Preplanned Studies

Infection and Genomic Characteristics of *Campylobacter jejuni* from a Patient Without Diarrhea — China, 2018

Xiangdong Yang[1,2]; Wen Wang[1]; Chajin Cui[3]; Binbin Yu[2]; Qing Zhang[2]; Yanhua Wang[1,#]

Summary

What is already known about this topic?

A 20-month-old boy was admitted to the hospital with a maximum temperature of 40 °C and a single convulsion. Unexpectedly, blood culture detected *Francisella tularensis* (*F. tularensis*) using the VITEK 2 Compact System.

What is added by this report?

After incubation of the patient's blood for 48 hours, the cultured strain was identified as *Campylobacter jejuni*, named L8, excluding *F. tularensis*. In the genome sequence of L8, we found a novel Type VI Secretion System (T6SS), of which the conserved C-terminal VgrG domain from positions 561 to 884 showed significant changes.

What are the implications for public health practice?

It should be underscored that relying solely on automatic bacterial identification instruments for accurate strain identification is unreliable. Moreover, our study suggests that the potential effect of T6SS should be considered when studying the genetic features of a patient's clinical phenotypes.

Campylobacter jejuni (*C. jejuni*) is widely recognized as the primary causative agent of global foodborne bacterial diarrheal disease (*1*). Despite a limited number of documented *C. jejuni* outbreaks in China (*2*), a young patient in Yunnan Province presented with suspected tularemia. The patient presented with high fever and convulsions rather than typical diarrheal symptoms, but the isolated strain was biochemically identified as *C. jejuni*.

To elucidate the genetic features contributing to the clinical phenotype in this case, the isolate underwent comprehensive whole-genome sequencing. This investigation aimed to discern the genetic relationships between this strain and previously sequenced *C. jejuni* strains, considering population structure, differences in genomic composition, and common virulence factors in comparison to reference strains. Additionally, the presence of specific virulence factors was investigated. Furthermore, 13 major components of the Type VI Secretion System (T6SS) and *Campylobacter jejuni* Integrated Element 3 (CJIE3) were identified and analyzed.

A 20-month-old boy was admitted to the hospital with a maximum temperature of 40 °C, which had fluctuated around 39 °C for eight hours prior. The patient exhibited no respiratory or gastrointestinal symptoms but experienced a single, one-minute convulsion five minutes prior to admission, after which he regained consciousness. The initial diagnosis was herpes pharyngitis with febrile convulsions. The patient had no history of infection. His white blood cell count was within the normal range at 10.8×10^9/L (with elevated neutrophils at 78.1% and decreased lymphocytes at 14.0%). Hypersensitive C-reactive protein levels were elevated at 41.5 mg/L. Procalcitoninogen and erythrocyte sedimentation rate were both within normal limits at 0.134 ng/mL and 11 mm/h, respectively. Electroencephalogram and digital computed tomography scan of the head and lungs revealed no abnormalities. Unexpectedly, blood culture detected *Francisella tularensis* using the VITEK 2 Compact System based on the positive PyrA. Intracranial infection and central nervous system disease were ruled out based on the patient's signs and symptoms and clinical test results. The study was approved by the Ethics Committee of the Yunnan Institute of Endemic Diseases Control & Prevention (Ethics Committee approval number 2021-09). Informed consent was obtained from legal guardians.

In the reported clinical case of suspected tularemia, blood was inoculated onto cysteine heart agar blood plates suitable for *F. tularensis* in a 5% CO_2 incubator at 37 °C. After 48 hours of incubation, the culture medium exhibited several single colonies, 1–2 mm in diameter, with a teardrop shape and consistent morphology. Tularemia was ruled out in this patient based on negative tests for *F. tularensis*-specific antigens and antibodies. The colonies were Gram-stain-negative and elongated in an S-shape without spores, and all

were identified as *C. jejuni* through positive reactions for oxidase, catalase, and hippurate hydrolysis. One strain was selected for high-quality whole-genome sequencing and confirmed as *C. jejuni*. This strain, designated L8, was tested for susceptibility to 12 antibiotics. The results showed that L8 was resistant to ciprofloxacin and tetracycline and sensitive to the other antibiotics. Comparisons using ResFinder (https://cge.food.dtu.dk/services/ResFinder/) and CARD (https://card.mcmaster.ca/analyze), a database of drug resistance genes, revealed that L8 expressed *cmeABC* and *cmeR*, which are closely related to macrolide, fluoroquinolone, cephalosporin, and fusidane resistance. The tetracycline, doxycycline, and minocycline resistance gene *tet(O)* was also expressed.

The *C. jejuni* L8 genome was completely sequenced (GenBank accession no. CP139640) and comprised a single contig of 1,732,398 bp with no plasmids (Figure 1A). The L8 genome exhibited a G+C content of 30.29% and an ANI of 98.44%. Although L8 shared similar genomic characteristics with NCTC 11168, collinear analyses indicated that L8 possessed an additional fragment of 93,816 bp (Figures 1C, 1D), housing approximately 90 more genes, of which 55 had functional annotations (Supplementary Table S1, available at https://weekly.

