Vital Surveillances

Genetic and Drug Resistance Characteristics of *Campylobacter* Isolated — China, 2020–2023

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ABSTRACT

Introduction: This study aimed to characterize the genetic diversity and antimicrobial resistance patterns of *Campylobacter* isolates collected throughout China from 2020 to 2023.

Methods: *Campylobacter* isolates analyzed in this study were obtained from the National Pathogen Identification Network Center database, maintained by the National Institute for Infectious Disease Control and Prevention of the Chinese Center for Disease Control and Prevention. Antimicrobial susceptibility testing (AST) was performed against eleven antimicrobial agents. Genomic characteristics were analyzed through comprehensive genome sequence analysis.

Results: Between 2020 and 2023, the National Pathogen Identification Network documented 1,077 *Campylobacter jejuni* (*C. jejuni*) and 221 *Campylobacter coli* (*C. coli*) isolates. Most isolates originated from patients presenting with diarrhea. Antimicrobial susceptibility testing was conducted on 634 *C. jejuni* and 165 *C. coli* isolates. The tested isolates demonstrated high resistance rates to nalidixic acid (78.22%), ciprofloxacin (78.07%), and tetracycline (71.96%). Longitudinal analysis of antimicrobial susceptibility testing results revealed a declining resistance trend from 2020 to 2023. Whole genome sequences were obtained for 540 *C. jejuni* and 125 *C. coli* isolates within the database. Virulence factors and antibiotic resistance determinants were identified using the VFDB and CARD databases, respectively. Phylogenetic relationships were established through Snippy 4.0 software analysis based on core genome comparisons.

Conclusions: This comprehensive analysis describes the antibiotic resistance profiles and genetic characteristics of *Campylobacter* isolates collected through the Identification Network Database from 2020 to 2023, establishing a foundational framework for campylobacteriosis control and prevention strategies in China.

Campylobacter spp. represents one of the most significant foodborne pathogens globally, ranking as the leading cause of foodborne illness in Europe, with *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) constituting the predominant pathogenic species (*1*–*2*). Beyond gastroenteritis, *Campylobacter* infections can precipitate severe complications, including Guillain-Barré syndrome, reactive arthritis, sepsis, and other serious health conditions (*3*). Recent surveillance reports and the expanding number of strains documented in databases indicate that *Campylobacter* infections are increasing throughout China. The frequent emergence of *C. jejuni* outbreaks particularly demands heightened attention (*4*). Understanding *Campylobacter*'s genetic diversity and antimicrobial resistance characteristics provides essential theoretical foundations for effective disease control strategies. This study aims to analyze temporal trends in the genetic and drug resistance characteristics of *Campylobacter* isolates collected in China from 2020 to 2023.

METHODS

Data Sources

Campylobacter data utilized in this study were obtained from the National Pathogen Identification Network Center database, maintained by the National Institute for Infectious Disease Control and Prevention of the Chinese Center for Disease Control and Prevention. Data collection occurred annually and encompassed isolation location, source, and isolation date. All isolates were collected from 20 provincial-level administrative divisions (PLADs) between January 1, 2020, and December 31, 2023.

Antimicrobial Susceptibility Testing (AST)

A total of 799 *Campylobacter* isolates were obtained from 9 PLADs and cities during 2020–2023, comprising 634 *C. jejuni* isolates and 165 *C. coli* isolates. Minimal inhibitory concentrations (MICs) were determined using the agar dilution method against eleven antimicrobial agents representing seven classes: erythromycin (ERY), azithromycin (AZI), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), streptomycin (STR), chloramphenicol (CHL), florfenicol (FLO), tetracycline (TET), telithromycin (TEL), and clindamycin (CLI).

Genomic Analysis

Genome annotation was performed using the Prokka pipeline v1.14.6 (VicBioinformatics, University of Melbourne, Australia) for gene prediction and functional annotation. Sequence types (STs) and clonal complexes (CCs) were determined using the pubMLST database (https://pubmlst.org/). Antimicrobial resistance genes and point mutations conferring antibiotic resistance were identified using the Resistance Gene Identifier (RGI). Virulence genes were detected across all genomes using the virulence factor database (VFDB). Core genome single nucleotide polymorphisms (cg-SNPs) were extracted using Snippy 4.0 (Wellcome Sanger Institute, Wellcome Genome Campus, UK), with Gubbins 2.4 (Earlham Institute, UK) employed for recombination removal to obtain pure SNP data. Phylogenomic trees were constructed using FastTree 1.6 (Physical Biosciences Division, Lawrence Berkeley National Laboratory, CA, USA) and visualized with iTOL 6.9 (https://itol.embl.de) (*5*).

