/30/13	B	breast	*C. jejuni*	S13	K13	P13
		B	carcass	*C. jejuni*	S8	K8	P8
				C. jejuni	S3	K3	P3
		B	thigh	*C. jejuni*	S13	K13	P13
				C. jejuni	S13	K14	P14
		B	liver	*C. jejuni*	S8	K8	P8
	1/2/14	A	breast	*C. jejuni*	S5	K5	P5
		A	drumstick	*C. jejuni*	S5	K5	P5
				C. jejuni	S12	K12	P12
		A	thigh	*C. jejuni*	S5	K5	P5
		A	drumette	*C. jejuni*	S5	K5	P5
		B	drumstick	*C. coli*	S21	K22	P22
		B	drumette	*C. jejuni*	S14	K15	P15
				C. jejuni	S17	K18	P18

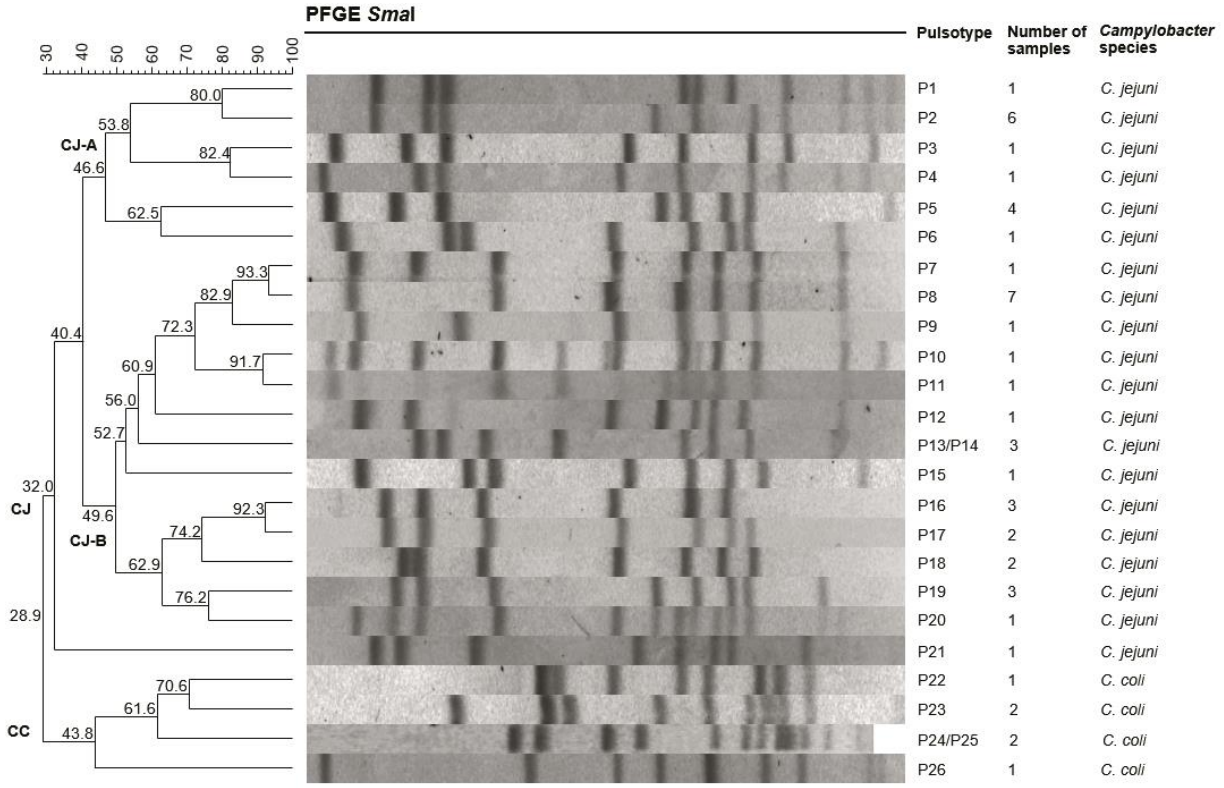

Figure 1. Dendrogram representing the genetic relationship among *Campylobacter* isolated from poultry meat products evaluated by PFGE using the restriction enzyme *Sma*I. The dendrogram was generated by cluster analysis using the unweighted pair group method with arithmetic averages (UPGMA). The similarity between PFGE patterns was expressed as a Dice coefficient (1% tolerance in band position).

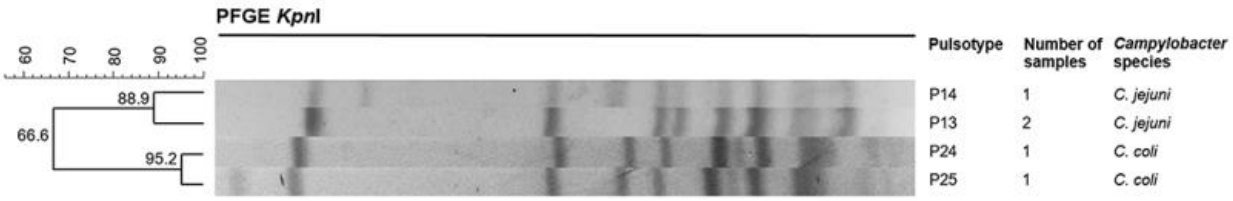

Figure 2. Dendrogram representing the genetic relationship among *Campylobacter* isolated from poultry meat products evaluated by PFGE using the restriction enzyme *Kpn*I. The dendrogram was generated by cluster analysis using the unweighted pair group method with arithmetic averages (UPGMA). The similarity between PFGE patterns was expressed as a Dice coefficient (1% tolerance in band position).

3.2 Artigo 2

Comprehensive characterization reveals a diversity of antimicrobial resistant and potentially virulent *Campylobacter* isolates contaminating poultry meat products in Brazil

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Comprehensive characterization reveals a diversity of antimicrobial resistant and potentially virulent *Campylobacter* isolates contaminating poultry meat products in Brazil

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Abstract

The aim of this study was to perform a comprehensive characterization of 48 *Campylobacter* isolates obtained from poultry meat products in Brazil, which were previously typed by pulsed-field gel electrophoresis (PFGE). Antimicrobial resistance (AMR) and presence of 30 virulence-associated genes (VAG) were investigated. The highest levels of resistance were observed to tetracycline (75%), followed by doxycycline (66.7%), ciprofloxacin (60.4%), and 35.4% to macrolides. Multidrug resistance (MDR) was found in 35.4% of the isolates. Our results revealed six AMR patterns and 26 VAG patterns. All 30 VAG were detected among the *Campylobacter* isolates, and each isolate carried at least 13 VAG. The *cadF*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *dnaJ*, *flaA*, *flaB*, *flhA*, and *luxS* genes were detected in all *Campylobacter* isolates. Moreover, all *C. jejuni* isolates carried the *ahpC*, *cj1000*, *clpP*, *docA*, *docB*, *flhB*, *fliA*, *fliM*, *fliY*, *pldA*, *racR*, *rpoN*, and *sodB* genes. Only the *wlaN* gene was more prevalent among *C. coli* isolates (66.7%). Data analysis for the combination of AMR and VAG profiles indicated that the antimicrobial resistance was no related to a particular species. The addition of AMR to PFGE data provided little additional discrimination to *Campylobacter* isolates, whereas adding VAG to PFGE results increased the discrimination power. However, the combination of the methods improved the characterization of the isolates. The presence of a diversity of antimicrobial resistant *C. jejuni* and *C. coli* isolated from poultry meat products in Brazil carrying several virulence factors represent a potential risk to health of consumers in many countries, since Brazil is the world's largest exporter of chicken meat. The findings demonstrate the need of more rigorous control measures for *Campylobacter* in poultry production chain from Brazil.

Keywords: *Campylobacter*; poultry meat; antimicrobial resistance; virulence factors

1 Introduction

Campylobacter is a foodborne pathogen considered to be the most common bacterial cause of human gastroenteritis (Shen et al., 2018; WHO, 2018) and is responsible for as many as 400–500 million cases worldwide each year (Wieczorek and Osek, 2013). The majority of the cases is associated to *C. jejuni* and *C. coli* species, and the poultry meat is recognized as one of the main sources of campylobacteriosis in humans (Bolton, 2015; Silva et al., 2018; WHO, 2013). In Brazil, as in most developing countries, there are few studies and scarce epidemiological data concerning campylobacteriosis. As national surveillance programs do not include campylobacteriosis, it is difficult to estimate the burden of this foodborne disease (Silva et al., 2018; WHO, 2013). However, the high isolation rates of *C. jejuni* and *C. coli* from chicken and chicken meat products in Brazil demonstrate the importance of controlling this pathogen in the country (Silva et al., 2018; Würfel et al., 2018), which is the world's largest exporter and the second largest producer of chicken meat (ABPA, 2017).

The clinical spectrum of campylobacteriosis ranges from self-limiting gastroenteritis to serious complications such as Guillain-Barré Syndrome (GBS) and Miller-Fisher Syndrome (MFS), acute transverse myelitis, and reactive arthritis (Bolton, 2015; EFSA/ECDC, 2018). Severe cases are usually treated with macrolides, fluoroquinolones or tetracycline, which represents a world public health concern as the efficacy of such treatments is currently compromised by increasing resistance of *C. jejuni* and *C. coli* to these antimicrobials (Bolton, 2015). According to World Health Organization (WHO, 2017), *Campylobacter* spp. are among the "priority pathogens" that pose greatest threat to human health, for which new antibiotics are urgently needed. Therefore, monitoring of antimicrobial resistance is essential for identification

of emerging or specific patterns of resistance, providing relevant data for risk assessments and targeted interventions (EFSA/ECDC, 2018).

The pathogenesis of *Campylobacter* infection is complex and poorly understood (Koolman et al., 2015) and the exact mechanisms by which *C. jejuni* or *C. coli* cause infection are unknown (WHO, 2013; Wieczorek et al., 2015). It is believed that complex multifactorial systems involving motility and chemotaxis (involving the *docB*, *docC*, *flaA*, *flaB*, *fliA*, *fliM*, *fliY*, and *rpoN* genes), adhesion (involving the *cadF*, *cj0588*, and *pldA* genes), toxin production (involving the *cdtA*, *cdtB*, and *cdtC* genes), cell invasion (involving the *ciaB*, *flhA*, and *flhB* genes), lipo-oligosaccharides (LOS) (involving the *wlaN* and *cgtB* genes), capsule (involving the *kpsM* gene), stress response (involving the *ahpC*, *cj1000*, *clpP*, *dnaJ*, *docA*, *katA*, *luxS, racR*, and *sodB* genes), and type-6 secretion system (T6SS; involving the *hcp* gene) are primordial for the success of *Campylobacter* growth in broilers, survival during food processing and virulence in humans (Bolton, 2015; Ghunaim et al., 2015; Harrison et al., 2014; Koolman et al., 2016; Krutkiewicz and Klimuskzo, 2010). Moreover, the wide genetic diversity among strains may contribute for differences in phenotypic responses of the pathogen (Silva et al., 2018).

Knowledge about the virulence potential of antimicrobial resistant *Campylobacter* isolates would contribute for risk assessments and development of control strategies. Therefore, the aim of this study was to perform a comprehensive characterization of *C. jejuni* and *C. coli* isolated from poultry meat products sold on the retail market in Brazil, which were previously typed by pulsed-field gel electrophoresis (PFGE). Antimicrobial resistance (AMR) and presence of virulence-associated genes (VAG) were investigated.

2 Material and Methods

2.1 *Campylobacter* isolates

Altogether, 48 *Campylobacter* isolates obtained from poultry meat products sold on the retail market in Southern Brazil were used in this study. They were previously characterized by polymerase chain reaction (PCR) as *C. jejuni* (n=42) and *C. coli* (n=6), and 26 DNA profiles were determined by PFGE (Würfel et al., 2018). All *Campylobacter* isolates were cultivated in blood agar base no. 2 (Oxoid, Basingstoke, Hampshire, UK) with 5% lysed horse blood under microaerophilic conditions (5% O_2, 10% CO_2, and balance N_2; White Martins, Rio de Janeiro, Brazil) for 48 h at 42 °C.

2.2 Antimicrobial resistance

A set of antimicrobials belonging to three different classes, considered as first- and second-choice for treatment of human campylobacteriosis, i.e. macrolides (azithromycin - AZI, clarithromycin - CLA, and erythromycin - ERY) fluoroquinolones (ciprofloxacin - CIP), and tetracyclines (doxycycline - DOX and tetracycline - TET) was used for determine the AMR profiles of the *C. jejuni* and *C. coli* isolates. The disk diffusion method was used according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017). Briefly, colonies were suspended in 0.85% NaCl (wt/vol) and adjusted to 1.5 X 10^8 $CFU.ml^{-1}$, followed by inoculation on Mueller-Hinton agar (Acumedia, Lansing, Michigan, USA) supplemented with 5% lysed horse blood and 20 $mg.L^{-1}$ β-NAD (Sigma-Aldrich, St. Louis, Missouri, USA). Afterwards, the disks impregnated with the antimicrobials agents (Laborclin, Paraná, Brazil) were placed on the agar surface. The plates were then incubated at 42 °C for 24 h under microaerophilic conditions. AMR of the *Campylobacter* isolates to each antimicrobial agent was measured, and the results were interpreted in accordance with interpretative

criteria provided by EUCAST (2018). The reference strain *Campylobacter jejuni* ATCC 33560 was used as control.

2.3 Detection of virulence-associated genes

The virulence potential of the *Campylobacter* isolates was evaluated by PCR amplification of 30 putative VAG, which are related to motility and chemotaxis (*docB*, *docC*, *flaA*, *flaB*, *fliA*, *fliM*, *fliY*, and *rpoN*), adhesion (*cadF*, *cj0588*, and *pldA*), cell invasion (*ciaB*, *flhA*, *flhB*, and *pldA*), toxin production (*cdtA*, *cdtB*, and *cdtC),* LOS biosynthesis (*cgtB* and *wlaN*), capsule biosynthesis (*kpsM*), stress response (*ahpC*, *docA*, *cj1000*, *clpP*, *dnaJ*, *katA*, *luxS, racR*, and *sodB*), and T6SS (*hcp*) (Table 1). Genomic DNA was isolated from a microaerophilic 24 h culture grown in blood agar base no. 2 (Oxoid) with 5% lysed horse blood using the illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, Little Chalfont, UK) according to manufacturer's protocol.

The amplification reactions of 25 µL were performed using 12.5 µL of 2x GoTaq Colorless Master Mix (Promega, Madison, Wisconsin, USA), 10 pmol/µL of each primer, and 10 ng of genomic DNA. PCR was performed in a thermocycler (T100 Thermal Cycler, Bio-Rad Laboratories, Hercules, California, USA) using standardized cycling parameters: 95 ºC for 1 min for initial denaturation followed by 35 cycles of denaturation at 95 ºC for 1 min, variable annealing (Table 1) for 1 min, primer extension step at 72 ºC for 1 min, and final extension step at 72 ºC for 5 min. The amplification products were submitted to agarose gel electrophoresis stained with Blue Green loading dye I (LGC Biotecnologia, São Paulo, Brazil) and visualized using a transilluminator L-Pix Touch (Loccus Biotecnologia, São Paulo, Brazil). The length of the expected PCR products are shown in Table 1. *Campylobacter jejuni* 100 (GenBank

CP023446.1), *C. jejuni* 104 (GenBank CP023343.1), and the reference strain *C. jejuni* ATCC 33560 were used as positive control. A mixture without the addition of DNA was incorporated as a negative control reaction.

2.4 Statistical analysis

Statistical differences in the AMR and prevalence of VAG among *Campylobacter* isolates, as well as in the prevalence of particular AMR and VAG profile, were evaluated using a 2 x 2 contingency table and Fisher's Least Significant Difference (LSD). Spearman's nonparametric test was used to evaluate correlations. The size of the correlation coefficient was interpreted according to Hinkle et al. (2003). *P* values of <0.05 were considered significant. The discriminatory ability of the methods was measured using the Simpson's index of diversity (SID) and 95% confidence interval (CI_{95}) was calculated as previously described (Simpson, 1949; Hunter and Gaston, 1988; Grundmann et al., 2001). The congruence among the methods was calculated using the Wallace (W) coefficient and CI_{95} (Wallace, 1983; Pinto et al., 2008). This coefficient indicates the probability that a pair of isolates which is assigned to the same type by one typing method is also typed as identical by the other method. *P* values were calculated for assessing whether differences between the coefficient values exist. All these parameters were generated using online tool Comparing Partitions (http://www.comparingpartitions.info).

3 Results

3.1 Antimicrobial resistance

AMR analysis of the 48 *Campylobacter* isolates revealed six different resistance patterns (Table 2). Overall, 38 (79.2%) *Campylobacter* isolates were resistant to one

or more antimicrobial agents, whereas 10 (20.8%) isolates were susceptible to all antimicrobials tested. Six (6%) isolates were resistant to a single antimicrobial agent (either CIP or TET), 5 (10.4%) isolates showed resistance to two antimicrobial agents (DOX and TET), and 27 (56.3%) isolates were resistant to more than two antimicrobial agents. Multidrug resistance (MDR) was found in 17 (35.4%) isolates. The highest levels of resistance were observed to TET (75%), followed by DOX (66.7%), and CIP (60.4%). Less frequent, 35.4% of the *Campylobacter* isolates were resistant to macrolides (AZI, CLA, and ERY). Antimicrobial resistance to tetracyclines and CIP was significantly more frequent than resistance to macrolides (P <0.05). All *Campylobacter* isolates that displayed resistance to macrolides were also resistant to the other antimicrobial classes tested.

It was found that *C. jejuni* isolates showed more resistance to TET (71.4%), followed by DOX (61.9%), CIP (57.1%), and macrolides (38.1%). *Campylobacter coli* isolates revealed greater rates of resistance to tetracyclines (100%), followed by CIP (83.3%), and macrolides (16.7%). Moreover, the *C. coli* isolates showed higher levels of CIP and tetracyclines resistance than *C. jejuni* isolates, whereas the *C. jejuni* isolates showed higher levels of resistance to macrolides than *C. coli* isolates (Fig. 1). Altogether, 16 (38.1%) *C. jejuni* and 1 (16.7%) *C. coli* isolates showed MDR. There was statistically significant difference among the AMR profiles (P <0.05). The pattern prevalent for *C. jejuni* was the MDR (profile 6, 38.1% of the isolates), which was significantly more frequent than profiles 2, 3, 4, and 5 (P <0.05). *Campylobacter coli* isolates showed CIP, DOX and TET (profile 5, 66.7% of the isolates) as prevalent profile, which was significantly more frequent than other profiles (P <0.05). Furthermore, none *C. coli* isolate was susceptible to all antimicrobials tested (profile 1) (Table 2).

3.2 Virulence-associated genes

All 30 VAG were detected among *C. jejuni* and *C. coli* isolates, and no VAG was detected in only one species. Except the *wlaN* gene, all genes were found in higher percentage among *C. jejuni* than among *C. coli* isolates. All 48 *Campylobacter* isolates carried the *cadF*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *dnaJ*, *flaA*, *flaB*, *flhA*, and *luxS* genes, which were significantly more frequent than *cgtB*, *cj0588*, *docC*, *hcp*, *katA*, *kpsM*, and *wlaN* genes (P <0.05). Moreover, 100% of *C. jejuni* isolates also carried others VAG (*ahpC*, *cj1000*, *clpP*, *docA*, *docB*, *flhB*, *fliA*, *fliM*, *fliY*, *pldA*, *racR*, *rpoN*, and *sodB*) (Fig. 2). The 26 different VAG patterns identified in the *Campylobacter* isolates are present in Table 3. All isolates carried at least 13 VAG but no profile was common to both *Campylobacter* species. The presence of the virulence markers varied between *C. jejuni* and *C. coli* species, however, the *C. jejuni* isolates carried at least 24 VAG, whereas *C. coli* isolates carried 13 to 15 VAG, with the exception of one isolate that carried 29 VAG. The predominant VAG pattern was the profile 1 composed of eight *C. jejuni* isolates, whereas no profile was predominant among *C. coli* isolates. The VAG profile 1 was significantly more frequent than other profiles, with exception of VAG profile 10 (P <0.05). The VAG profile 3 included only isolates susceptible to all antimicrobials tested, whereas the VAG profiles 18 and 19 included only MDR isolates.

