GENOME SEQUENCES





Draft Genome Sequences of Four Strains of *Campylobacter jejuni* Isolated from Patients with Axonal Variant of Guillain-Barré Syndrome in Bangladesh

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ABSTRACT Four *Campylobacter jejuni* strains (Z191005RS, Z191005SS, Z201020RS, and Z201020SS) isolated from the axonal variant of Guillain-Barré syndrome (GBS) were sequenced using Illumina technology. The average genome size was from 1.61 to 1.63 gb, with a very high coverage ranging from $654 \times$ to $758 \times$, which facilitates the possibility of rare variant calling.

C ampylobacter jejuni is the predominant preceding infectious agent in the axonal variant of Guillain-Barré syndrome (GBS) (1). Certain *C. jejuni* strains have a ganglioside-like structure in lipo-oligosaccharides (LOSs) and induce molecular mimicrydriven GBS (1–3). The detailed mechanism of this cross-reactive autoimmunity is still unknown (1, 4). Bacterial genetics are entailed to be assessed in evaluating the virulence factors responsible for inducing axonal GBS (4). Periodical genome profiling of *C. jejuni* strains isolated from a patient with an axonal variant of GBS would provide features revealing their detailed versatile pathogenesis mechanism. It also helps in comparing the mechanisms among different *C. jejuni* strains causing different types of campylobacteriosis and variation in subtypes of GBS. The study was reviewed and approved under protocol number PR-19048 by the institutional review board (IRB) of icddr,b, Bangladesh.

Four C. jejuni strains were isolated from rectal swabs or stool specimens (Z191005RS and Z191005SS from one and Z201020RS and Z201020SS from another patient) of two patients with acute motor axonal neuropathy (AMAN), using standard microbiological procedures (4). The C. jejuni isolates were selected and enriched for 48 h at 42°C in Brucella agar and blood agar with 5% sheep blood, respectively. Selected C. jejuni isolates were confirmed by species-specific PCR. Genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega) followed by quality and quantity check using a NanoDrop spectrophotometer (Thermo Scientific) and Qubit 2.0 fluorimeter (Life Technologies), respectively (5). The sequencing library was constructed using 1 ng of genomic DNA employing Illumina Nextera XT DNA library preparation kit. Sequencing was performed using the Illumina NextSeq 500 platform employing the Illumina NextSeq v2.5 reagent kit (2,150 bp) (Illumina platform) (6). Quality checks on the paired-end sequencing reads (150 bp) were performed using FastQC v0.11.9 (7). The genome coverage was found to be $654 \times$ to $758 \times$ by mapping the reads against the reference genome *Campylobacter jejuni* ATCC 700819 (GenBank accession number NC_002163.1) using BWA v0.7.17-r1188 and SAMtools v1.14 (8, 9). Trimmomatic v0.36 and fastp v0. 19.5 were used for adapter trimming and quality trimming, respectively (10, 11). De novo assembly was performed using SPAdes v3.14.1 adding "--isolate" flag and QUAST v5.0.2 to assess assembly statistics (12, 13). NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.1 was used to annotate the assembled draft genomes (14). Clustered regularly interspaced short palindromic repeats (CRISPR) arrays were interpreted

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The authors declare no conflict of interest.

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		Genome size		content	No. of contigs		Total no. of	No. of rRNAs	No. of		Genome
Strain	Source	(pb) ^a	No. of reads	(mol %)	(N ₅₀ [bp] ^a)	GenBank accession no.	genes	(5S, 16S, 23S)	tRNAs	SRA ^b accession no	coverage (×)
Z191005RS	Rectal swab	1,628,993	3,897,758	30.57	55 (153,957)	JAGIQH00000000	1,718	1, 1, 1	40	SRR14206027	682
Z191005SS	Stool	1,631,208	4,319,713	30.62	55 (153,957)	JAGIQ1000000000	1,720	1, 1, 1	40	SRR14205973	758
Z201020RS	Rectal swab	1,622,648	4,526,623	30.64	60 (183,722)	JAGIQJ000000000	1,714	1, 2, 1	41	SRR14206028	730
Z201020SS	Stool	1,616,053	4,057,623	30.55	35 (183,722)	JAGIQK010000000	1,692	1, 1, 1	39	SRR14205974	654
a bp, base pair.											

and analyzed on the CRISPRCasFinder and the CRISPRTarget web tools (15, 16). Default parameters were applied for all software unless otherwise specified.