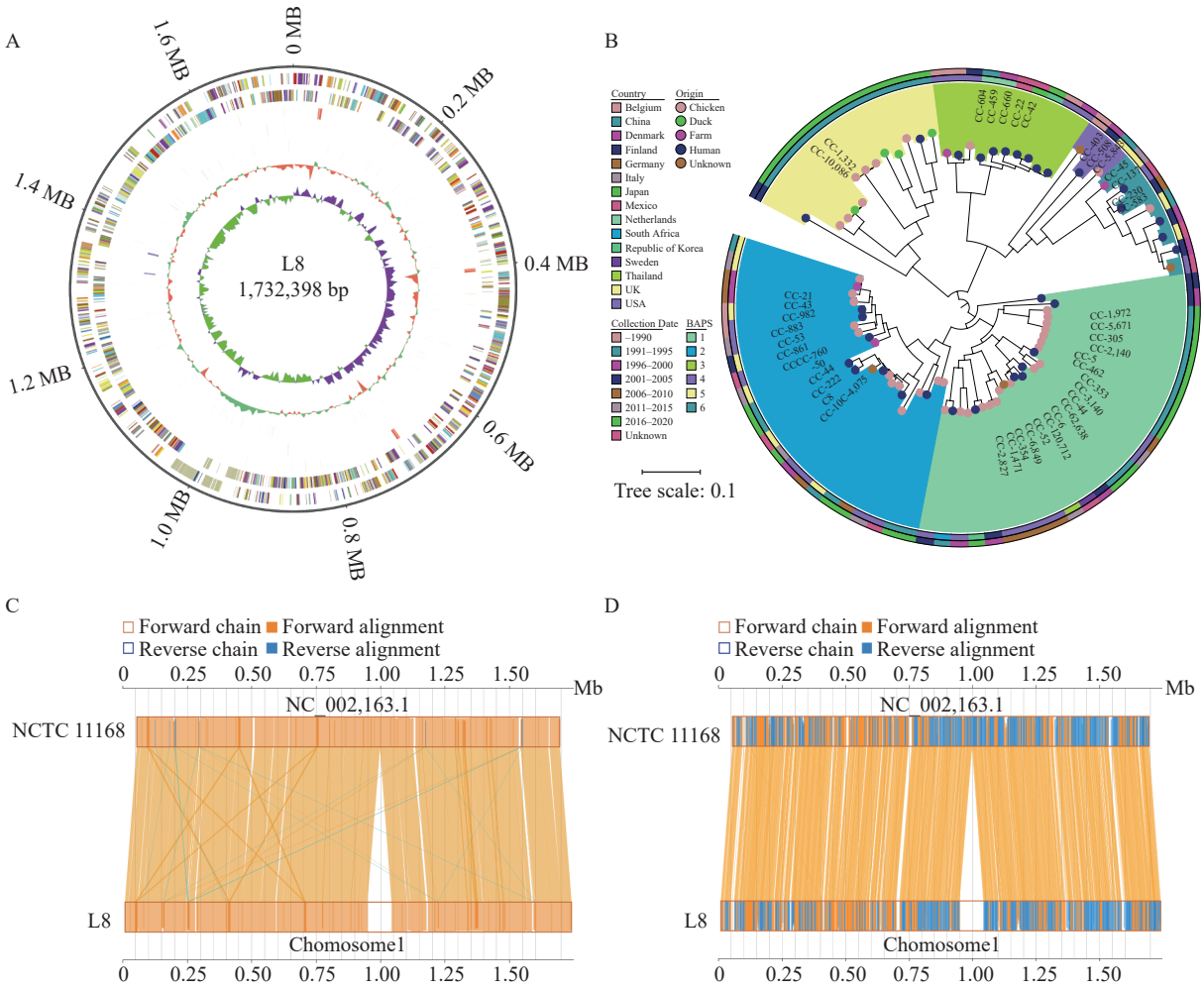

FIGURE 1. Complete genomes of *C. jejuni* L8, comparative genomic analysis with NCTC 11168, and population structure of 84 *C. jejuni* strains based on the core genome alignment with BAPS clusters. (A) Circular representation of the chromosome from *C. jejuni* L8. (B) Population structure of 84 C. jejuni strains based on the core genome alignment with BAPS clusters. (C) Co-linear analysis of the genome between C. jejuni L8 and C. jejuni NCTC 11168 using MUMmer. (D) Co-linear analysis of the proteome between *C. jejuni* L8 and *C. jejuni* NCTC 11168 using MUMmer.

Note: In panel B, the clonal complexes are color-coded in the inner ring; the country of genome origin is coded in the second ring; and the collection date of the genome is described in the outer ring. The leaves are colored by the origin of each sample. In panel C, the yellow connecting lines in the middle region indicate high sequence identity of the forward alignment, and the blue lines represent the reverse complementary alignment.

chinacdc.cn).

