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Antimicrobial Resistance Profiles and Phylogenetic Analysis of *Campylobacter jejuni* Strains Isolated in Brazil by Whole Genome Sequencing

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Aims: The objectives of this work were to use whole genome sequencing (WGS) to determine the antimicrobial resistance genotypes of 116 *Campylobacter jejuni* strains isolated in Brazil and to compare it with the results obtained by antimicrobial susceptibility testing (AST). In addition, WGS was used to uncover the phylogenetic relationship among those strains.

Results: By AST, the *C. jejuni* strains resistant to ciprofloxacin, tetracycline, doxycycline, and erythromycin were 51 (44%), 41 (35.3%), 41 (35.3%), and 6 (5.2%), respectively. By WGS, the genes *aph*(3′)III, *aadE*, $bla_{\text{OXA-449}}$, $bla_{\text{OXA-184}}$, $bla_{\text{OXA-61}}$, and *tet*(O) were detected in 6 (5.2%), 3 (2.6%), 1 (0.9%), 10 (8.6%), 55 (47.4%), and 44 (38%) strains, respectively. Fifty-four (46.6%) strains showed the mutation T86I in the *gyrA* gene, and four (3.4%) strains presented the mutation A2075G in the 23S rRNA gene. The correlation between AST and WGS was 100% for ciprofloxacin, 97.5% for tetracyclines, and 66.7% for erythromycin. The whole genome single nucleotide polymorphism (SNP) tree clustered the *C. jejuni* strains into two clades comprising strains that were highly related from different sources, places, and years.

Conclusion: The high rates of *C. jejuni* strains resistant to ciprofloxacin and tetracyclines are of concern and may represent a public health problem. WGS has a potential to be a powerful tool for the prediction of resistance of antibiotics used to treat campylobacteriosis. The results obtained by whole genome SNP analysis suggested the potential for transmission between clinical and nonclinical sources and between human and animal sources over the course of 20 years in Brazil.

Keywords: *Campylobacter jejuni*, antimicrobial resistance profiles, whole genome sequencing, antimicrobial susceptibility testing, phylogenetic analysis

Introduction

CAMPYLOBACTER JEJUNI HAS BEEN REPORTED as the most common bacterial pathogen that causes foodborne gastroenteritis in humans in many countries.[1,2] In the United States, *Campylobacter* causes 1.5 million illnesses per year and it is the most common cause of diarrhea in humans.[3] According to the European Food Safety Authority in 27 European countries in 2017, it was estimated that there are 246,000 cases of campylobacteriosis with a rate of 64.8 per 100,000 population, ranking this bacterial pathogen as the most commonly reported gastrointestinal cause in humans.[4]

The disease caused by *C. jejuni* is usually self-limiting and does not require the use of antimicrobials. However, the antimicrobial treatment is indicated in immunocompromised patients or in severe cases of the disease, fluoroquinolones or macrolides being the drugs of choice.[5]

In recent years, antimicrobial resistance (AMR) in *Campylobacter* has become a significant public health problem, and increasing numbers of *Campylobacter* strains have developed resistance to fluoroquinolones and other antimicrobials such as macrolides, tetracyclines, beta-lactams, and aminoglycosides.[6] According to the Centers for Disease Control and Prevention, 448,400 cases of infection each

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year are caused by drug resistant *Campylobacter*, and the percentage of *Campylobacter* strains resistant to ciprofloxacin has almost doubled in the past 20 years, limiting treatment options.[7]

The advent of whole genome sequencing (WGS) has revolutionized genomic research through the possibility of sequencing entire genomes of diverse organisms.[8,9] WGS is becoming a powerful and highly attractive tool for epidemiological investigations, as well as to characterize the AMR profile of specific genes and/or point mutations associated with resistance.[8,10] Furthermore, with WGS it is possible to predict bacterial antibiotic resistance and to correlate these results with resistant phenotypes identified by *in vitro* antimicrobial susceptibility testing (AST).[11,12]

In Brazil, cases of campylobacteriosis have been underreported and underdiagnosed, and studies of *C. jejuni* isolates have been scarce.[13–19] In this way, additional studies that assess the AMR profiles and the molecular genotyping would help to assess the characteristics of *C. jejuni* strains isolated in Brazil.

The aims of this work were to use WGS to determine AMR genotypes of *C. jejuni* strains isolated from diverse sources in Brazil and to compare it with the results obtained by AST against some important antimicrobials in clinical use. In addition, WGS was used to uncover the phylogenetic relationship among these strains.

