Vital Surveillances

Genomic Analysis and Antimicrobial Resistance of *Campylobacter jejuni* Isolated from Diarrheal Patients — Beijing Municipality, China, 2019–2021

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ABSTRACT

Introduction: *Campylobacter jejuni (C. jejuni*) is the leading cause of human bacterial gastroenteritis worldwide and has a major impact on global public health. The objective of the present study was to conduct whole genome sequencing (WGS) to determine the genetic diversity, virulence factors, and determinants of antimicrobial resistance of *C. jejuni* during a 3-year surveillance period in Beijing, China.

Methods: A total of 184 clinical isolates were obtained from sentinel hospital surveillance between 2019 and 2021. Antimicrobial susceptibility testing was conducted using the agar dilution method. WGS was employed to characterize the 184 *C. jejuni* strains.

Results: Multilocus sequence typing analysis revealed high genetic diversity among the 184 *C. jejuni* strains, identifying 71 sequence types (STs) and 19 clonal complexes (CCs). The most prevalent ST was ST760 (6.5%), and the most common CC was CC21 (24.5%), consisting of 11 STs. High resistance rates were observed for ciprofloxacin (76.6%), nalidixic acid (76.1%), and tetracycline (71.2%). A total of 77 *C. jejuni* isolates (41.8%) exhibited multidrug resistance with 43 resistance patterns. Virulome analysis disclosed the differential distribution of virulence factors related to adherence, colonization, chemotaxis, as well as lipo-oligosaccharide and capsular polysaccharide biosynthesis. Resistome analysis demonstrated widespread resistance to quinolones and tetracycline, but low rates of macrolides resistance. The phylogeny, based on whole genome single nucleotide polymorphisms, indicated a high degree of clonality and grouped the *C. jejuni* strains into six clades. Closely related isolates that were part of a genetic cluster mostly shared a homogenous clonal complex.

Conclusions: The present study emphasizes the rising resistance to quinolones and tetracycline, as well as the virulence potential and diverse genotypes identified among *C. jejuni* strains isolated from diarrheal patients in Beijing.

Campylobacter jejuni (*C. jejuni*) is a prevalent foodborne zoonotic pathogen and has been identified as the primary cause of human gastroenteritis globally (*1*–*2*). Besides gastrointestinal illness, *C. jejuni* is also associated with autoimmune conditions such as Guillain–Barré syndrome and Miller Fisher syndrome (*1*,*3*). Although infections caused by *C. jejuni* are often self-limiting and typically resolve within a few days without antibiotic intervention, effective antimicrobial treatment is essential for immunocompromised patients or severe cases of the disease. The rapid increase in antimicrobial resistance (AMR) among *C. jejuni* strains has become a critical public health concern. Consequently, the World Health Organization has listed fluoroquinolone-resistant *Campylobacter* spp. as one of the six high-priority antimicrobial-resistant pathogens posing the greatest threat to human health (*4*).

Whole genome sequencing (WGS) has the capacity to generate vast amounts of precise data rapidly, which can subsequently be utilized for species identification, typing, phylogenetic analyses, and determining virulence and resistance characteristics (*5*). WGS is progressively utilized as the foremost approach for foodborne pathogen surveillance in public health laboratories, supplanting prior conventional typing methods.

Recently, *Campylobacter* spp. has emerged as the most prevalent bacteria causing human diarrhea in Beijing, China, with an alarmingly high burden on human health (*6*). Only a few reports have analyzed the genetic diversity and AMR profiles of clinical strains of *Campylobacter* in Beijing (*6*–*7*), and such studies have lacked the resolution provided by WGS-based typing methods. Therefore, larger genomic

epidemiology studies are needed to elucidate the molecular characteristics of *Campylobacter* strains circulating in Beijing and more broadly in China.

In this investigation, a total of 184 *C. jejuni* strains were collected from patients with diarrhea during active surveillance in Beijing throughout a three-year period (2019–2021). This study presents a comprehensive genomic analysis, examining the genetic diversity, virulence potential, and AMR profiles of the isolated strains.

METHODS

Sample Collection and *Campylobacter* Isolation

Hospital-based active surveillance of *Campylobacter* has been conducted since 2010 in Beijing, China. A *Campylobacter* isolation kit incorporating a membrane filter method (ZC-CAMPY-002, Sinova Biotechnology Co., Ltd., Qingdao, China) was employed to isolate *Campylobacter*. In brief, a 1 mL stool specimen suspension was transferred into 4 mL of enrichment medium provided in the kit. The enriched suspension was incubated at 37 ℃ for 24 hours under microaerophilic conditions, consisting of 5% O_2, 10% CO_2, and 85% N_2. Approximately 300 μL of the enriched culture was spotted onto the membrane filter surface and then pasted onto both Karmali and Columbia agar plates. The plates were subsequently incubated in a microaerophilic atmosphere at 37 ℃ for 48 hours. Suspected colonies were picked and identified using a real-time PCR assay targeting the *hipO* gene of *C. jejuni*. From 2019 to 2021, 184 *C. jejuni* isolates were obtained from this surveillance program.