All four sequences have a coverage >650 with a G+C content around 30%. PGAP annotated a total of 1,692 to 1,720 genes, including 45 to 48 RNAs in the *C. jejuni* strains. Moreover, Z191005RS and Z191005SS strains had similar genome sequences (99.99%) calculated by the average nucleotide identity (ANI) calculator (17). Both genomes possessed the same CRISPR arrays and spacer sequences. The protospacer from *Campylobacter* phage DA10 (GenBank accession number MN530981) was commonly targeted by both strains, which is similar to the previously announced *C. jejuni* strains (18). Finally, these draft genome sequences would help to determine strain-level differences and identify genetic variants/ genes associated with axonal GBS pathogenesis.

Data availability. All mentioned draft genomes were deposited in GenBank under BioProject accession no. PRJNA717137 (Table 1).

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REFERENCES

- Islam Z, Gilbert M, Mohammad QD, Klaij K, Li J, van Rijs W, Tio-Gillen AP, Talukder KA, Willison HJ, van Belkum A, Endtz HP, Jacobs BC. 2012. Guillain-Barré syndrome-related Campylobacter jejuni in Bangladesh: ganglioside mimicry and cross-reactive antibodies. PLoS One 7:e43976. https:// doi.org/10.1371/journal.pone.0043976.
- Perera VN, Nachamkin I, Ung H, Patterson JH, McConville MJ, Coloe PJ, Fry BN. 2007. Molecular mimicry in Campylobacter jejuni: role of the lipo-oligosaccharide core oligosaccharide in inducing anti-ganglioside antibodies. FEMS Immunol Med Microbiol 50:27–36. https://doi.org/10.1111/j .1574-695X.2007.00225.x.
- Guirado P, Paytubi S, Miró E, Iglesias-Torrens Y, Navarro F, Cerdà-Cuéllar M, Stephan-Otto Attolini C, Balsalobre C, Madrid C. 2020. Differential distribution of the wlaN and cgtB genes, associated with Guillain-Barré syndrome, in Campylobacter jejuni isolates from humans, broiler chickens, and wild birds. Microorganisms 8:325. https://doi.org/10.3390/microorganisms8030325.
- Islam Z, Sarker SK, Jahan I, Farzana KS, Ahmed D, Faruque ASG, Guerry P, Poly F, Heikema AP, Endtz HP. 2018. Capsular genotype and lipooligosaccharide locus class distribution in Campylobacter jejuni from young children with diarrhea and asymptomatic carriers in Bangladesh. Eur J Clin Microbiol Infect Dis 37:723–728. https://doi.org/10.1007/s10096-017-3165-7.
- Asad A, Hayat S, Nabila FH, Begum R, Nusrin S, Islam Z. 2021. Draft genome sequences of multidrug-resistant *Shigella* strains isolated from diarrheal patients in Bangladesh. Microbiol Resour Announc 10:e00854-21. https://doi.org/10.1128/MRA.00854-21.
- Mazumder R, Abdullah A, Hussain A, Ahmed D, Mondal D. 2020. Draft genome sequence of Chromobacterium violaceum RDN09, isolated from a patient with a wound infection in Bangladesh. Microbiol Resour Announc 9:e00957-20. https://doi.org/10.1128/MRA.00957-20.
- 7. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham Institute, Cambridge, UK.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303.3997 [q-bio.GN]. https://arxiv.org/abs/ 1303.3997v2.
- Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H. 2021. Twelve years of SAMtools

and BCFtools. Gigascience 10:giab008. https://doi.org/10.1093/gigascience/ giab008.

- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/ bty560.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- Zhao Y, Wu J, Yang J, Sun S, Xiao J, Yu J. 2012. PGAP: pan-genomes analysis pipeline. Bioinformatics 28:416–418. https://doi.org/10.1093/bioinformatics/ btr655.
- Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. 2018. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res 46: W246–W251. https://doi.org/10.1093/nar/gky425.
- Biswas A, Gagnon JN, Brouns SJJ, Fineran PC, Brown CM. 2013. CRISPRTarget: bioinformatic prediction and analysis of crRNA targets. RNA Biol 10: 817–827. https://doi.org/10.4161/rna.24046.
- Yoon SH, Min Ha S, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4.
- Islam Z, Nabila FH, Asad A, Begum R, Jahan I, Hayat S, Endtz HP. 2021. Draft genome sequences of three strains of Campylobacter jejuni isolated from patients with Guillain-Barré syndrome in Bangladesh. Microbiol Resour Announc 10:e00005-21. https://doi.org/10.1128/MRA.00005-21.