3.3 Relationship between antimicrobial resistance and virulence factors

Comparing the data of VAG and AMR, there was a low significant negative correlation between MDR isolates and detection of *cgtB*, *docC* and *kpsM* genes (r =-0.4004 to -0.452; P <0.05), whereas a low positive correlation between MDR isolates and detection of *hcp* gene (r =0.3897; P <0.05) was observed. Moreover, there was a

significant positive correlation between susceptible isolates and detection of *cgtB* (r =0.5737; P <0.05) gene, in addition to a low positive correlation between susceptible isolates and detection of *docC* (r =0.4524; P <0.05) gene. Among macrolide resistant *Campylobacter* isolates, none of them carried the *cgtB* gene (r =-0.4752; P <0.05), and most of them did not carry the *kpsM* gene (r =-0.4004; P <0.05). However, most macrolide resistant isolates carried the *hcp* gene (r =0.3897; P <0.05). Among CIP resistant isolates, most of them did not carry the *cgtB* (r =-0.3241; P <0.05) and *docC* (r =-0.4562; P <0.05) genes, whereas most of CIP resistant isolates carried *hcp* gene (r =0.3167; P <0.05). Among the isolates resistant to tetracyclines, most of them did not carry the *cgtB* (r =-0.3241 DOX and r =-0.4763 TET; P <0.05) and *docC* (r =0.4454 DOX and r =-0.4122 TET; P <0.05) genes, whereas most of DOX resistant isolates carried the *hcp* (r =0.3563; P <0.05) gene.

3.4 Data of PFGE typing comparing to AMR and VAG profiles

Data of PFGE typing, AMR and VAG profiles of the *Campylobacter* isolates are summarizes in Table S1 (available in the online Supplementary Material). It was observed that the combination of the methods increased the diversity of the isolates evaluated. Only three PFGE patterns were composed of *Campylobacter* isolates sensitive to all antimicrobials tested, 24 PFGE patterns were composed of isolates resistant to at least one antimicrobial tested, whereas 11 PFGE patterns were exclusively composed of MDR isolates. Moreover, all PFGE patterns contained *Campylobacter* isolates carrying at least 13 VAG.

To assess the congruence among typing methods the W index was calculated (Table 4). A directional correlation between PFGE and AMR results was found, since the probability of two isolates having the same PFGE type also sharing the same AMR

profile was 78.8%. By contrast, the chance that two isolates sharing the same AMR profile also shared the same PFGE typing was 16.9% (Table 4). This difference can be explained by higher discriminatory power of PFGE compared to AMR. Indeed, most isolates that shared the same PFGE typing also shared the same AMR profile. The coefficients calculated for other combination of methods (PFGE and VAG; VAG and PFGE; AMR and VAG; VAG and AMR) were lower than 0.400 (Table 4). These results indicate that the addition of the AMR to PFGE provided little additional discrimination, whereas adding VAG to PFGE results in a higher discriminatory power.

3.5 Evaluation of the methods

The discriminatory power of the three methods was calculated by Simpson's Index of Diversity (SID) applied to the test population. The SID for PFGE typing, VAG and AMR profiles was 0.954 (CI_{95}, 0.927 to 0.981), 0.950 (CI_{95}, 0.920 to 0.980), and 0.785 (CI_{95}, 0.725 to 0.844), respectively. Difference between the SID of PFGE typing and VAG profile was not significant (P >0.05). However, there was a significant difference between the SID of PFGE typing and AMR profile, as well as between the SID of VAG and AMR profiles (P <0.05). These results indicate that PFGE typing and VAG profile had greater discriminatory capacity than AMR profile.

4 Discussion

A total of 48 *Campylobacter* isolates from poultry meat products from Southern Brazil were evaluated regarding AMR and VAG patterns and related to PFGE typing results (Würfel et al., 2018). Antimicrobial resistance in *Campylobacter* strains originated from food of animal origin has become a major public health concern, and there is a clear correlation between use of antimicrobials in the animal production and resistant strains

in humans (Wieczorek and Osek, 2013). High levels of resistance to tetracyclines (75% to TET and 66.7% to DOX) followed by CIP (60.4%) were found in our study, whereas resistance to macrolides (AZI, CLA, and ERY) was less frequent (35.4%) (Fig. 1).

The macrolides (primarily AZI, CLA, and ERY) remain the frontline agents to confirmed campylobacteriosis cases and the emergence of widespread resistance to this antimicrobial class would be of great clinical concern (Iovine, 2013). The low levels of *Campylobacter* resistance to macrolides such as found in our study are frequently reported (Fraqueza et al., 2014; Ghunaim et al., 2015; Proietti et al., 2018; Whitehouse et al., 2018; Wieczorek et al., 2013; Wieczorek et al., 2015). In a study performed by Woźniak-Biel et al. (2018) in Poland, all *Campylobacter* isolates from broilers were susceptible to macrolides. According to the authors, this result was attributed to rare use of these antimicrobials in Polish poultry production. The low rate of resistance to macrolides found in our study may be also explained by the non-use of macrolides in Brazilian poultry production, which reduces the selective pressure, such as was also observed by Ferro et al. (2005) in *Campylobacter* isolates from broiler carcasses in Southern Brazil.

In contrast to acquisition of macrolide resistance in *Campylobacter*, which is a stepwise process and requires prolonged exposure, the fluoroquinolone resistance is rapidly developed (Wieczorek and Osek, 2013) and does not incur a fitness cost, since *Campylobacter* is highly mutable to treatment with this antimicrobial class (Shen et al., 2018). Indeed, an increasing number of *Campylobacter* isolates have developed resistance to fluoroquinolones in recent years (Shen et al, 2018; Wieczorek and Osek, 2013), limiting its usage for the treatment of campylobacteriosis (Shen et al, 2018). This is worrisome, since the fluoroquinolones include the most commonly used antibiotics to treat acute bacterial diarrhea (Iovine, 2013). High levels of resistance to

fluoroquinolones, such as found in our study (60.4%) has been reported. Lopes et al. (2018) found 72.2% of resistance to CIP in *Campylobacter* isolated from raw chicken in Brazil. In study performed by Ghunaim et al. (2015) in Qatar, 63.2% of *Campylobacter* isolates from stools of patients with severe diarrhea showed resistance to CIP, whereas Woźniak-Biel et al. (2018) detected resistance to CIP in all *Campylobacter* isolates from broiler and turkey in Poland.

The high resistance levels of *Campylobacter* isolates to TET (75%) found in our study were previously reported by Ferro et al. (2015) in Brazil, as well as in other countries. In Poland, Woźniak-Biel et al. (2018) detected resistance to TET in 78.6% of *Campylobacter* isolates from broiler, whereas Proietti et al. (2018) found higher levels of TET resistance (90%) in *Campylobacter* isolates from chicken meat products in Central Italy. Fraqueza et al. (2014) found 67.1% of resistance to TET in cecum, carcass, and breast meat of poultry in Portugal. According Shen et al. (2018), tetracyclines are used in some cases to treat systemic infection with *Campylobacter*. However, the widespread resistance due to their heavy use in the past has somewhat limited their use today (Iovine, 2013).

In the U.S.A., as part of the NARMS (National Antimicrobial Resistance Monitoring System) retail meat surveillance program evaluated 589 *Campylobacter* isolates obtained in 2015 from retail poultry meats. The isolates displayed phenotypic resistance most frequently to TET (44.4%) and 7.1% were MDR (Whitehouse et al., 2018). In our study, MDR, defined as resistant to at least three different antimicrobial classes, was found in 35.4% of *Campylobacter* isolates. Similar levels of MDR (26.1%) were found by Wieczorek et al. (2013) in *Campylobacter* isolated from cattle in Poland. Woźniak-Biel et al. (2018) and Ghunaim et al. (2015) did not find MDR in *Campylobacter* isolates, whereas Ferro et al. (2015) reported 75% of MDR.

The higher prevalence of MDR among *C. jejuni* than among *C. coli* isolates found in our study differed of previously studies (Lopes et al., 2018; Proietti et al., 2018; Whitehouse et al., 2018; Wieczorek et al., 2013, 2015). The resistance to macrolides was also more prevalent among *C. jejuni* than among *C. coli* isolates. Furthermore, all *Campylobacter* isolates that displayed resistance to macrolides were also resistant to other antimicrobial classes, which is in accordance to recent studies (Fraqueza et al., 2014; Lopes et al., 2018; Proietti et al., 2018). Our results are also in accordance with Lehtopolku et al. (2010), which found that *Campylobacter* isolates resistant to macrolides were uniformly MDR. This is worrisome since MDR bacteria lead to a significant reduction in the therapeutic arsenal to treat campylobacteriosis caused by these strains.

The effort to reduce *Campylobacter* infections in humans is directly linked to a better understanding of the virulence mechanisms of this pathogen (Dasti et al., 2010). However, little is known about the virulence factors in *Campylobacter* (Bolton, 2015). Some genes have been recognized as responsible for pathogenicity expression (Datta et al., 2003). In our study, all 30 VAG investigated were detected among *C. jejuni* and *C. coli* isolates, and no VAG was associated to a single species (Fig. 2). Despite the existence of data concerning this, the genes evaluated, PCR primers used, and samples tested are different, which makes comparison of results difficult. However, some genes as *cadF*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *dnaJ*, *flaA*, *flaB*, and *flhA*, which were detected in all *Campylobacter* isolates in our study, are frequently found in high levels (Frazão et al., 2017; Koolman et al., 2015; Muller et al., 2006; Nguyen et al., 2016; Wieczorek et al., 2015).

Overall, most genes were found in higher percentage among *C. jejuni* than among *C. coli* isolates suggesting a higher virulence potential among *C. jejuni* isolates, which

would justify the higher rates of human infections associated to this species (over 90%) (Bolton, 2015; Koolman et al., 2016). Only the *wlaN* gene was more prevalent among *C. coli* isolates (66.7%). This gene was detected in lower levels among the *Campylobacter* isolates (41.7%), however, it was more prevalent in our study than in previous studies (Datta et al., 2003; Frazão et al., 2017; Koolman et al., 2015; Wieczorek et al., 2013, 2015). Muller et al. (2006) found the *wlaN* gene in 54.5% of *C. jejuni* strains, including two strains with strong colonization and invasion ability. In Brazil, Gomes et al. (2018) did not detecte the *wlaN* gene in *Campylobacter* isolates from human feces, animals, environment and chicken meat as well as Nguyen et al. (2016) in Vietnam. The *wlaN* and *cgtB* genes encodes ß-1,3 galactosyltransferases, which are responsible for specific LOS structures that are considered to be a critical factor in the triggering of the GBS and MFS neuropathies after *Campylobacter* infection (Muller et al., 2006).

Although *C. jejuni* isolates carried a higher number VAG (at least 24 VAG), the *C. coli* isolates carried at least 13 VAG and, curiously, one *C. coli* isolate carried 29 VAG. No profile was common to both *Campylobacter* species. Data analysis for the combination of AMR and VAG profiles indicated that the VAG profile 3 included only isolates susceptible to all antimicrobials tested, whereas the VAG profiles 18 and 19 included only MDR isolates. However, these profiles were composed by only *C. jejuni* isolates, suggesting that antimicrobial resistance was not related to a particular species. We also observed that the *Campylobacter* isolates included in the VAG profile 3 contained the *cgtB*, *docC*, *katA*, and *kpsM* genes, which were not detected in isolates of the VAG profiles 18 and 19. Moreover, there was a significant negative correlation between MDR isolates and the *cgtB*, *docC*, and *kpsM* genes, in addition to a significant positive correlation between susceptive isolates and the *cgtB* and *docC* genes, suggesting a

higher prevalence of the *cgtB* and *docC* genes among isolates susceptible to all antimicrobials tested. However, this correlation was statistically low to moderate.

Ghunaim et al. (2015) found that the prevalence of resistance to both ERY and CIP was higher among the *Campylobacter* isolates carrying *ciaB* but not *clpP* gene. This result was not found in our study, since all *Campylobacter* isolates carried the *ciaB* gene and only one isolate did not carry the *clpP* gene. There was a significant positive correlation between *Campylobacter* MDR isolates, resistant to macrolides, resistant to CIP and DOX, and the presence of the *hcp* gene, but this correlation was low. According to Harrison et al. (2014), *Campylobacter* strains possessing a complete T6SS cluster could be distinguished by presence of the *hcp* gene, which seems be a marker associated to severe campylobacteriosis. Thus, our results indicate that the presence of antimicrobial resistant T6SS-positive *Campylobacter* isolates into the Brazilian food chain may represent a potential emerging threat to public health.

In our study, the combination of the methods improved the characterization of the isolates. Wieczorek et al. (2013) found a poor correlation between virulence patterns, PFGE profiles and antimicrobial resistance of the *Campylobacter* isolates, that is, most of isolates with the same virulence markers belonged to different PFGE types and resistance patterns. The presence of a diversity of antimicrobial resistant *C. jejuni* and *C. coli* isolated from poultry meat products in Brazil carrying several virulence factors represent a potential risk to health of consumers in many countries, since Brazil is the world's largest exporter of chicken meat. The findings demonstrate the need of more rigorous control measures for *Campylobacter* in poultry production chain from Brazil.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Characteristics of PCR primers used in the study.

Gene	Sequence (5' → 3')	Annealing temperature (°C)	PCR product (bp)	Reference
Motility and chemotaxis				
docB	CGGAGAATTTAGAGGCACC CCGCAAATTCCATAGCAG	55	1418	Muller et al. (2006)
docC	TGAGCTACGCTATCATTG GCTTACGCTATGGGTTGG	50	1835	Muller et al. (2006)
flaA	GGATGGCGATAGCAGATAGTTT CTCATCCATAGCCTTATCAGCA	50	113	Chaisowwong et al. (2012)
flaB	ACACCAACATCGGTGCATTA CATCCCTGAAGCATCATCTG	50	128	Chaisowwong et al. (2012)
fliA	TATCGTAACGGCGTGAAAGC TCTTACATGCCAGCTCTGCG	55	127	Koolman et al. (2016)
fliM	TCATCCTCCTCTTCAGGCTC CACCGACACACCCATAGCCTC	55	1011	Muller et al. (2006)
fliY	GAACGCCGAAGTTACCATCG TGCATCAGCACCATTGAAGG	60	757	Muller et al. (2006)
rpoN	ATCGGGCTCTTTGCTTGCTA AATCGGCAACCAAGAGCGTA	55	171	Koolman et al. (2016)
Adhesion				
cadF	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	45	400	Konkel et al. (1999)
cj0588	ATGAGATTTGATTTTTTTGTTTCA ATTTTTGATATAGTAGTAAA	50	770	Krutkiewicz and Klimuskzo (2010)
pldA	AAGAGTGAGGCGAAATTCCA GCAAGATGGCAGGATTATCA	58	385	Zheng et al. (2006)
Cell invasion				
ciaB	TGCGAGATTTTTCGAGAATG TGCCCGCCTTAGAACTTACA	53	527	Zheng et al. (2006)
flhA	GGAGCGATTAAAGGCCCCAA AGTGGTGGCACTTGTCCAAA	55	183	Koolman et al. (2016)
flhB	CAGGTGCGGATGTGGTGATC CACTCCTTTGGCAACAACCCT	52	101	Muller et al. (2006)
Toxin production				
cdtA	CCTTGTGATGCAAGCAATC ACACTCCATTTGCTTTCTG	49	370	Hickey et al. (2000)
cdtB	CAGAAAGCAAATGGAGTGTT AGCTAAAAGCGGTGGAGTAT	51	620	Datta et al. (2003)
cdtC	CGATGAGTTAAAACAAAAAGATA TTGGCATTATAGAAAATACAGTT	53	182	Datta et al. (2003)
Lipo-oligosaccharides (LOS) biosynthesis				
cgtB	TTAAGAGCAAGATATGAAGGTG GCACATAGAGAACGCTACAA	58	561	Linton et al. (2000)
wlaN	TGCTGGGTATACAAAGGTTGTG AATTTTGGATATGGGTGGGG	47	330	Wassenaar et al. (2002)
Capsule biosynthesis				
kpsM	AGCAAAGGACGAGGAGTTAGC TATGGGTAGTTGGGGAGCCT	45	517	Koolman et al. (2015)
Stress response				

ahpC	CATGATAGTTACTAAAAAAGCTTTAG GTTAAAGTTTAGCTTCGTTTTTGCC	55	599	Oh and Jeon (2014)
cj1000	TTCGCTCTCAAACGCAGAAT TCCCTTAAGCCTGAGCCTACT	51	250	Koolman et al. (2016)
clpP	TGGGAGCATTTTTGCTTAGTTG CTCCACCTAAAGGTTGATGAATCAT	50	90	Ghunaim et al. (2015)
dnaJ	ATTGATTTTGCTGCGGGTAG ATCCGCAAAAGCTTCAAAAA	55	177	Chansiripornchai and Sasipreeyajan (2009)
docA	ATAAGGTGCGGTTTTGGC GTCTTTGCAGTAGATATG	55	725	Muller et al. (2006)
katA	CTGAACGCGATGTGAGAGGT TCACTTCGCTTTTTGCACGA	55	774	Koolman et al. (2015)
luxS	AAAATGCCAGCTCCTGCTGT GTGCGACAACCCATAGGTGA	45	218	Koolman et al. (2016)
racR	GATGATCCTGACTTTG TCTCCTATTTTTACCC	45	584	Datta et al. (2003)
sodB	AAGTACAGGCTGTGGCTGTG AAATAAGCAGGGCGTGCATTG	45	300	Koolman et al. (2015)
Type VI secretion system				
hcp	CAAGCGGTGCATCTACTGAA TAAGCTTTGCCCTCTCTCCA	60	463	Harrison et al. (2014)

Table 2. Distribution of antimicrobial resistance profiles (AMR) among the *Campylobacter* isolates.

AMR profile	Antimicrobial resistance pattern	Number (%) of isolates	
		C. jejuni (n = 42)	*C. coli* (n = 6)
1	Susceptible to all antimicrobials	10 (23.8)	-
2	CIP	2 (4.8)	-
3	TET	4 (9.5)	-
4	DOX, TET	4 (9.5)	1 (16.7)
5	CIP, DOX, TET	6 (14.3)	4 (66.7)
6	AZI, CLA, ERY, CIP, DOX, TET	16 (38.1)	1 (16.7)

AZI - azithromycin; CLA - clarithromycin; ERY - erythromycin; CIP - ciprofloxacin; DOX - doxycycline; TET - tetracycline.

Table 3. Distribution of virulence-associated genes (VAG) profiles among the *Campylobacter* isolates.