Antimicrobial Susceptibility Testing (AST)

The minimum inhibitory concentration (MIC) of all *C. jejuni* isolates was determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute document (CLSI M45-P). Six classes of 11 antimicrobial agents (Sinova Biotechnology Co., Ltd., Qingdao, China) were used for AST: erythromycin (ERY), azithromycin (AZI), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), streptomycin (STR), chloramphenicol (CHL), florfenicol (FLO), tetracycline (TET), telithromycin (TEL), and clindamycin (CLI). The breakpoints for resistance used in this study were based on standards used in the National Antimicrobial Resistance Monitoring System (NARMS), except for ERY, CIP, and TET, which were based on CLSI guidelines. The following MIC values were determined for *C. jejuni*: ERY ≥32 μg/mL, AZI ≥1 μg/mL, NAL ≥32 μg/mL, CIP ≥4 μg/mL, GEN ≥4 μg/mL, STR ≥16 μg/mL, CHL ≥32 μg/mL, FLO ≥8 μg/mL, TET ≥16 μg/mL, TEL ≥8 μg/mL, and CLI ≥1 μg/mL. *C. jejuni* ATCC 33,560 was included in the test as a quality control strain. Multidrug resistance was defined as resistance to at least three classes of antimicrobials in this study.

WGS and Genomic Analysis

DNA was extracted utilizing a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The quantification of the extracted genomic DNA (gDNA) was determined through agarose gel electrophoresis and fluorometric analysis (Qubit 2.0). WGS was performed on an Illumina PE150 platform with 100× coverage (Novogene Technology Co., Ltd., Beijing, China). Raw sequencing data were assessed for quality, trimmed, and subsequently assembled *de novo* into a draft genome sequence using the SPAdes 3.13 software.

Multilocus sequence typing (MLST) was conducted utilizing WGS data in accordance with the *Campylobacter* PubMLST scheme (https://pubmlst.org/). STs and CCs were identified for each isolate. AMR genes were analyzed using the NCBI AMRFinderPlus tool 3.1.1b (https://ftp.ncbi.nlm.nih.gov/pathogen/Antimicrobial_resistance/AMRFinder/). Virulence genes were detected via the virulent factors of pathogenic bacteria (VFDB) database (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi). Whole genome single nucleotide polymorphisms (wgSNPs) analysis was performed on all draft genomes with the reference strain NCTC 11168 (GenBank ID: NC_002163.1) using the parsnp software. Finally, the phylogenetic tree and heatmap of resistance and virulence genes were visualized using the ChiPlot tool (https://www.chiplot.online/).

RESULTS

Diversity of MLST Genotype STs and CCs

MLST analysis of the 184 *C. jejuni* isolates produced 71 distinct STs, categorized into 19 CCs and unassigned (not belonging to any known CC) categories (Supplementary Table S1, available in https://weekly.chinacdc.cn/). Out of these, 23 isolates belonged to 16 unassigned STs. The most prevalent STs were ST760 (6.5%), followed by ST49 (6.0%) and ST22 (5.4%). Thirty STs were represented by only

one isolate each. The most common CC, CC21 (24.5%), consisted of 11 STs: ST21, ST50, ST298, ST760, ST1811, ST3597, ST6500, ST8261, ST9873, ST9960, and ST10075. The second most frequent CC, CC45, comprised six STs and 13 isolates (7.1%). The remaining CCs included relatively few STs or a small number of strains.

AST

In the present study, 13 *C. jejuni* isolates (7.1%) demonstrated susceptibility to all 11 tested antimicrobials. The highest recorded resistance rates were observed for CIP (76.6%), with NAL (76.1%) and TET (71.2%) following closely behind. Conversely, resistance rates for ERY, TEL, and CHL were relatively low at 3.8%, 7.6%, and 8.2% respectively. The resistance rates for other antimicrobials included FLO (27.3%), CLI (18.5%), STR (11.4%), and both AZI and GEN (10.9%) (Table 1).

Regarding multidrug resistance, 77 *C. jejuni* isolates (41.8%) were resistant to three or more classes of

TABLE 1. MIC distribution and AMR phenotype and genotype in 184 *C. jejuni* strains.

Class	Antibiotic agent	MIC (μg/mL)												Resistant strains
	AMR gene	Breakpoint	Range	≤0.25	0.5	1	2	4	8	16	32	64	>64	*n* (%)
Macrolides	ERY	≥32	0.5–64		84	69	14	7	1	2	1	2	4	7 (3.8)
	*50S rRNA*_L22_A103V													0
	23S rRNA_A2075G												1	1
	cmeABC											2	3	5
	AZI	≥1	0.5–64		164	5	2	6	1	1	1	2	2	20 (10.9)
	*50S rRNA*_L22_A103V							1						1
	23S rRNA_A2075G												1	1
	cmeABC					3	2	4	1	1	1	1	2	15
Quinolones	NAL	≥32	0.5–64		3	19	6	4	8	4	5	36	99	140 (76.1)
	*gyrA*_T86I										5	34	98	137
	cmeABC										3	21	58	82
	CIP	≥4	0.5–64		17	15	11	5	26	34	41	22	13	141 (76.6)
	*gyrA*_T86I							5	26	34	39	22	12	138
	cmeABC							3	25	27	11	11	11	88
Aminoglycosides	GEN	≥4	0.5–64		144	13	7	4	0	3	2	3	8	20 (10.9)
	aph(3')–IIIa							2			1	1	2	6
	aph(2'')–If									1	1	3		5
	aac(6')–Ie/aph(2'')–Ia							2					1	3
	STR	≥16	0.5–64		104	23	23	4	9	6	4	5	6	21 (11.4)
	ant(6)-Ia									1	1			2
	aadE											1		1
Tetracyclines	TET	≥16	0.5–64		28	12	6	3	4	4	7	37	83	131 (71.2)
	tetO									3	7	35	78	123
	cmeABC									4	5	23	46	78
Phenicols	CHL	≥32	0.5–64		16	27	25	43	21	37	12	1	2	15 (8.2)
	FLO	≥8	0.5–64		25	24	50	35	30	8	8	4		50 (27.3)
Ketolides	TEL	≥8	0.25–32	45	33	30	50	12	4	3	3	4		14 (7.6)
Lincosamides	CLI	≥1	0.25–32	107	43	16	4	4	1	4	1	4		34 (18.5)

