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Authors

Halka, Ihor

Shchur, Natalia

Bortz, Eric

et al.

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Genome sequences of antimicrobial-resistant *Campylobacter coli* and *Campylobacter jejuni*, isolated from poultry in Ukraine

Ihor Halka,¹ Natalia Shchur,^{1,2} Eric Bortz,³ Svitlana Mandyhra,⁴ Vitalii Nedosekov,² Orest Katsaraba,⁵ Ian Goodfellow,⁶ Devin M. Drown,^{7,8} Ganna Kovalenko^{3,6}

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Genomes of a *Campylobacter coli* OR12-like strain ChP2023 (1,713,995 bp) isolated from broiler chicken and *Campylobacter jejuni* subsp. *jejuni* NCTC 11168R-like strain KF2023 (1,729,995 bp) isolated from turkey, from poultry production facilities in Ukraine in 2023, were sequenced using Oxford Nanopore Technologies. Both genomes included antibiotic resistance genes and other virulence factors.

KEYWORDS *Campylobacter jejuni*, *Campylobacter coli*, nanopore sequencing, genome analysis, AMR, Ukraine

We sequenced two *Campylobacter* spp. genomes isolated from the cecal contents randomly collected during the slaughter of poultry on farms to understand genetic diversity and antimicrobial resistance (AMR) in bacterial zoonoses in Ukraine (1–3). A *Campylobacter coli* strain ChP2023 was isolated from a broiler chicken in May 2023 in Cherkasy Oblast (49°04'14"N, 31°44'35"E) and a *Campylobacter jejuni* strain KF2023 was from a turkey in June 2023 in Kyiv Oblast (49°23'29"N, 30°11'30"E). Selective isolation followed ISO 10272-1:2017 (Procedure C) with direct plating on mCCD agar and incubation at 41.5°C for 24 h under microaerobic conditions (4, 5). Presumptive colonies were confirmed using the VITEK MS system (BioMerieux, France). The isolates were cryopreserved at –70°C. Bacterial biomass enrichment followed Procedure A: cultures were inoculated in Bolton broth with 5% lysed horse blood, incubated under microaerobic conditions at 37°C for 4 h and 41.5°C for 24 h (5). Cultures were then plated onto mCCD agar and incubated for another 24 h at 41.5°C before harvesting the colonies for DNA extraction.

DNA was extracted using IndiSpin Pathogen Kit (Indical Bioscience, Germany), yielding 16.8 ng/μL for *C. coli* and 2.73 ng/μL for *C. jejuni*. A library was prepared with the Rapid PCR Barcoding Kit (SQK-RPB004, Oxford Nanopore Technologies [ONT]) using barcodes BC01 and BC02 for the *C. coli* and BC03–BC05 for *C. jejuni*. Barcodes were pooled for each corresponding isolate and cleaned with AMPure XP beads (0.6x), resulting in final concentrations of 40.5 ng/μL for *C. coli* and 169.1 ng/μL for *C. jejuni*. A total of 1 μg of DNA (8 μL of *C. coli* and 4 μL of *C. jejuni*) was sequenced for 24 h on a GridION using an R9.4.1 flow cell.

The data were basecalled using Dorado version 7.2.13 (ONT) with the super-accuracy model (dna_r9.4.1_450bps_sup.cfg), filtering reads with a quality score of 10. Reads from barcodes corresponding to each species were then combined for downstream analysis (Table 1). Reads were assembled utilizing the EPI2ME wf-bacterial-genomes version 1.1.1 workflow (<https://github.com/epi2me-labs/wf-bacterial-genomes>). Briefly, this workflow used fastcat version 0.15.1 to combine input, Flye version 2.9.3 (6) for *de novo* assembly, Medaka version 1.11.3 (<https://github.com/nanoporetech/medaka>) for polishing, and MLST version 2.23.0 (<https://github.com/tseemann/mlst>) (7) for multilocus sequence typing (MLST). Default parameters were used except where otherwise noted.