VAG profile	Virulence-associated genes pattern	Number of genes	Number (%) of isolates *C. jejuni* (n = 42)	*C. coli* (n = 6)
1	*ahpC, cadF, cdtA, cdtB, cdtC, cgtB, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB*	28	8 (19.0)	-
2	*ahpC, cadF, cdtA, cdtB, cdtC, cgtB, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	29	-	1 (16.7)
3	*ahpC, cadF, cdtA, cdtB, cdtC, cgtB, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, katA, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	29	3 (7.1)	-
4	*ahpC, cadF, cdtA, cdtB, cdtC, cgtB, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB*	27	1 (2.4)	-
5	*ahpC, cadF, cdtA, cdtB, cdtC, cgtB, ciaB, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, luxS, pldA, racR, rpoN, sodB, wlaN*	27	1 (2.4)	-
6	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, katA, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	29	1 (2.4)	-
7	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	28	1 (2.4)	-
8	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, luxS, pldA, racR, rpoN, sodB, wlaN*	27	1 (2.4)	-
9	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, katA, kpsM, luxS, pldA, racR, rpoN, sodB*	27	4 (9.5)	-
10	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, katA, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	28	5 (11.9)	-
11	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, katA, luxS, pldA, racR, rpoN, sodB*	26	1 (2.4)	-
12	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, docC, flaA, flaB, flhA, flhB, fliA, fliM, fliY, kpsM, luxS, pldA, racR, rpoN, sodB*	26	1 (2.4)	-
13	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, katA, kpsM, luxS, pldA, racR, rpoN, sodB*	27	1 (2.4)	-

14	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, katA, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	28	1 (2.4)	-
15	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, katA, luxS, pldA, racR, rpoN, sodB*	26	1 (2.4)	-
16	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB*	26	2 (4.8)	-
17	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, kpsM, luxS, pldA, racR, rpoN, sodB, wlaN*	27	1 (2.4)	-
18	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, luxS, pldA, racR, rpoN, sodB*	25	4 (9.5)	-
19	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, luxS, pldA, racR, rpoN, sodB, wlaN*	26	2 (4.8)	-
20	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj0588, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, kpsM, luxS, pldA, racR, rpoN, sodB*	25	2 (4.8)	-
21	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, cj1000, clpP, dnaJ, docA, docB, flaA, flaB, flhA, flhB, fliA, fliM, fliY, hcp, luxS, pldA, racR, rpoN, sodB*	24	1 (2.4)	-
22	*ahpC, cadF, cdtA, cdtB, cdtC, ciaB, dnaJ, docB, flaA, flaB, flhA, fliA, fliY, katA, luxS*	15	-	1 (16.7)
23	*cadF, cdtA, cdtB, cdtC, ciaB, cj1000, clpP, dnaJ, flaA, flaB, flhA, fliY, luxS, racR, sodB*	15	-	1 (16.7)
24	*cadF, cdtA, cdtB, cdtC, ciaB, clpP, dnaJ, flaA, flaB, flhA, fliY, katA, luxS, racR, sodB, wlaN*	16	-	1 (16.7)
25	*cadF, cdtA, cdtB, cdtC, ciaB, clpP, dnaJ, flaA, flaB, flhA, fliY, katA, luxS, racR, wlaN*	15	-	1 (16.7)
26	*cadF, cdtA, cdtB, cdtC, ciaB, clpP, dnaJ, flaA, flaB, flhA, luxS, sodB, wlaN*	13	-	1 (16.7)

Table 4. Congruence of isolates typing results as indicated by Wallace coefficients.

Method	Wallace coefficient (95% CI)[a]		
	PFGE typing	AMR profile	VAG profile
PFGE typing		0.788 (0.651-0.327)	0.327 (0.173-0.481)
AMR profile	0.169 (0.059-0.278)		0.086 (0.019-0.154)
VAG profile	0.304 (0.155-0.452)	0.375 (0.230-0.520)	

[a]Data were generated using online tool Comparing Partitions (http://www.comparingpartitions.info). CI, confidence interval.

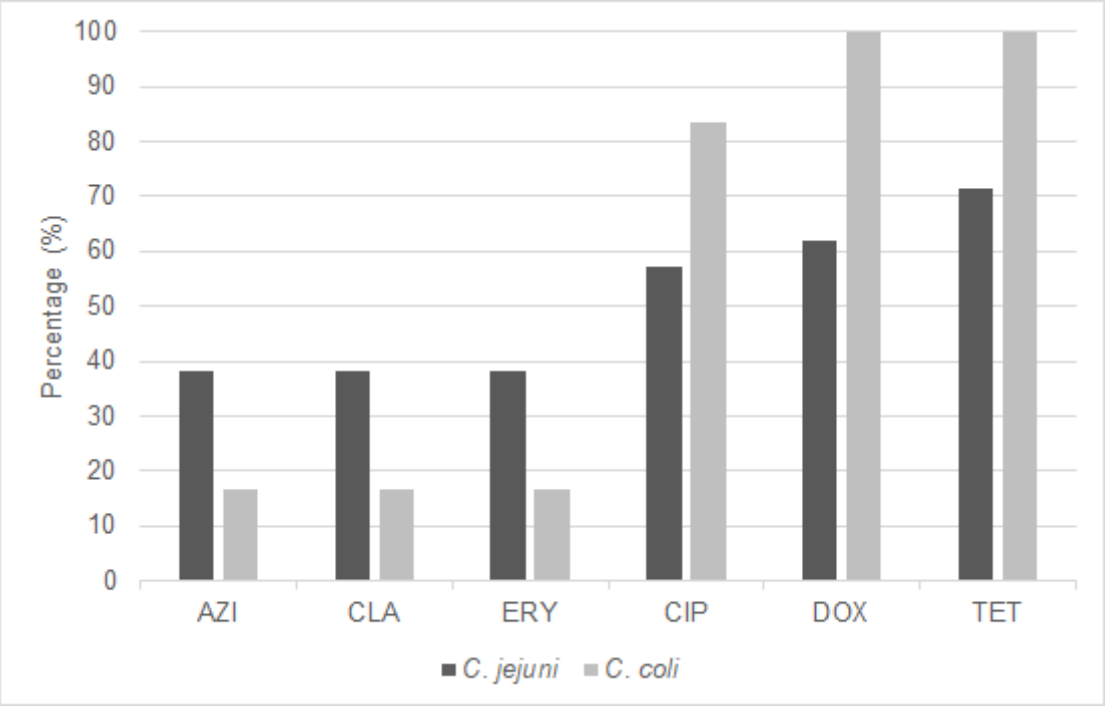

Fig. 1. Prevalence of antimicrobial resistance genes among *Campylobacter jejuni* and *Campylobacter coli* isolates.

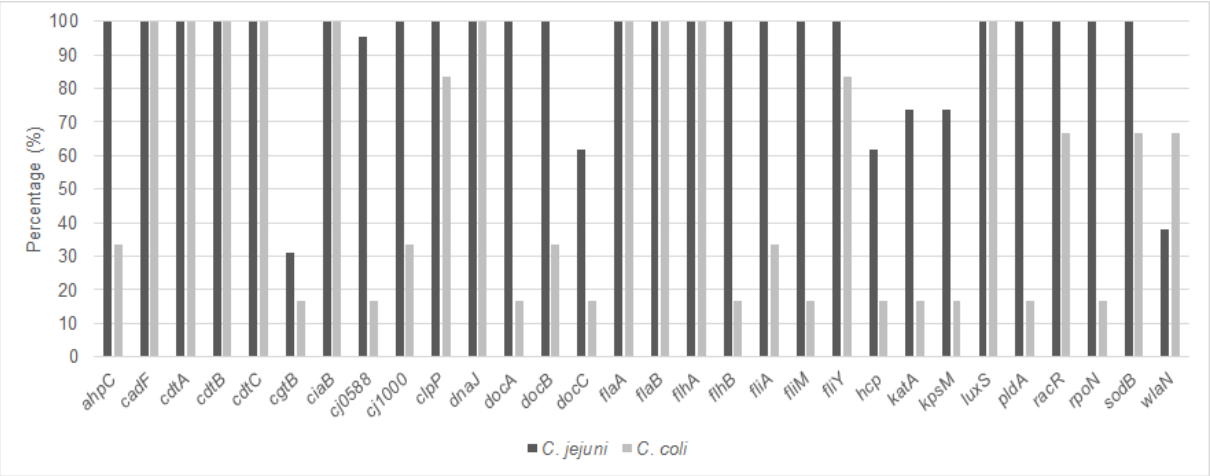

Fig. 2. Prevalence of virulence-associated genes among *Campylobacter jejuni* and *Campylobacter coli* isolates.

3.3 Artigo 3

Whole-genome sequence and comparative analysis of *Campylobacter jejuni* isolated from poultry meat in Brazil

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Whole-genome sequence and comparative analysis of *Campylobacter jejuni* isolated from poultry meat in Brazil

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Abstract

Campylobacter jejuni is the most common bacterial cause of foodborne diarrheal disease worldwide. This pathogen is a common inhabitant of the intestinal tract of broilers and frequently causes human disease via contamination of poultry meat products intended for human consumption. In this study, two genomes of *C. jejuni* isolated from poultry meat in Brazil were sequenced, assembled, and subjected to genomic analysis. The isolates *C. jejuni* 100 and 104 (CJ100 and CJ104) showed distinct multilocus sequence typing (MLST), belonging to the ST-353 and ST-607 complexes, respectively. The comparative analyses reference revealed a large number of single nucleotide polymorphisms (SNPs), rearrangements, and inversions in both genomes, in addition to virulence factors, genomic islands, prophage sequences, and insertion sequences. A circular 103-kilobase megaplasmid carrying virulence factors related to motility, toxin production, and the iron uptake system was identified in the genome of CJ100. Moreover, the genes *blaOXA-61* and *tetO*, conferring resistance to beta-lactams and tetracyclines, respectively, were identified, as well as *aad9*, *aphA-3*, *aadE*, and *sat4* genes, which are related to aminoglycoside resistance. In addition, a mutation in the quinolone resistance determining region (QRDR) of the *gyrA* gene, which confers high-level resistance to quinolones was found in the genome of CJ100. Herein, we reported the first genomic analysis of *C. jejuni* isolated in Brazil. The genomic data of *C. jejuni* isolated in Brazil and the analysis of a novel virulence megaplasmid provide a basis for pan-genome studies and further investigations. The presence of potentially virulent *C. jejuni* isolates in the poultry meat in this country represent a potential risk to health of consumers, since the Brazil is the world's largest exporter of chicken meat. This demonstrate the need of approaches to control this pathogen in poultry production chain.

Keywords: *Campylobacter jejuni*, genomics, bioinformatics, megaplasmid, antimicrobial resistance, virulence factors

Introduction

Campylobacter is a foodborne pathogen considered to be the most common bacterial cause of human gastroenteritis (Kinana et al. 2006; Parkhill et al. 2000; WHO 2018). Most *Campylobacter* infections are associated with ingestion of poultry meat, often contaminated during slaughter and processing as a result of intestinal leakage, since *C. jejuni* is a common inhabitant of the intestinal tract of broilers (Young et al. 2007). The clinical spectrum of campylobacteriosis ranges from uncomplicated gastroenteritis to peripheral neuropathies such as Guillain-Barré and Miller-Fisher syndromes (Fouts et al. 2005). These variations in clinical manifestations of campylobacteriosis could be due to the wide genetic diversity that exists between strains of *Campylobacter* (Silva et al. 2018).

Brazil is the world's largest exporter and the second largest producer of chicken meat, and Southern Brazil produces and exports more than 50% of this chicken meat (ABPA 2017). Despite the importance of *Campylobacter* infections, the disease remains underdiagnosed and underreported in Brazil. Moreover, molecular studies of local *Campylobacter* isolates are scarce (Frazão et al. 2017; Gomes et al. 2016; Silva et al. 2018).

Campylobacter jejuni present an extensive genetic variation due to intragenomic mechanisms and genetic exchange between strains (Young et al. 2007). The complete genomic sequence of the *C. jejuni* strain NCTC 11168 revealed the presence of several genes encoding proteins with virulence potential and survival properties, in addition to hypervariable sequences found in regions that encode proteins involved in the biosynthesis or modification of surface-accessible carbohydrate structures (Parkhill et al. 2000). These structural variations arise from mechanisms such as point mutations, gene duplication and deletion, frameshifts, and phase variation (Young et al. 2007).

Nevertheless, the poor understanding of the genetic, physiologic, and virulence mechanisms of *Campylobacter* hinders efforts to control it effectively in the food chain and to design disease prevention strategies (Parkhill et al. 2000).

In this study, we report the whole genome sequences and genetic features of two *C. jejuni* isolates from poultry meat in Southern Brazil. We aimed to compare the genomes in order to characterize potential factors of virulence, survival, and antimicrobial resistance mechanisms, and to get insights into the mechanisms of pathogenicity. Moreover, we identified a novel megaplasmid carrying virulence-related genes.

Materials and Methods

Strains selection, growth conditions, and genomic DNA extraction

Two *C. jejuni* isolates from poultry meat sold on the retail market in Southern Brazil (Würfel et al., 2018) were selected for whole genome sequencing and comparative genomic analysis. The isolates were characterized as multidrug-resistant (CJ100) and susceptible (CJ104) to six antimicrobials of three different classes: macrolides (azithromycin, clarithromycin, and erythromycin), fluoroquinolones (ciprofloxacin), and tetracyclines (doxycycline and tetracycline) (unpublished observations). Genomic DNA from both isolates was extracted using the illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, Uppsala, Sweden) from a microaerophilic (5% oxygen, 10% carbon dioxide, 85% nitrogen) 24 h culture grown in blood agar base no. 2 (Oxoid, Basingstoke, Hampshire, UK) with 5% lysed horse blood.

Whole-genome sequencing

Bacterial genome sequencing was performed using the Ion Torrent PGM (Life Technologies, Carlsbad, CA, USA). Briefly, DNA libraries were constructed using

enzymatic fragmentation and adaptor ligation with the Ion Xpress Plus fragment library kit (Life Technologies). Fragment size selection was performed using E-Gel® SizeSelect 2% (Invitrogen, Carlsbad, CA, USA). Template preparation, emulsion PCR, and ion sphere particle (ISP) enrichment were performed using Ion One Touch template kit (Life Technologies). The ISPs were loaded and sequenced on a 314 chip (Life Technologies).

Genome assembly and annotation

De novo genome assembly of strains 100 and 104 was performed using Newbler (https://www.roche.com/), MIRA (http://www.chevreux.org/), SPAdes (Bankevich et al. 2012), and CLC Genome Workbench (https://www.qiagenbioinformatics.com/). For each isolate, the results of the assemblers were merged using CISA (Lin and Liao 2013) to generate a consensus assembly, which was scaffolded by the tool CAR (Lu et al. 2014) using the genome of the *C. jejuni* strain NCTC 11168 – ATCC 700819 (GenBank accession number: AL111168.1) as the reference. Unmapped sequences were searched against the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). The assembly gaps were closed using GMCloser (Kosugi et al. 2015) and FGAP (Piro et al. 2014). The final assemblies were annotated using Genix (Kremer et al. 2016) and manually curated using Artemis (Rutherford et al. 2000).

Detection in silico of antimicrobial resistance mechanisms, virulence, and survival factors

The genomes of CJ100 and CJ104 were searched for antimicrobial resistance mechanisms automatically using the ResFinder 3.0 webserver (https://cge.cbs.dtu.dk/services/ResFinder/) and the CARD database

(https://card.mcmaster.ca/faq), and manually using BLAST at the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Virulence and survival factors were manually identified using BLAST searches against the Uniprot database (www.uniprot.org/).

Identification of prophage sequences and genomic islands (GIs)

The identification of prophage sequences in the genomes of CJ100 and CJ104 was performed using the PHAge search tool, PHAST (Zhou et al. 2011), whereas the GIs were identified using the IslandViewer 4 (Bertelli et al. 2017).

Clustered regularly interspaced short palindromic repeats (CRISPR) analysis

The identification of CRISPR *loci* in the genomes of CJ100, CJ104, and the reference strain NCTC 11168 was performed using the CRISPRone webserver (http://omics.informatics.indiana.edu/CRISPRone/). The repeat regions identified by the program were aligned using MUSCLE (Edgar 2004) to evaluate their conservation.

Analysis of variants and in silico prediction of the biological impacts

Single nucleotide polymorphisms (SNPs) and insertions and deletions (INDELs) in the genomes of CJ100 and CJ104 were identified based on the genome of NCTC 11168. The sequencing reads were aligned to the reference genome using Segemehl (Hoffmann et al. 2009), and alignments were processed using SAMTools (with the argument “-m 10” to avoid false-negative INDELs caused by homopolymer errors), BCFTools, and VCFUtils.pl (Li et al. 2009). Thus, the resulting VCF file was processed using an in-house Python script to filter the variants based on Phred quality (Q) by applying a minimum Q of 60 for SNPs. SnpEff (Reumers et al. 2005) was used to predict the biological impact of all variants based on the genes present in the original

annotation of the NCTC 11168 genome. Finally, affected genes were annotated using the COG database (Tatusov et al. 2000), and the mutations that affect each functional group were summarized using an in-house Python script.

Comparative sequence, phylogenetic, and in silico multilocus sequence typing (MLST) analysis

The whole-genome sequences of CJ100 and CJ104 were aligned with the reference genome strain NCTC 11168 using the Artemis Comparison Tool (ACT) (Rutherford et al. 2000) to identify structural variations (e.g., translocations, large insertions/deletions, inversions). For phylogenetic analysis, the genomes of CJ100 and CJ104 were compared and aligned with a dataset composed of 40 *C. jejuni* strains using Mugsy (Angiuoli and Salzberg 2011). The alignments were processed and filtered using an in-house Python script (https://www.python.org/) that selected only the syntenic blocks conserved among all strains, merged them into a single alignment, and exported it as a .phylip file. Thus, PhyML was used to generate a phylogenetic tree using a maximum-likelihood (ML) algorithm, and the final plot was created with iToL (Letunic and Bork 2016). MLST types were assigned using the *Campylobacter* MLST database, and new allele sequences were submitted to the *Campylobacter jejuni/coli* MLST database (http://pubmlst.org/campylobacter/).

Results

Genomes features

The genomes of CJ100 and CJ104 contained a circular chromosome of 1,766,193 bp and 1,558,306 bp in size, respectively. CJ100 also contained a megaplasmid of

103,409 bp. The chromosome of CJ100 contained 1,763 predicted coding sequences (CDS) with 42 tRNAs and 3 rRNAs, while the chromosome of CJ104 contained 1,567 CDS with 37 tRNAs and 3 rRNAs. Antisense DNA (*purD* gene) was identified in the chromosome of CJ100, and riboswitches were found in the chromosomes of both the CJ100 and CJ104 isolates. The data are summarized in Table 1. The G+C content of the genomes of CJ100 and CJ104 each was 30.3%, similar to reference genome NCTC 11168 (30.5%).

The genomes of isolates CJ100 and CJ104 had 86.12% and 88.72% symmetric identities, respectively, with reference genome NCTC 11168. The closest genome neighbor of CJ104 was a *C. jejuni* isolate (GenBank assembly accession: GCA_001407815.1; 91.39% symmetric identity), while the closest genome neighbor of CJ100 was *C*. *jejuni* M129 (GenBank assembly accession: GCA_001865595.1; 89.78% symmetric identity).

Comparative sequence analysis and phylogenetics

The structural comparison based on the reference genome *C. jejuni* NCTC 11168 is presented in Fig. 1. The structural analysis facilitated the identification of some potential events of genomic inversions, translocations, and deletions in both genomes of CJ100 and CJ104. The phylogenetic analysis performed based on alignment of the syntenic regions suggests that CJ100 and CJ104 are more related to each other than to the strain *C. jejuni* NCTC 11168 (Fig. 2). In the phylogenetic tree, CJ100 and CJ104 are clustered with isolates from different sources, but both are more related to clinical isolates. CJ100 is closely related to *C. jejuni* M129.

MLST profiling

The results of the *in silico* MLST analysis are presented in Table 2. As demonstrated by the combination alleles, CJ100 and CJ104 belong to the ST-353 and ST-607 complexes, respectively.