Materials and Methods

Bacterial strains

A total of 116 *C. jejuni* strains were studied. Those strains were isolated from humans (47 strains), monkey feces (20 strains), chicken feces (15 strains), chicken meat (32 strains), and sewage (02 strains) from cities of São Paulo, Minas Gerais, Rio de Janeiro, and Rio Grande do Sul States located in the Southeast and South regions of Brazil between 1996 and 2016. Specifically, the strains isolated from monkeys were isolated from captive individuals of the species *saimiri*, *rhesus*, and *cynomolgus*. In addition, some strains were isolated from wild marmosets. These strains were selected from the collections of the *Campylobacter* References Laboratories of the Oswaldo Cruz Institute of Rio de Janeiro (Fiocruz-RJ) and of the Adolfo Lutz Institute of Ribeirão Preto (IAL-RP) in Brazil. They were systematically chosen to represent isolates from sporadic cases from different clinical and nonclinical samples of the two collections of the reference laboratories mentioned above that occurred during different years. Specifically, 28 *C. jejuni* strains isolated from humans were provided by the IAL-RP, and the other 88 *C. jejuni* strains were provided by the Oswaldo Cruz Institute of Rio de Janeiro (Fiocruz-RJ). Supplementary Table S1 summarizes the characteristics of the 116 *C. jejuni* strains used in this study.

DNA extraction and quantification

The genomic DNA of the strains listed in Supplementary Table S1 was extracted according to Campioni and Falcão,[20] with a few modifications. Specifically, the strains were cultured at 42°C on BBL™ Columbia Agar Base (Becton Dickinson), supplemented with charcoal (Neon) and FBP [(0.5% ferrous sulfate (Labsynth), 0.5% sodium pyruvate (Vetec), and 0.5% sodium metabisulfite (Labsynth) diluted in sterile water] under microaerobic conditions (10% carbon dioxide, 5% oxygen, and 85% nitrogen), and the growth of the strains was placed directly in Solution 1 (20% sucrose, 50 mM Tris/HCl, pH 8.0, 50 mM EDTA) of the extraction protocol. The quality of the DNAs was checked using NanoDrop 1000 (Thermo Scientific, Rockford, IL), and the concentrations were determined by Qubit double-stranded DNA BR Assay Kit and Qubit fluorometer (Life Technologies, Grand Island, NY) according to each manufacturer's instructions.

Antimicrobial susceptibility testing

Minimal inhibitory concentrations were performed for the 116 *C. jejuni* strains listed in Supplementary Table S1 as recommended by the Clinical Laboratory and Standards Institute M45-Ed3.[21] The bacterial suspension was adjusted to match the 0.5 McFarland (Probac, Brazil) turbidity standard as recommended by the Clinical and Laboratory Standards Institute,[21] seeded in Mueller Hinton agar supplemented with blood (bioMérieux, France), and then the Etest® (bioMérieux) of the antimicrobial agents ciprofloxacin, doxycycline, tetracycline, and erythromycin was used. After inoculation, the plates were incubated at 42°C under microaerophilic atmosphere for 24 hours and then screened. The *C. jejuni* strain ATCC 33291 was included as quality positive control.

Genome sequencing, assembly, and annotation

All isolates were prepared using 1 ng of genomic DNA with the Nextera Sample Preparation Kit (Illumina, San Diego, CA) and then sequenced on a MiSeq or a NextSeq (Illumina) using a 2×250-bp or a 2×150-bp paired-end MiSeq or NextSeq Reagent Kit, respectively. *De novo* assemblies were generated from all raw sequence data. The Illumina reads were assembled with CLC Genomics Workbench version 10.0.1 (CLC Bio, Aarhus, Denmark). The total lengths of the genomes ranged from 1.6 to 1.8 Mb; the number of contigs per assembly for each isolate ranged from 24 to 338, with an average guanine and cytosine (GC) content of 30.35%.[22] The contigs for each isolate (draft genome) were annotated using National Center for Biotechnology Information (NCBI)'s Prokaryotic Genomes Automatic Annotation Pipeline.[23]

Resistance genetic profile

The presence of resistance genes, as well as point mutations in the 23S, Quinolone Resistance-Determining Region (QRDR) of the *gyrA*, *rpsL*, and *cmeR* genes, was determined using ResFinder (Center for Genomic Epidemiology) with settings of threshold of 90% and minimum length of 60%.

Phylogenetic data analysis

To analyze the phylogenetic relationships among the strains studied, a matrix of single nucleotide polymorphisms (SNPs) was constructed using the CFSAN SNP pipeline[24] and the *C. jejuni* strain ATCC 33291 (GenBank accession GCA_009939125.1) as the reference genome. Genetic Algorithm for Rapid Likelihood Inference (GARLI) v2.01 program was used to construct maximum-likelihood

phylogenetic tree (rate matrix = 6 rate; ratehetmodel = gamma). Multiple runs were performed ($n = 100$) to ensure that results were consistent. To estimate support for each node, phylogenies were created for 1,000 bootstrap replicates of the data set from GARLI. Python program SumTrees was used to generate one consensus tree with bootstrap values at a 70% threshold, and FigTree v 1.4.3 was used to export the figures.