Abbreviation: MIC=minimum inhibitory concentration; AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; ERY=erythromycin; AZI=azithromycin; NAL=nalidixic acid; CIP=ciprofloxacin; GEN=gentamicin; STR=streptomycin; TET=tetracycline; CHL=chloramphenicol; FLO=florfenicol; TEL=telithromycin; CLI=clindamycin.

* The gray shade indicates resistance strains and red numbers indicate the number of resistance strains.

antimicrobials and showed 43 different resistance patterns. Among these, the predominant resistance pattern was NAL-CIP-TET-FLO (12.0%). Three isolates were resistant to at least 10 antimicrobials (Supplementary Figure S1, available in https://weekly.chinacdc.cn/).

Detection of Antimicrobial Resistance Genes

Genomic analysis revealed a total of 29 AMR determinants among 184 *C. jejuni* isolates. These included 26 acquired AMR genes and three resistance-conferring point mutations (Table 2). The *gyrA*_T86I point mutation, associated with resistance to quinolones, was the most prevalent, identified in 96.2% of the isolates. The *tetO* gene, conferring resistance to tetracycline, was also highly prevalent, found in 82.6% of isolates.

A variety of 11 known bla_{OXA} variants were detected in 147 strains (79.9%), with $bla_{OXA\text{-}193}$, $bla_{OXA\text{-}461}$, and $bla_{OXA\text{-}591}$ being the most prevalent (40.2%,

TABLE 2. Distribution of AMR and virulence genes in 184 *C. jejuni* strains.

Genes	Class	Gene	No. of isolates (%)
Resistance genes	Quinolones	*gyrA*_T86I	177 (96.2)
	Tetracyclines	*tetO*	152 (82.6)
	ß-Lactams	Any of the following	147 (79.9)
		$bla_{OXA\text{-}193}$	74 (40.2)
		$bla_{OXA\text{-}461}$	19 (10.3)
		$bla_{OXA\text{-}591}$	17 (9.2)
		$bla_{OXA\text{-}184}$	12 (6.5)
		$bla_{OXA\text{-}460}$	9 (4.9)
		$bla_{OXA\text{-}592}$	7 (3.8)
		$bla_{OXA\text{-}583}$	3 (1.6)
		$bla_{OXA\text{-}631}$	3 (1.6)
		$bla_{OXA\text{-}465}$	1 (0.5)
		$bla_{OXA\text{-}594}$	1 (0.5)
		bla_{EC}	1 (0.5)
	Macrolides	Any of the following	37 (20.1)
		*50S rRNA*_L22_A103V	35 (19.0)
		23S rRNA_A2075G	2 (1.1)
	Aminoglycosides	Any of the following	67 (36.4)
		aph(3')-IIIa	18 (9.8)
		ant(6)-Ia	14 (7.6)
		sat4	12 (6.5)
		aph(2'')-If	9 (4.9)
		aac(6')-Ie/aph(2'')-Ia	5 (2.7)
		aadE	4 (2.2)
		aad9	1 (0.5)
	Phenicols	*catA13*	2 (1.1)
	Arsenics	*arsP*	95 (51.6)
		acr3	73 (39.7)
	Lincosamides	*lnuC*	6 (3.3)
	Multidrug efflux pump	*cmeA*	183 (99.5)
		cmeB	114 (62.0)
		cmeC	184 (100.0)
		cmeABC	114 (62.0)

TABLE 2. (Continued)

Genes	Class	Gene	No. of isolates (%)
Virulence genes	Invasion	*ciaB; ciaC; flhA; flhB; fliP; fliQ; fliR;*	184 (100.0)
		flaC	182 (98.9)
	Adhesion	*cadF; jlpA; pebA;*	184 (100.0)
		porA	112 (60.9)
	Toxin	*cdtB*	184 (100.0)
		cdtA	182 (98.9)
		cdtC	182 (98.9)
	Chemotaxis	*cheA; cheV; cheW; cheY;*	184 (100.0)
	Motility	*flgG; flgH; fliF; fliM;*	184 (100.0)
		fliY	183 (99.5)
		flgI	183 (99.5)
		flgE	181 (98.4)
		fliK	178 (96.7)
		flaB	22 (12.0)
		flaA	21 (11.4)
	LOS	*hldD; waaC;*	184 (100.0)
		htrB	183 (99.5)
		gmhA	66 (35.9)
		hldE	55 (29.9)
		cstIII	41 (22.3)
		neuA	41 (22.3)
		neuB	41 (22.3)
		neuC	41 (22.3)
	CPS	*kpsS*	183 (99.5)
		kpsD	183 (99.5)
		kpsE	177 (96.2)
		Cj1417c	158 (85.9)
		Cj1419c	158 (85.9)
		Cj1420c	154 (83.7)
		waaF	130 (70.7)
		kpsF	71 (38.6)
		kpsT	36 (19.6)

Abbreviation: AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; LOS=lipo-oligosaccharide; CPS=capsule polysaccharide.