Identification of variants and biological impacts

The variant calling analysis based on the reference genome *C. jejuni* NCTC 11168 identified 18.521 and 19.306 variations in the genomes of CJ100 (Fig. 3a) and CJ104 (Fig. 3b), respectively. Among the SNPs identified in the genome of CJ100, 5.246 led to missense mutations and 12.458 led to synonymous mutations in comparison to the *C. jejuni* NCTC 11168 reference genome. In the genome of CJ104, 5.430 SNPs led to missense mutations and 12.959 led to synonymous mutations in comparison to the reference genome. In this analysis, most of the synonymous mutations for both isolates were found in genes related to cell wall/membrane biogenesis, amino acid metabolism and transport, and translation, ribosomal structure, and biogenesis. The identified variants and their biological impacts are showed in Fig. 3a,b.

CRISPR-Cas (CRISPR-associated) system

Three predicted genes of the CRISPR-Cas system were found in the genomes of both the CJ100 and CJ104 isolates. They encoded Cas2 subtype-II-C, Cas1 subtype-II-C, and Cas9 subtype-II-C proteins, respectively. We also identified similar sequences of CRISPR-repeats longer than 21-bp in four regions of chromosomes in both CJ100 and CJ104, which were similar to CRISPR-repeats in the reference strain *C. jejuni* NCTC 11168 (Fig. 4).

Prophage sequences and genomic islands

Incomplete prophage regions were identified in the chromosomes of both CJ100 and CJ104. In the chromosome of CJ104, a prophage region (region I) of 8.1kb (29.78% GC) containing 9 CDS (position 137819-145920) was found (Fig. S1). In the chromosome of CJ100, a prophage region (region I) of 15.3kb (29.56% G+C content) containing 14 CDS (position 1247404-1262759) was identified (Fig. S2), in addition to a prophage region (region II) of 8.1kb (29.76% G+C content) containing 9 CDS (position 1665823-1673923) (Fig. S3). Interestingly, region I of chromosome of CJ104 and region II of chromosome of CJ100 contained the same incomplete prophages. Phage-like genes were also found in other regions of the chromosomes of both isolates CJ100 and CJ104, and a prophage sequence was inserted in a GI in the chromosome of CJ100. The incomplete prophage regions identified in CJ100 and CJ104 are summarized in Table 3.

GIs were found in the chromosomes of both CJ104 (Fig. S4) and CJ100 (Fig. S5). In the chromosome of CJ104, a GI containing 8 genes (Table S1) encoding proteins involved in the iron uptake system and heat shock, and an insertion sequence (IS) element (IS*Cco1*, transposase orfB) was found. In the chromosome of CJ100, 12 GIs containing several genes were identified (Table S2). Among them, antimicrobial resistance genes, genes related to virulence and survival, phage-like genes, and genes related to the type IV secretion system (T4SS), among others were found in GI regions of CJ100. Genes encoding homologues of VirB (VirB2, VirB3/VirB4, VirB5, VirB6, VirB8, VirB9, VirB10, and VirB11) and VirD4 proteins were found as well, together with phage-like genes and resistance genes to aminoglycosides and tetracyclines. However, some GIs overlapped due to the use of more than one identification method (IslandPath-DIMOB, SIGI-HMM, and IslandPick). The

chromosome of CJ100 contains also two copies of an IS element (IS*Cco1*, transposase orfB).

Antimicrobial resistance mechanisms

The acquired antimicrobial resistance genes and chromosomal point mutations identified in CJ100 and CJ104 are showed in Table 4a,b. A mutation in the quinolone resistance-determining region (QRDR) of the *gyrA* gene, which leads to the Thr-86-Ile substitution in gyrase was found in CJ100 (resistant to ciprofloxacin) but not in CJ104 (susceptible to ciprofloxacin). Unknown mutations in the *gyrA* gene were also identified in both isolates CJ100 and CJ104, but their relation to quinolone resistance is unknown. No mutations were found in the 23S rRNA genes of both isolates, but unknown mutations were found in the *rplV* gene that encodes the L22 ribosomal protein, which is related to macrolides resistance. Among them, the chromosome of CJ100 carried the amino acid substitution A103V.

Isolate CJ100 carried the genes encoding the CmeABC efflux pump and its transcriptional regulator CmeR, whereas CJ104 carried the *cmeABC* genes, but the *cmeR* gene contained unknown mutations. The *tetO* gene was located on the chromosome of CJ100, in addition to *blaOXA-61* gene, which are related to tetracyclines and beta-lactams resistance, respectively. In addition, CJ100 also carried the *aad9*, *aphA-3*, *aadE*, and *sat4* genes, which are related to aminoglycoside resistance. These genes were found in the same GI as the *tetO* gene.

Virulence and survival factors

A large number of related virulence and survival genes were found in the sequenced genomes of CJ100 and CJ104. The annotation results indicated the presence of genes

related to motility, chemotaxis, adhesion, invasion, toxin production, lipooligosaccharide (LOS) biosynthesis, extracellular polysaccharide (EP) biosynthesis, iron uptake system, and stress response, among others. The presence and location of the genes varied between the genomes (Table S3). Two copies of the *cheY* gene that encodes a chemotaxis regulatory protein were found in the chromosomes of both isolates CJ100 and CJ104. Besides that, CJ100 carried two copies of the *fliI* gene (flagellum-specific ATP synthase) and *clpP* gene (ATP-dependent Clp protease proteolytic subunit), which are related to motility and stress response, respectively.

Some genes related to chemotaxis (*docC*), adhesion (*pldA*), iron uptake system (*ceuE*), and stress response (*katA*) were found in the genome of CJ100 only. Similarly, genes related to invasion (*cipA*), LOS biosynthesis (*cgtB* and *htrB*), and iron uptake system (*exbB2*) were present in the genome of CJ104 only. The *clpP* gene was found in the prophage region of CJ104, whereas the *docC* gene was present in the GI of CJ100. In our study, the LOS biosynthesis locus was located in approximately the same region of the genomes of both CJ100 (position 1085290-1100366) and CJ104 (position 1076941-1090940). However, the LOS biosynthesis locus of the genome of CJ100 contained 4 gaps.

Plasmid

A novel 103-kilobase megaplasmid denominated pJc100 was found in the genome of CJ100. It was the third-largest plasmid deposited at DDBJ/EMBL/GenBank and contained 104 CDS encoding some virulence factors related to motility, toxin production, and iron uptake system, none of which were present in the chromosome of CJ100 (Fig. 5; Table S3). Among them was the *cdt* gene cluster that encodes the

cytolethal distending toxin (CDT). Interestingly, the complete genomic sequence of the pJc100 plasmid showed 99% nucleotide identity with a region of 89,386 bp (position 54200-143585) of *C. jejuni* M129.

Discussion

Campylobacter is regarded as the most common bacterial cause of human gastroenteritis worldwide (WHO 2018). Despite the great importance of *Campylobacter* as foodborne pathogen, this microorganism has not been widely studied in Brazil when compared to other pathogens and molecular studies of local *Campylobacter* isolates are very limited (Silva et al. 2018). In this study, we report the whole genome sequences and genetic features of two *C. jejuni* isolates from poultry meat in Southern Brazil.

The structural comparison based on the reference genome *C. jejuni* NCTC 11168 (Fig. 1) indicated that *C. jejuni* might present high plasticity in genomic organization. Moreover, the SNPs analysis identified a high number of variations in the genomes of both CJ100 (Fig. 3a) and CJ104 (Fig. 3b), which led to missense and synonymous mutations. According to Young et al. (2007), the genome plasticity and spread of new factors may be due to natural transformation since *C. jejuni* is naturally competent and can take up DNA from the environment, which leads to recombination between strains and increased genetic diversity. There is a high rate of recombination due to horizontal gene transfer (HGT), which results in increased allelic diversity across *C. jejuni* genomes and varying degrees of disruption of the overall clonal population structure, such that the introduction of large numbers of polymorphisms can generate novel phenotypes (Sheppard and Maiden 2015).

The HGT contributes greatly to the evolution of bacterial species and the genes acquired laterally account for a large proportion of bacterial genomes. Approximately 75% of the genes in each genome have been acquired by HGT during evolution. They are disseminated by mobile genetic elements, such as plasmids, transposons, bacteriophages or genomic islands (Juhas 2015). In our study, incomplete prophage regions and phage-like genes were identified in the chromosomes of both CJ100 and CJ104 (Table 3; Fig. S1, S2, S3). Moreover, a prophage sequence was inserted in a GI in the chromosome of CJ100. Prophages are clusters of phage-like genes within a bacterial genome and the prophage sequences can account for a significant fraction of the variation within bacterial species or clades since bacterial genomes can contain around 20% of bacteriophage genes (Zhou et al. 2011). According to Juhas (2015), bacteriophages are vehicles for the horizontal transfer of virulence genes between bacteria, which can play a role in many aspects of bacterial virulence (Fouts et al. 2005), in addition to facilitate transfer of mobile genetic elements as GIs (Juhas 2015). The presence of prophage sequences may also allow the existence in new environmental niches and the acquisition of antimicrobial resistance (Zhou et al. 2011). GIs are mobile regions found in bacterial genomes containing genes of probable horizontal origin. They are a major driver of genome evolution since often provide adaptive traits that enhance the fitness of bacteria within a niche (Bertelli et al. 2017). GIs containing several genes were found in the chromosomes of both CJ100 and CJ104 (Fig. S4, S5; Table S1, S2). Among them, genes related to virulence and survival, antimicrobial resistance, phage-like genes, and genes related to the type IV secretion system (T4SS), which has been identified in numerous pathogenic bacteria and plays diverse roles including DNA export, bacterial conjugation, and protein secretion (Cao and Saier 2001). In *C. jejuni*, this DNA-uptake system is denominated

C. jejuni Cjp/VirB system and is composed of homologues of the VirB core proteins (Cascales and Christie 2003). VirD4 homologues are thought to assist VirB2–11 homologues as coupling proteins, linking DNA–protein substrates to membrane pores. Homologues of VirB8, 9, 10, and 11 found in *C. jejuni* may serve virulence-related functions not requiring a complete type IV secretion system (Cao and Saier 2001).

In contrast with *C. jejuni* NCTC 11168, which is notable for the apparent absence of prophages and functional IS elements in the genome, with the exception of one copy of a degenerate transposase resembling IS605 (Parkhill et al. 2000), the chromosomes of both isolates CJ100 and CJ104 contains IS elements (IS*Cco1*, transposase orfB). According to Juhas (2015), the transposase has been shown to be indispensable for the catalysis of the insertion and excision of the IS element, as mobile genetic elements often undergo excision and reintegration into the host's chromosome. These findings suggest that part of genetic material of CJ100 and CJ104 may has been acquired by HGT, such as the virulence factors, adaptation, bacterial survival mechanisms, and multidrug-resistance.

Acquired antimicrobial resistance genes and chromosomal point mutations were found in the chromosomes of both isolates CJ100 (Table 4a) and CJ104 (Table 4b). The Thr-86-Ile substitution in *gyrA* gene found in the chromosome of CJ100 confers high-level resistance to quinolones (Iovine 2013; Wieczorek and Osek 2013). According to Kinana et al. (2006), there seems to be a relationship between the ST-353 complex and the isolates carrying the Thr-86-Ile substitution in the GyrA protein. A high level of macrolide resistance is attributed to mutations at positions 2074 and 2075 of the peptidyl encoding region in domain V of 23S rRNA, while mutations in ribosomal proteins L4/L22 likely have only minor contributions to macrolide resistance (Iovine 2013). However, no mutations were found in the 23S rRNA genes of CJ100, but were

found in the *rplV* gene that encodes the L22 ribosomal protein. Among them, the amino acid substitution A103V, which was also described by Corcoran et al. (2006) in two high-level erythromycin-resistant *Campylobacter* isolates.

The chromosome of isolate CJ100 also carried the genes encoding the CmeABC efflux pump and its transcriptional regulator CmeR. The chromosomally-encoded multidrug efflux pump CmeABC consists of three components: an outer membrane protein (encoded by *cmeC*), an inner membrane drug transporter (encoded by *cmeB*), and a periplasmic protein (encoded by *cmeA*) that bridges CmeB and CmeC. This efflux pump usually works in synergy with a second resistance mechanism and can contribute to antimicrobial resistance to several antimicrobials, including tetracyclines (Iovine 2013; Wieczorek and Osek 2013).

The resistance to tetracyclines in CJ100 likely is related to the presence of the *tetO* gene, which can act in synergy with efflux pump CmeABC. According to Iovine (2013), TetO alone has been shown to confer high-levels of tetracycline resistance; however, the contribution of efflux to tetracycline resistance is demonstrated by the increase in tetracycline MIC when efflux pumps are genetically inactivated. In our study, the *tetO* gene was located on the chromosome, but it is more commonly found on the plasmid pTet in *C. jejuni* (Iovine 2013). Another gene identified in CJ100 was *blaOXA-61,* which is related to beta-lactams resistance. According to Iovine (2013), enzymes of class D β-lactamase OXA-61 mediate resistance to penicillin, oxacillin, ampicillin, amoxicillin-clavulanate, piperacillin, and carbenicillin. A most of *C. jejuni* isolates is able to produce beta-lactamases, which act inactivating the beta-lactam molecule by hydrolyzing the structural lactam ring (Wieczorek and Osek 2013).

The chromosome of CJ100 also carried the *aad9*, *aphA-3*, *aadE*, and *sat4* genes, which are related to aminoglycoside resistance. *aad9* is a streptomycin modification

gene and encodes an aminoglycoside 3''-adenylyltransferase. The *aphA-3* gene, which encodes a 3'-aminoglycoside phosphotransferase and confers kanamycin resistance, remains the most common source of aminoglycoside resistance in *Campylobacter* (Gibreel et al. 2004; Iovine 2013). In some isolates, such as CJ100, *aphA-3* is found with genes encoding streptomycin resistance (encoded by *aadE*, a 6'-adenylyl transferase) and streptothricin resistance (encoded by *sat*, a streptothricin acetyltransferase) (Iovine 2013). The genetic organization of this resistance cluster suggests that *C. jejuni* acquired it from a Gram-positive organism via horizontal transfer (Gibreel et al. 2004). In study performed by Gibreel et al. (2004), the kanamycin-resistance phenotype was transferred along with tetracycline resistance by conjugation in *C. jejuni* strains. According to Iovine (2013), some *Campylobacter* strains may acquire plasmids containing various aminoglycoside resistance genes along with *tetO* gene from Gram-negative or Gram-positive sources, becoming multidrug resistant. As *Campylobacter* to possess genetic mechanisms for natural transformation and conjugation, any acquired antimicrobial resistance genes may be transferred rapidly between strains (Alfredson and Korolik 2007). According to Bertelli et al. (2017), some antimicrobial resistance genes are known to be commonly found in GIs. Indeed, the resistance genes to aminoglycosides found in CJ100 were in the same GI as the *tetO* gene, however, were located in the chromosome.

A large number of related virulence and survival genes were found in the genomes of CJ100 and CJ104 (Table S3). Some genes were found in the genome of CJ100 only, whereas other genes such as *cgtB* and *htrB*, which are related to LOS biosynthesis (Gilbert et al. 2000; Parkhill et al. 2000), were present in the genome of CJ104 only. The identification of the associated genes to LOS biosynthesis is of considerable interest for a better understanding of the pathogenesis mechanisms used by

Campylobacter, since the bacteria evade the immune response by using the saccharide portion of LOS for molecular mimicry of host structures (Gilbert et al. 2000). Hypervariable sequences are found in the region that encodes proteins involved in LOS biosynthesis (Parkhill et al. 2000), and there are major differences in the organization of the LOS biosynthesis locus between strains, suggesting that genetic rearrangements and heterologous DNA uptake can contribute to the diversity of LOS structures (Gilbert et al. 2000). In our study, the LOS biosynthesis locus was located in approximately the same region of the genomes of both CJ100 and CJ104. However, the LOS biosynthesis locus of the genome of CJ100 contained 4 gaps, which may account for the absence of some genes, such as *cgtB* and *htrB* that were found only in CJ104.

The presence of virulence genes in prophage regions and GI of the genomes of CJ100 and CJ104 may suggest acquisition of genes by HGT. According to Juhas (2015), CRISPR was shown to play a role in the HGT in *C. jejuni*. The CRISPR-Cas system equips prokaryotes with an effective defense against mobile genetic elements as bacteriophages, plasmids, and transposons (Sampson and Weiss 2014). Three predicted genes of the CRISPR-Cas system, which are encoded predominantly in the genomes of pathogenic bacteria that interact with eukaryotic hosts (Sampson and Weiss 2014), were found in the genomes of both CJ100 and CJ104, in addition to sequences of CRISPR-repeats longer than 21-bp in four regions of chromosomes of both isolates (Fig. 4), being, therefore, considered CRISPR-positive according to Fouts et al. (2005).

Plasmids are frequently associated with virulence attributes in pathogenic bacteria and play a major role in the ability of bacteria to exploit new environments, particularly under selective pressure (Batchelor et al. 2004). According to Krutkiewicz and

Klimuszko (2010), 19–53% of *Campylobacter* strains contain plasmids of various sizes. The isolate CJ100 contained a 103-kilobase megaplasmid carrying some virulence factors (Fig. 5; Table S3) as the gene cluster that encodes the CDT, which has been shown to arrest epithelial cells in the G2/M phase of the cell cycle and to induce the release of the pro-inflammatory cytokine IL-8 (Hickey et al. 2000). In a study performed by Hickey et al. (2000), a shuttle plasmid encoding the *C. jejuni cdt* operon induced IL-8 activity in *C. coli* strains that naturally lacked CDT.

The first evidence of the involvement of plasmids in the virulence of *C. jejuni* was related by Bacon et al. (2000), which identified the plasmid pVir in strain 81-176 containing several genes that encoded orthologues of the T4SS (Bacon et al. 2000, 2002). According to Fouts et al. (2005), the presence of a T4SS is a conserved feature of all of the large *Campylobacter* plasmids. In our study, did not find evidence of a T4SS in the megaplasmid pJc100, only in the chromosome of the isolate CJ100. Nevertheless, the presence of virulence-associated genes suggests that pJc100 may play a role in the pathogenesis of CJ100. Moreover, the complete genomic sequence of the pJc100 plasmid showed 99% nucleotide identity with a region of *C. jejuni* M129 strain, which is closely related to genome of isolate CJ100. Interestingly, *C. jejuni* M129 was originally isolated in 1990 from a clinical case of human gastroenteritis in the Unites States of America and was highly invasive *in vitro* (Babakhani et al. 1993; Konkel et al. 1992).

The results of the *in silico* MLST analysis demonstrated that CJ100 and 104 belong to the ST-353 and ST-607 complexes, respectively (Table 2). According to the *Campylobacter* MLST database, the majority of the isolates of the ST-607 complex was obtained from chicken and human stools. Similarly, the majority of the isolates of ST-353, including *C. jejuni* M129, was obtained from human diseases. Other studies

also report that the ST-353 complex contains isolates associated with human gastroenteritis or systemic disease (Cody et al. 2012; Collado et al. 2018; Dingle et al. 2002). In a study performed by Lévesque et al. (2008) in Canada, both the ST-353 and ST-607 complexes comprised exclusively or predominantly human and chicken isolates. However, these researchers found that almost all clonal complexes contained human isolates, suggesting that potentially pathogenic strains are not restricted to specific lineages.

We report the first genomic analysis of *C. jejuni* isolates in Brazil. The genetic features detected in CJ100 explain its multidrug-resistant phenotype. We identified differences in the genomic structure, some genes related to virulence, and the presence of mobile genetic elements in both isolates CJ100 and CJ104 compared with the genome of reference strain NCTC 11168. The genomic data of *C. jejuni* isolated in Brazil and the analysis of a novel virulence megaplasmid provide a basis for pan-genome studies and further investigations. The presence of potentially virulent *C. jejuni* isolates in the poultry meat in this country represent a potential risk to health of consumers, since the Brazil is the world's largest exporter of chicken meat. This demonstrate the need of approaches to control this pathogen in poultry production chain.