Nucleotide sequence accession numbers

WGS assemblies of 116 *Campylobacter jejuni* strains of this study were submitted to the NCBI, and the GenBank accession numbers of each strain are listed in Supplementary Table S1.

Results

Antimicrobial susceptibility testing

The phenotypic AMR patterns of the 95 *C. jejuni* strains that showed some genotypic resistance are presented in Table 1. Sixty-six (56.9%) strains were phenotypically resistant to at least one of the antimicrobials tested. The number of *C. jejuni* strains resistant to ciprofloxacin, tetracycline, doxycycline, and erythromycin was 51 (44%), 41 (35.3%), 41 (35.3%), and 6 (5.2%), respectively. Specifically, 22 *C. jejuni* strains isolated from animals (12), humans (7), and food (3) were resistant to ciprofloxacin, tetracycline, and doxycycline, simultaneously. Two *C. jejuni* strains isolated from humans were resistant to tetracycline, doxycycline, and erythromycin, simultaneously, and four strains isolated from food were considered multidrug resistant because they were phenotypically resistant to all antimicrobial agents tested (Table 1).

Genotypic resistance profiles

A total of six AMR genes were identified in the genomes of the 116 *C. jejuni* strains studied. Ninety-five (81.9%) strains presented at least one resistance gene or point mutation. Two aminoglycoside resistance genes [*aph*(3′)III and *aadE*] were detected in six (5.2%) strains and three (2.6%) strains, respectively. The genes $bla_{\text{OXA-61}}$, $bla_{\text{OXA-184}}$, and $bla_{\text{OXA-449}}$ that confer resistance to beta-lactams were detected in 55 (47.4%), 10 (8.6%), and 1 (0.9%) strain, respectively. Forty-four (38%) strains presented the *tet*(O) gene that confers resistance to tetracyclines. Regarding the point mutations, 54 (46.6%) strains showed the mutation T86I in the QRDR of the *gyrA* gene, and 4 (3.4%) strains presented the mutation A2075G in the domain V of the 23S rRNA gene (Table 1).

Correlation between AMR phenotype and genotype

The correlation of AMR phenotype and genotype was assessed for the antimicrobials tetracyclines, ciprofloxacin, and erythromycin. Forty of the 41 phenotypically Tet^r strains carried the *tet*(O) gene showing a correlation of 97.5% among the Tet^r strains, and 4 of 75 Tet^s strains in the AST carried this gene with a correlation of 94.6% among the Tet^s strains. All the 51 Cip^r strains in the AST had a *gyrA* T86I point of mutation, with a correlation of 100% among the Cip^r strains, and three of 65 Cip^s in the AST presented this mutation showing a correlation of 95.4% among the Cip^s strains. Four of six Ery^r strains in the AST showed the 23S rRNA A2075G mutation with a correlation of 66.7% among the Ery^r strains, and none of the 110 Ery^s strains presented any mutation, showing a correlation of 100% among the Ery^s strains. The discrepancies between phenotypic and genotypic resistance are marked with asterisk in the Table 1.

Phylogenetic analysis

The phylogenetic tree generated with the whole genome SNP analysis is shown in Fig. 1. The 116 *C. jejuni* strains studied were distributed into 2 major clades designated A and B (Fig. 1). Clade A was composed of 53 (46%) strains isolated from humans (29), animals (20), food (3), and the environment (1) between 1996 and 2016, in Minas Gerais, Sao Paulo, and Rio de Janeiro States. The *C. jejuni* ATCC 33291 reference strain was allocated in clade A. Clade A was subdivided into two subclades named A1 and A2. Specifically, subclade A1 included 19 strains isolated from humans (10) and animals (9) between 1996 and 2009, in Sao Paulo and Rio de Janeiro States. Subclade A2 was composed of 30 strains isolated from humans (18), animals (9), and food (3) between 1997 and 2016, in Minas Gerais, Sao Paulo, and Rio de Janeiro States. Clade B comprised 63 (54%) strains isolated from food (29), humans (18), animals (15), and the environment (1) between 1996 and 2016, in Minas Gerais, Sao Paulo, Rio de Janeiro, and Rio Grande do Sul States. This clade B was subdivided into two subclades named B1 and B2. Specifically, subclade B1 included 49 strains isolated from humans (17), animals (14), food (17), and the environment (1) between 1996 and 2009, in Sao Paulo and Rio de Janeiro States. Subclade B2 was composed of 14 strains isolated from humans (1), animal (1), and food (12) between 2007 and 2015, in Minas Gerais, Sao Paulo, and Rio Grande do Sul States.