The 84 *C. jejuni* genomes, including L8, were isolated from poultry, human, and environmental samples from various countries. These genomes comprised 600 core genes, covering 54.6% of the average genome size of 1,694,827 bp. Phylogenetic analysis revealed six distinct branches (*1*–*6*), confirmed by BAPS clustering (Figure 1B). These branches were characterized by their clonal complexes (CCs), sequence type, geographic location, isolation source, and collection year (Supplementary Table S2, available at https://weekly.chinacdc.cn/). L8 belonged to sequence type ST-464, assigned to BAPS cluster 1, which included isolates from eight countries. A total of 126 common virulence genes and 83 shared virulence factors were identified in L8, NCTC 11168, RM 1221, and 81-176, representing the fundamental elements contributing to *C. jejuni* virulence (Figure 2A). The distribution of virulence factors was most similar between L8 and NCTC 11168. L8 harbored 27 virulence genes, exhibiting the greatest diversity compared to 81-176 (15 virulence genes), excluding RM 1221, which contained the fewest virulence factors (Figure 2B). The previously identified virulence factor CDT was present in L8. Phylogenetic analysis of the *cdtA* gene indicated significant differences in L8 compared to other strains (Figure 3, *cdtA*), although no distinct variations were observed for *cdtB* and *cdtC* (Figure 3, *cdtB*, *cdtC*). Additionally, other virulence traits, such as lipooligosaccharide sialylation and the metabolism-related virulence factors γ-glutamyl transpeptidase (GGT) and fucose permease (Cj0486), were absent in L8.

Strain L8 harbored the 13 T6SS core components. Comparisons with T6SS-positive *C. jejuni* 108 and 488 revealed a strongly conserved T6SS cluster, sharing synteny in the genomic arrangement among *C. jejuni* strains (Figure 4A). In L8, the C-terminal *tssI* (*vgrG*) domain from positions 561 to 884 differed observably from those in 108 and 488. Notably, in L8, 25 amino acids were inserted at positions 566 to 590 and 16 amino acids at positions 596 to 611, respectively (Figure 4C). In contrast, the amino acid sequences at the same positions of 108 and 488 showed high consistency of 99.38%. Furthermore, L8 exhibited consecutive or scattered mutations outside of the two insertion segments from 561 to 884. Additionally, CJIE3 was identified in L8.

DISCUSSION

Here, we isolated strain L8 from a clinically reported case of suspected tularemia and identified it as *C. jejuni* through biochemical characteristics and whole-genome

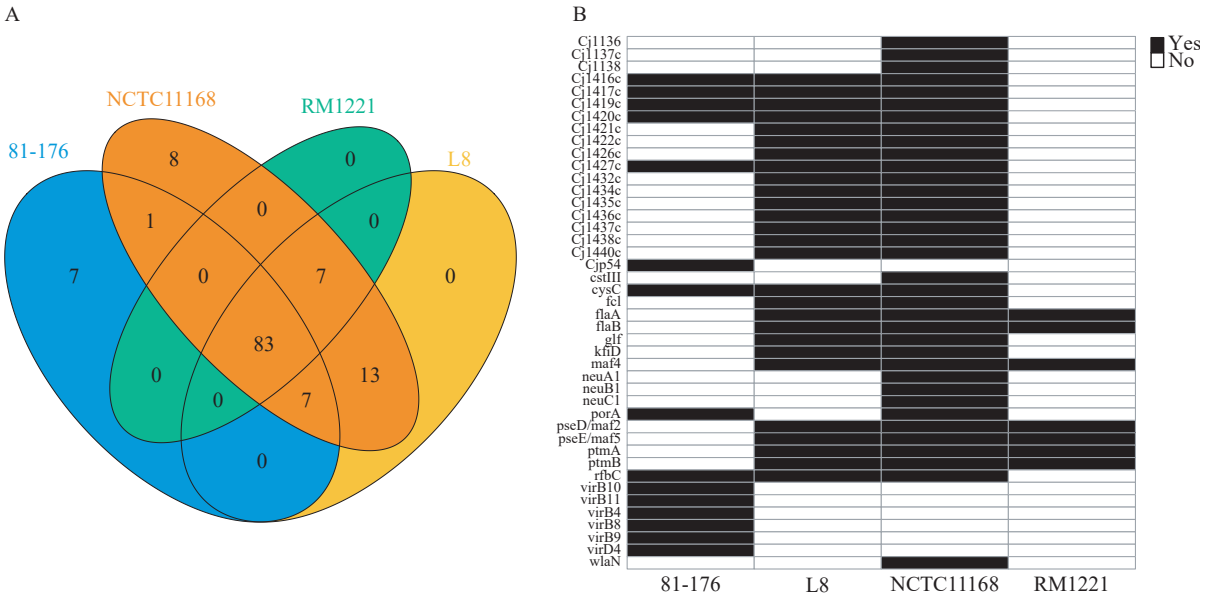

FIGURE 2. Distribution comparison of 126 virulence genes in the four strains. (A) Venn diagram of the relationship between the 126 genes identified in the four strains. (B) Heat map of the distribution of the remaining 43 virulence factors except the 83 shared.
Note: A total of 98, 119, 90, and 110 genes were detected in *C. jejuni* 81-176, NCTC 11168, RM 1221, and L8, respectively. L8 shared 110 genes with NCTC 11168, 90 genes with RM 1221, and 90 genes with 81-176.