Nucleotide sequence accession numbers

The whole genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accessions CP023343.1 (chromosome, *C. jejuni* strain 104), CP023446.1 (chromosome, *C. jejuni* strain 100), and CP023447.1 (plasmid pJc100).

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Author Contribution

SFRW, SJ, FSK, WPS, and OAD designed the study and wrote the manuscript. SFRW, SJ, NRO, and VFC performed the sequencing run. SFRW, CDS, LSP, and FSK performed the bioinformatics analysis and created the figures and tables. SFRW obtained the *C. jejuni* isolates. All authors contributed to and revised the manuscript.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Table 1

Genome features of *Campylobacter jejuni* 100 and 104

Genomic information	*C. jejuni* 100	*C. jejuni* 104
Length	1,869,602 bp	1,558,306 bp
# contigs	12	9
N50	238,245	228,820
L50	4	3
CG%	30.3	30.3
CDSs[a]	1,867	1,567
tRNA	42	37
rRNA	3	3
ncRNA[b]	1	1
tmRNA[c]	1	1
Other features	riboswitch antisense DNA (*purD*) plasmid	riboswitch

[a]Coding DNA sequences

[b]Other non-coding RNA genes that are not tRNAs nor rRNAs

[c]Transfer-messenger RNAs

Table 2

Alleles identified in the *in silico* MLST analysis of *Campylobacter jejuni* 100 and 104

Isolate	*aspA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*	ST
100	7	**658**[a]	5	2	10	3	6	ST-353 complex[b]
104	8	2	5	503	900	3	1	ST-607 complex[c]

[a]Imperfect match with 97% of identity

[b]Closest profile with 6 matches

[c]Closest profile with 5 matches

Table 3

Incomplete prophages regions identified in the genomes of *Campylobacter jejuni* 100 and 104

Incomplete prophages regions	Isolate/Locus tag	
	100	104
	Region I	
PHAGE_Helico_KHP30_NC_019928	CMV38_1395	
PHAGE_Campyl_CP21_NC_019507	CMV38_1396	
PHAGE_Vibrio_qdvp001_NC_029057	CMV38_1397	
PHAGE_Cellul_phi46:1_NC_021800	CMV38_1398	
PHAGE_Campyl_NCTC12673_NC_015464	CMV38_1399	
PHAGE_Clostr_phiCD211_NC_029048	CMV38_1401	
PHAGE_Shewan_1/44_NC_025463	CMV38_1406	
PHAGE_Liston_phiHSIC_NC_006953	CMV38_1407	
PHAGE_Lister_LP_030_3_NC_024384	CMV38_1408	
	Region II	Region I
PHAGE_Pseudo_NP1_NC_031058	CMV38_1852	CLH93_0162
PHAGE_Bacill_G_NC_023719	CMV38_1854	CLH93_0160
PHAGE_Caulob_CcrColossus_NC_019406	CMV38_1855	CLH93_0159
PROPHAGE_Escher_Sakai	CMV38_1856	CLH93_0158
PHAGE_Prochl_P_SSM7_NC_015290	CMV38_1859	CLH93_0155
PHAGE_Bacill_CP_51_NC_025423	CMV38_1860	CLH93_0154

Table 4

Antimicrobial resistance mechanisms identified in the genomes of *Campylobacter jejuni* **(a)** 100 and **(b)** 104

(a)

Drug class	Resistance gene or mutation	Locus tag	GenBank Code	Identity (%)
Aminoglycoside	*aad9*	CMV38_1909	KQH92572.1	100
	aadE	CMV38_1910	AAU10334.1	100
	sat4	CMV38_1911	AAB53445.1	98.9

	aphA-3	CMV38_1912	AGV10830.1	100
Beta-lactam	*blaOXA-61*	CMV38_0320	AAT01092.1	99.6
Macrolide	*rplV*[a]	CMV38_0059	WP_002869681.1	100
Quinolone	*gyrA*[b]	CMV38_1104	YP_002344422.1	99.5
Tetracycline	*tetO*	CMV38_1905	AAA23033.1	93.4
	tetO	CMV38_1964	AAA23033.1	93.4
Multidrug efflux pump	*cmeC*	CMV38_0388	BAO79432.1	98.6
	cmeB	CMV38_0389	ABS43151.1	97.9
	cmeA	CMV38_0390	ABS43901.1	95.4
	cmeR	CMV38_0391	YP_002343805.1	100

[a]Unknown mutations in *rplV* (50S ribosomal protein L22): P13S, A103V, S109A, and V137A

[b]Unknown mutations in *gyrA* (DNA gyrase subunit A): S22G, N203S, R285K, and Q863* - premature stop codon; Known mutation in *gyrA* T86I conferring high level resistance to nalidixic acid and ciprofloxacin

(b)

Drug class	Resistance gene or mutation	Locus tag	GenBank Code	Identity (%)
Macrolide	*rplV*[a]	CLH93_1672	WP_002869681.1	97.0
Quinolone	*gyrA*[b]	CLH93_1101	YP_002344422.1	99.7
Multidrug efflux pump	*cmeR*[c]	CLH93_0325	YP_002343805.1	98.6
	cmeA	CLH93_0324	ABS43901.1	96.5
	cmeB	CLH93_0323	ABS43151.1	98.1
	cmeC	CLH93_0322	BAO79432.1	98.6

[a]Unknown mutations in *rplV* (50S ribosomal protein L22): I65V, S109A, K118_T119insAPAAKK, and V137A

[b]Unknown mutations in *gyrA* (DNA gyrase subunit A): S22G, N203S, R285K, and Q863* - premature stop codon

[c]Unknown mutations in *cmeR* (transcriptional regulator CmeR): G144D, P183R, and S207G

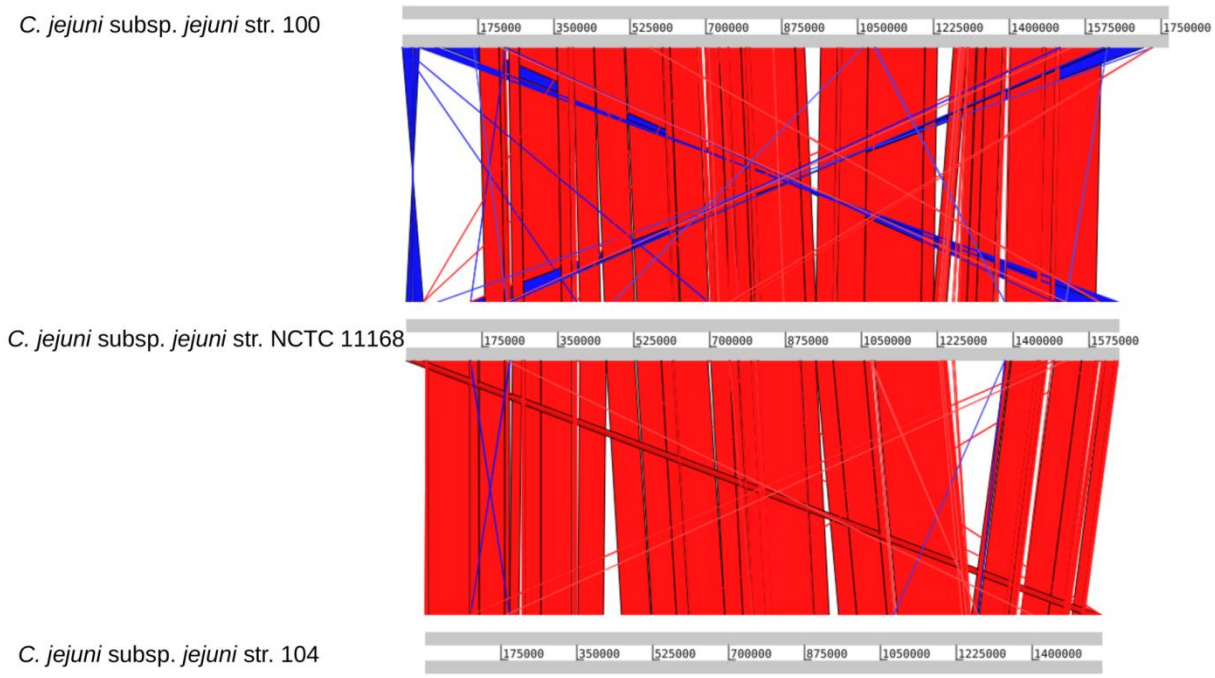

Fig. 1

Structural comparison generated by Artemis Comparison Tool (ACT) of the genomes of *Campylobacter jejuni* 100 and 104 based on the reference genome *Campylobacter jejuni* NCTC 11168

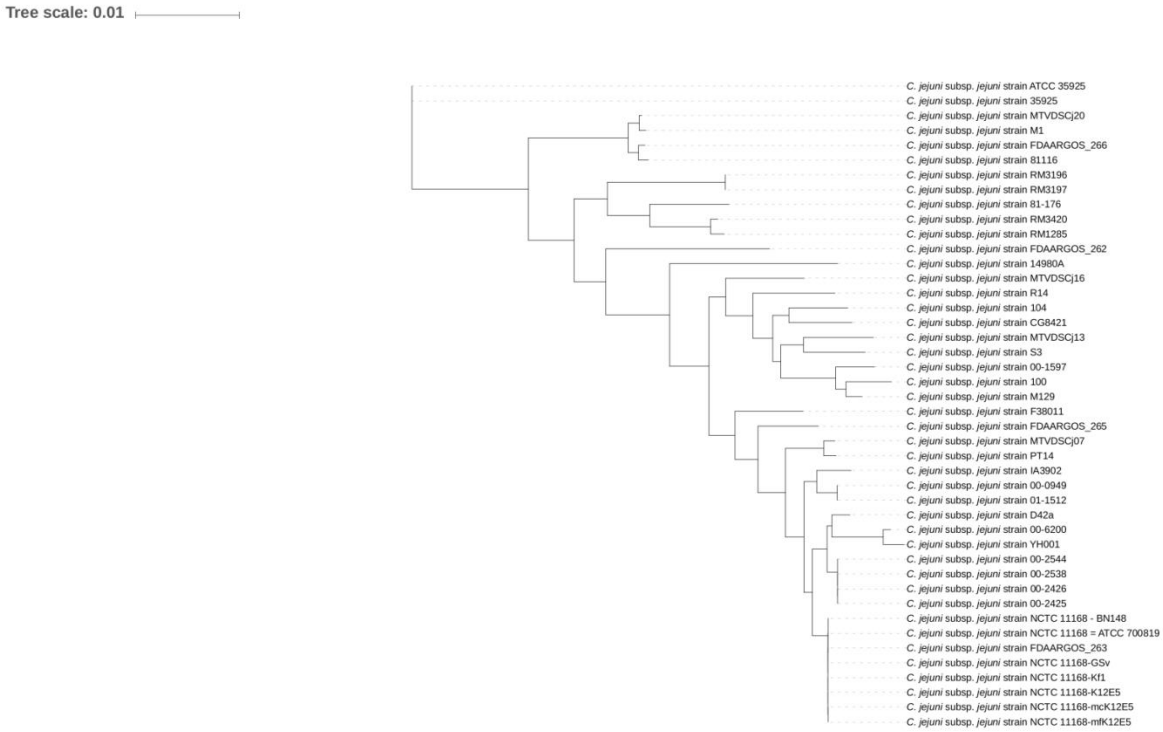

Fig. 2

The findings from the Neighbour-Joining tree generated by iTOL (interactive tree of life) constructed based on the multiple alignment of syntenic regions reveal the relationship and genetic diversity among *Campylobacter jejuni* 100 and 104, and others whole-genome sequenced strains

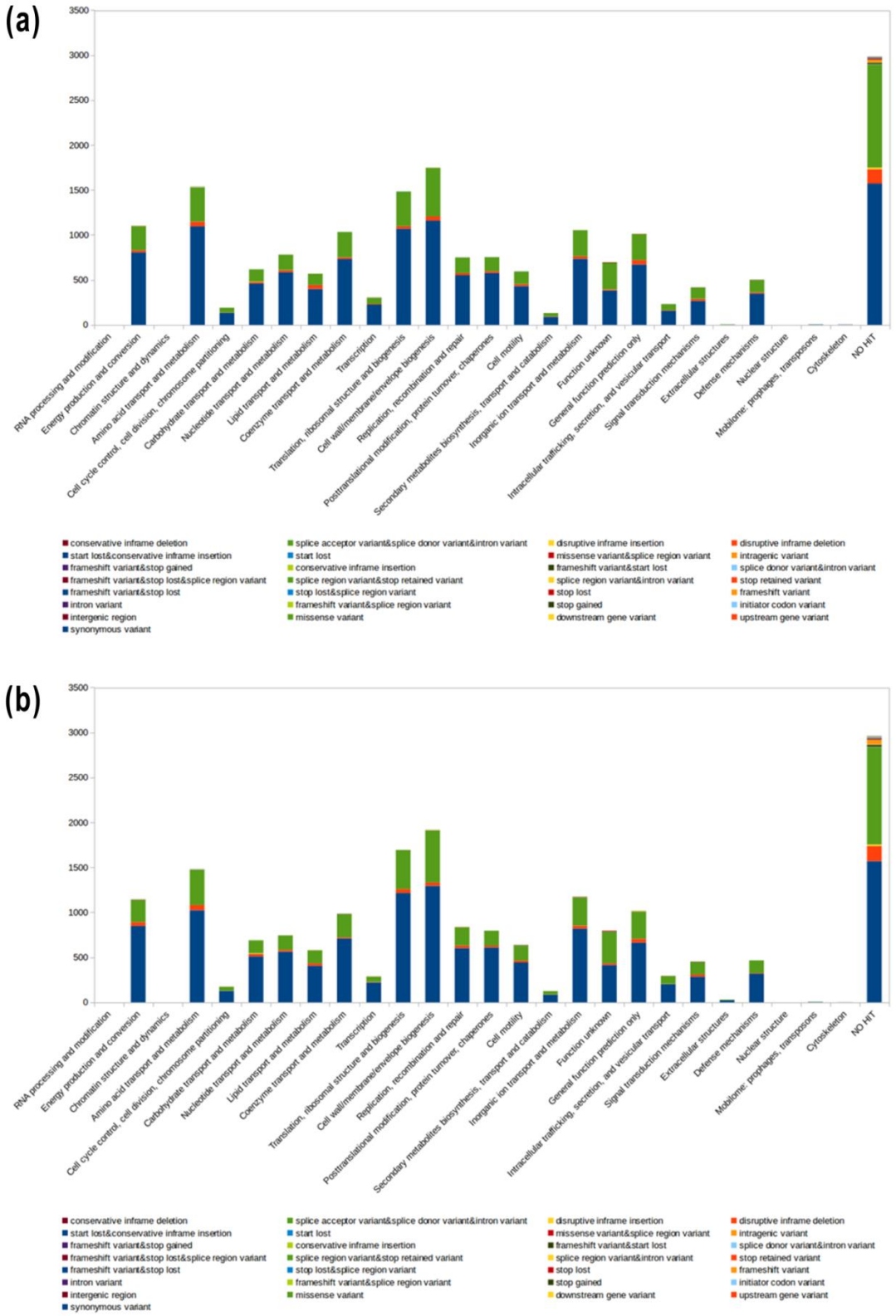

Fig. 3

Single-Nucleotide Polymorphisms identified in the genomes of *Campylobacter jejuni* **(a)** 100 and **(b)** 104, and their biologic impacts, generate by LibreOffice Calc

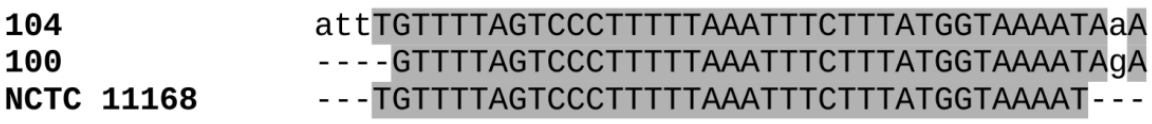

Fig. 4

CRISPR Sequence CRISPR-repeat in both genomes of *Campylobacter jejuni* 100 and 104 generated by MUSCLE using the genome of *Campylobacter jejuni* NCTC 11168 as reference

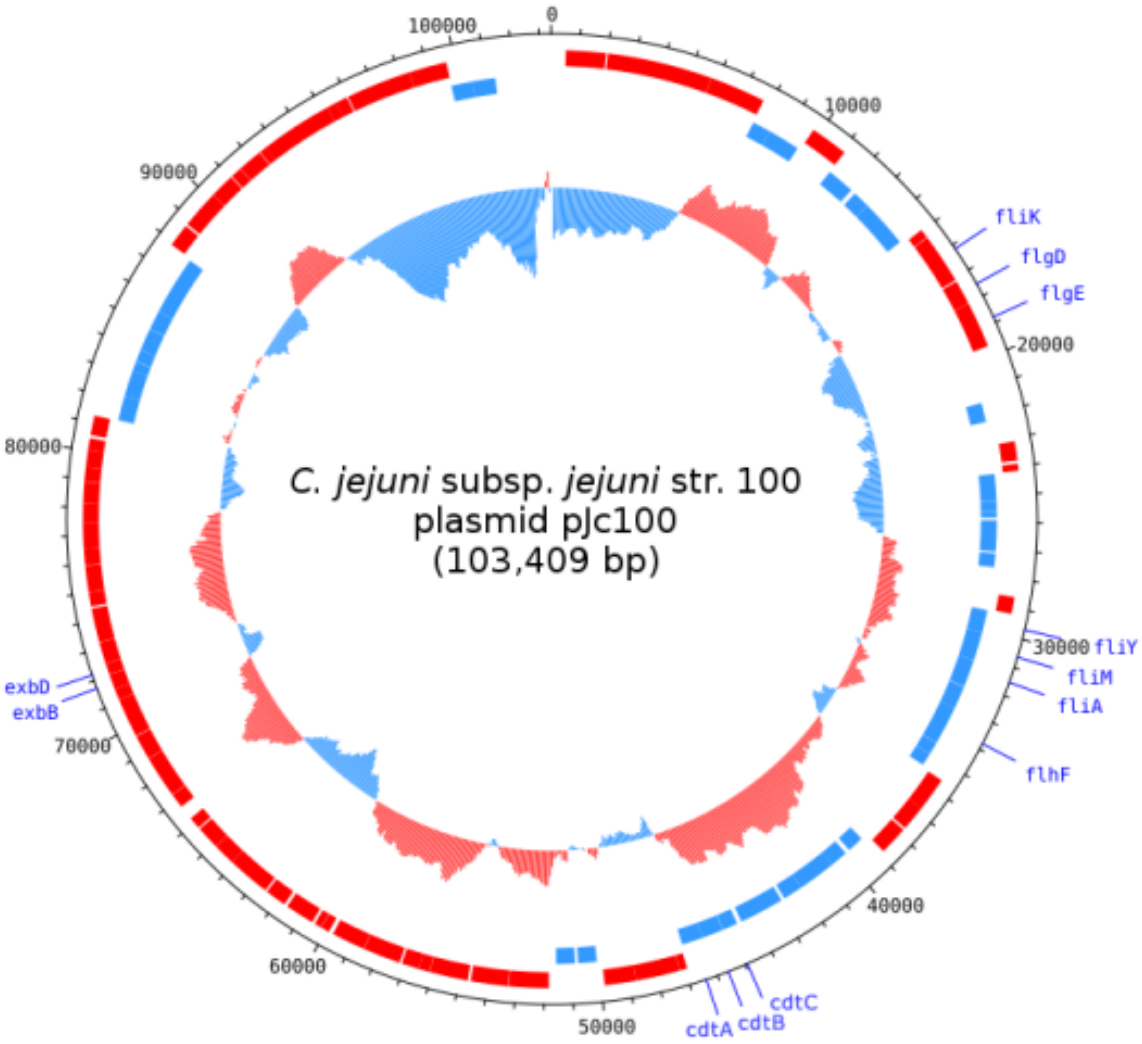

Fig. 5

Circular representation generated by DNAPlotter of the megaplasmid pJc100 identified in the genome of *Campylobacter jejuni* 100 and the virulence-associated genes found

