GENOME SEQUENCES





Draft Genome Sequences of Multidrug-Resistant Campylobacter jejuni Strains Isolated from Chickens in Central China

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ABSTRACT Campylobacter jejuni is a major foodborne pathogen that plays an important role in spreading drug resistance. We report the draft genome sequences of two multidrug-resistant *C. jejuni* isolates which contained similar mutations in the CmeR box. This will improve the understanding of *C. jejuni* antimicrobial resistance and genetic characteristics.

Campylobacter jejuni is a major foodborne pathogen that causes bacterial gastroenteritis in humans (1–3). Poultry has been recognized as the most important reservoir of this pathogen. Recently, the antibiotic resistance of *C. jejuni* has been increasing due to the prophylactic or therapeutic application of antimicrobials in animal husbandry (4). The availability of genomic sequences would provide valuable knowledge for understanding the antimicrobial resistance and genetic characteristics of *C. jejuni*.

In 2014, two *C. jejuni* strains, XZJ48 and XZJ52, were isolated from anal swabs from chickens in wet markets in Hubei Province, China. The *C. jejuni* strains were cultured at 42°C in Bolton broth containing *Campylobacter* growth supplements and *Campylobacter* selective supplements (Oxoid, England), under microaerobic conditions. Antimicrobial susceptibility was analyzed according to the Clinical and Laboratory Standards Institute guidelines (5), and the results showed that XZJ48 and XZJ52 were resistant to at least six antimicrobial agents, including cefoperazone (64 mg/liter for XZJ48 and 128 mg/liter for XZJ48 and XZJ52), tetracycline (64 mg/liter for XZJ48 and XZJ52), sulfamethoxazole (8 mg/liter for XZJ48 and XZJ52), ciprofloxacin (128 mg/liter for XZJ48 and XZJ52), erythromycin (16 mg/liter for XZJ48 and 512 mg/liter for XZJ52), and clindamycin (8 mg/liter for XZJ48 and XZJ52).

To isolate the genome DNA, the strains were cultured for 24 h at 42°C in Bolton broth containing *Campylobacter* growth supplements, under microaerobic conditions. Genomic DNA was extracted using the MiniBEST universal genomic DNA extraction kit (TaKaRa, Dalian, China), according to the manufacturer's instructions. The sequencing library was generated using the TruSeq DNA sample preparation kit (Illumina, San Diego, CA). Sequencing of *C. jejuni* XZJ48 and XZJ52 was performed using the TruSeq SBS kit (Illumina), on an Illumina MiSeq PE150 platform (run configuration, 2×150 bp). The paired-end reads were adapter trimmed and assembled with SOAPdenovo v2.04 (6) and GapCloser v1.12 (7). Default parameters were used for all software unless otherwise specified. All relevant sequencing and assembly statistics are summarized in Table 1.

Multilocus sequence typing was carried out based on the sequences of seven housekeeping genes and showed that the sequence types (STs) of XZJ48 and XZJ52 were ST-7510 and ST-7512, respectively (8). ST-7510 and ST-7512 belong to the ST-353 clonal complex, which is the most common clonal complex worldwide (9). Resistance

Citation Cheng Y, Zhang W, Lu Q, Wen G, Luo Q, Shao H, Zhang T. 2020. Draft genome sequences of multidrug-resistant *Campylobacter jejuni* strains isolated from chickens in central China. Microbiol Resour Announc 9:e01303-19. https://doi.org/10.1128/MRA.01303-19.

Editor David Rasko, University of Maryland School of Medicine

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Received 19 October 2019 Accepted 14 November 2019 Published 2 January 2020

TABLE 1 Sequencing ar	d assembly statistics f	for <i>C. jejuni</i> strain genomes
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	No. of reads	No. of total bases in	No. of reads	No. of total bases in	Avg coverage	No. of	No. of bases in all	GC content		
Strain	in raw data	raw data	in clean data	clean data	(fold)	contigs	contigs	(%)	N ₅₀ (bp)	N ₉₀ (bp)
XZJ48	15,205,214	2,280,782,100	14,350,745	2,116,736,070	1,156	90	1,831,780	30.76	246,974	92,068
XZJ52	25,601,202	3,840,180,300	24,417,852	3,607,441,610	1,910	110	1,889,087	31.28	188,109	92,149

genes in strains XZJ48 and XZJ52 were screened using ARG-ANNOT (10), which showed that both of them contained the tetracycline resistance gene tetO and the fluoroquinolone-resistance-related Thr-86-Ile substitution in GyrA (11) but not other known resistance genes or mutations (12, 13). The CmeABC efflux pump has been characterized as contributing to antimicrobial resistance in C. jejuni; it is negatively regulated by CmeR binding to the CmeR box (TGTAATATTTATTACA [inverted repeats are underlined]) in the promoter of the *cmeABC* operon (14, 15). Both XZJ48 and XZJ52 were found to contain an adenine point deletion between two repeats of the CmeR box (TGTAAT-TTTATTACA), and this point deletion was confirmed by PCR and sequencing, as reported previously (16, 17). In addition, this point deletion was further identified, by PCR and sequencing, in 11 multidrug-resistant isolates of 143 Campylobacter isolates in our laboratory. As reported previously, a mutation in the CmeR box could cause derepression and overexpression of *cmeABC* and then accelerate the excretion of antimicrobial agents in C. jejuni (17). We think that the adenine deletion in the CmeR box is responsible for multidrug resistance in these two C. jejuni isolates, and we will further investigate the role of this point deletion in multidrug resistance.

Data availability. The whole-genome shotgun sequencing projects were deposited in GenBank under accession numbers SZOP00000000 (XZJ48) and SZWG00000000 (XZJ52). The raw sequence data were deposited under SRA accession numbers SRR10297828 (XZJ48) and SRR10297827 (XZJ52).

ACKNOWLEDGMENTS

This work was supported by the Chinese Key Research and Development Plan (grant 2016YFD0501305) and the China Agriculture Research System (grant CARS-41-G13).

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