10.3%, and 9.2%, respectively). In contrast, a much smaller fraction of strains harbored resistance markers for other antimicrobials. Resistance to macrolides via two point mutations, *50S rRNA*_L22_A103V and *23S rRNA_A2075G*, was observed in 19.0% and 1.1% of isolates, respectively.

Additionally, seven genes for resistance to aminoglycosides (i.e., *aph(3')-IIIa*, *ant(6)-Ia*, *sat4*, *aph(2'')-If*, *aac(6')-Ie/aph(2'')-Ia*, *aadE*, and *aad9*) were detected in fewer than 10% of the isolates. High prevalence of arsenic resistance genes, *arsP* and *acr3*, was observed. Notably, the *cmeABC* operon, encoding a multidrug efflux pump and consisting of *cmeA*, *cmeB*, and *cmeC* genes, was present in 62% of *C. jejuni* isolates. The prevalence of remaining resistance genes was no greater than 3.3%. Detailed prevalence rates of the aforementioned AMR genes can be found in Table 2.

Correlation of Phenotypic and Genotypic Resistance

Of the seven strains exhibiting ERY-resistant phenotypes, one strain carried the *A2075G* mutation

in the *23S rRNA* gene, and five strains contained the *cmeABC* multi-efflux pump operon gene (Table 1). Among the 20 strains with AZI-resistant phenotypes, one displayed the *23S rRNA_A2075G* mutation, another exhibited the *50S rRNA*_L22_A103V mutation, and fifteen possessed the *cmeABC* operon. These two isolates with the *23S rRNA_A2075G* point mutation demonstrated high-level resistance to macrolides (ERY, AZI MIC >64 μg/mL).

Out of 141 CIP-resistant strains, 97.9% (*n*=138) presented the *gyrA*_T86I mutation, and 62.4% (*n*=88) carried the *cmeABC* operon. Interestingly, the *gyrA*_T86I point mutation was present across the entire range of CIP resistance (MIC 4–64 μg/mL). Of the 131 TET-resistant strains, 93.9% (*n*=123) harbored the *tetO* gene. Moreover, 59.5% (*n*=78) of the TET-resistant strains contained the *cmeABC* operon.

Detection of Virulence Factors

Virulome analysis identified 47 virulence genes across 184 *C. jejuni* isolates. These genes were classified into seven categories based on their roles in pathogenesis/colonization (Table 2). The prevalence rates of cell invasion-associated genes included: *ciaB*, *ciaC*, *flhA*, *flhB*, *flip*, *fliQ*, and *fliR* (all at 100%), and *flaC* (98.9%). Adherence and colonization-related genes demonstrated prevalence rates of *cadF*, *jlpA*, *pebA* (all at 100%), and *porA* (60.9%). The cytolethal distending toxin (CDT) genes encoded by the *cdtABC* operon showed prevalence rates of *cdtB* (100%) and *cdtA* and *cdtC* (both at 98.9%). All 184 isolates contained chemotaxis-associated genes: *cheA*, *cheV*, *cheW*, and *cheY*. Genes involved in motility displayed prevalence rates of: *flgG*, *flgH*, *fliF*, *fliM* (all at 100%), *flgI*, *fliY* (both at 99.5%), *flgE* (98.4%), *fliK* (96.7%), *flaB* (12.0%), and *flaA* (11.4%). Moreover, the analysis identified various genes implicated in the biosynthesis of lipo-oligosaccharide (LOS) and capsule polysaccharide (CPS). Percentages of all detected virulence genes categorized by function are outlined in Table 2.

Phylogenetic Analysis and Heatmap of AMR and Virulence Genes

The wgSNP phylogenetic analysis of 184 *C. jejuni* strains identified six major clades, designated A–F (Figure 1), with no association between the lineages and the years of isolation. Clade A consisted of 62 strains from CC21, CC206, or CC48, while the largest clade, clade B, included 68 isolates forming five sub-clusters (B1–B5) with diverse CCs as follows: B1: CC257, CC354, CC460; B2: CC52, CC353, CC464; B3: CC443, CC574; B4: CC257; B5: CC828, CC1034. Notably, CC257 appeared in both B1 and B4, indicating the differentiation and expansion of clonal groups. Lineages C, D, and E each contained unique independent CCs, specifically CC49, CC58, and CC403, respectively. Additionally, 33 strains from CC22, CC42, or CC45 formed a single lineage, lineage F.

The heatmap of virulence genes revealed that all strains were divided into two clusters related to CC, based on the distribution of virulence factors. Strains from CC21 exhibited a wide distribution of virulence determinants, particularly those related to LOS (Figure 2A). CC354 and CC257 strains possessed fewer CPS genes compared to other strains. Although no correlation was found between the observed AMR genes and their distribution of CCs on the heatmap (Figure 2B), interestingly, 12 isolates from CC49 displayed the least resistance genes and were sensitive to most antimicrobial agents.