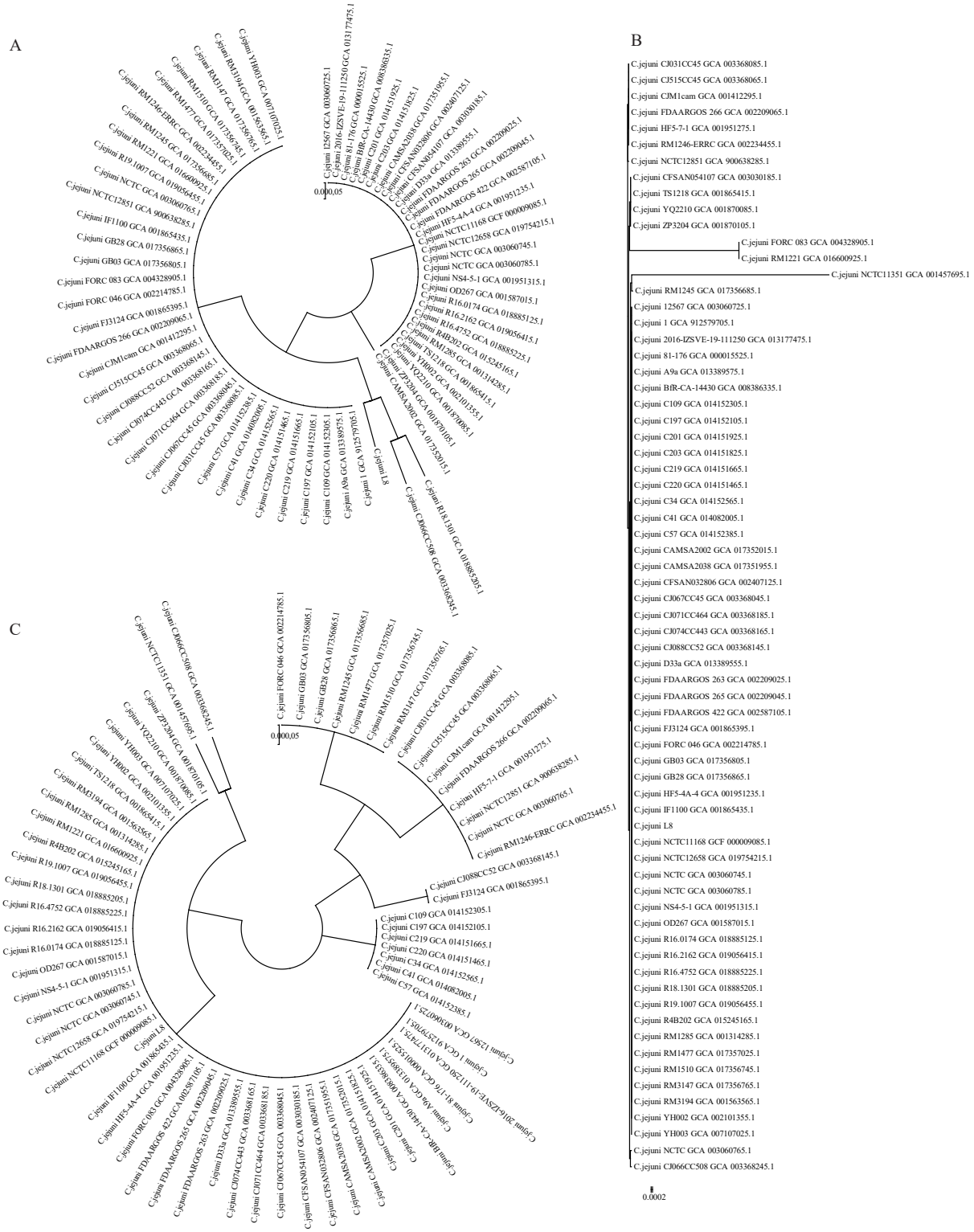

FIGURE 3. Phylogenetic tree of predicted amino acid sequence variants encoded by (A) *cdtA*, (B) *cdtB*, and (C) *cdtC* in the 84 *C. jejuni* strains from different geographic backgrounds.
Note: CdtA was predicted in 67 of the 84 *C. jejuni* strains, and both CdtB and CdtC in 69. The L8 strain is denoted with a black triangle.