Discussion

Campylobacter jejuni is an important zoonotic pathogen that has been causing foodborne gastroenteritis in many countries.[1–4] In Brazil, campylobacteriosis has been underdiagnosed and underreported; in this way, there is a paucity of studies about this pathogen.[13–19]

The aims of this study were to use WGS to assess the phylogenetic relationship, to determine the AMR genotypes and to compare AMR with the results obtained by AST against four important antimicrobials in clinical use for 116 *C. jejuni* strains isolated from humans, animals, food, and the environment between 1996 and 2016 in Brazil.

Some studies using AST performed worldwide corroborated the present work and also showed that *C. jejuni* strains are resistant to ciprofloxacin, tetracycline, and erythromycin.[25–29] Duarte *et al.*[27] studied 89 *C. jejuni* strains isolated from humans, animals, and food and observed resistance to ciprofloxacin, tetracycline, and erythromycin in 82, 59, and 6 strains, respectively. Fifteen of the 39 *C. jejuni* strains isolated from poultry in Côte d'Ivoire were resistant to ciprofloxacin, and seven strains were resistant to erythromycin.[25] A study performed in three regions of Peru evaluated

Table 1. Phenotypic and Genotypic Resistance Profiles of the 95 *Campylobacter jejuni* Resistant Strains

CFSAN no.	*Isolate name*	*Source*	*Genotypic resistance profile (identity %)*	*Points of mutation*	*Phenotypic resistance profile*	*SNP clade*
CFSAN065295	Cj 02	Human	bla_{OXA-61} (99.87), *tet*(O) (99.84)	—	Dox, Tet, Ery*	A2
CFSAN065296	Cj 03	Human	*tet*(O) (94.95)	—	Dox, Tet, Ery*	A2
CFSAN065297	Cj 04	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065298	Cj 06	Human	*aadE* (100)	*gyrA* p.T86I*	—	B1
CFSAN065299	Cj 07	Human	*aadE* (100)	*gyrA* p.T86I	Cip	B1
CFSAN065300	Cj 09	Human	bla_{OXA-61} (99.87), *tet*(O) (99.9)	—	Dox, Tet	A1
CFSAN065301	Cj 11	Human	*tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065302	Cj 12	Human	bla_{OXA-61} (99.87)	*gyrA* p.T86I	Cip	A2
CFSAN065303	Cj 13	Human	*tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065305	Cj 15	Human	*tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065306	Cj 16	Human	bla_{OXA-61} (100), *tet*(O) (94.95)*	*gyrA* p.T86I*	—	B1
CFSAN065307	Cj 17	Human	$bla_{OXA-184}$ (99.87), aph(3′)III (100)	—	—	A
CFSAN065309	Cj 19	Human	—	*gyrA* p.T86I	Cip	A2
CFSAN065310	Cj 20	Human	*tet*(O) (94.95)*	—	—	B1
CFSAN065312	Cj 22	Human	*tet*(O) (94.95)	—	Dox, Tet	B2
CFSAN065313	Cj 23	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065314	Cj 24	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065315	Cj 25	Human	—	*gyrA* p.T86I	Cip	A1
CFSAN065316	Cj 26	Human	bla_{OXA-61} (99.87)	—	—	B1
CFSAN065317	Cj 27	Human	bla_{OXA-61} (99.87)	—	—	B1
CFSAN065319	Cj 29	Human	bla_{OXA-61} (100)	—	—	B1
CFSAN065320	Cj 30	Human	bla_{OXA-61} (100)	—	—	A1
CFSAN065321	Cj 31	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065322	Cj 32	Human	bla_{OXA-61} (99.87)	—	—	A1
CFSAN065323	Cj 33	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065324	Cj 34	Human	—	*gyrA* p.T86I	Cip	B1
CFSAN065325	CCAMP 81	Animal	bla_{OXA-61} (99.74)	—	—	B1
CFSAN065327	CCAMP 162	Animal	bla_{OXA-61} (99.74)	—	—	B1
CFSAN065328	CCAMP 163	Animal	bla_{OXA-61} (99.74)	—	—	B1
CFSAN065329	CCAMP 470	Animal	*tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065330	CCAMP 471	Animal	*tet*(O) (94.27)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065331	CCAMP 472	Animal	*tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065332	CCAMP 473	Animal	bla_{OXA-61} (100), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065333	CCAMP 476	Animal	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065334	CCAMP 478	Animal	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065335	CCAMP 479	Animal	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065336	CCAMP 480	Animal	bla_{OXA-61} (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065337	CCAMP 481	Animal	bla_{OXA-61} (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065338	CCAMP 487	Human	bla_{OXA-61} (100)	—	—	B1
CFSAN065340	CCAMP 489	Human	bla_{OXA-61} (99.87), *tet*(O) (99.9)	—	Dox, Tet	A2

(*continued*)