RESULTS

Background Information

Between 2020 and 2023, the National Pathogen Identification Network documented a total of 1,298 *Campylobacter* isolates (Table 1), comprising 1,077 *C. jejuni* and 221 *C. coli* strains. The *C. jejuni* isolates originated from 20 PLADs, with the highest concentrations observed in Beijing (50.79%, 547/1,077), Shanghai (33.05%, 356/1,077), and Zhejiang PLADs (6.50%, 70/1,077). Clinical isolates from diarrhea patients constituted the predominant source (92.57%, 997/1,077). The 221 *C. coli* isolates were distributed across 6 PLADs: Beijing (50.68%, 112/221), Shanghai (33.03%, 73/221), Fujian (13.57%, 30/221), Zhejiang (1.35%, 3/221), Guangdong (0.90%, 2/221), and Sichuan (0.45%, 1/221). Among these *C. coli* isolates, 83.26% (184/221) were recovered from diarrhea patients, while 9.05% (20/221) originated from environmental samples, 4.98% (11/221) from animal sources, and 2.71% (6/221) from food samples.

Antibiotic Resistance

Among all *Campylobacter* spp. combined, 7.26% (58/799) of isolates demonstrated susceptibility to all antimicrobial agents tested, though several isolates lacked resistance results for certain antibiotics. The isolates exhibited a high prevalence of resistance to NAL (78.22%), CIP (78.07%), and TET (71.96%), while demonstrating lower resistance rates to CLI (22.45%), GEN (19.92%), AZI (18.30%), FLO (16.77%), TEL (15.01%), STR (13.37%), ERY (12.67%), and CHL (6.14%).

Among the 634 *C. jejuni* isolates examined over the four-year period, resistance to NAL was most prevalent (77.92%, 494/634), followed by CIP (77.44%, 491/634) and TET (70.98%, 450/634). However, these isolates exhibited considerably lower resistance rates to other tested antibiotics, with CHL resistance reaching only 4.89%. In contrast, the 165 *C. coli* isolates analyzed demonstrated higher resistance levels than *C. jejuni* across multiple antimicrobials. Resistance rates for NAL, CIP, and TET exceeded 75.00% in *C. coli* isolates. Additional antibiotics, including ERY, AZI, GEN, STR, TEL, and CLI, also exhibited higher resistance rates in *C. coli* compared to *C. jejuni*. Notably, resistance rates for CHL and FLO remained relatively low, staying below 25.00%. These corresponding results are illustrated in Supplementary Table S1 (available at https://weekly.chinacdc.cn/) and Figure 1.

Longitudinal comparison of susceptibility testing results revealed an overall declining trend in resistance rates across all 11 antibiotics. Significant temporal trends in antibiotic resistance were observed throughout the three-year study period, including FLO resistance in *C. jejuni* and ERY, STR, FLO, and TEL resistance in *C. coli*. However, the limited sample size in 2020 may not accurately represent the resistance patterns for that year.

Genomic Characteristics

Between 2020 and 2023, we obtained whole-genome sequencing data for 665 *Campylobacter* isolates

TABLE 1. Categories of *Campylobacter* isolates, 2020–2023.

Categories		*Campylobacter jejuni*					*Campylobacter coli*				
		2020	2021	2022	2023	Total	2020	2021	2022	2023	Total
Province	Beijing	71	146	123	207	547	11	31	20	50	112
	Shanghai	26	125	12	193	356	8	32	4	29	73
	Zhejiang	1	–	61	8	70	–	–	2	1	3
	Fujian	–	–	–	5	5	–	–	10	20	30
	Jiangsu	–	8	8	4	20	–	–	–	–	–
	Sichuan	12	–	–	5	17	1	–	–	–	1
	Chongqing	–	1	12	3	16	–	–	–	–	–
	Guangdong	–	6	2	1	9	–	–	1	1	2
	Shandong	–	–	8	–	8	–	–	–	–	–
	Guangxi	–	1	1	4	6	–	–	–	–	–
	Neimenggu	5	–	1	–	6	–	–	–	–	–
	Jiangxi	–	2	–	3	5	–	–	–	–	–
	Hubei	–	–	–	3	3	–	–	–	–	–
	Anhui	–	–	2	–	2	–	–	–	–	–
	Guizhou	–	–	2	–	2	–	–	–	–	–
	Henan	–	–	1		1	–	–	–	–	–
	Hainan	–	–	1	–	1	–	–	–	–	–
	Hunan	–	–	1	–	1	–	–	–	–	–
	Ningxia	–	–	1	–	1	–	–	–	–	–
	Heilongjiang	1	–	–	–	1	–	–	–	–	–
Source	Human	108	279	187	423	997	18	58	27	81	184
	Food	8	10	49	–	67	2	4	–	–	6
	Animal	–	–	–	12	12	–	1	10	–	11
	Environment	–	–	–	1	1	–	–	–	20	20
Total		116	289	236	436	1077	20	63	37	101	221