Supplementary material

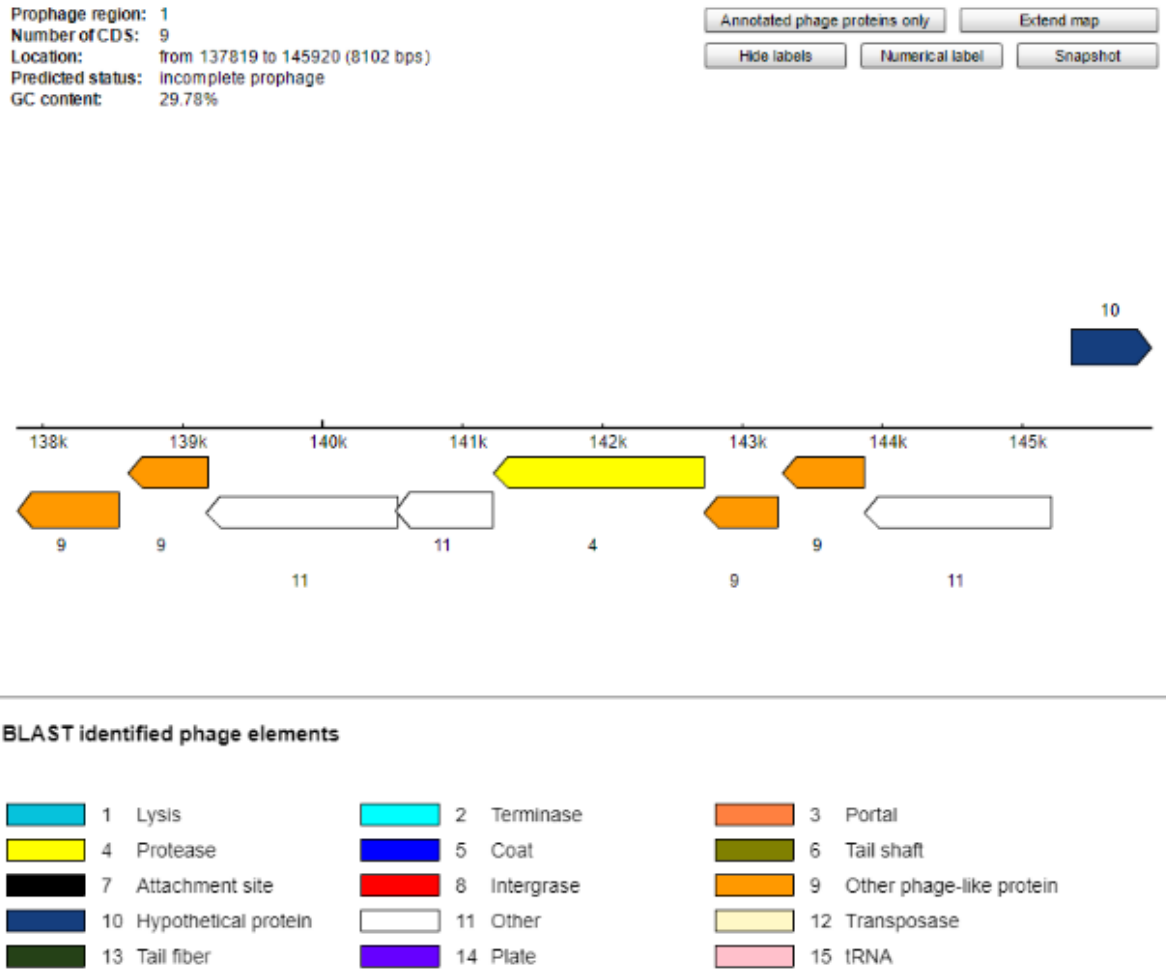

Fig. S1. Prophage region I identified in the chromosome of *Campylobacter jejuni* 104 by PHAST.

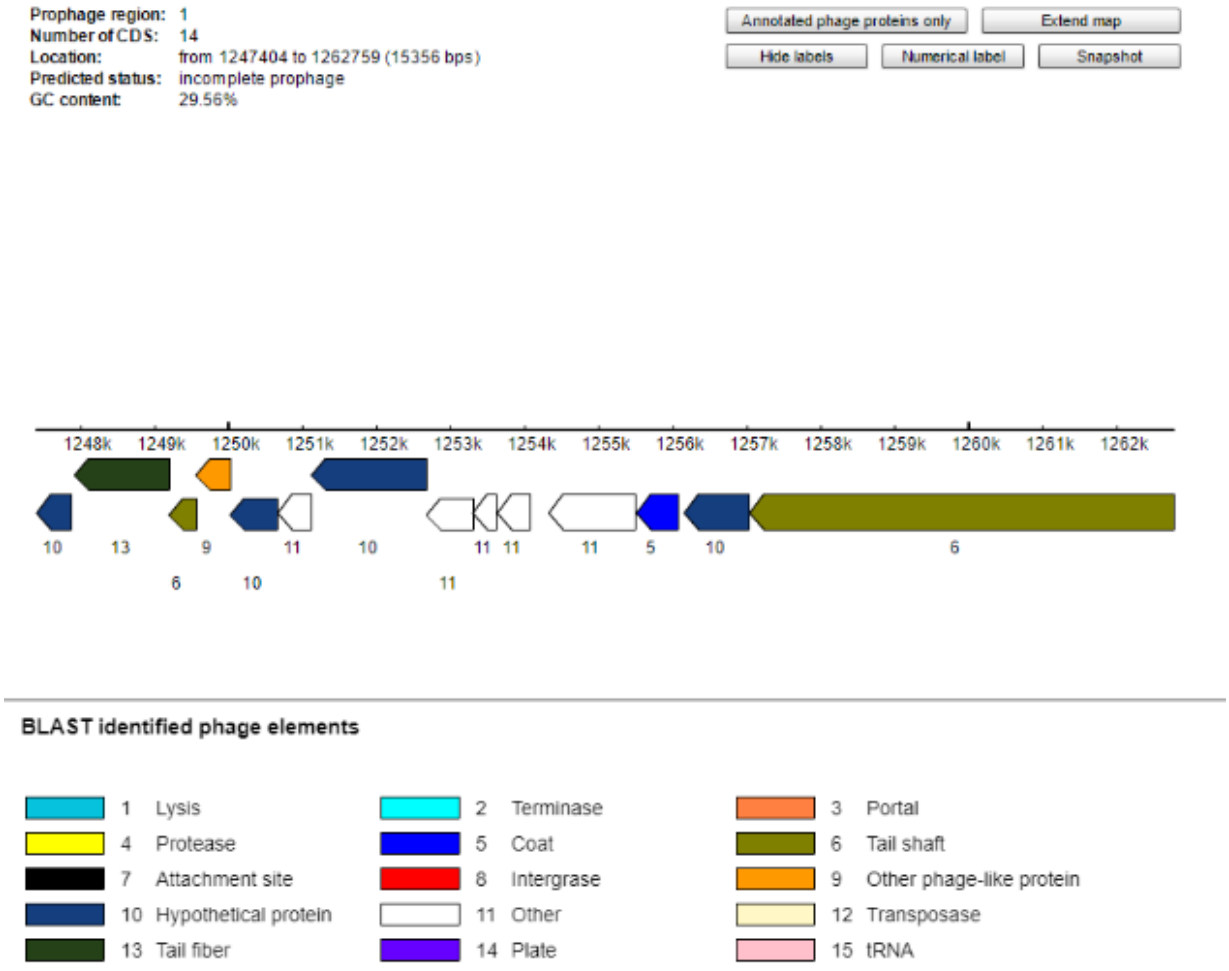

Fig. S2. Prophage region I identified in the chromosome of *Campylobacter jejuni* 100 by PHAST.

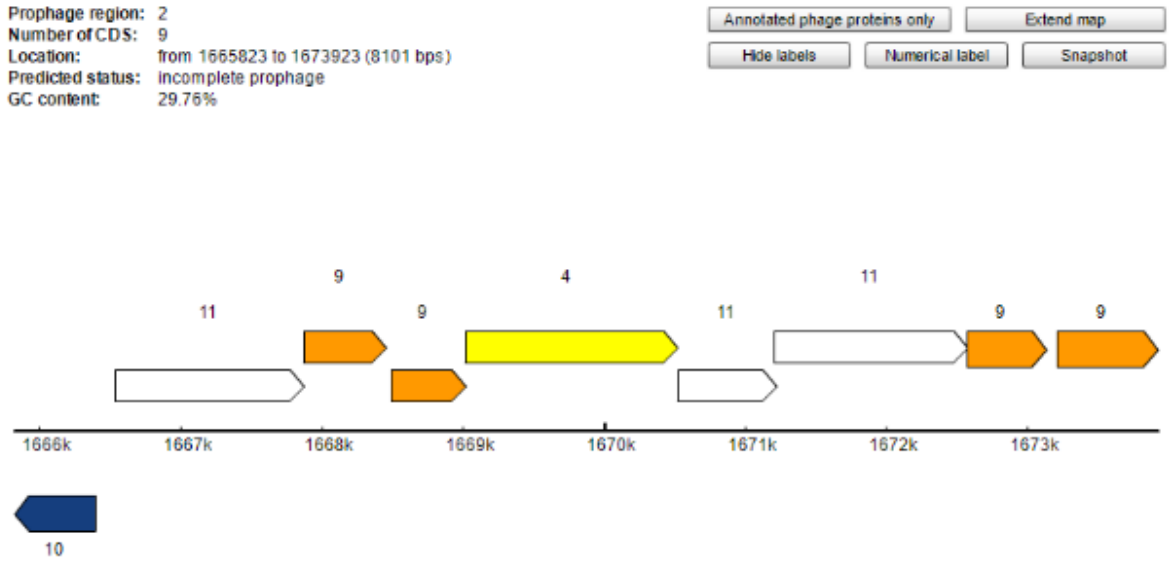

Fig. S3. Prophage region II identified in the chromosome of *Campylobacter jejuni* 100 by PHAST.

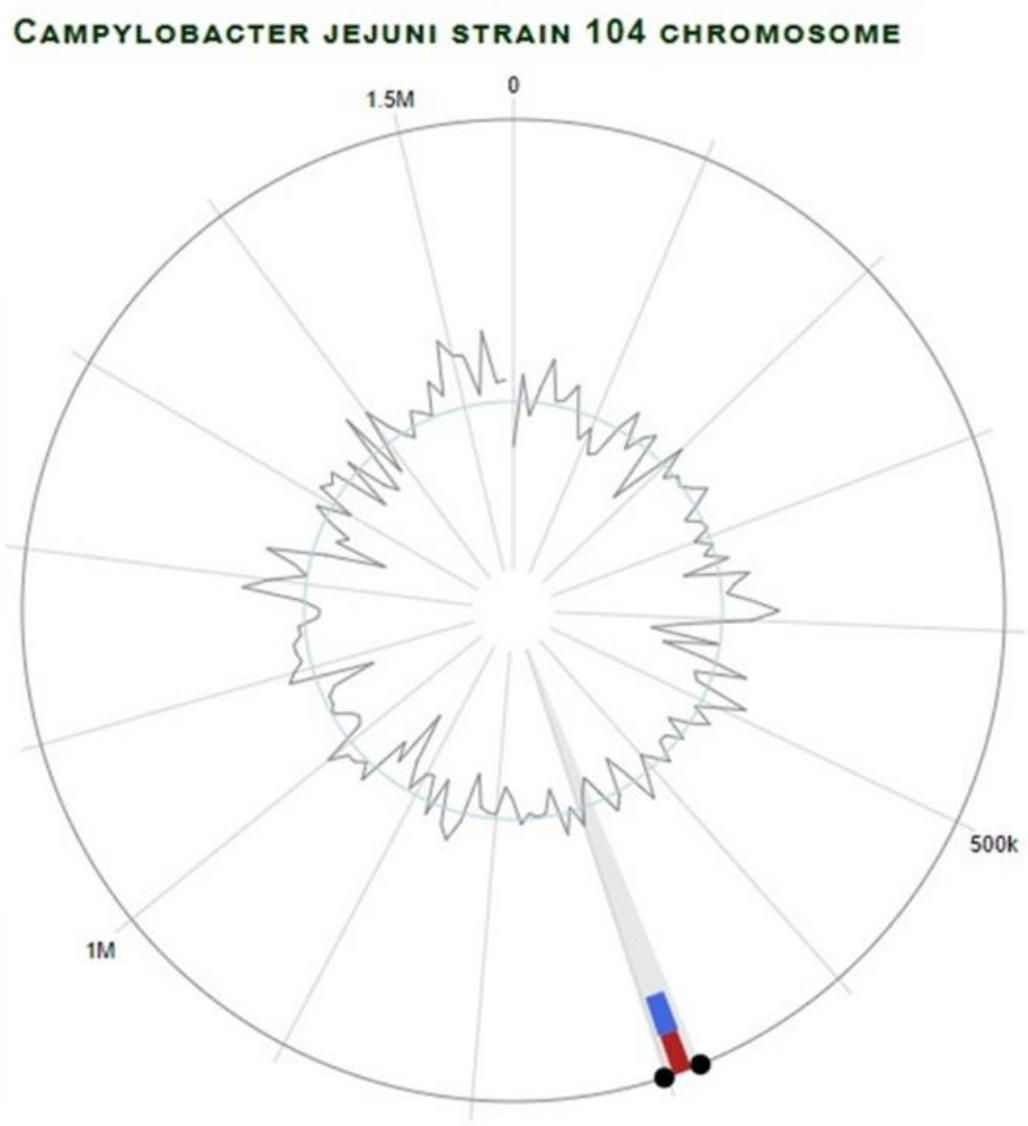

Fig. S4. Prediction of genomic islands in the chromosome of *Campylobacter jejuni* 104 by IslandViewer 4. Circular visualization of predicted genomic islands are shown with blocks colored according to the prediction method: IslandPick (green), IslandPathe-DIMOB (blue), SIGI-HMM (orange) as well as the integrated results (dark red).

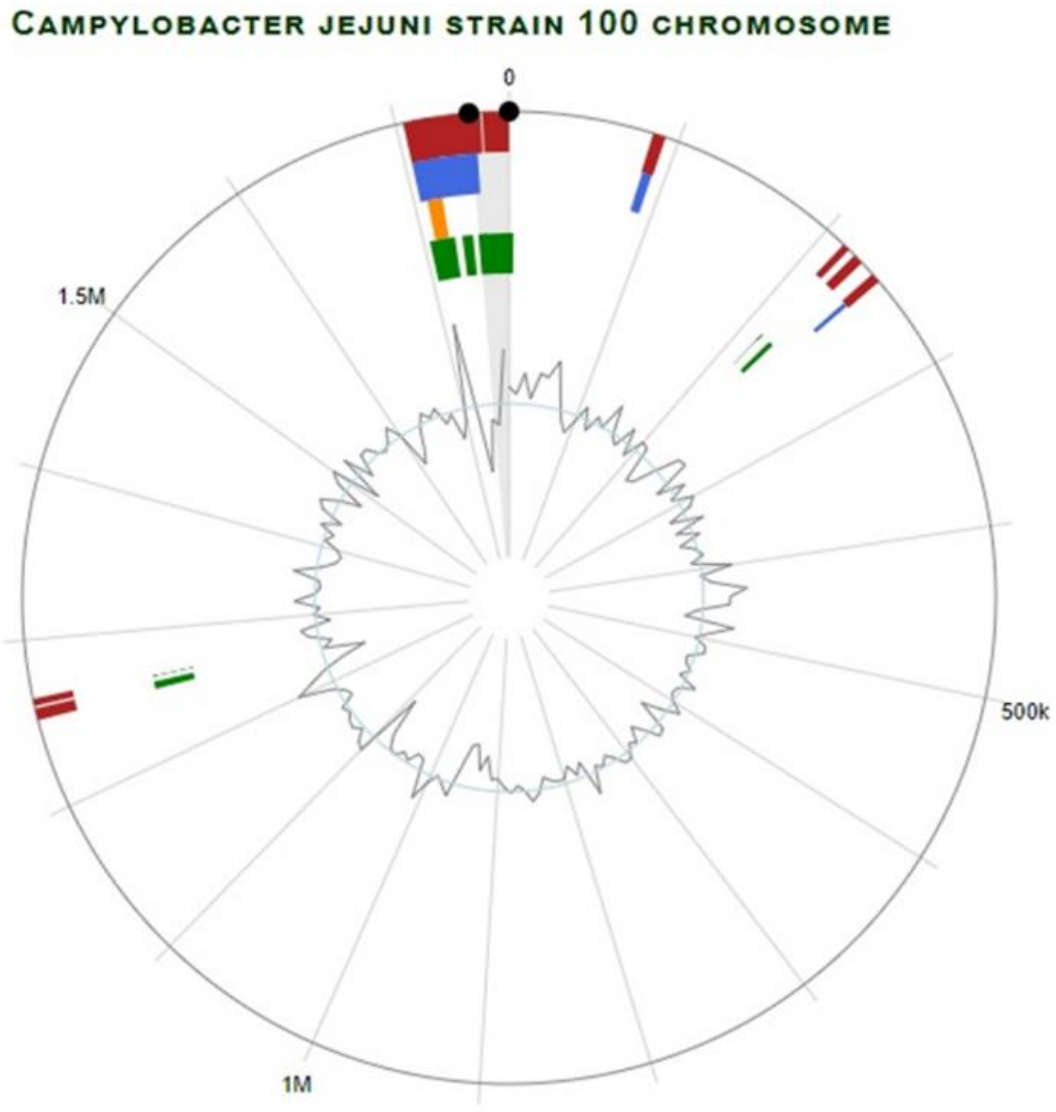

Fig. S5. Prediction of genomic islands in the chromosome of *Campylobacter jejuni* 100 by IslandViewer 4. Circular visualization of predicted genomic islands are shown with blocks colored according to the prediction method: IslandPick (green), IslandPathe-DIMOB (blue), SIGI-HMM (orange) as well as the integrated results (dark red).