Table 1. (Continued)

CFSAN no.	*Isolate name*	*Source*	*Genotypic resistance profile (identity %)*	*Points of mutation*	*Phenotypic resistance profile*	*SNP clade*
CFSAN065343	CCAMP 501	Human	—	*gyrA* p.T86I	Cip	B1
CFSAN065344	CCAMP 506	Human	bla_{OXA-61} (99.87), *aadE* (100)	*gyrA* p.T86I	Cip, Dox, Tet*	A2
CFSAN065345	CCAMP 512	Human	bla_{OXA-61} (99.87)	*gyrA* p.T86I	Cip	A2
CFSAN065346	CCAMP 588	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065347	CCAMP 594	Human	*tet*(O) (99.9)	—	Dox, Tet	B1
CFSAN065348	CCAMP 601	Human	bla_{OXA-61} (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065349	CCAMP 612	Human	bla_{OXA-61} (99.87), *tet*(O) (99.9)	—	Dox, Tet	A1
CFSAN065351	CCAMP 672	Animal	bla_{OXA-61} (99.87), *tet*(O) (99.9)	—	Dox, Tet	A2
CFSAN065352	CCAMP 674	Animal	bla_{OXA-61} (99.87), *tet*(O) (99.87)	—	Dox, Tet	A2
CFSAN065353	CCAMP 675	Animal	bla_{OXA-61} (99.87), *tet*(O) (99.87)	—	Dox, Tet	A2
CFSAN065354	CCAMP 678	Human	bla_{OXA-61} (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065355	CCAMP 685	Animal	bla_{OXA-61} (99.87)	—	—	A1
CFSAN065360	CCAMP 699	Human	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065362	CCAMP 730	Animal	bla_{OXA-61} (99.87)	—	—	A2
CFSAN065363	CCAMP 764	Environment	bla_{OXA-61} (99.74)	—	—	B1
CFSAN065366	CCAMP 828	Animal	bla_{OXA-61} (100)	—	—	B1
CFSAN065367	CCAMP 830	Environment	bla_{OXA-61} (99.87)	—	—	A
CFSAN065369	CCAMP 980	Animal	bla_{OXA-61} (99.87)	—	—	A
CFSAN065370	CCAMP 991	Animal	bla_{OXA-61} (99.87)	—	—	A
CFSAN065371	CCAMP 1013	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065373	CCAMP 1015	Food	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	—	Dox, Tet	B1
CFSAN065374	CCAMP 1016	Food	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	—	Dox, Tet	B1
CFSAN065375	CCAMP 1018	Food	bla_{OXA-61} (100), *tet*(O) (94.95)*	*gyrA* p.T86I	Cip	B2
CFSAN065376	CCAMP 1019	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065377	CCAMP 1020	Food	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	—	Dox, Tet	B1
CFSAN065378	CCAMP 1021	Food	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	—	Dox, Tet	B1
CFSAN065379	CCAMP 1023	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065380	CCAMP 1024	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065381	CCAMP 1025	Food	$bla_{OXA-184}$ (100), *tet*(O) (94.95)	—	Dox, Tet	B1
CFSAN065382	CCAMP 1032	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065383	CCAMP 1039	Food	bla_{OXA-61} (99.87), *tet*(O) (93.02)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065384	CCAMP 1047	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065385	CCAMP 1048	Food	bla_{OXA-61} (99.87), *tet*(O) (94.95)*	*gyrA* p.T86I	Cip	A2
CFSAN065389	CCAMP 1053	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065390	CCAMP 1054	Food	—	*gyrA* p.T86I*	—	B2
CFSAN065391	CCAMP 1055	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065394	CCAMP 1058	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065395	CCAMP 1059	Food	—	*gyrA* p.T86I	Cip	B2
CFSAN065397	CCAMP 1061	Food	bla_{OXA-61} (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065399	CCAMP 1080	Animal	bla_{OXA-61} (100)	*gyrA* p.T86I	Cip	B1
CFSAN065400	CCAMP 1140	Animal	bla_{OXA-61} (99.74)	*gyrA* p.T86I	Cip	B1

(*continued*)

Table 1. (Continued)