Note: "–" indicates absence of data.

from 12 different provinces and cities, comprising 540 *C. jejuni* and 125 *C. coli* isolates. The majority of these isolates originated from human patients (91.88%, 611/665), while the remaining specimens were derived from poultry (3.46%, 23/665), environmental samples (3.16%, 21/665), and food samples (1.50%, 10/665).

We identified a total of 53 resistance genes in *C. jejuni* and *C. coli*, predominantly associated with *gyrA* mutations (T86I), *cmeABC* efflux systems, and *tet* genes. More than 99% (99.07%, 535/540) of the analyzed *C. jejuni* isolates harbored genes linked to the multidrug efflux systems *cmeR* and *cmeC*, demonstrating a higher carriage rate than the *cmeB* gene (77.60%, 97/125) detected in *C. coli* isolates. Nearly all *C. coli* isolates possessed the *gyrA* mutation (T86I) (98.40%, 123/125), which occurred more frequently than in *C. jejuni* (95.56%, 516/540). These findings align with the elevated resistance levels observed against ciprofloxacin and nalidixic acid in both species. Additional antibiotic-resistant genes and mutations were detected across isolates, as illustrated in (Supplementary Figure S1, available at https://weekly.chinacdc.cn/).

We identified numerous virulence genes associated with *Campylobacter* adherence, colonization, immune evasion, invasion, motility, export apparatus, secretion systems, and toxin production. All categories of virulence genes were detected, with certain genes related to motility and export apparatus present in all isolates, including *flgB*, *flgC*, and *fliE* in *C. jejuni*, and *cheY*, *flgP*, *fliR*, and *fliW* in *C. coli*. Three genes — *cdtA*, *cdtB*, and *cdtC* — encoding the A, B, and C subunits of cytolethal distending toxin (CDT), respectively, were confirmed in *C. jejuni* isolates.

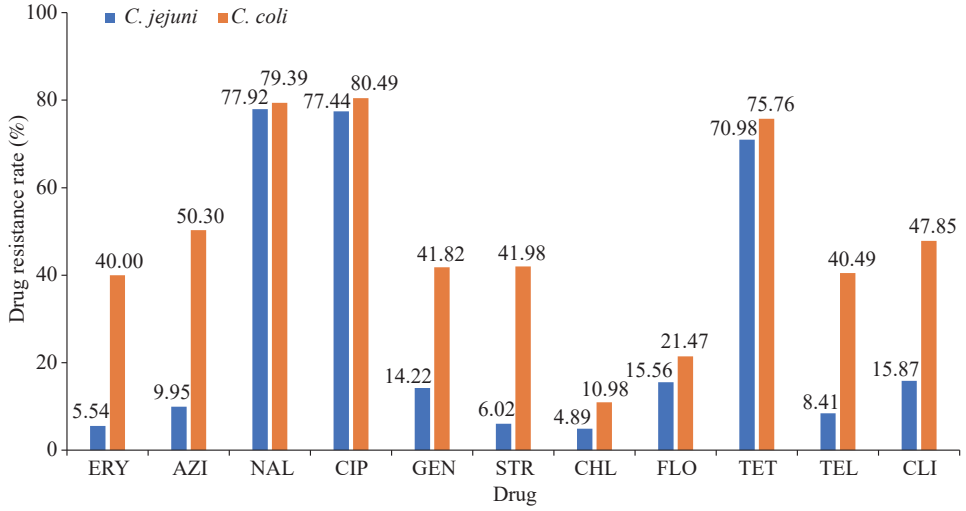

FIGURE 1. Comparison of drug resistance patterns between *Campylobacter jejuni* and *Campylobacter coli*. Blue indicates *C. jejuni*, and orange indicates *C. coli*.
Abbreviation: ERY=erythromycin; AZI=azithromycin; NAL=nalidixic acid; CIP=ciprofloxacin; GEN=gentamicin; STR=streptomycin; CHL=chloramphenicol; FLO=florfenicol; TET=tetracycline; TEL=telithromycin; CLI=clindamycin.