DISCUSSION

In recent decades, the dramatic increase in AMR in *Campylobacter* worldwide has prompted research on the prevalence and molecular determinants of resistance. In this study, *C. jejuni* isolates exhibited a high rate of resistance to certain antimicrobials (CIP, NAL, and TET) and a low rate of resistance to ERY, which concurs with findings previously reported in China (*8*) and elsewhere (*9*–*12*). The high percentage of quinolone-resistant *C. jejuni* strains (97.9%) with point mutation *gyrA*_T86I was frequently observed, which contributed to the high-level resistance to quinolones (*9*–*10*,*12*). The *tetO* gene was detected in 93.9% of TET-resistant *C. jejuni* isolates, which also corroborates previous studies (*9*–*10*,*12*). The two markers of AMR to macrolides, *50S rRNA*_L22_A103V and *23S rRNA_A2075G*, were detected at low prevalence (19.0% and 1.1%, respectively) among *Campylobacter* isolates. Notably, *C. jejuni* isolates (62.0%) harbored the *cmeABC* operon, which encodes a member of the resistance-nodulation cell division superfamily of multidrug efflux transporters and functions synergistically with other mechanisms to contribute to resistance to quinolones, macrolides, and tetracyclines (*9*–*10*). In this study, three strains with phenotypic resistance to

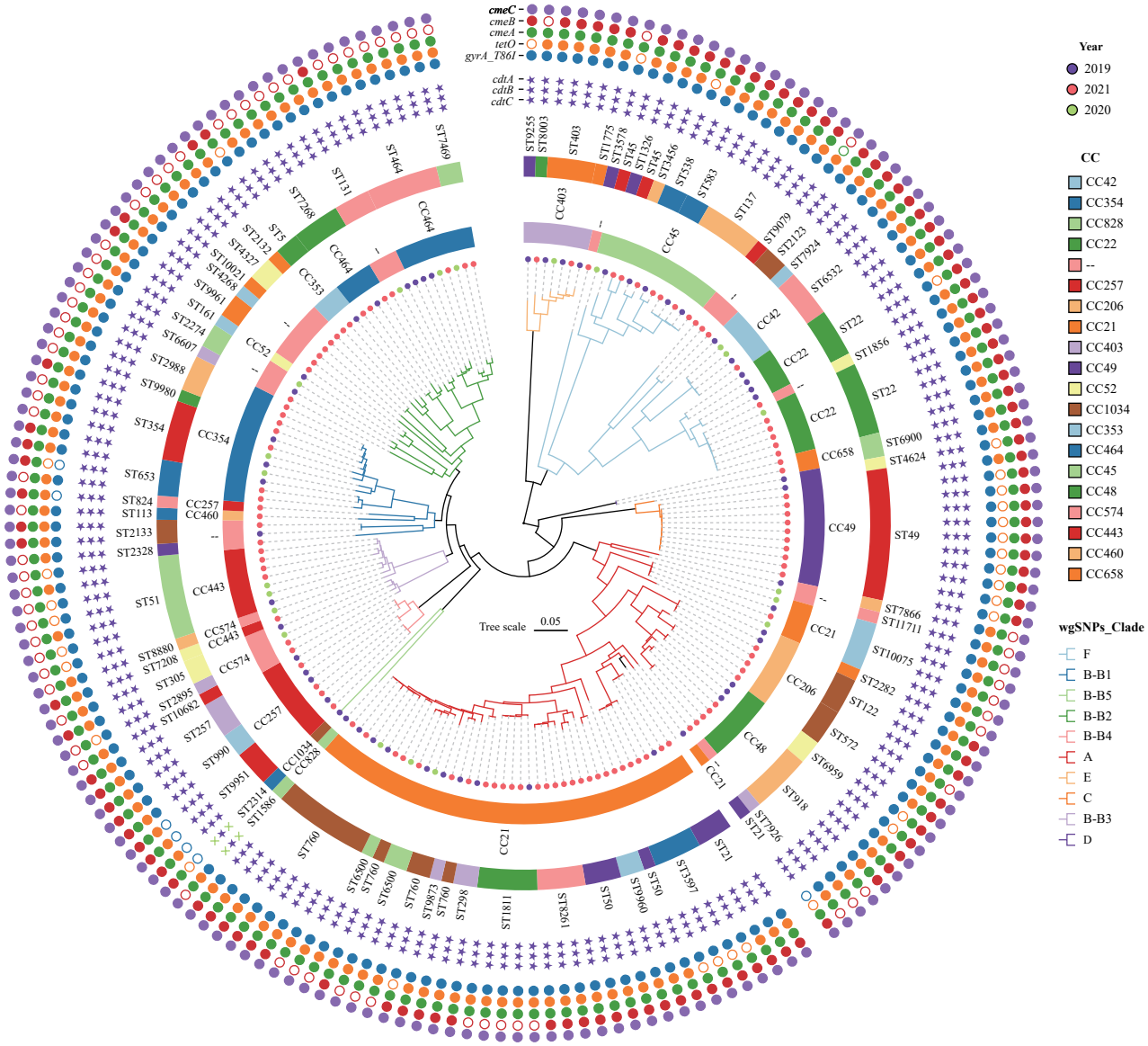

FIGURE 1. Phylogenetic analysis of 184 *C. jejuni* strains in Beijing from 2019 to 2021.
Note: The phylogenetic tree is based on wgSNPs analysis. CCs and STs are shown in colored rings for each strain, and tree branches are color coded to highlight *C. jejuni* strains from clades A, C, D, E, F, and sub-cluster of clade B (B1-B5). The isolation year of each strain is indicated by colored dots. Unassigned CC is denoted by two short lines (--). The presence of 5 main resistance genes is denoted by colored solid circles: *gyrA*_T86I (blue), *tetO* (orange), *cmeA* (green), *cmeB* (red) and *cmeC* (purple). The absence is indicated by hollow circles. The presence of toxin genes (*cdtA*, *cdtB* and *cdtC*) is denoted by purple star symbol and the absence is denoted by cross symbol.
Abbreviation: *C. jejuni=Campylobacter jejuni*; wgSNPs=whole genome single nucleotide polymorphisms; CC=clonal complex; ST=sequence type.