sequencing. Genomic comparisons revealed the presence of a T6SS-containing CJIE3 in L8, considered a novel variant of the pathogenicity island (*3*). Notably, the insertion of two long segments and

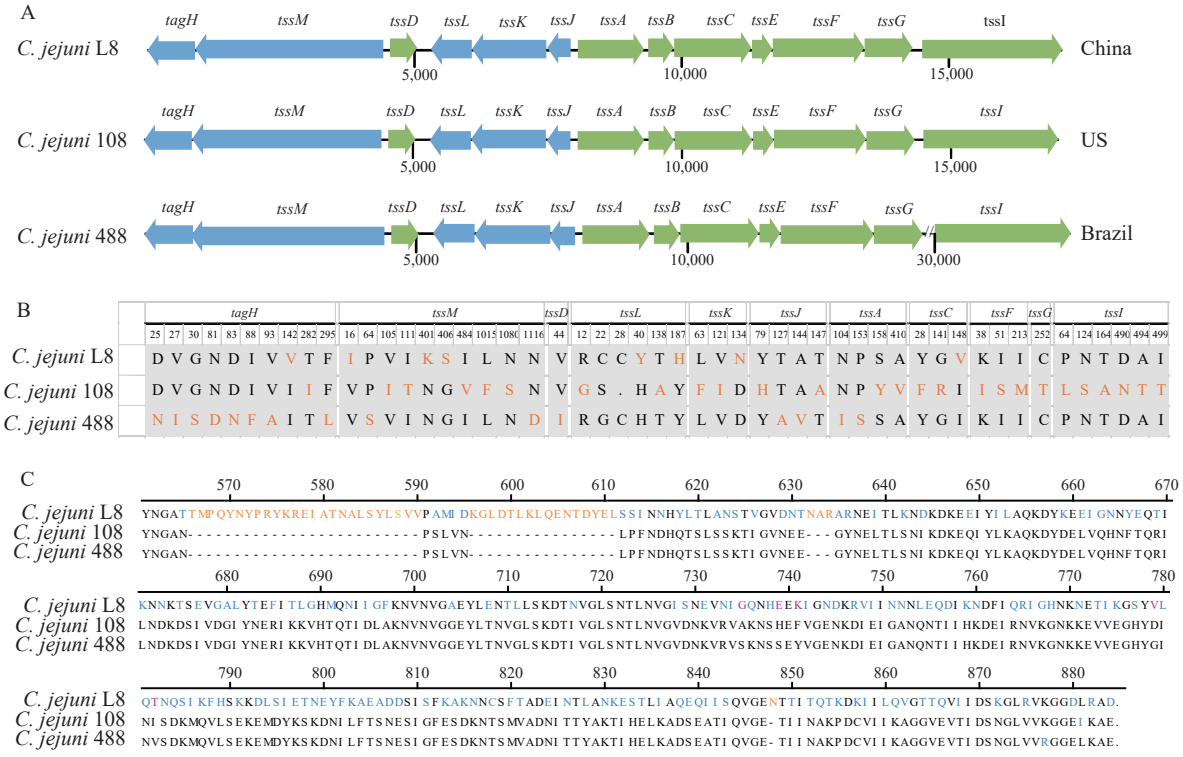

FIGURE 4. Comparison of 13 T6SS core components among L8, 108, and 488. (A) Organization of T6SS genes encoded by *C. jejuni* L8, 108, and 488. (B) Alignment of amino acid sequences of 11 genes. (C) Alignment of the amino acid sequence of TssI (VgrG) from the three *C. jejuni* strains after 560.
Note: The sequence alignment of TssI (VgrG) was truncated at 560. There was no change in the sequence alignment of TssB and TssE. Insertion sequences only in L8 are highlighted orange, and variant sequences are highlighted blue compared with the other two strains.

multiple consecutive or scattered mutations were present in L8's C-terminal VgrG, a crucial effector of the conserved T6SS. In the search for specific virulence factors, we found that L8 contained the complete CDT gene, with CdtA showing significant differences from those in other strains.

In bacterial infections, a crucial virulence determinant is the T6SS, which forms a nano-crossbow-like structure in the attacker cell's cytoplasm that propels an arrow composed of a haemolysin co-regulated protein tube and a VgrG spike to puncture the prey's cell wall (*4*). VgrG is an essential and conserved structural component in all reported T6SSs to date (*5*–*6*), and the C terminus of VgrG is widely conserved and necessary for functional T6SS assembly (*5*). Surprisingly, L8's C-terminal VgrG domain exhibited significant changes from positions 561 to 884, including the insertion of two long fragments and multiple consecutive or scattered mutations on their exterior. These alterations are very rare in previously reported T6SS structures (*3*). In contrast, the amino acid sequences between positions 108 and 488 showed 99.38% consistency. We hypothesize that this C-terminal VgrG domain was likely transformed into a toxin protein that may be associated with the patient's clinical phenotype. Notably, this study did not elucidate the specific role of this unique structure in VgrG function. Nevertheless, this finding implies that this unusual VgrG structure may enhance the initial impact of T6SS on bacterial antagonism, subversion of host cells, and niche colonization, raising the possibility that the injection of toxin proteins into host cells led to high fever and convulsions in the child.