However, only *cdtB* and *cdtC* genes were detectable in *C. coli* isolates. The virulence gene *wlaN*, associated with Guillain-Barré syndrome, was detected exclusively in *C. jejuni* but not in *C. coli*. Additionally, 71 *C. jejuni* isolates harbored a cluster of virulence genes related to capsule formation, including *Cj1421c*, *Cj1422c*, *Cj1426c*, *Cj1427c*, *Cj1429c*, *Cj1432c*, *Cj1433c*, *Cj1435c*, *Cj1436c*, *Cj1437c*, and *Cj1440c*. Genes associated with adhesion, invasion, and motility were present in nearly all isolates, while genes linked to the type IV secretion system were detected less frequently (Supplementary Figure S1).

Multilocus sequence typing (MLST) identified 159 distinct sequence types (STs) among 420 *C. jejuni* isolates, while 44 different multilocus sequence typing locus combinations were identified in the remaining 120 isolates (Figure 2). Among these, 23 STs were characterized, with the most prevalent clonal complexes being ST-21 (33.81%, 142/420), ST-45 (9.29%, 39/420), ST-464 (6.19%, 26/420), ST-354 (5.48%, 23/420), and ST-443 (5.24%, 22/420). For *C. coli*, all detected isolates belonged to the clonal complex ST-828, and one unclassified clonal complex (Figure 2). Among *C. jejuni* isolates, ST-403 exhibited exceptionally high resistance gene carriage rates, with 78.57% (11/14) simultaneously harboring *aac(6')-Ie-aph(2'')-Ia*, *ant(6)-Ia*, *aph(3')-IIIa*, and *aad(6)* (aminoglycoside resistance genes), *sat-4* (streptomycin resistance), and *InuC* (lincosamide resistance), while *tet(O/M/O)* demonstrated an even higher carriage rate of 85.71% (12/14). In contrast, specific β-lactam resistance genes (*OXA-583*, *OXA-591*) were exclusively identified in ST-21. Unlike the resistance gene patterns, *C. jejuni* ST-464 demonstrated the most diverse virulence gene profile, particularly showing enriched carriage of capsular polysaccharide (CPS) cluster genes *Cj1413c-Cj1448c* compared to other sequence types (Supplementary Figure S2, available at https://weekly.chinacdc.cn/).

DISCUSSION

This study leveraged genetic and drug resistance surveillance data to examine trends in *Campylobacter* infections across China from 2020 to 2023. The number of *Campylobacter* isolates reported through the National Pathogen Identification Network Center database increased substantially during this period. This upward trend likely reflects enhanced surveillance focus on *Campylobacter* infections combined with improved detection methodologies that have increased pathogen identification rates. Notably, fewer *Campylobacter* isolates were collected in 2020 and 2022 compared to 2021 and 2023, which may be attributed to disruptions caused by the COVID-19 pandemic (*6*).

While *Campylobacter* infections typically resolve without intervention, antimicrobial treatment becomes essential in severe or prolonged cases. Fluoroquinolones and macrolides serve as first-line therapeutic agents for human *Campylobacter* infections in clinical practice (*7*). Previous investigations of antibiotic resistance in *Campylobacter*-associated