quinolones did not present the mutation in *gyrA*_T86I; however, the presence of the *cmeABC* operon was observed, indicating that the efflux pumps could be conferring resistance to quinolones in these three strains. Also, six ERY-resistant strains did not carry the mutation *A2075G* in the *23S rRNA* gene; however, the presence of the *cmeABC* operon was observed in five strains, which would likely be responsible for the resistance to ERY in strains that did not present the mutation in the *23S rRNA*. Finally, eight TET-resistant strains did not harbor the *tetO* gene; similarly, the presence of the *cmeABC* operon was observed in all eight strains, which would also be responsible for the resistance to TET. Taken together, these data contribute to our understanding of the *Campylobacter* resistome, which will support the development of AMR surveillance programs in China.

The mechanisms underlying the pathogenic

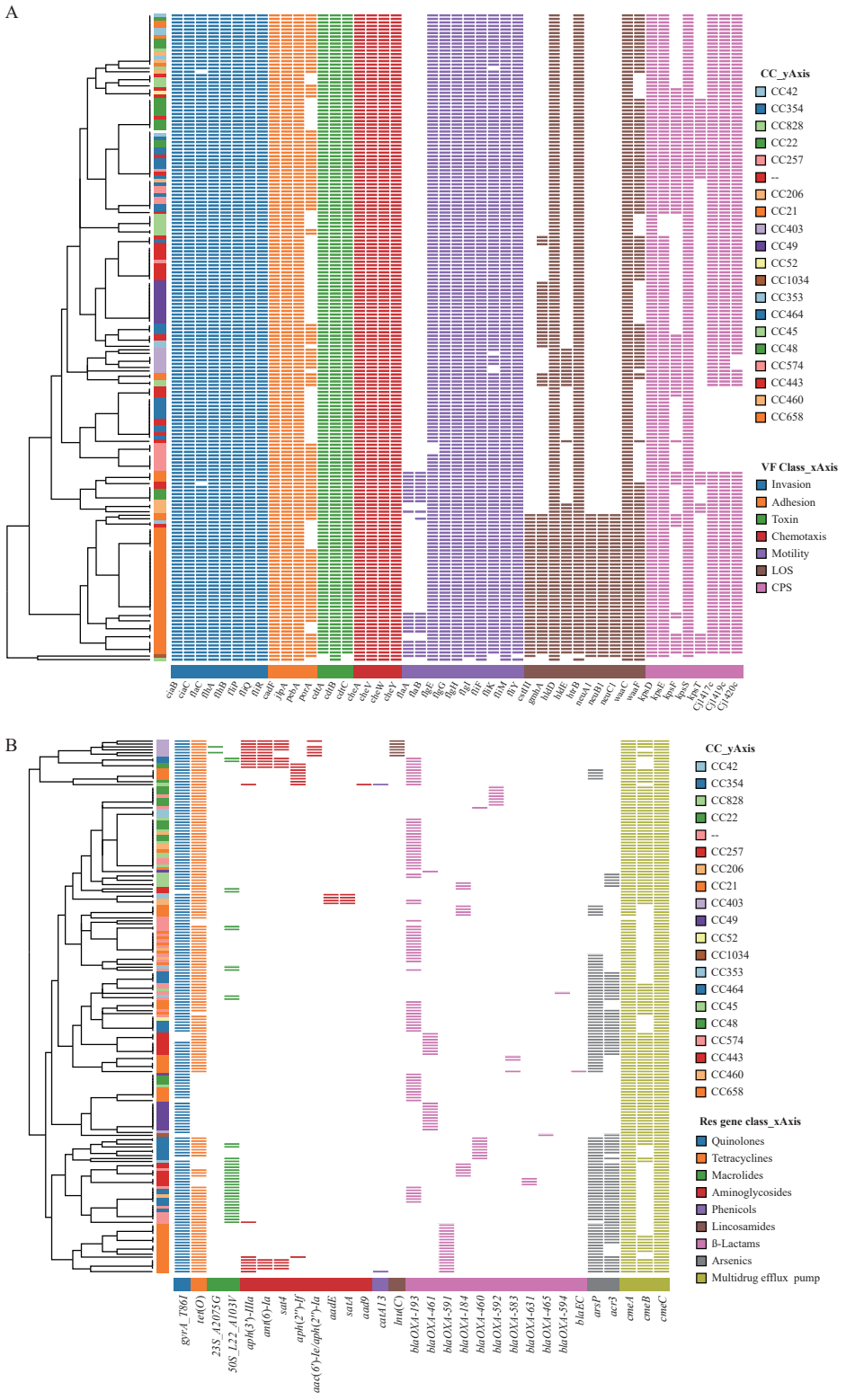

FIGURE 2. Annotation heatmap of virulence and AMR genes among 184 *C. jejuni* strains in Beijing from 2019 to 2021. (A) Virulence genes; (B) AMR genes.

Note: The color strips of Y-axis represent CCs corresponding to each strain. The color blocks of X-axis represent the categories of genes. Binary heatmaps show the presence and absence of virulence and AMR genes. Colored cells represent the presence of genes and white cells represent the absence of the genes. Unassigned CC is denoted by two short lines (--).