Table S1. Genomic island identified in the chromosome of *Campylobacter jejuni* 104

Island start	Island end	Length	Method	Gene name	Locus_tag	Gene start	Gene end	Strand	Product
687132	696832	9700	Predicted by at least one method	ATD41362.1	CLH93_0786	687132	687329	1	Uncharacterized protein
687132	696832	9700	Predicted by at least one method	ATD41363.1	CLH93_0789	688161	688343	1	Uncharacterized protein
687132	696832	9700	Predicted by at least one method	ATD41364.1	CLH93_0792	689778	690032	1	Uncharacterized protein
687132	696832	9700	Predicted by at least one method	ATD41365.1	CLH93_0793	691712	691978	1	Transposase
687132	696832	9700	Predicted by at least one method	ATD41366.1	CLH93_0794	692623	693321	-1	Protein TonB
687132	696832	9700	Predicted by at least one method	ATD41367.1	CLH93_0795	693647	695737	1	Ferric enterobactin receptor
687132	696832	9700	Predicted by at least one method	ATD41368.1	CLH93_0796	696041	696832	1	Heat-inducible transcription repressor HrcA
687132	696832	9700	Predicted by at least one method	ATD41369.1	CLH93_0797	696829	697356	1	Protein GrpE

Table S2. Genomic islands identified in the chromosome of *Campylobacter jejuni* 100

Island start	Island end	Length	Method	Gene name	Locus_tag	Gene start	Gene end	Strand	Product
84582	92153	7571	Predicted by at least one method	ATE68118.1	CMV38_0094	84582	85721	1	Integrase
84582	92153	7571	Predicted by at least one method	ATE68119.1	CMV38_0095	85724	85936	1	Tgh84
84582	92153	7571	Predicted by at least one method	ATE68120.1	CMV38_0096	85924	86364	1	Uncharacterized protein
84582	92153	7571	Predicted by at least one method	ATE68121.1	CMV38_0097	86483	86710	1	Uncharacterized protein
84582	92153	7571	Predicted by at least one method	ATE68122.1	CMV38_0098	86707	87075	1	Hypothetical protein
84582	92153	7571	Predicted by at least one method	ATE68123.1	CMV38_0099	87086	87223	1	Uncharacterized protein
84582	92153	7571	Predicted by at least one method	ATE68124.1	CMV38_0100	87261	88115	1	putative preotein
84582	92153	7571	Predicted by at least one method	ATE68125.1	CMV38_0101	88419	88601	1	Uncharacterized protein
84582	92153	7571	Predicted by at least one method	ATE68126.1	CMV38_0102	88612	88857	1	Hypothetical protein
84582	92153	7571	Predicted by at least one method	ATE68127.1	CMV38_0103	88909	90024	1	Uncharacterized protein
84582	92153	7571	Predicted by at least one method	ATE68128.1	CMV38_0104	90021	91214	1	Replicative helicase
84582	92153	7571	Predicted by at least one method	ATE68129.1	CMV38_0105	91469	91675	-1	Hypothetical protein

84582	92153	7571	Predicted by at least one method	ATE68130.1	CMV38_0106	91926	92153	-1	Uncharacterized protein
212963	217476	4513	Predicted by at least one method	ATE68260.1	CMV38_0252	211954	212976	-1	Ribonucleoside-diphosphate reductase subunit beta
212963	217476	4513	Predicted by at least one method	ATE68261.1	CMV38_0253	213181	213402	-1	Membrane protein
212963	217476	4513	Predicted by at least one method	ATE68262.1	CMV38_0254	213403	214011	-1	Orotate phosphoribosyltransferase
212963	217476	4513	Predicted by at least one method	ATE68263.1	CMV38_0255	214014	214571	-1	Ribosome-recycling factor
212963	217476	4513	Predicted by at least one method	ATE68264.1	CMV38_0256	214587	214958	-1	Preprotein translocase SecYEG, SecG subunit
212963	217476	4513	Predicted by at least one method	ATE68265.1	CMV38_0257	215062	215757	-1	Uncharacterized protein
212963	217476	4513	Predicted by at least one method	ATE68266.1	CMV38_0258	215918	216553	1	Carbonic anhydrase
212963	217476	4513	Predicted by at least one method	ATE68267.1	CMV38_0259	216550	218433	1	Mechanosensitive ion channel family protein
221737	228041	6304	Predicted by at least one method	ATE68271.1	CMV38_0263	221336	222520	-1	Uncharacterized protein
221737	228041	6304	Predicted by at least one method	ATE68272.1	CMV38_0264	222661	222852	1	50S ribosomal protein L35
221737	228041	6304	Predicted by at least one method	ATE68273.1	CMV38_0265	222946	223299	1	50S ribosomal protein L20
221737	228041	6304	Predicted by at least one method	ATE68274.1	CMV38_0266	224107	224433	-1	Uncharacterized protein

221737	228041	6304	Predicted by at least one method	ATE68275.1	CMV38_0267	224916	225773	1	HDOD domain-containing protein
221737	228041	6304	Predicted by at least one method	ATE68276.1	CMV38_0268	225804	226280	1	Uncharacterized protein
221737	228041	6304	Predicted by at least one method	ATE68277.1	CMV38_0269	226249	227559	-1	Putative transmembrane transport protein
221737	228041	6304	Predicted by at least one method	ATE68278.1	CMV38_0270	227562	227717	-1	Uncharacterized protein
221737	228041	6304	Predicted by at least one method	ATE68279.1	CMV38_0271	227810	228283	1	Cyclic pyranopterin monophosphate synthase
236856	242769	5913	Predicted by at least one method	ATE68288.1	CMV38_0280	236856	237569	-1	SAM-dependent methyltransferase
236856	242769	5913	Predicted by at least one method	ATE68289.1	CMV38_0281	237650	238240	-1	Methyl-accepting chemotaxis protein
236856	242769	5913	Predicted by at least one method	ATE68290.1	CMV38_0282	238737	239168	-1	Uncharacterized protein
236856	242769	5913	Predicted by at least one method	ATE68291.1	CMV38_0283	239224	239409	-1	Uncharacterized protein
236856	242769	5913	Predicted by at least one method	ATE68292.1	CMV38_0284	239406	239597	-1	RNA polymerase subunit sigma-70
236856	242769	5913	Predicted by at least one method	ATE68293.1	CMV38_0285	239600	240523	-1	Bacteriocin
236856	242769	5913	Predicted by at least one method	ATE68294.1	CMV38_0286	240694	242769	-1	Bacteriophage DNA transposition protein A, putative
236856	242769	5913	Predicted by at least one method	ATE68295.1	CMV38_0287	242766	242966	-1	Uncharacterized protein

1252861	1259983	7122	Predicted by at least one method	ATE69280.1	CMV38_1402	1252670	1253302	-1	Uncharacterized protein
1252861	1259983	7122	Predicted by at least one method	ATE69281.1	CMV38_1403	1253299	1253616	-1	Head-tail adaptor protein
1252861	1259983	7122	Predicted by at least one method	ATE69282.1	CMV38_1404	1253629	1254066	-1	Uncharacterized protein
1252861	1259983	7122	Predicted by at least one method	ATE69283.1	CMV38_1405	1254325	1255491	-1	Capsid protein
1252861	1259983	7122	Predicted by at least one method	ATE69284.1	CMV38_1406	1255508	1256065	-1	Peptidase
1252861	1259983	7122	Predicted by at least one method	ATE69285.1	CMV38_1407	1256151	1257020	-1	Uncharacterized protein
1252861	1259983	7122	Predicted by at least one method	ATE69286.1	CMV38_1408	1257033	1262759	-1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69286.1	CMV38_1408	1257033	1262759	-1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69287.1	CMV38_1409	1262819	1263157	1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69288.1	CMV38_1410	1263149	1263364	-1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69289.1	CMV38_1411	1263445	1263801	-1	DNA repair protein
1261447	1265721	4274	Predicted by at least one method	ATE69290.1	CMV38_1412	1263798	1264778	-1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69291.1	CMV38_1413	1264950	1265300	-1	Uncharacterized protein
1261447	1265721	4274	Predicted by at least one method	ATE69292.1	CMV38_1414	1265301	1265843	-1	Uncharacterized protein

1704421	1746311	41890	Predicted by at least one method	ATE69735.1	CMV38_1901	1704421	1705359	1	Thioredoxin reductase
1704421	1746311	41890	Predicted by at least one method	ATE69736.1	CMV38_1902	1705397	1707178	-1	Putative TAT (Twin-Arginine Translocation) pathway signal sequence domain protein
1704421	1746311	41890	Predicted by at least one method	ATE69737.1	CMV38_1903	1707754	1709175	1	Cpp50
1704421	1746311	41890	Predicted by at least one method	ATE69738.1	CMV38_1904	1709194	1709373	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69739.1	CMV38_1905	1709732	1711651	1	TetO protein
1704421	1746311	41890	Predicted by at least one method	ATE69740.1	CMV38_1906	1712426	1712998	1	Cpp3
1704421	1746311	41890	Predicted by at least one method	ATE69741.1	CMV38_1907	1713786	1714271	1	Repa
1704421	1746311	41890	Predicted by at least one method	ATE69742.1	CMV38_1908	1714296	1715066	1	Pnp protein
1704421	1746311	41890	Predicted by at least one method	ATE69743.1	CMV38_1909	1715091	1715870	1	Aad9
1704421	1746311	41890	Predicted by at least one method	ATE69744.1	CMV38_1910	1716070	1716690	1	Adenyltransferase
1704421	1746311	41890	Predicted by at least one method	ATE69745.1	CMV38_1911	1716687	1717229	1	Streptothricin resistance protein
1704421	1746311	41890	Predicted by at least one method	ATE69746.1	CMV38_1912	1717322	1718116	1	Apha-3 aminoglycosidase
1704421	1746311	41890	Predicted by at least one method	ATE69747.1	CMV38_1913	1718291	1718596	1	Cpp10

1704421	1746311	41890	Predicted by at least one method	ATE69748.1	CMV38_1914	1718601	1718867	1	Cpp11
1704421	1746311	41890	Predicted by at least one method	ATE69749.1	CMV38_1915	1718926	1719486	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69750.1	CMV38_1916	1719489	1719755	1	Cpp13
1704421	1746311	41890	Predicted by at least one method	ATE69751.1	CMV38_1919	1725621	1726325	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69752.1	CMV38_1920	1726631	1727074	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69753.1	CMV38_1921	1727181	1728428	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69754.1	CMV38_1922	1728460	1729026	-1	Peptidase C39
1704421	1746311	41890	Predicted by at least one method	ATE69755.1	CMV38_1923	1729038	1729892	-1	HmcD domain-containing protein
1704421	1746311	41890	Predicted by at least one method	ATE69756.1	CMV38_1924	1729988	1731376	-1	Spore coat protein CotH
1704421	1746311	41890	Predicted by at least one method	ATE69757.1	CMV38_1925	1731376	1731927	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69758.1	CMV38_1926	1732121	1732210	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69759.1	CMV38_1927	1732164	1732403	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69760.1	CMV38_1928	1732432	1733043	1	NTPase
1704421	1746311	41890	Predicted by at least one method	ATE69761.1	CMV38_1929	1733047	1733706	1	Uncharacterized protein

1704421	1746311	41890	Predicted by at least one method	ATE69762.1	CMV38_1930	1733788	1735020	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69763.1	CMV38_1931	1735135	1735398	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69764.1	CMV38_1932	1735558	1735767	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69765.1	CMV38_1933	1735917	1737089	-1	Protein kinase
1704421	1746311	41890	Predicted by at least one method	ATE69766.1	CMV38_1934	1737101	1737280	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69767.1	CMV38_1935	1737817	1738035	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69768.1	CMV38_1936	1738126	1739919	1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69769.1	CMV38_1937	1739923	1740375	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69770.1	CMV38_1938	1740379	1740570	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69771.1	CMV38_1939	1740672	1741286	-1	Cpp27
1704421	1746311	41890	Predicted by at least one method	ATE69772.1	CMV38_1940	1741283	1741660	-1	Vapd
1704421	1746311	41890	Predicted by at least one method	ATE69773.1	CMV38_1941	1741651	1741974	-1	Uncharacterized protein
1704421	1746311	41890	Predicted by at least one method	ATE69774.1	CMV38_1942	1742145	1742408	1	Conjugal transfer protein TraC
1704421	1746311	41890	Predicted by at least one method	ATE69775.1	CMV38_1943	1742427	1745195	1	Cmgb3/4

1704421	1746311	41890	Predicted by at least one method	ATE69776.1	CMV38_1944	1745206	1745763	1	Antirepressor
1704421	1746311	41890	Predicted by at least one method	ATE69777.1	CMV38_1945	1745760	1746311	1	Uncharacterized protein
1707378	1721570	14192	Predicted by at least one method	ATE69737.1	CMV38_1903	1707754	1709175	1	Cpp50
1707378	1721570	14192	Predicted by at least one method	ATE69738.1	CMV38_1904	1709194	1709373	1	Uncharacterized protein
1707378	1721570	14192	Predicted by at least one method	ATE69739.1	CMV38_1905	1709732	1711651	1	TetO protein
1707378	1721570	14192	Predicted by at least one method	ATE69740.1	CMV38_1906	1712426	1712998	1	Cpp3
1707378	1721570	14192	Predicted by at least one method	ATE69741.1	CMV38_1907	1713786	1714271	1	Repa
1707378	1721570	14192	Predicted by at least one method	ATE69742.1	CMV38_1908	1714296	1715066	1	Pnp protein
1707378	1721570	14192	Predicted by at least one method	ATE69743.1	CMV38_1909	1715091	1715870	1	Aad9
1707378	1721570	14192	Predicted by at least one method	ATE69744.1	CMV38_1910	1716070	1716690	1	Adenyltransferase
1707378	1721570	14192	Predicted by at least one method	ATE69745.1	CMV38_1911	1716687	1717229	1	Streptothricin resistance protein
1707378	1721570	14192	Predicted by at least one method	ATE69746.1	CMV38_1912	1717322	1718116	1	Apha-3 aminoglycosidase
1707378	1721570	14192	Predicted by at least one method	ATE69747.1	CMV38_1913	1718291	1718596	1	Cpp10
1707378	1721570	14192	Predicted by at least one method	ATE69748.1	CMV38_1914	1718601	1718867	1	Cpp11

1707378	1721570	14192	Predicted by at least one method	ATE69749.1	CMV38_1915	1718926	1719486	1	Uncharacterized protein
1707378	1721570	14192	Predicted by at least one method	ATE69750.1	CMV38_1916	1719489	1719755	1	Cpp13
1709194	1719755	10561	Predicted by at least one method	ATE69738.1	CMV38_1904	1709194	1709373	1	Uncharacterized protein
1709194	1719755	10561	Predicted by at least one method	ATE69739.1	CMV38_1905	1709732	1711651	1	TetO protein
1709194	1719755	10561	Predicted by at least one method	ATE69740.1	CMV38_1906	1712426	1712998	1	Cpp3
1709194	1719755	10561	Predicted by at least one method	ATE69741.1	CMV38_1907	1713786	1714271	1	Repa
1709194	1719755	10561	Predicted by at least one method	ATE69742.1	CMV38_1908	1714296	1715066	1	Pnp protein
1709194	1719755	10561	Predicted by at least one method	ATE69743.1	CMV38_1909	1715091	1715870	1	Aad9
1709194	1719755	10561	Predicted by at least one method	ATE69744.1	CMV38_1910	1716070	1716690	1	Adenyltransferase
1709194	1719755	10561	Predicted by at least one method	ATE69745.1	CMV38_1911	1716687	1717229	1	Streptothricin resistance protein
1709194	1719755	10561	Predicted by at least one method	ATE69746.1	CMV38_1912	1717322	1718116	1	Apha-3 aminoglycosidase
1709194	1719755	10561	Predicted by at least one method	ATE69747.1	CMV38_1913	1718291	1718596	1	Cpp10
1709194	1719755	10561	Predicted by at least one method	ATE69748.1	CMV38_1914	1718601	1718867	1	Cpp11
1709194	1719755	10561	Predicted by at least one method	ATE69749.1	CMV38_1915	1718926	1719486	1	Uncharacterized protein

1709194	1719755	10561	Predicted by at least one method	ATE69750.1	CMV38_1916	1719489	1719755	1	Cpp13
1729891	1738485	8594	Predicted by at least one method	ATE69755.1	CMV38_1923	1729038	1729892	-1	HmcD domain-containing protein
1729891	1738485	8594	Predicted by at least one method	ATE69756.1	CMV38_1924	1729988	1731376	-1	Spore coat protein CotH
1729891	1738485	8594	Predicted by at least one method	ATE69757.1	CMV38_1925	1731376	1731927	-1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69758.1	CMV38_1926	1732121	1732210	1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69759.1	CMV38_1927	1732164	1732403	1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69760.1	CMV38_1928	1732432	1733043	1	NTPase
1729891	1738485	8594	Predicted by at least one method	ATE69761.1	CMV38_1929	1733047	1733706	1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69762.1	CMV38_1930	1733788	1735020	1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69763.1	CMV38_1931	1735135	1735398	1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69764.1	CMV38_1932	1735558	1735767	-1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69765.1	CMV38_1933	1735917	1737089	-1	Protein kinase
1729891	1738485	8594	Predicted by at least one method	ATE69766.1	CMV38_1934	1737101	1737280	-1	Uncharacterized protein
1729891	1738485	8594	Predicted by at least one method	ATE69767.1	CMV38_1935	1737817	1738035	1	Uncharacterized protein

1729891	1738485	8594	Predicted by at least one method	ATE69768.1	CMV38_1936	1738126	1739919	1	Uncharacterized protein
1741938	1749491	7553	Predicted by at least one method	ATE69773.1	CMV38_1941	1741651	1741974	-1	Uncharacterized protein
1741938	1749491	7553	Predicted by at least one method	ATE69774.1	CMV38_1942	1742145	1742408	1	Conjugal transfer protein TraC
1741938	1749491	7553	Predicted by at least one method	ATE69775.1	CMV38_1943	1742427	1745195	1	Cmgb3/4
1741938	1749491	7553	Predicted by at least one method	ATE69776.1	CMV38_1944	1745206	1745763	1	Antirepressor
1741938	1749491	7553	Predicted by at least one method	ATE69777.1	CMV38_1945	1745760	1746311	1	Uncharacterized protein
1741938	1749491	7553	Predicted by at least one method	ATE69778.1	CMV38_1946	1746345	1746767	1	Single-stranded DNA-binding protein
1741938	1749491	7553	Predicted by at least one method	ATE69779.1	CMV38_1947	1746783	1747058	1	Uncharacterized protein
1741938	1749491	7553	Predicted by at least one method	ATE69780.1	CMV38_1948	1747062	1748051	1	Type IV secretion system protein VirB5
1741938	1749491	7553	Predicted by at least one method	ATE69781.1	CMV38_1949	1748048	1749043	1	Conjugal transfer protein TrbL
1741938	1749491	7553	Predicted by at least one method	ATE69782.1	CMV38_1950	1749033	1749200	1	hypothetical protein
1741938	1749491	7553	Predicted by at least one method	ATE69783.1	CMV38_1951	1749190	1749852	1	Conjugal transfer protein TraJ
1750672	1766194	15522	Predicted by at least one method	ATE69784.1	CMV38_1952	1749849	1750736	1	CmgB9
1750672	1766194	15522	Predicted by at least one method	ATE69785.1	CMV38_1953	1750763	1751938	1	CmgB10

1750672	1766194	15522	Predicted by at least one method	ATE69786.1	CMV38_1954	1751919	1752911	1	PTet26
1750672	1766194	15522	Predicted by at least one method	ATE69787.1	CMV38_1955	1752912	1754723	1	Sodium:calcium antiporter
1750672	1766194	15522	Predicted by at least one method	ATE69788.1	CMV38_1956	1754704	1755141	1	Cag pathogenicity island protein
1750672	1766194	15522	Predicted by at least one method	ATE69789.1	CMV38_1957	1755153	1755917	1	PTet29
1750672	1766194	15522	Predicted by at least one method	ATE69790.1	CMV38_1958	1755920	1756717	1	Cpp46
1750672	1766194	15522	Predicted by at least one method	ATE69791.1	CMV38_1959	1756788	1757408	1	Cpp47
1750672	1766194	15522	Predicted by at least one method	ATE69792.1	CMV38_1960	1757671	1759737	1	Uncharacterized protein
1750672	1766194	15522	Predicted by at least one method	ATE69793.1	CMV38_1961	1759970	1760098	1	Cpp50
1750672	1766194	15522	Predicted by at least one method	ATE69794.1	CMV38_1962	1760073	1761392	1	Cpp50
1750672	1766194	15522	Predicted by at least one method	ATE69795.1	CMV38_1963	1761411	1761590	1	Uncharacterized protein
1750672	1766194	15522	Predicted by at least one method	ATE69796.1	CMV38_1964	1761949	1763868	1	TetO protein
1750672	1766194	15522	Predicted by at least one method	ATE69797.1	CMV38_1965	1764643	1765215	1	Cpp3