Comparative analysis of genes within the T6SS core components showed greater similarities between L8 and 108 for *tagH*, *tssD*, and *tssA*, while L8 and 488 were more similar for *tssM*, *tssK*, *tssC*, *tssF*, *tssG*, and *tssI* (<500). Multiple scattered single amino acid mutations identified within the T6SS core components of each strain indicate individual divergences. These findings suggest that these genes warrant further investigation to elucidate T6SS function.

Although L8 and NCTC 11168 exhibited high similarity in the distribution of common virulence genes, their clinical phenotypes differed significantly. Unfortunately, no specific virulence factors were

identified in L8, unlike the potential virulence factors clearly observed in 81-176. However, phylogenetic analysis revealed that the subunit CdtA of CDT in L8 formed a distinct branch, indicating that its amino acid sequences were highly divergent from those of the other 66 strains. The toxic effects of CDT are primarily reflected in its ability to induce cell death and regulate the inflammatory response in human epithelial cells. *C. jejuni* CDT comprises three subunits: CdtA, CdtB, and CdtC. CdtA and CdtC bind to membrane lipid rafts, a crucial step for CdtB entry into cells (*7*). We hypothesize that the binding of CdtA variants and CdtC to membrane lipid rafts may facilitate CdtB entry into cells, potentially enhancing apoptosis and inflammation. Consequently, this series of processes likely exacerbated the patient's clinical presentation, although further functional verification is required.

The sequence type of L8 was ST-464, assigned to BAPS cluster 1, where 25 of the 26 genomes originated from either chickens (17 genomes) or humans (8 genomes), with one exception isolated from a farm (Figure 1B). This suggests the patient was likely infected by a chicken, a conclusion supported by epidemiological investigation. The child had come into contact with chicken feces on his mouth due to thrush treatment using a folk remedy. The patient was initially admitted on acyclovir and then switched to cefoperazone/sulbactam a week later, and was discharged after another week. Interestingly, a similar case was reported in China, where a strain isolated from the blood of a child with bacteremia was initially identified as "*Francisella*" by the automatic bacterial identification instrument VITEK2.0, later confirmed as *C. jejuni*. Another case clinically suspected as tularemia was later identified as *Paenibacillus assamensis* (*8*). These instances underscore the unreliability of depending solely on automatic bacterial identification instruments for accurate strain identification. Additionally, a case of bloodstream infection by *C. jejuni* was reported in Guizhou Province, in which the patient presented with syncope and high fever without diarrhea (*9*).

This work might expand our understanding of the clinical manifestations of campylobacteriosis. Furthermore, we recommend considering the potential significance of T6SS in the pathogenesis of *C. jejuni* when studying the genetic characterization of clinical phenotypes (*10*).

Conflicts of interest: No conflicts of interest.

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Corresponding author: Yanhua Wang, wangyanhua@icdc.cn.

[1] Ecological Medicine Research Center, National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [2] Department of Zoonotic Disease Control and Prevention, Yunnan Institute of Endemic Diseases Control & Prevention, Dali Bai Autonomous Prefecture, Yunnan Province, China; [3] Medical Laboratory Department, Yunnan Luxi County People's Hospital, Honghe Hani-Yi Autonomous Prefecture, Yunnan Province, China.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Specific ninety genes in *C. jejuni* L8 chromosome compared with NCTC 11168.

Start	End	Orientation	Gene name	Predicted function
940127	940471	+	L8GL000983	Hypothetical protein
940522	940968	−	L8GL000984	Recombinase XerC
940977	941459	−	L8GL000985	Recombinase XerC
941620	941850	+	L8GL000986	Mobilization protein
942097	942213	−	L8GL000987	Hypothetical protein
942404	942547	−	L8GL000988	Hypothetical protein
942559	942690	−	L8GL000989	Hypothetical protein
943032	943181	−	L8GL000990	Hypothetical protein
943453	943680	+	L8GL000991	Hypothetical protein
943961	944083	+	L8GL000992	Hypothetical protein
944059	944196	+	L8GL000993	Hypothetical protein
944217	944375	−	L8GL000994	Hypothetical protein
944447	944743	−	L8GL000996	Hypothetical protein
944745	944921	−	L8GL000995	Hypothetical protein
944937	945149	−	L8GL000997	Hypothetical protein
945333	945791	−	L8GL000998	Hypothetical protein
945845	947050	−	L8GL000999	Conjugal transfer protein TraG
947966	948769	−	L8GL001000	Hypothetical protein
948823	950124	−	L8GL001001	Hypothetical protein
950136	951584	−	L8GL001002	Hypothetical protein
951638	953605	−	L8GL001003	Hypothetical protein
953602	955536	−	L8GL001004	lipase family protein
955599	956096	−	L8GL001005	Hypothetical protein
956097	956228	−	L8GL001006	Uncharacterized protein
956215	956718	−	L8GL001007	Uncharacterized protein
956719	958251	−	L8GL001008	Hypothetical protein
958393	958710	−	L8GL001009	Uncharacterized protein
958718	960241	−	L8GL001010	Hypothetical protein
960256	960372	−	L8GL001011	Hypothetical protein
960498	960890	−	L8GL001012	Hypothetical protein
960887	961354	−	L8GL001013	Hypothetical protein
961424	962416	−	L8GL001014	Hypothetical protein
962908	963492	+	L8GL001015	Lysozyme
963521	963880	+	L8GL001016	Uncharacterized protein
964147	968079	+	L8GL001017	Hypothetical protein
968098	969366	+	L8GL001018	Hypothetical protein
969420	970223	+	L8GL001019	Hypothetical protein
971204	972076	+	L8GL001020	Hypothetical protein
972073	972486	+	L8GL001021	DNA-binding protein
972901	973275	+	L8GL001022	Hypothetical protein

