



# Whole-Genome Sequences of Agricultural, Host-Associated Campylobacter coli and Campylobacter jejuni Strains

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We report here the genome sequences of four agricultural, multidrug-resistant *Campylobacter* spp.: *C. coli* 11601 and *C. jejuni* 11601MD, isolated from turkey cecum and jejunum, respectively, and *C. coli* 6067 and *C. coli* 6461, isolated from turkey-house water and swine feces, respectively. The genomes provide insights on *Campylobacter* antimicrobial resistance and host adaptations.

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**C**ampylobacter jejuni and *C*. coli are leading agents of human foodborne disease that frequently colonize the mammalian and avian intestine (1–3). While certain *C*ampylobacter lineages colonize diverse animal hosts, others exhibit preference for certain hosts or environments (3–7). Antimicrobial resistance (AMR) is frequently encountered among *C*ampylobacter spp. (8). Much remains to be elucidated about the genomic basis of host preference and AMR in *C*ampylobacter spp. of agricultural origin.

We determined draft genomes of four MDR strains from a longitudinal study of *Campylobacter* spp. from conventional turkey and swine farms in eastern North Carolina (9). *C. coli* 11601, resistant to tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), nalidixic acid (N) and ciprofloxacin (C), was isolated in 2006 from the cecum of a turkey. On the other hand, *C. jejuni* 11601MD (resistant to T, K, N, C) was isolated from the jejunum of the same turkey. *C. coli* 6067 (resistant to T, N, C) was isolated in 2003 from drinker water in a turkey house and belonged to the turkey-associated "cluster II" lineage (10), while *C. coli* 6461 (resistant to T, S, E) was isolated in 2004 from swine feces and its DNA resisted digestion by MboI, an attribute found frequently in *C. coli* from swine, but not turkeys (11).

Genomic DNA was extracted using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA). DNA library preparation used the GS FLX Titanium rapid library preparation kit (Roche, Basel, Switzerland), and sequencing employed the 454 GS FLX high-throughput DNA sequencer (Roche). The raw reads were *de novo* assembled using Newbler version 1.1.03.24 with >30× estimated coverage, <77 contigs,  $N_{50}$  > 135,251 bp, providing draft genomes with total sizes of 1,695,750, 1,792,449, 1,957,712, and 1,736,776 bp for *C. coli* (6067, 6461, and 11601) and *C. jejuni* (11601MD) strains, respectively. Annotations were performed using the NCBI Prokaryotic Genome Annotation Pipeline (http: //www.ncbi.nlm.nih.gov/genome/annotation\_prok) and an updated version of the GAMOLA annotation suite (12). Genome annotation identified 1,618, 1,770, 1,939, 1,695 coding sequences, 76 to 123 pseudogenes, 1 to 4 rRNAs, and 35 to 36 tRNAs in *C. coli* 6067, 6461, and 11601 and *C. jejuni* 11601MD, respectively. In *C. coli* 11601 and *C. jejuni* 11601MD, the tetracycline resistance determinant *tet*(O) was plasmid-borne, with one contig of 11601MD harboring exclusively plasmid-associated sequences (13). As previously reported (14), *tet*(O) was found in the chromosome in *C. coli* 6067 and (in a different chromosomal locus) in *C. coli* 6461. Plasmids were also detected in *C. coli* 6067 and 6461.

Potential host association and AMR attributes of such agricultural strains remain poorly understood, with especially limited information from eastern North Carolina, a major turkey- and swine- producing region in the United States. The genome sequences in this announcement can be used to further elucidate population structure, adaptations, and evolutionary biology of agricultural *C. coli* and *C. jejuni* and inform analyses of *Campylobacter* transmission from agricultural reservoirs to the human food supply.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers LKCQ00000000, LKCR00000000, LKCS00000000, and LKCT000000000. The versions described in this paper are the first versions.