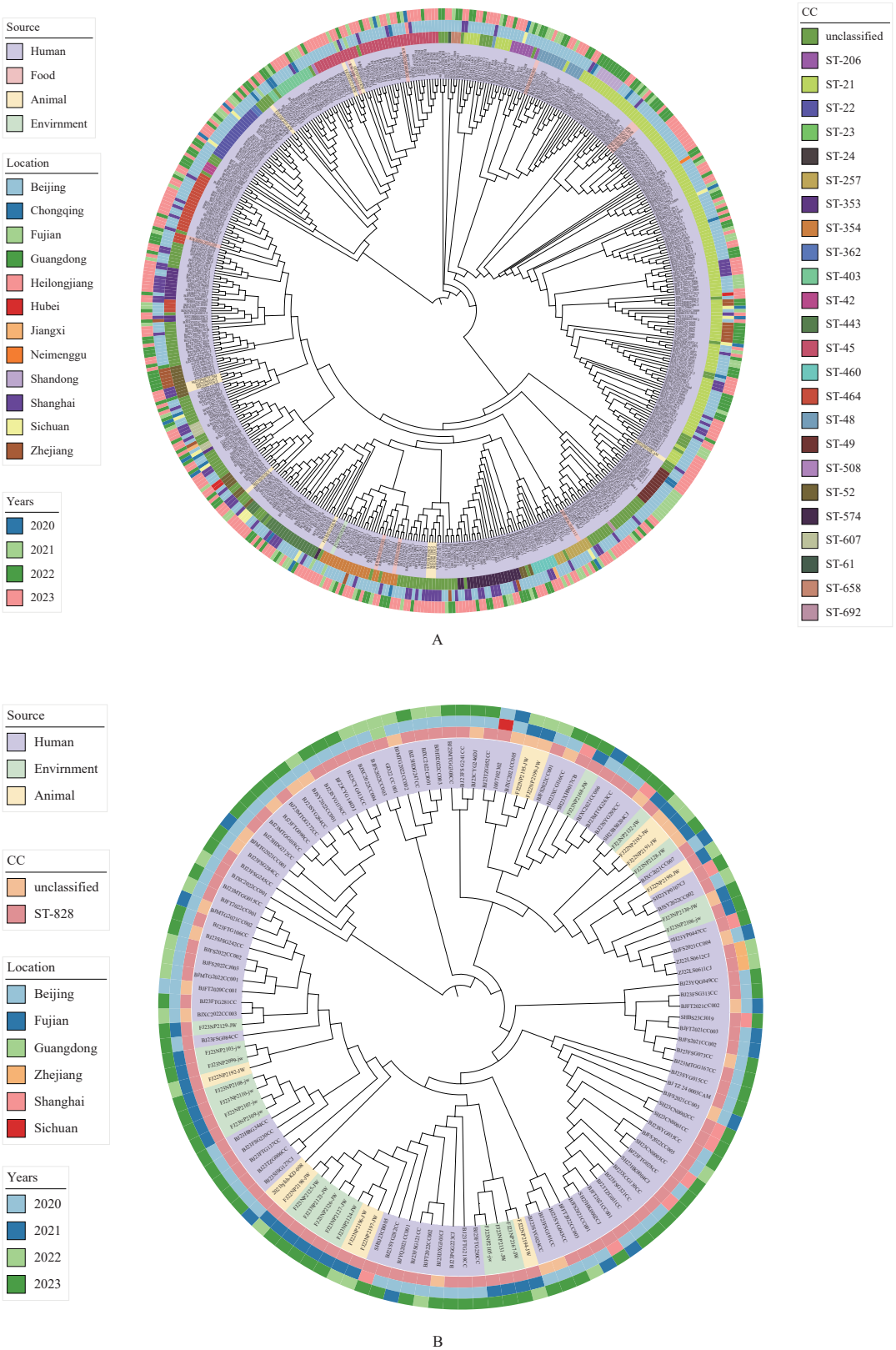

FIGURE 2. Phylogenomic tree based on cg-SNPs in (A) *C. jejuni* and (B) *C. coli*.
Note: The first ring represents the strains and sources, the second ring represents the CCs, the third ring represents the isolated location, and the fourth ring represents the isolated date.
Abbreviation: cg-SNP=core genome single nucleotide polymorphism; CC=clonal complex.

gastroenteritis have documented variable resistance patterns (*8*). Our analysis of resistance trends from 2020 to 2023 revealed persistent high-level resistance to multiple antibiotics, particularly nalidixic acid, ciprofloxacin, and tetracycline. Although overall resistance trends showed a declining pattern over the four-year period, the decreases for most antibiotics lacked statistical significance, indicating that multidrug resistance in *Campylobacter* remained consistently elevated. These findings underscore the continued need for more stringent antibiotic stewardship measures. Consistent with previous research, *C. coli* demonstrated higher resistance rates than *C. jejuni* (*9*). Given the emergence and dissemination of novel resistance mechanisms in *Campylobacter*, China must strengthen antimicrobial regulation while maintaining robust pathogen resistance surveillance programs.