Continued

Start	End	Orientation	Gene name	Predicted function
973358	974590	+	L8GL001023	Hypothetical protein
974749	974931	+	L8GL001024	Hypothetical protein
975105	975689	−	L8GL001025	Hypothetical protein
976064	976177	+	L8GL001026	Hypothetical protein
976181	976690	+	L8GL001027	Methyltransferase
976684	977322	+	L8GL001028	Hypothetical protein
977367	981617	+	L8GL001029	lipase family protein
981607	982353	+	L8GL001030	Hypothetical protein
982398	986330	+	L8GL001031	Hypothetical protein
986349	987617	+	L8GL001032	Hypothetical protein
987621	988520	−	L8GL001033	Hypothetical protein
988517	992044	−	L8GL001034	Hypothetical protein
992162	992677	+	L8GL001035	Hcp protein
992927	993700	−	L8GL001036	Asp/Glu-ADT subunit B
993697	995094	−	L8GL001037	Type VI secretion protein
995104	995550	−	L8GL001038	Type VI secretion protein
995676	996923	+	L8GL001039	Nucleobase: cation symporter
996992	997477	+	L8GL001040	Type VI secretion protein
997479	998933	+	L8GL001041	Type VI secretion protein
998936	999328	+	L8GL001042	Tgh104
999325	1001046	+	L8GL001043	Type VI secretion protein
1001043	1001951	+	L8GL001044	type VI secretion protein
1002120	1004753	+	L8GL001045	Type VI secretion protein VgrG
1004807	1005760	+	L8GL001046	Hypothetical protein
1005753	1007375	+	L8GL001047	Hypothetical protein
1007386	1008324	+	L8GL001048	Hypothetical protein
1008370	1008834	+	L8GL001049	Uncharacterized protein
1008831	1010081	+	L8GL001050	Hypothetical protein
1010074	1011165	+	L8GL001051	Hypothetical protein
1011179	1011733	+	L8GL001052	Hypothetical protein
1011788	1012741	+	L8GL001053	Hypothetical protein
1012731	1013117	+	L8GL001054	Hypothetical protein
1013132	1013689	+	L8GL001055	Tgh121
1013744	1014520	+	L8GL001056	Hypothetical protein
1014538	1015086	+	L8GL001057	Hypothetical protein
1015171	1015521	+	L8GL001058	Hypothetical protein
1015521	1016153	+	L8GL001059	Hypothetical protein
1016315	1018813	+	L8GL001060	type VI secretion protein VgrG
1018813	1020804	+	L8GL001061	Hypothetical protein
1020806	1021417	+	L8GL001062	Hypothetical protein
1022163	1022942	+	L8GL001063	Hypothetical protein
1022998	1025274	+	L8GL001064	Hypothetical protein

Continued

Start	End	Orientation	Gene name	Predicted function
1025241	1025471	+	L8GL001065	Hypothetical protein
1025625	1026233	+	L8GL001066	VgrG protein
1026292	1027011	+	L8GL001067	Hypothetical protein
1026995	1027168	+	L8GL001068	Hypothetical protein
1027184	1028044	+	L8GL001069	Uncharacterized protein
1028032	1029984	+	L8GL001070	Hypothetical protein
1029977	1031224	+	L8GL001071	Hypothetical protein
1031226	1032473	+	L8GL001072	Hypothetical protein

SUPPLEMENTARY TABLE S2. Basic information, BAPS clusters, and sequence types of 84 *C. jejuni* strains.