CFSAN no.	*Isolate name*	*Source*	*Genotypic resistance profile (identity %)*	*Points of mutation*	*Phenotypic resistance profile*	*SNP clade*
CFSAN065401	CCAMP 1266	Animal	$bla_{\text{OXA-61}}$ (99.87), *tet*(O) (99.9)	*gyrA* p.T86I	Cip, Dox, Tet	A2
CFSAN065402	CCAMP 1466	Animal	$bla_{\text{OXA-449}}$ (100)	—	—	A1
CFSAN065403	CCAMP 1478	Human	—	*gyrA* p.T86I	Cip	B1
CFSAN065404	CCAMP 1491	Human	$bla_{\text{OXA-184}}$ (100), *tet*(O) (99.9)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065405	CCAMP 1493	Animal	$bla_{\text{OXA-61}}$ (99.87)	*gyrA* p.T86I	Cip	A2
CFSAN065406	CCAMP 1497	Human	$bla_{\text{OXA-61}}$ (99.87)	*gyrA* p.T86I	Cip	A2
CFSAN065407	CCAMP 1518	Food	$bla_{\text{OXA-61}}$ (100), *tet*(O) (99.38), aph(3′)III (100)	*gyrA* p.T86I; 23S rRNA A2075G	Cip, Dox, Tet, Ery	B1
CFSAN065408	CCAMP 1519	Food	$bla_{\text{OXA-61}}$ (99.87), *tet*(O) (94.9), aph(3′)III (100)	*gyrA* p.T86I	Cip, Dox, Tet	B1
CFSAN065409	CCAMP 1520	Food	$bla_{\text{OXA-61}}$ (94.37), *tet*(O) (99.38), aph(3′)III (100)	*gyrA* p.T86I; 23S rRNA A2075G	Cip, Dox, Tet, Ery	B1
CFSAN065410	CCAMP 1521	Food	$bla_{\text{OXA-61}}$ (100), *tet*(O) (99.38), aph(3′)III (100)	*gyrA* p.T86I; 23S rRNA A2075G	Cip, Dox, Tet, Ery	B1
CFSAN065411	CCAMP 1523	Food	$bla_{\text{OXA-61}}$ (100), *tet*(O) (99.38), aph(3′)III (100)	*gyrA* p.T86I; 23S rRNA A2075G	Cip, Dox, Tet, Ery	B1
CFSAN065412	CCAMP 1538	Animal	$bla_{\text{OXA-61}}$ (94.37), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	B2
CFSAN065413	CCAMP 1555	Animal	—	*gyrA* p.T86I	Cip	B1
CFSAN065414	CCAMP 1574	Animal	$bla_{\text{OXA-61}}$ (99.87), *tet*(O) (94.95)	*gyrA* p.T86I	Cip, Dox, Tet	A2

*Indicates the discrepancies between phenotypic and genotypic resistance.

CFSAN, Center for Food Safety and Applied Nutrition; Cip, ciprofloxacin; Dox, doxycycline; Ery, erythromycin; SNP, single nucleotide polymorphism; Tet, tetracycline.

the ciprofloxacin resistance of *C. jejuni* strains isolated from humans in two different periods. These authors observed a significant increase in the resistant strains in all the regions, including 72.6% to 82.8% in Cusco, 24.1% to 48.9% in Iquitos, and 73.1% to 89.8% in Lima.[26] Carev and colleagues[29] studied 153 *C. jejuni* strains isolated from humans in Croatia and showed that 60% of the strains were resistant to ciprofloxacin, 24% resistant to tetracycline, and 0.7% resistant to erythromycin. In Brazil, Sierra-Arguello *et al.*[28] analyzed 50 *C. jejuni* strains isolated from broiler slaughterhouses in southern Brazil and showed that 94% and 2% of the strains were resistant to ciprofloxacin and erythromycin, respectively.

The World Health Organization (WHO) published in 2017 a list of bacteria resistant to some antimicrobials that represent a threat to human health to promote research and development of new drugs to treat infections caused by these bacteria. According to the WHO, *Campylobacter* strains resistant to fluoroquinolones have a high priority in the development of new antibiotics.[30] Macrolides, such as erythromycin, are one of the few available therapies to treat serious *Campylobacter* infections, particularly in children, for whom quinolone therapy is not recommended.[31,32]

The use of fluoroquinolones in veterinary industry, especially poultry production, has been highly associated with the spread of resistant *Campylobacter* strains, representing a significant public health problem with potential effects on human health and food safety.[1,27]

Comparing the results obtained in the present work by AST and by *in silico* search of AMR genetic profiles, a correlation was observed between phenotype and genotype profiles of 100%, 97.5%, and 66.7% for ciprofloxacin, tetracycline, and erythromycin, respectively (Table 1).

All the Cip^r strains presented a T86I mutation in the *gyrA* gene showing 100% correlation between AMR phenotype and genotype. This mutation in the *gyrA* gene was reported in other studies as the major mechanism of fluoroquinolone resistance for *Campylobacter* strains.[11,33,34]

Interestingly, incongruence between AMR phenotype and genotype was observed among some *C. jejuni* strains of this study suggesting either new genes present confirming resistance when AMR is absent but AST is present or new alleles that have lost AMR when a gene is present but AST is absent. Specifically, Cj 02 and Cj 03 strains were phenotypically erythromycin resistant with no mutation in the 23S rRNA gene suggesting that a new gene is providing the resistance. Other points of mutation, such as amino acid substitution in the L4 and L22 ribosomal proteins and efflux pumps, also play a role in the mechanism of resistance to erythromycin, and this could be an explanation for this observation.[35]

Zhao et al.[11] evaluated the correlation between resistance genotypes and phenotypes using WGS and *in vitro* antimicrobial susceptibility. These authors analyzed 82 *C. coli* and 32 *C. jejuni* strains isolated from diverse sources between 2000 and 2013 in the United States. Eighteen resistance genes and two different points of mutation were observed, and the phenotype to genotype correlation was 100% for ciprofloxacin, tetracycline, and erythromycin.