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Department of Biological Sciences, Wellesley,
Massachusetts, USA

Address correspondence to Devin M. Drown,
dmdrown@alaska.edu, or Ganna Kovalenko,
ak2388@cam.ac.uk.

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TABLE 1 Genomic characteristics of *Campylobacter coli* and *C. jejuni* isolates from poultry in Ukraine

Sequencing run report		
Total output	6,948,216,666 bp	
Total read count	2,151,311	
Total read N_{50}	3,910 bp	
Assembly report		
Metrics	<i>C. coli</i> OR12-like strain ChP2023 (BC01 and BC02 combined)	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168R-like strain KF2023 (BC03, BC04, and BC05 combined)
SRA experiment accession	SRX25150463	SRX25150464
Sequencing yield (Gb)	1.59	1.02
Total reads number	400,976	215,867
Median read length (bp)	3,943	4,593
Median Q score	14	14
Contig count	4	9
Contigs N_{50} (bp)	1,035,987	359,068
Mean contig coverage depth (x)	915	386.14
Circular contigs number	0	2
Assembled genome size (bp)	1,713,995	1,729,995
GC content (%)	31.50	30.56
CDS	1,720	1,770
tRNA	50	48
rRNA	17	12
Completeness (%)	99.6	95.2
Contamination (%)	2.7	5.2
MLST ^a	2,273	Not determined
Antibiotic resistance genes	7	7
Virulence factors ^b	63	102
AMR mechanism	Putative genes	
Beta-lactam antibiotic inactivation enzymes ^c	OXA-61 family (bla-resistant)	OXA-184 family
Efflux pump conferring antibiotic resistance	CmeABC, CmeDEF, MacB, YkkCD	CmeABC, CmeDEF, MacB, YkkCD
Antibiotic target replacement or absence of antibiotic target	7a-HSDH-like, <i>gidB</i>	7a-HSDH-like, <i>gidB</i>
Protein altering cell wall charge conferring antibiotic resistance	PgsA	PgsA
DNA topoisomerase (gyrase) subunit A genotype: prediction of ciprofloxacin resistance	<i>gyrA</i> : T86, D90 (susceptible)	<i>gyrA</i> : T86I, D90 (resistant)

^aMLST, multilocus sequence typing.

^bIdentified by the Virulence Factor Database (10).

^cIdentified by the ResFinder prediction (11).

Each genome was analyzed by comprehensive genome analysis at BV-BRC version 1.040 (8) and annotated with PGAP version 6.7 (9) as part of the submission pipeline.

Genome distance analysis, using Mash (12) on BV-BRC showed that the *C. coli* strain ChP2023 grouped with *Campylobacter coli* strain OR12 (CP013733.1), an aerotolerant strain from a chicken farm (13). The *C. jejuni* strain KF2023 is closely related to *Campylobacter jejuni* subsp. *jejuni* NCTC 11168 (SZUC0000000.1), isolated from human infection.

PATRIC predicted genomic markers of AMR and virulence factors (8). AMR genes include multidrug efflux pumps that can impact resistance to fluoroquinolones, macrolides, and aminoglycosides (14–16). The *C. jejuni* strain KF2023 encoded a T86I mutation in *gyrA*, which in conjunction with the multidrug efflux pump CmeABC confers resistance to ciprofloxacin. Ciprofloxacin resistance has been observed in other *Campylobacter* sp. isolates from poultry in Ukraine and human infections and poultry in Europe (4, 14, 17–19).