Abbreviation: AMR=antimicrobial resistance; *C. jejuni*=*Campylobacter jejuni*; CC=clonal complex; VF=virulence factors.

processes by which *Campylobacter* species cause diarrhea remain unclear (*1*). This study found that a large proportion of *Campylobacter* strains possessed genes linked to bacterial motility, invasion, adhesion, and chemotaxis to epithelial cells, all of which play vital roles in the onset of *Campylobacter* infection (*2*). These results support earlier research indicating that adherence, colonization, and invasion genes (e.g., *cadF*, *ciaB*, *ciaC*, *flaC*, *jlpA*, *pebA*, *porA*) are highly conserved among *C. jejuni* strains and present in the majority of clinical isolates (*13*–*14*), emphasizing the potential virulence of these *Campylobacter* strains in causing human infections.

Furthermore, virulence marker determinants *cdtABC*, which encode CDT and significantly contribute to diarrhea by interfering with the division and differentiation of intestinal crypt cells, were also detected in most examined isolates (>98.9%). Previous investigations have reported a high prevalence of CDT in *Campylobacter* strains isolated from patients experiencing life-threatening diarrhea (*10*,*14*–*15*). While the functions of individual LOS genes (*cstIII*, *gmhA*, *hldD*, *hldE*, *trB*, *neuA*, *neuB*, *neuC*, *waaC*, and *waaF*) have yet to be clearly established, prior studies have suggested that these genes are crucial for forming human ganglioside-like LOS structures capable of inducing Guillain–Barré syndrome (*3*). In this study, the majority of strains carried at least four genes associated with LOS biosynthesis.

This study emphasized the extensive diversity of clinical *C. jejuni* isolates circulating in Beijing. Our findings demonstrated that 184 *C. jejuni* strains were classified into 71 STs, with strains belonging to CC21 as the predominant group. CC21 is the largest and most widely distributed CC globally, representing 17.9% of all *C. jejuni* strains submitted to the PubMLST database. Numerous studies have reported varying major CCs of *Campylobacter* in different countries and regions, but CC21, CC45, CC48, and CC353 are consistently the predominant CCs among isolates in many investigations. The high prevalence of ST760 in our study was unexpected, given its scarce representation within the PubMLST database (0.033%, as of February 2023). ST760 was first reported in 2000 from a human gastroenteritis case in England. Subsequently, it spread to several neighboring European countries but not to North or South America. In Asia, there were four reports of ST760 in Jiangsu Province in China in 2006, but no reports in other regions of China or other Asian countries (based on the PubMLST database). These data highlight the wide-range transmission of these strains across regions and indicate that ST diversity varies among countries and regions.

Previous research has demonstrated that associations between clades and the presence of resistance and virulence determinants are prevalent, although not all clades within a phylogenetic tree are characterized by factors such as geographic distribution or year of isolation (*11*). To date, few studies have focused on the phylogenetic analysis of *Campylobacter* strains in Beijing. In this study, wgSNPs phylogenetic analysis revealed that the *C. jejuni* population from diarrheal patients in Beijing encompasses a wide range of lineages and genotypes, with strains belonging to the same ST and/or CC grouping together in distinct clusters. Although this study presents limited literature regarding virulence factors linked to specific CCs or STs, it emphasizes the virulence potential of the investigated *Campylobacter* isolates. Additionally, one limitation of this study is that all *Campylobacter* strains were isolated from human samples, with none originating from alternative sources such as food or animals.

In conclusion, this study represents one of the most extensive genomic analyses of *C. jejuni* in Beijing, offering valuable insights into the prevalence of virulence genes, AMR markers, as well as the phylogenetic relationships and circulating genotypes from 2019 to 2021. Moreover, we report the high resistance rates to quinolone and tetracycline alongside the low resistance rate to erythromycin among the *C. jejuni* strains identified in Beijing. This information proves crucial for the development of monitoring, control, and prevention strategies to address the growing concern of resistance posed by this pathogen.

Conflicts of interest: No conflicts of interest.

doi: 10.46234/ccdcw2023.080

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Submitted: March 14, 2023; Accepted: May 07, 2023