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## REFERENCES

- Huang H, Brooks BW, Lowman R, Carrillo CD. 2015. Campylobacter species in animal, food, and environmental sources, and relevant testing programs in Canada. Can J Microbiol 61:701–721. http://dx.doi.org/ 10.1139/cjm-2014-0770.
- Fitzgerald C. 2015. Campylobacter. Clin Lab Med 35:289–298. http:// dx.doi.org/10.1016/j.cll.2015.03.001.
- Sheppard SK, Maiden MC. 2015. The evolution of *Campylobacter jejuni* and *Campylobacter coli*. Cold Spring Harb Perspect Biol 7:a018119. http:// dx.doi.org/10.1101/cshperspect.a018119.
- Ogden ID, Dallas JF, MacRae M, Rotariu O, Reay KW, Leitch M, Thomson AP, Sheppard SK, Maiden M, Forbes KJ, Strachan NJ. 2009. *Campylobacter* excreted into the environment by animal sources: prevalence, concentration shed, and host association. Foodborne Pathog Dis 6:1161–1170. http://dx.doi.org/10.1089/fpd.2009.0327.
- Griekspoor P, Colles FM, McCarthy ND, Hansbro PM, Ashhurst-Smith C, Olsen B, Hasselquist D, Maiden MC, Waldenström J. 2013. Marked host specificity and lack of phylogeographic population structure of *Campylobacter jejuni* in wild birds. Mol Ecol 22:1463–1472. http://dx.doi.org/ 10.1111/mec.12144.
- Sheppard SK, Colles FM, McCarthy ND, Strachan NJ, Ogden ID, Forbes KJ, Dallas JF, Maiden MC. 2011. Niche segregation and genetic structure of *Campylobacter jejuni* populations from wild and agricultural host species. Mol Ecol 20:3484–3490. http://dx.doi.org/10.1111/j.1365 -294X.2011.05179.x.
- Sheppard SK, Cheng L, Méric G, de Haan CP, Llarena AK, Marttinen P, Vidal A, Ridley A, Clifton-Hadley F, Connor TR, Strachan NJ, Forbes K, Colles FM, Jolley KA, Bentley SD, Maiden MC, Hänninen ML, Parkhill J, Hanage WP, Corander J. 2014. Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals. Mol Ecol 23: 2442–2451. http://dx.doi.org/10.1111/mec.12742.

- Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q. 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol 4:189–200. http://dx.doi.org/10.2217/ 17460913.4.2.189.
- Wright SL, Carver DK, Siletzky RM, Romine S, Morrow WE, Kathariou S. 2008. Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. J Food Prot 71:1791–1796.
- Miller WG, Englen MD, Kathariou S, Wesley IV, Wang G, Pittenger-Alley L, Siletz RM, Muraoka W, Fedorka-Cray PJ, Mandrell RE. 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. Microbiology 152:245–255. http://dx.doi.org/10.1099/mic.0.28348-0.
- Wright S, Wilson S, Miller WG, Mandrell RE, Siletzky RM, Kathariou S. 2010. Differences in methylation at GATC sites in genomic DNA of *Campylobacter coli* from turkeys and swine. Appl Environ Microbiol 76: 7314–7317. http://dx.doi.org/10.1128/AEM.00934-10.
- Altermann E, Klaenhammer TR. 2003. GAMOLA: a new local solution for sequence annotation and analyzing draft and finished prokaryotic genomes. Omics 7:161-169. http://dx.doi.org/10.1089/ 153623103322246557.
- Crespo MD, Altermann E, Olson J, Miller WG, Chandrashekhar K, Kathariou S. 2016. Novel plasmid conferring kanamycin and tetracycline resistance in the turkey-derived *Campylobacter jejuni* strain 11601MD. Plasmid 86:32–37. http://dx.doi.org/10.1016/ j.plasmid.2016.06.001.
- Crespo MD, Olson JW, Altermann E, Siletzky RM, Kathariou S. 2012. Chromosomal *tet*(O)-harboring regions in *Campylobacter coli* isolates from turkeys and swine. Appl Environ Microbiol 78:8488–8491. http:// dx.doi.org/10.1128/AEM.02258-12.