Table S3. List of virulence and survival factors identified in the genomes of *Campylobacter jejuni* 100 and 104

Gene	Protein	Virulence or survival factor	Locus_tag		Reference
			***C. jejuni* 100**	***C. jejuni* 104**	
Motility and chemotaxis					
cetA	bipartate energy taxis response protein CetA	energy taxis protein	CMV38_1680	CLH93_1267	(Bolton 2015; Gundogdu et al. 2007; Lertsethtakarn et al. 2011; van Putten et al. 2009; Young et al. 2007)
cetB	bipartate energy taxis response protein CetB	energy taxis protein	CMV38_1271	CLH93_1266	(Bolton 2015; Lertsethtakarn et al. 2011; van Putten et al. 2009; Young et al. 2007)
cheA	chemotaxis histidine kinase	chemotaxis protein	CMV38_0307	CLH93_0238	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cheB	protein-glutamate methylesterase	chemotaxis protein	CMV38_0948	CLH93_0961	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cheR	MCP protein methyltransferase	chemotaxis protein	CMV38_0947	CLH93_0960	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)

cheV	chemotaxis signal transduction	chemotaxis protein	CMV38_0308	CLH93_0239	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cheW	purine-binding chemotaxis	chemotaxis protein	CMV38_0306	CLH93_0237	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; Young et al. 2007)
cheY	chemotaxis regulatory protein	response regulator controlling flagellar rotation	CMV38_1199 CMV38_1305	CLH93_1198 CLH93_1300	(Bolton 2015; Dasti et al. 2010; Lertsethtakarn et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
docB	MCP-type signal transduction protein	methyl-accepting chemotaxis protein (MCP) also called transducer-like protein (Tlp)	CMV38_0011	CLH93_1720	(Bolton 2015; Hendrixson and DiRita 2004; Lertsethtakarn et al. 2011; Parkhill et al. 2000; Young et al. 2007)
docC	MCP-type signal transduction protein	methyl-accepting chemotaxis protein (MCP) also called transducer-like protein (Tlp)	CMV38_0281[c]		(Bolton 2015; Hendrixson and DiRita 2004; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flaG	flagellar protein FlaG	length control of the polar flagella	CMV38_0566	CLH93_0578	(Parkhill et al. 2000; Inoue et al. 2018)
flgB	flagellar basal body rod protein FlgB	flagellar basal-body rod	CMV38_0546	CLH93_0559	(Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgC	flagellar basal body rod protein FlgC	flagellar basal-body rod	CMV38_0545	CLH93_0558	(Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)

flgD	flagellar hook assembly protein FlgD	flagellar hook assembly	CMV38_1977[a]	CLH93_0016	(Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgE	flagellar hook protein FlgE	flagellar hook	CMV38_1978[a]	CLH93_0017	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgE2	flagellar hook protein FlgE	flagellar hook subunit	CMV38_0030	CLH93_1701	(Parkhill et al. 2000)
flgH	flagellar basal body L-ring protein FlgH	flagellar basal body L-ring	CMV38_0715	CLH93_0731	(Bolton 2015; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgI	flagellar basal body P-ring protein FlgI	flagellar basal body P-ring	CMV38_1583	CLH93_1426	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgR	sigma-54 associated transcriptional activator	sigma-54 activating two-component system	CMV38_1101	CLH93_1098	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flgS	signal transduction histidine kinase	sigma-54 activating two-component system	CMV38_0820	CLH93_0833	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flhF	flagellar biosynthesis regulator	flagellar biosynthesis	CMV38_1999[a]	CLH93_0034	(Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliA	flagellar biosynthesis RNA polymerase sigma fator	sigma-28 promoter regulates *flaA* gene expression	CMV38_1996[a]	CLH93_0031	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000; Young et al. 2007)
fliD	flagellar hook-associated protein FliD	flagellar hook-associated	CMV38_0566	CLH93_0579	(Parkhill et al. 2000)

fliF	flagellar MS-ring protein FliF	flagella hook-basal body	CMV38_0340	CLH93_0272	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliG	flagellar motor switch protein FliG	flagellar motor switch	CMV38_0341	CLH93_0273	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliK	flagellar hook-length control protein FliK	flagellar hook-length control	CMV38_1976[a]	CLH93_0015	(Bolton 2015; Gundogdu et al. 2007; Lertsethtakarn et al. 2011)
fliM	flagellar motor switch protein FliM	flagella motor switch	CMV38_1995[a]	CLH93_0030	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliN	flagellar motor switch protein FliN	flagellar motor switch	CMV38_0374	CLH93_0309	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliY	flagellar motor switch protein FliY	flagellar motor switch	CMV38_1994[a]	CLH93_0029	(Bolton 2015; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
motA	flagellar motor protein MotA	flagellar motor componente	CMV38_0359	CLH93_0292	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
motB	flagellar motor protein MotB	flagellar motor componente	CMV38_0358	CLH93_0291	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
pseA	pseudaminic acid biosynthesis protein PseA	flagellin glycosylation	CMV38_1457	CLH93_1399	(Gilbreath et al. 2011; Guerry et al. 2006; Gundogdu et al. 2007)
pseB	UDP-N-acetylglucosamine 4,6-dehydratase	flagellin glycosylation	CMV38_1432	CLH93_1375	(Gilbreath et al. 2011; Guerry et al. 2006; Gundogdu et al. 2007)

pseF	pseudaminic acid cytidylyltransferase	flagellin glycosylation	CMV38_1452	CLH93_1392	(Gilbreath et al. 2011; Guerry et al. 2006; Parkhill et al. 2000)
pseG	UDP-2,4-diacetamido-2,4,6-trideoxy-beta-L-altropyranose hydrolase	flagellin glycosylation	CMV38_1453	CLH93_1393	(Gilbreath et al. 2011; Guerry et al. 2006; Gundogdu et al. 2007)
pseH	N-acetyltransferase	flagellin glycosylation	CMV38_1454	CLH93_1396	(Gilbreath et al. 2011; Guerry et al. 2006; Gundogdu et al. 2007)
pseI	pseudaminic acid synthase	flagellin glycosylation	CMV38_1458	CLH93_1400	(Gilbreath et al. 2011; Guerry et al. 2006; Gundogdu et al. 2007)
rpoN	RNA polymerase sigma-54 factor	sigma-54 promoter regulates *flaB* gene expression	CMV38_0697	CLH93_0712	(Bolton 2015; Dasti et al. 2010; Parkhill et al. 2000)
Adhesion					
cadF	outer membrane fibronectin-binding protein CadF	outer membrane protein	CMV38_1598	CLH93_1441	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
jlpA	surface-exposed lipoprotein	lipoprotein involved in adhesion to Hep-2 cells	CMV38_1021	CLH93_1032	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
pldA	phospholipase A	phospholipase A	CMV38_1469		(Bolton 2015; Parkhill et al. 2000)
Invasion					

ciaB	invasion antigen CiaB	protein involved in invasion	CMV38_0938	CLH93_0950	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cipA	invasion protein CipA	invasion associated protein		CLH93_0729	(Javed et al. 2010)
flaC	flagellin C	protein secreted into the host cells and essential for colonisation and invasion	CMV38_0749	CLH93_0766	(Bolton 2015; Dasti et al. 2010; Gilbreath et al. 2011; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
flhA	flagellar biosynthesis protein FlhA	component of the flagellar T3SS	CMV38_0904	CLH93_0917	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
flhB	flagellar biosynthesis protein FlhB	component of the flagellar T3SS	CMV38_0357	CLH93_0290	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliI	flagellum-specific ATP synthase	flagellar protein export	CMV38_0224 CMV38_1851	CLH93_0163	(Gilbreath et al. 2011; Parkhill et al. 2000)
fliP	flagellar biosynthesis protein FliP	component of the flagellar T3SS	CMV38_0846	CLH93_0859	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliQ	flagellar biosynthesis protein FliQ	component of the flagellar T3SS	CMV38_0082	CLH93_1640	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)
fliR	flagellar biosynthesis protein FliR	component of the flagellar T3SS	CMV38_1261	CLH93_1256	(Bolton 2015; Gilbreath et al. 2011; Lertsethtakarn et al. 2011; Parkhill et al. 2000)

htrA	serine protease	chaperone involved in the proper folding of adhesins	CMV38_1311	CLH93_1306	(Bolton 2015; Parkhill et al. 2000)
iamA	ABC transporter ATP-binding protein	Invasion associated marker	CMV38_1768	CLH93_1608	(Bolton 2015; Parkhill et al. 2000)
Toxin production					
cdtA	cytolethal distending toxin A	cytolethal distending toxin (CDT) subunit A	CMV38_2013[a]	CLH93_0047	(Bolton 2015; Dasti et al. 2010; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cdtB	cytolethal distending toxin B	cytolethal distending toxin (CDT) subunit B	CMV38_2012[a]	CLH93_0046	(Bolton 2015; Dasti et al. 2010; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
cdtC	cytolethal distending toxin C	cytolethal distending toxin (CDT) subunit C	CMV38_2011[a]	CLH93_0045	(Bolton 2015; Dasti et al. 2010; Parkhill et al. 2000; van Putten et al. 2009; Young et al. 2007)
Lipooligosaccharide (LOS) and extracellular polysaccharide (EP) biosynthesis					
cgtB	beta-1,3 galactosyltransferase	lipopolysaccharide production		CLH93_1218	(Bolton 2015; Gilbert et al. 2000; Linton et al. 2000a)
htrB	lipid A biosynthesis lauroyl acyltransferase	synthesis of lipid A		CLH93_1213	(Parkhill et al. 2000)
kpsE	capsule polysaccharide ABC transporter permease	capsule biosynthesis gene	CMV38_1566	CLH93_1408	(Bolton 2015; Parkhill et al. 2000)
kpsM	capsule polysaccharide ABC transporter permease	capsular polysaccharide transport gene M	CMV38_1568	CLH93_1410	(Bacon et al. 2001; Bolton 2015; Parkhill et al. 2000)

neuA1	bifunctional beta-1,4-N-acetylgalactosaminyltransferase/CMP-Neu5Ac synthase	sialylation of lipooligosaccharide	CMV38_1227	CLH93_1222	(Linton et al. 2000b; Parkhill et al. 2000)
neuB1	sialic acid synthase	sialylation of lipooligosaccharide	CMV38_1225	CLH93_1220	(Linton et al. 2000b; Parkhill et al. 2000)
neuC1	UDP-N-acetylglucosamine 2-epimerase	sialylation of lipooligosaccharide	CMV38_1226	CLH93_1221	(Linton et al. 2000b; Parkhill et al. 2000)
waaC	heptosyltransferase I	lipooligosaccharide inner core biosynthesis	CMV38_1214	CLH93_1212	(Linton et al. 2000b; Parkhill et al. 2000)
waaF	heptosyltransferase II	lipooligosaccharide inner core biosynthesis	CMV38_1231	CLH93_1226	(Oldfield et al. 2002; Parkhill et al. 2000)
Iron uptake system					
ceuE	enterochelin uptake substrate-binding protein	lipoprotein involved in iron acquisition	CMV38_1473		(Bolton 2015; Gilbreath et al. 2011; Miller et al. 2009; Parkhill et al. 2000)
cfrA	ferric enterobactin uptake receptor	outer membrane ferric enterobactin FeEnt receptors	CMV38_0773	CLH93_0795[c]	(Bolton 2015; Gilbreath et al. 2011; Gundogdu et al. 2007; Miller et al. 2009; Parkhill et al. 2000)
chuA	hemin uptake system outer membrane recept	outer membrane receptor for hemin and haemoglobin	CMV38_1729	CLH93_1572	(Bolton 2015; Gilbreath et al. 2011; Miller et al. 2009; Parkhill et al. 2000)
ctuA	TonB-denpendent outer membrane receptor	putative transferring-bound iron utilization outer-membrane receptor	CMV38_1868	CLH93_0143	(Bolton 2015; Miller et al. 2009; Parkhill et al. 2000)
exbB2	ExbB/TolQ family transport protein	transport of iron compounds over the outer membrane		CLH93_1590	(Miller et al. 2009; Parkhill et al. 2000)

exbB3	MotA/TolQ/ExbB proton channel family protein	transport of iron compounds over the outer membrane	CMV38_2042[a]	CLH93_0076	(Miller et al. 2009; Parkhill et al. 2000)
exbD1	biopolymer transport protein	transport of iron compounds over the outer membrane	CMV38_1866	CLH93_0148	(Miller et al. 2009; Parkhill et al. 2000)
exbD2	ExbD/TolR family transport protein	transport of iron compounds over the outer membrane	CMV38_1749	CLH93_1591	(Miller et al. 2009; Parkhill et al. 2000)
exbD3	ExbD/TolR family transport protein	transport of iron compounds over the outer membrane	CMV38_2043[a]	CLH93_0077	(Miller et al. 2009; Parkhill et al. 2000)
fur	ferric uptake regulator	ferric uptake regulator	CMV38_0422	CLH93_0357	(Bolton 2015; Gilbreath et al. 2011; Miller et al. 2009; Parkhill et al. 2000)
tonB1	TonB transport protein	transport of iron compounds over the outer membrane	CMV38_1865	CLH93_0149	(Miller et al. 2009; Parkhill et al. 2000)
tonB3	TonB transport protein	transport of iron compounds over the outer membrane	CMV38_0772	CLH93_0794[c]	(Miller et al. 2009; Parkhill et al. 2000)
Stress response and survival					
ahpC	alkyl hydroperoxide reductase	detoxification of reactive oxygen species (ROS)	CMV38_0356	CLH93_0289	(Atack et al. 2008; Bolton 2015; Dufour et al. 2013; Gilbreath et al. 2011; Palyada et al. 2009; Parkhill et al. 2000)
bcp	bacterioferritin comigratory protein	detoxification of reactive oxygen species (ROS)	CMV38_0295	CLH93_0226	(Atack et al. 2008; Bolton 2015; Dufour et al. 2013; Parkhill et al. 2000)
cj0358	cytochrome C551 peroxidase	detoxification of reactive oxygen species (ROS)	CMV38_0382	CLH93_0316	(Atack and Kelly 2009; Bolton 2015; Hendrixson and DiRita 2004; Parkhill et al. 2000)

cj1000	transcriptional regulator	LysR-type transcriptional regulator Cj1000	CMV38_1037	CLH93_1049	(Dufour et al. 2013; Parkhill et al. 2000)
cj1545c	MdaB protein, NADPH quinine reductase	defense against reactive oxygen species (ROS) and aerobic stress	CMV38_1664	CLH93_1507	(Atack and Kelly 2009; Bolton 2015; Parkhill et al. 2000)
clpP	ATP-dependent Clp protease proteolytic subunit	heat shock response	CMV38_0221 CMV38_1854	CLH93_0160[b]	(Cohn et al. 2007; Parkhill et al. 2000; Poli et al. 2012)
csrA	carbon storage regulator	post-transcriptional regulator of oxidative stress defenses	CMV38_1183	CLH93_1182	(Atack and Kelly 2009; Dufour et al. 2013; Parkhill et al. 2000; van Putten et al. 2009)
dnaJ	chaperone protein DnaJ	heat shock response	CMV38_1346	CLH93_1340	(Bolton 2015; Dasti et al. 2010; Parkhill et al. 2000)
docA	cytochrome C551 peroxidase	detoxification of reactive oxygen species (ROS)	CMV38_0010	CLH93_1721	(Atack and Kelly 2009; Bolton 2015; Hendrixson and DiRita 2004; Parkhill et al. 2000)
katA	catalase	detoxification of reactive oxygen species (ROS)	CMV38_1506		(Atack et al. 2008; Bolton 2015; Dufour et al. 2013; Gilbreath et al. 2011; Palyada et al. 2009; Parkhill et al. 2000)
luxS	S-ribosylhomocysteine lyase	AI-2 biosynthesis enzyme	CMV38_1281	CLH93_1277	(Bolton 2015; Gundogdu et al. 2007; van Putten et al. 2009)
mrsA	methionine sulfoxide reductase A	protection against oxidative stress	CMV38_0666	CLH93_0679	(Atack and Kelly 2009; Parkhill et al. 2000)
mrsB	methionine sulfoxide reductase B	protection against oxidative stress	CMV38_1193	CLH93_1192	(Atack and Kelly 2009; Parkhill et al. 2000)
perR	peroxide stress regulator	peroxide stress regulator	CMV38_0344	CLH93_0276	(Atack and Kelly 2009; Dufour et al. 2013; Gilbreath et al. 2011; Miller et al. 2009;

					Palyada et al. 2009; Parkhill et al. 2000)
racR	two-component regulator	temperature-responsive regulator	CMV38_1347	CLH93_1341	(Atack and Kelly 2009; Bolton 2015; Parkhill et al. 2000)
racS	two-component sensor histidine kinase	heat shock response	CMV38_1348	CLH93_1342	(Bolton 2015; Parkhill et al. 2000)
rrc	non-heme iron protein	defense against reactive oxygen species (ROS) and aerobic stress	CMV38_0018	CLH93_1713	(Atack and Kelly 2009; Bolton 2015; Parkhill et al. 2000)
sodB	superoxide dismutase	antioxidant protein	CMV38_1877	CLH93_0137	(Atack and Kelly 2009; Bolton 2015; Gilbreath et al. 2011; Palyada et al. 2009; Parkhill et al. 2000)
spoT	guanosine-3\',5\'-bis(diphosphate) 3\'-pyrophosphohydrolase	stringent control	CMV38_1359	CLH93_1353	(Bolton 2015; Parkhill et al. 2000)
tpx	thiol peroxidase	detoxification of reactive oxygen species (ROS)	CMV38_0803	CLH93_0818	(Atack et al. 2008; Bolton 2015; Dufour et al. 2013; Parkhill et al. 2000)

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4 Considerações Finais

Os resultados deste estudo forneceram informações importantes sobre a grande diversidade de *C. jejuni* e *C. coli* contaminando produtos cárneos de frango comercializados no sul do Brasil, embora tenha-se observado recorrência de alguns clones. Além disso, os isolados apresentaram níveis elevados de resistência às tetraciclinas e ciprofloxacina, enquanto que níveis menores de resistência aos macrolídeos e multirresistência foram identificados. Os isolados também portavam vários genes associados à virulência, mas nenhum gene específico foi relacionado com a resistência a antimicrobianos. A técnica de pulsed-field gel electrophoresis (PFGE) e a pesquisa de genes associados à virulência tiveram maior poder discriminatório que a análise de resistência a antimicrobianos, porém, a associação entre as três técnicas melhorou a diferenciação entre os isolados.

Realizamos a primeira análise gênomica de *C. jejuni* isolados no Brasil. Os dois isolados avaliados diferiram entre si na multilocus sequence typing (MLST). A análise comparativa de referência revelou diferenças na estrutura genômica (SNPs, rearranjos e inversões) em ambos os genomas, bem como a presença de alguns genes associados à virulência e de elementos genéticos móveis, como transposons, ilhas genômicas e sequências de profagos. Além disso, mecanismos de resistência a antimicrobianos e um novo megaplasmídeo de virulência foram identificados.

De modo geral, podemos concluir que a presença de uma diversidade de isolados de *Campylobacter* resistentes a antimicrobianos e potencialmente virulentos contaminando produtos cárneos de frango no Brasil representa um risco potencial à saúde dos consumidores, uma vez que este país é o maior exportador mundial de carne de frango. Isso demonstra a necessidade de medidas de controle mais rigorosas para *Campylobacter* na cadeia de produção avícola do Brasil.

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