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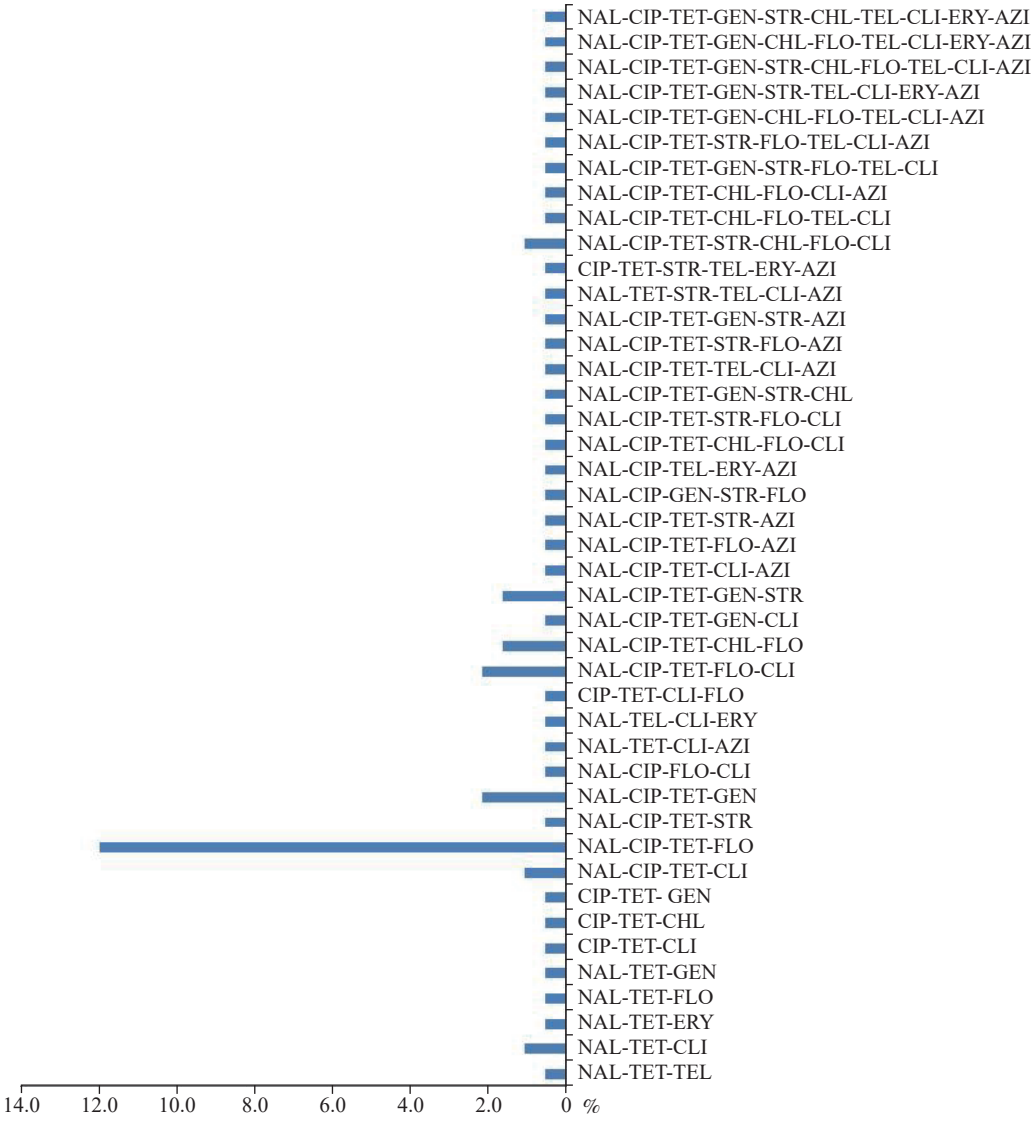
SUPPLEMENTARY TABLE S1. Distribution of CCs and STs in 184 *C. jejuni* strains.

CC type	ST type	No. of isolates (%)	CC type	ST type	No. of isolates (%)	CC type	ST type	No. of isolates (%)
CC21	ST21	4 (2.2)	CC52	ST161	1 (0.5)	CC574	ST305	2 (1.1)
	ST50	4 (2.2)	CC206	ST122	3 (1.6)		ST2895	1 (0.5)
	ST298	2 (1.1)		ST572	3 (1.6)		ST8880	1 (0.5)
	ST760	12 (6.5)		ST2282	1 (0.5)		ST10682	1 (0.5)
	ST1811	5 (2.7)	CC257	ST257	3 (1.6)	CC658	ST6900	2 (1.1)
	ST3597	4 (2.2)		ST824	1 (0.5)	CC828	ST1586	1 (0.5)
	ST6500	3 (1.6)		ST990	2 (1.1)	CC1034	ST2314	1 (0.5)
	ST8261	4 (2.2)		ST9951	3 (1.6)	unassigned	ST131	3 (1.6)
	ST9873	1 (0.5)	CC353	ST5	2 (1.1)		ST1856	1 (0.5)
	ST9960	2 (1.1)		ST2132	1 (0.5)		ST2123	2 (1.1)
	ST10075	4 (2.2)	CC354	ST354	5 (2.7)		ST2133	2 (1.1)
CC22	ST22	10 (5.4)		ST653	3 (1.6)		ST2274	2 (1.1)
CC42	ST6532	4 (2.2)		ST2988	3 (1.6)		ST2328	1 (0.5)
	ST7924	1 (0.5)		ST9980	1 (0.5)		ST3578	1 (0.5)
CC45	ST45	2 (1.1)	CC403	ST403	4 (2.2)		ST4268	1 (0.5)
	ST137	5 (2.7)		ST1775	1 (0.5)		ST4327	2 (1.1)
	ST538	2 (1.1)		ST8003	1 (0.5)		ST6607	1 (0.5)
	ST583	2 (1.1)		ST9255	1 (0.5)		ST7866	1 (0.5)
	ST1326	1 (0.5)	CC443	ST51	7 (3.8)		ST7926	1 (0.5)
	ST3456	1 (0.5)		ST7208	1 (0.5)		ST9079	1 (0.5)
CC48	ST918	5 (2.7)	CC460	ST113	1 (0.5)		ST9961	2 (1.1)
	ST6959	2 (1.1)	CC464	ST464	6 (3.3)		ST10021	1 (0.5)
CC49	ST49	11 (6.0)		ST7268	4 (2.2)		ST11711	1 (0.5)
	ST4624	1 (0.5)		ST7469	2 (1.1)			

Abbreviation: CC=clonal complex; ST=sequence type; *C. jejuni=Campylobacter jejuni*.



SUPPLEMENTARY FIGURE S1. MDR patterns of 184 *C. jejuni* strains to various antibiotic combinations.
Abbreviation: MDR=multidrug resistance; *C. jejuni*=*Campylobacter jejuni*.