Strain	BAPS	Sequence type	Location	Host	Collection year
NCTC 11168	2	43	UK	Human blood	1981
81-176	3	604	USA	Human blood	1981
L8	1	464	Yunnan, China	Human blood	2018
2016-IZSVE-19-111250	2	50	Italy	Human feces	2016
R19.1007	1	–	Taiwan, China	Human feces	2019
NCTC11351	4	403	Belgium	Unknown	1980
BC	5	–	Guangzhou, China	Chicken	2016
CC19PF065	5	–	Fujian, China	Human feces	2019
C57	1	2140	Henan, China	Chicken	2018
C220	1	2140	Henan, China	Chicken	2018
C219	1	2140	Henan, China	Chicken	2018
C34	1	2140	Henan, China	Chicken	2018
C197	1	2140	Henan, China	Chicken	2018
C41	1	2140	Henan, China	Chicken	2018
C201	2	10075	Henan, China	Chicken	2018
C203	2	10075	Henan, China	Chicken	2018
C109	1	305	Henan, China	Chicken	2018
C1922C72	5	10086	Henan, China	Chicken	2019
ZS004	5	–	Zhejiang, China	Duck	2019
ZS005	5	–	Zhejiang, China	Duck	2019
ZS007	5	–	Zhejiang, China	Duck	2019
ZH003	5	10086	Zhejiang, China	Chicken	2018
ZH006	5	10086	Zhejiang, China	Chicken	2018
ZJB021	5	–	Zhejiang, China	Chicken	2018
ZJB020	5	–	Zhejiang, China	Chicken	2018
ZJB023	5	–	Zhejiang, China	Chicken	2018
ZS006	5	10086	Zhejiang, China	Duck	2019
CAMSA2002	2	21	Denmark	Chicken	2008
CAMSA2038	2	21	Denmark	Chicken	2008
CJ018CCUA	1	1972	Finland	Human blood	2002
CJ071CC464	1	3140	Finland	Human blood	1999
CJ067CC45	6	137	Finland	Human blood	2000

Continued

Strain	BAPS	Sequence type	Location	Host	Collection year
CJ074CC443	1	5671	Finland	Human blood	1998
CJ017CCUA	4	5673	Finland	human blood	2001
CJ066CC508	4		Finland	Human blood	2000
CJ515CC45	6	45	Finland	Human feces	2006
CJ031CC45	6	230	Finland	Human blood	1999
CJ088CC52	1	52	Finland	Human blood	2000
CJ090CC1332	5	1332	Finland	Human blood	2001
BfR-CA-14430	2	44	Germany	Chicken	2016
RM1510	3	22	Japan	Human blood	Unknown
RM3147	3	22	Mexico	Human blood	Unknown
GB03	3	22	Netherlands	Human blood	1995
GB28	3	660	Netherlands	Human blood	1999
RM3194	1	1471	South Africa	Human feces	1994
FORC_083	1	6849	Republic of Korea	Chicken	2017
FORC_046	3	22	Republic of Korea	Human feces	2016
1	1	464	Sweden	Chicken	Unknown
R18.1301	1	5	Taiwan, China	Human feces	2018
R16.2162	2	–	Taiwan, China	Human feces	2016
R16.0174	2	760	Taiwan, China	Human feces	2016
R16.4752	2	760	Taiwan, China	Human feces	2016
CFSAN054107	1	6238	Thailand	Unknown	2014
HF5-4A-4	2	861	UK	Farm	2012
NS4-5-1	2	21	UK	Farm	2012
NCTC12658	2	50	UK	Unknown	Unknown
HF5-7-1	6	45	UK	Farm	2012
CJM1cam	6	137	UK	Human blood	Unknown
NCTC12851	6	45	UK	Chicken	1993
NCTC 12664	2	50	UK	Chicken	1992
NCTC 12661	4	5843	UK	Avian	1992
12567	2	53	UK	Chicken	2005
NCTC 12660	2	21	UK	Chicken	1992
RM1221	1	354	USA	Chicken	2000
F	1	1212	USA	Chicken	2009
YH003	1	353	USA	Chicken	2014
CFSAN032806	2	222	USA	Chicken	2012
FJ3124	1	–	USA	Chicken	2009
FDAARGOS_422	2	883	USA	Human blood	Unknown
OD267	2	50	USA	Chicken	2009
R4B202	3	42	USA	Field Isolate	1983
RM1285	2	50	USA	Chicken	1997
RM1246-ERRC	6	45	USA	Human blood	Unknown
RM1245	3	22	USA	Human blood	1996
RM1477	3	22	USA	Human blood	1983

Continued

Strain	BAPS	Sequence type	Location	Host	Collection year
D33a	3	459	USA	Chicken	2003
A9a	1	2827	USA	Chicken	2003
YH002	2		USA	Chicken	2014
TS1218	1	607	USA	Chicken	2009
YQ2210	1	1212	USA	Chicken	2009
IF1100	1	462	USA	Chicken	2009
FDAARGOS_265	2	48	USA	Human blood	Unknown
FDAARGOS_263	2	43	USA	Human feces	1977
FDAARGOS_266	6	583	USA	ATCC strain	Unknown

Note: "–" means none