Previous investigations have established strong correlations between antibiotic resistance phenotypes in *Campylobacter* and specific resistance genes or genetic mutations, with antimicrobial resistance genotypes serving as reliable predictors of resistance phenotypes (*10*). Our findings align with this principle, as the *gyrA* (T86I) mutation conferring quinolone resistance, the *tet(O)* gene responsible for tetracycline resistance, and the *cme* and *OXA* genes associated with β-lactam resistance were detected in most isolates, corresponding to the observed high resistance rates against nalidixic acid, ciprofloxacin, and tetracycline. *C. coli* exhibited more severe antibiotic resistance compared to *C. jejuni*. Recent studies have consistently identified various resistance genes in *C. coli*, including the *erm* gene associated with macrolide resistance and the *fexA* and *optrA* genes linked to phenicol resistance (*11*). These genes were also detected in our study, resulting in *C. coli* resistance to erythromycin, azithromycin, chloramphenicol, and florfenicol. Consequently, antimicrobial resistance in *C. coli* represents an increasingly significant public health threat, necessitating coordinated surveillance and management strategies to prevent the emergence and spread of resistant *C. coli* strains through food supply chains. The mechanisms underlying *Campylobacter*-induced diarrhea remain incompletely understood. Our analysis identified numerous *Campylobacter* isolates harboring genes associated with adhesion, colonization, motility, and invasion — factors critical for *Campylobacter* pathogenesis. Genes related to adherence, colonization, and invasion (including *cadF*, *ciaC*, *flgB*, *flgC*, *fliE*, *fliR*, *fliW*, *flgP*, *jlpA*, *cheY*, *Cj1279c*, and *pebA*) demonstrated high conservation among *Campylobacter* isolates and were present in the majority of clinical specimens. This conservation pattern highlights the substantial virulence potential of these *Campylobacter* isolates in human infections. Capsular polysaccharide plays a crucial role in *Campylobacter* pathogenicity (*12*). We identified a distinct cluster of isolates carrying virulence genes associated with capsular polysaccharide expression. Among the 26 isolates classified as ST-464, which represents the predominant sequence type in poultry reservoirs (*13*), 21 isolates (80.77%) clustered within this group. This finding indicates a strong phylogenetic association between ST-464 and capsular polysaccharide virulence determinants, potentially contributing to the pathogenicity and host adaptation characteristics of this sequence type. Additional research is needed to elucidate the specific genetic and evolutionary relationships involved.

The correlation between clonal complexes and factors such as sample sources and collection timeframes was relatively weak in this study. However, the predominance of human-derived samples introduces potential analytical bias. To address this limitation, future investigations should incorporate continuous, systematic surveillance of *Campylobacter* from diverse sources, which would strengthen epidemiological insights and inform more effective public health interventions.

CONCLUSIONS

As a critical foodborne pathogen affecting both developed and developing nations, systematic surveillance of *Campylobacter* remains essential for effective disease control and comprehensive food safety risk assessment. Our comprehensive analysis of the genetic characteristics and antibiotic resistance profiles of *Campylobacter* isolates collected across China from 2020 to 2023 provides crucial baseline data encompassing virulence gene distributions, antimicrobial resistance phenotypes and associated genetic markers, phylogenetic relationships, and the circulation patterns of resistance determinants. These findings reveal persistently high resistance rates to fluoroquinolones and tetracyclines, with *C. coli* demonstrating more extensive multidrug resistance compared to *C. jejuni*. The identification of specific sequence types associated with distinct virulence and resistance profiles, particularly the concentration of capsular polysaccharide genes in ST-464 isolates, highlights important epidemiological patterns that

warrant continued monitoring. However, a notable limitation of this investigation is that antimicrobial susceptibility testing and whole-genome sequencing were performed on only a subset of available isolates, which may not fully capture the complete epidemiological landscape of *Campylobacter* infections in China. Future surveillance efforts should incorporate more comprehensive sampling strategies to enhance the representativeness of resistance and genetic diversity assessments.

Conflicts of interest: No conflicts of interest.