Generally, the short read WGS data are in draft fragmented genomes with sequences assembled into numerous contigs. This fragmentation could make some resistance genes undetected if it is located in the gaps inside the contigs with the gene sequence interrupted. Also with these fragmented genomes, it is difficult to determine whether the resistance genes are located on a chromosome or mobile

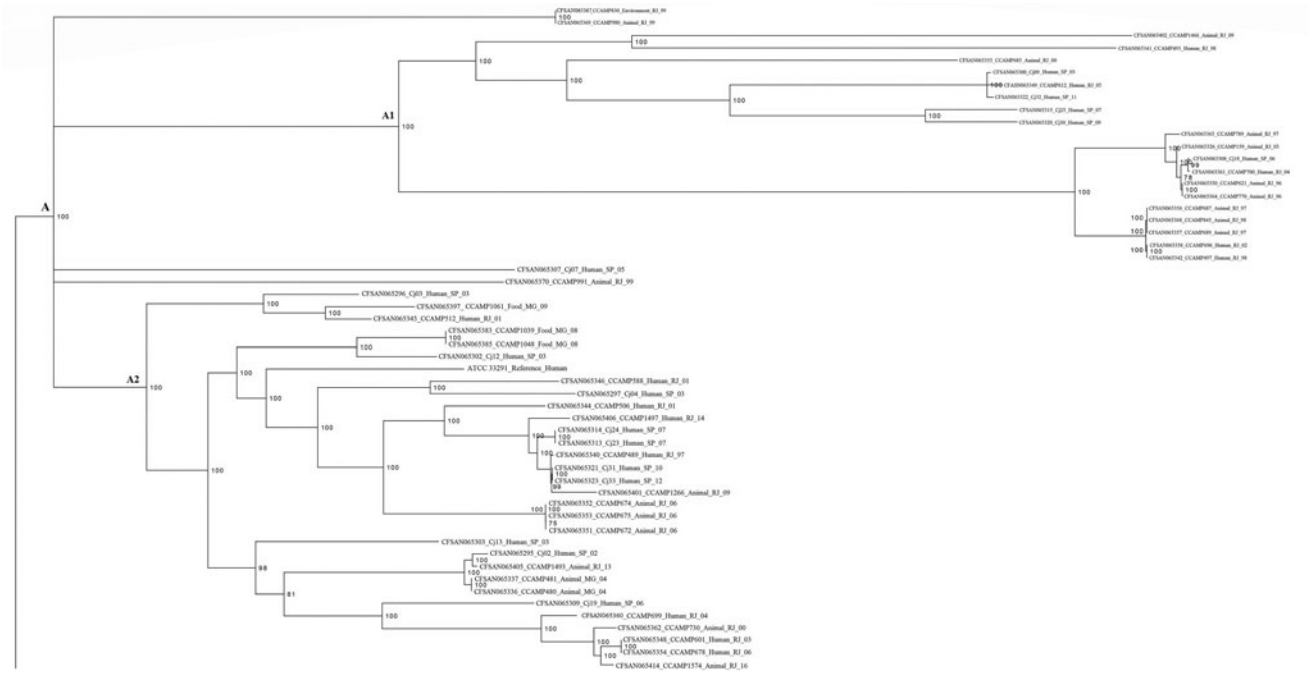

FIG. 1. Phylogenetic analysis based on SNPs of the 116 *Campylobacter jejuni* strains isolated in Brazil (In the branch of tree: CFSAN no_isolate number_source_year of isolation_state of isolation). CFSAN, Center for Food Safety and Applied Nutrition; SNP, single nucleotide polymorphism. Letter labels can be viewed online at www.liebertpub.com/mdr.

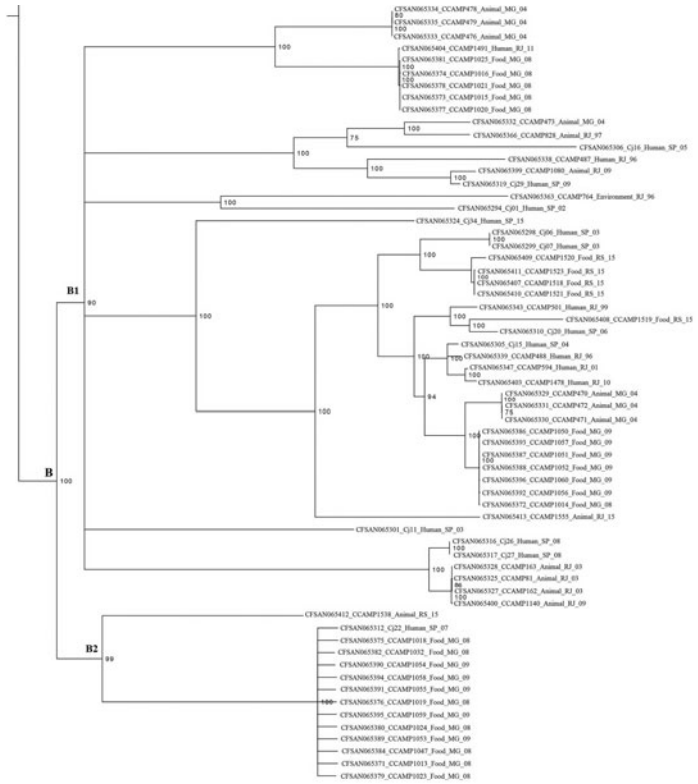

FIG. 1. (Continued).

element.[11,12] Furthermore, some false positive errors (genotypically resistant and phenotypically susceptible) or false negatives (genotypically susceptible and phenotypically resistant) in the genome prediction can occur and may cause consequences for efficient treatment.[12]

Nevertheless, it is possible to infer that WGS has the potential to be a powerful tool for prediction of resistance genes and points of mutation, especially if it is used in combination with AST. However, more studies are required to ensure a better correlation between phenotype and genotype results.

In a previous study 48 of the 116 *C. jejuni* strains from the current work were analyzed for the correlation between the AST and the presence of some resistance genes and points of mutation that were assessed by PCR and sequencing of the amplified gene fragments.[36] In the present study we analyzed more *C. jejuni* strains and also included additional genes by WGS and the ResFinder Database.

All 116 *C. jejuni* strains were sequenced by WGS, and the phylogenetic relationship among them was assessed based on SNP analysis. The whole genome SNP analysis tree allocated the *C. jejuni* strains into two major clades. Clade A was composed of 53 (46%) strains isolated from humans, animals, food, and the environment between 1996 and 2016, in Minas Gerais, Sao Paulo, and Rio de Janeiro States. Clade B comprised 63 (54%) strains isolated from food, humans, animals, and the environment between 1996 and 2016, in Minas Gerais, Sao Paulo, Rio de Janeiro, and Rio Grande do Sul States. Strains from all the sources were distributed in both clades; however, the majority of the food strains (90%) were allocated in clade B. All five strains isolated in Rio Grande do Sul States were allocated in the clade B, and the strains isolated in Minas Gerais, Sao Paulo, and Rio de Janeiro States were distributed across both clades (Fig. 1).

There were no correlations between the AMR profiles observed and their distribution on the whole genome SNP tree (Table 1; Fig. 1). Strains isolated from different sources, places, and years were highly related to each other, suggesting the potential for transmission between clinical and nonclinical sources and between humans and animal sources over the course of 20 years in four different States located in the Southeast and Southern regions of Brazil (Fig. 1). The same hypothesis was observed when these *C. jejuni* strains were typed by *flaA*—short variable region sequencing and pulsed field gel electrophoresis in a previous study.[17,37]

Our findings using whole genome SNP analysis improved the characterization of this important poultry-related pathogen circulating in Brazil, the first exporter and the second largest poultry meat producer worldwide.[38] According to the literature, this is the first study performed in Brazil that used the next generation sequencing technology to assess the phylogenetic relationship of *C. jejuni* based on whole genome SNP analysis.

In conclusion, the high rates of *C. jejuni* strains resistant to ciprofloxacin and tetracyclines are of concern and may represent a public health concern for *Campylobacter* infections in humans when the treatment is needed. WGS has the potential to be a powerful tool for prediction of AMR genes and point mutations, especially when used in combination with AST. In addition, the results obtained by whole genome SNP analysis showed that strains isolated from different sources, locations, and years were highly related among each other, suggesting the potential for transmission between clinical and nonclinical sources and

between humans and animal sources over the course of 20 years in four different States located in the Southeast and Southern regions of Brazil. This study contributes to better characterization of the AMR and molecular epidemiology of *C. jejuni* isolated during two decades from diverse sources in Brazil.

Ethics Statement

The authors declare that ethical approval was not required. The study was conducted using isolates belonging to culture collections of the Oswaldo Cruz Institute (FIOCRUZ-RJ) and Adolfo Lutz Institute (IAL-SP).

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Table S1

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