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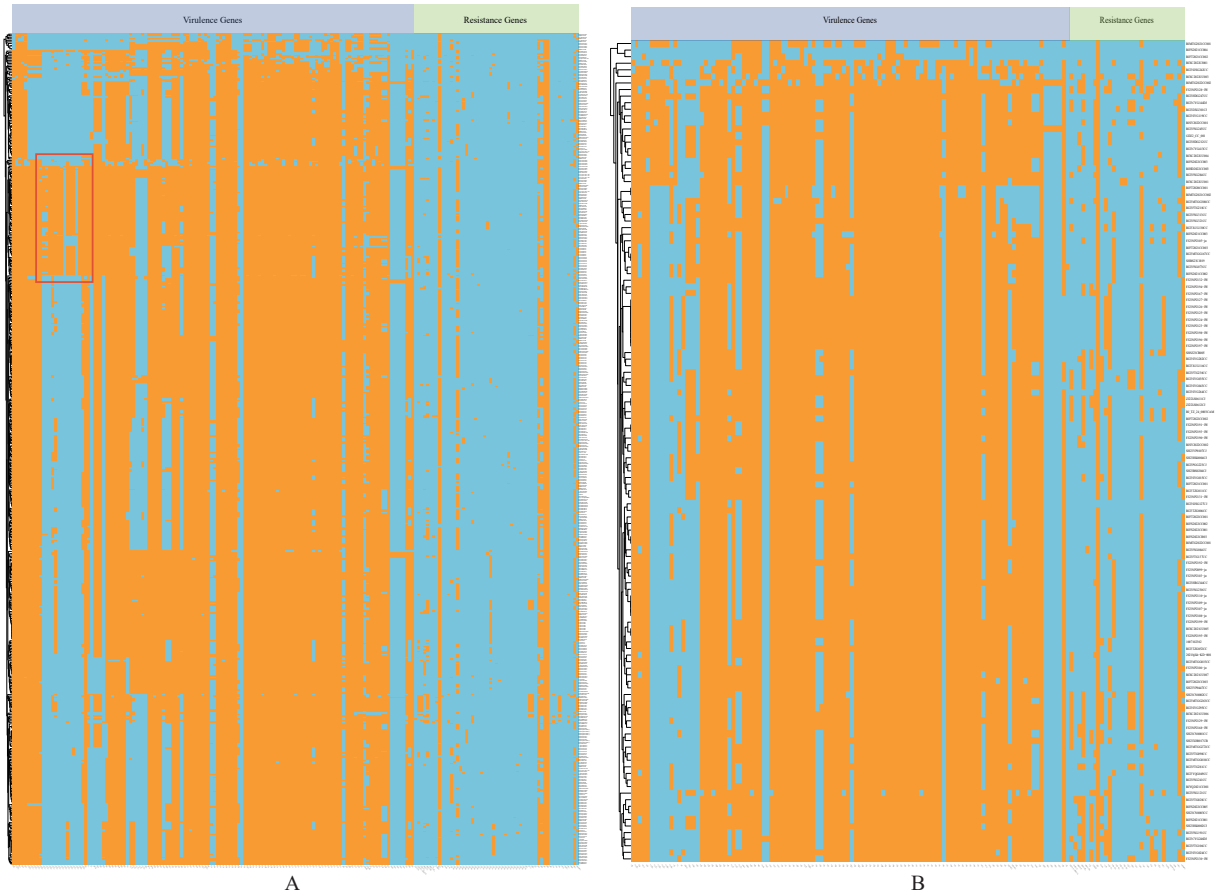
SUPPLEMENTARY MATERIALS

SUPPLEMENTARY TABLE S1. Resistance rates of *C. jejuni* and *C. coli*.

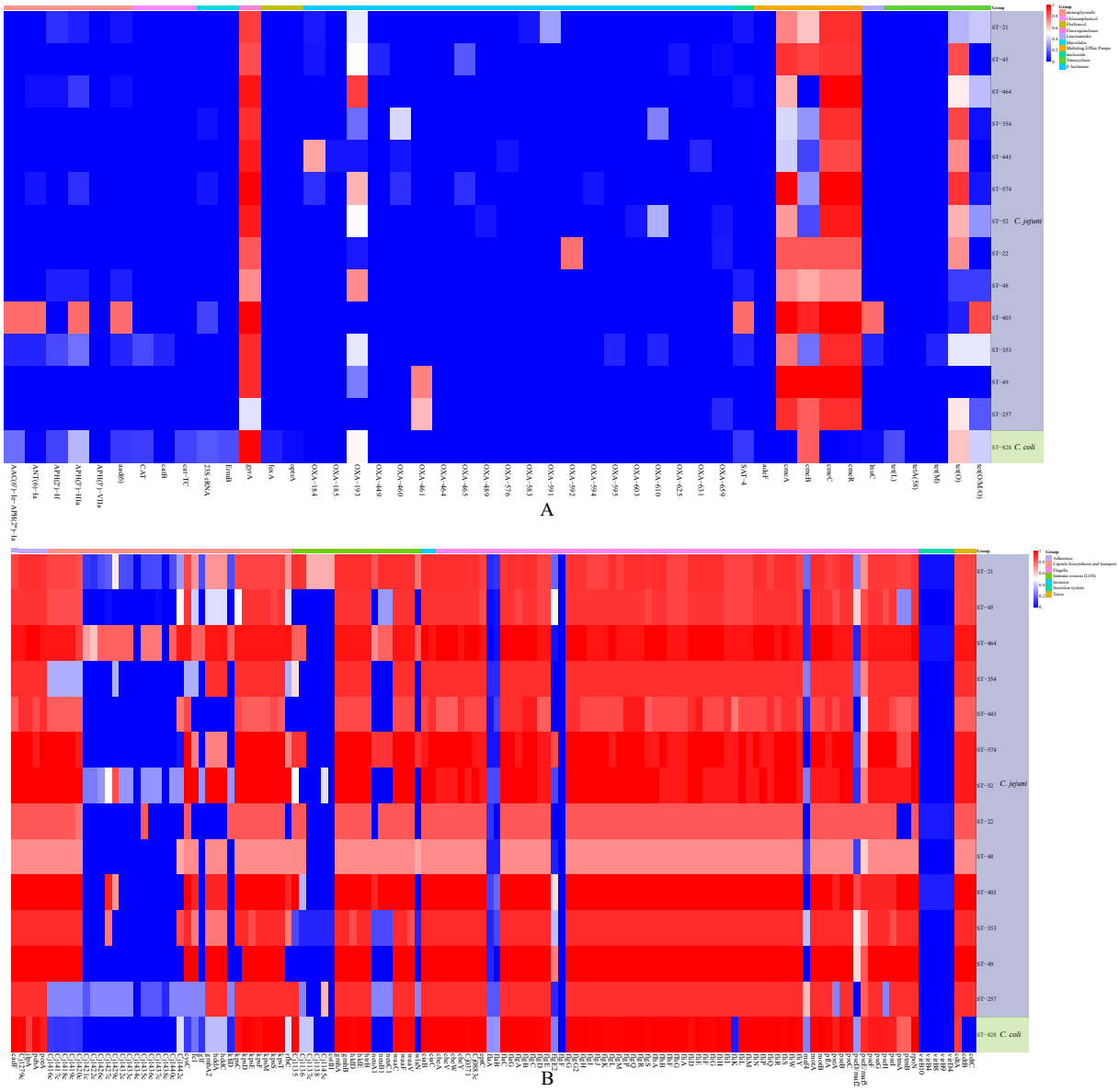
Antimicrobial agent	Resistance rates of *C. jejuni* (%)					Resistance rates of *C. coli* (%)				
	2020 *n*=33	2021 *n*=148	2022 *n*=131	2023 *n*=322	Total *n*=634	2020 *n*=8	2021 *n*=41	2022 *n*=28	2023 *n*=88	Total *n*=165
ERY	18.18	7.43	3.10	4.35	5.54	87.50	53.66	21.43	35.23	40.00
AZI	21.12	12.16	6.11	9.35	9.95	87.50	56.10	39.29	47.73	50.30
TET	75.76	65.54	69.47	73.60	70.98	87.50	80.49	75.00	72.73	75.76
NAL	96.97	72.30	76.34	79.19	77.92	100.00	87.80	78.57	73.86	79.39
CIP	93.94	81.08	78.63	73.60	77.44	100.00	90.00	67.86	78.41	80.49
GEN	27.27	16.22	11.54	13.04	14.22	100.00	48.78	25.00	38.64	41.82
STR	10.00	6.08	5.34	5.90	6.02	83.33	56.10	25.00	37.50	41.98
CHL	15.15	4.05	7.63	3.11	4.89	0.00	12.20	10.71	11.36	10.98
FLO	20.00	12.84	23.85	13.04	15.56	50.00	21.95	7.14	23.86	21.47
TEL	26.67	9.46	10.00	5.59	8.41	83.33	58.54	25.00	34.09	40.49
CLI	33.33	19.59	12.31	13.98	15.87	83.33	56.10	28.57	47.73	47.85

Abbreviation: ERY=erythromycin; AZI=azithromycin; NAL=nalidixic acid; CIP=ciprofloxacin; GEN=gentamicin; STR=streptomycin; CHL=chloramphenicol; FLO=florfenicol; TET=tetracycline; TEL=telithromycin; CLI=clindamycin.



A B

SUPPLEMENTARY FIGURE S1. Virulence genes and antibiotic resistance genes in (A) *C. jejuni* and (B) *C. coli*.
Note: Orange indicates the presence, and sky blue indicates the absence. The red box in Figure (A) highlights a distinct genetic cluster that harbors capsular polysaccharide (CPS)-associated virulence determinants.



SUPPLEMENTARY FIGURE S2. Heatmap illustrating the distribution of (A) resistance genes and (B) virulence genes across different STs.

Note: The top annotation bar indicates the categories of resistance genes (A) or virulence genes (B). The purple section in the right-side annotation bar denotes *C. jejuni*, while the green section represents *C. coli*. Blue indicates a carriage rate of 0%, while red indicates a carriage rate of 100%. We specifically selected sequence types with strain counts exceeding 10 isolates for analysis.

Abbreviation: ST=sequence type.