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AUTHOR AFFILIATIONS

¹Research Bacteriological Department, State Scientific Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

²Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

³Department of Biological Sciences, University of Alaska Anchorage, Anchorage, Alaska, USA

⁴Research Molecular and Genetical Department, State Scientific Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

⁵Department of Obstetrics, Gynecology and Biotechnology of Animal Reproduction, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Lviv, Ukraine

⁶Department of Pathology, University of Cambridge, Cambridge, United Kingdom

⁷Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska, USA

⁸Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska, USA

AUTHOR ORCIDs

Devin M. Drown  <http://orcid.org/0000-0002-2437-7019>

Ganna Kovalenko  <http://orcid.org/0000-0002-4929-0841>

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DATA AVAILABILITY

The genome project is indexed at GenBank under BioProject accession number [PRJNA1129571](https://ncbi.nlm.nih.gov/bioproject/PRJNA1129571). Genome assemblies are deposited under accession numbers [JBEWFL000000000.1](https://ncbi.nlm.nih.gov/assembly/JBEWFL000000000.1) and [JBEWFM000000000.1](https://ncbi.nlm.nih.gov/assembly/JBEWFM000000000.1). Direct SRA accessions for each data set are *Campylobacter jejuni* [SRX25150464](https://ncbi.nlm.nih.gov/sra/SRX25150464) and *Campylobacter coli* [SRX25150463](https://ncbi.nlm.nih.gov/sra/SRX25150463).

ETHICS APPROVAL

This study was determined to be exempt as it used samples from routine monitoring of poultry after slaughter.

REFERENCES

- Arefiev V, Kovalenko G, Frant M, Chumachenko T, Polyvianna Y, Pivnenko S, Bolotin V, Mayboroda O, Solodiankin O, Tarasov O, Bezymenni M, Lyon C, Redlinger M, Sapachova M, Mezhenyskiy AA, Ducluzeau A-L, Bortz E, Gerilovych A, Drown DM. 2020. Complete genome sequence of *Salmonella enterica* subsp. *enterica* serovar Kottbus strain Kharkiv, isolated from a commercial pork production facility in Ukraine. Microbiol Resour Announc 9:e01171-20. <https://doi.org/10.1128/MRA.01171-20>

2. Bolotin V, Kovalenko G, Marchenko N, Solodianskin O, Rudova N, Kutsenko V, Bortz E, Gerilovych A, Drown DM. 2021. Complete genome sequence of *Brucella abortus* 68, isolated from aborted fetal sheep in Ukraine. *Microbiol Resour Announc* 10:e01436-20. <https://doi.org/10.1128/MRA.01436-20>
3. Tarasov O, Kovalenko G, Muzykina L, Bezymennyi M, Bortz E, Drown DM. 2022. Genome sequence of *Erysipelothrix* sp. strain poltava, isolated from acute septic erysipelas of swine in Ukraine. *Microbiol Resour Announc* 11:e0043822. <https://doi.org/10.1128/mra.00438-22>
4. Shchur N, Chechet O, Mazur T, Martyniuk O, Gorbatiuk O, Buchkovska H, Musiets I, Ordynska D, Finkova O, Moskalenko L, Ponomaryova-Gerasimyyuk T, Lusta M, Nedosekov V. 2023. Prevalence and antimicrobial resistance of campylobacter isolated from animals and poultry in Ukraine. *AAVS* 11:852–863. <https://doi.org/10.17582/journal.aavs/2023/11.5.852.863>
5. ISO. 2017. 10272-1:2006 revised 2017, microbiology of the food chain - horizontal method for detection and enumeration of *Campylobacter* spp. - part 1: detection method. Available from: <https://www.iso.org/standard/63225.html>
6. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546. <https://doi.org/10.1038/s41587-019-0072-8>
7. Jolley KA, Maiden MCJ. 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 11:595. <https://doi.org/10.1186/1471-2105-11-595>
8. Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, Dempsey DM, Dickerman A, Dietrich EM, Kenyon RW, et al. 2023. Introducing the bacterial and viral bioinformatics resource center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res* 51:D678–D689. <https://doi.org/10.1093/nar/gkac1003>
9. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the prokaryotic genome annotation pipeline reach with protein family model curation. *Nucleic Acids Res* 49:D1020–D1028. <https://doi.org/10.1093/nar/gkaa1105>
10. Liu B, Zheng D, Zhou S, Chen L, Yang J. 2022. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res* 50:D912–D917. <https://doi.org/10.1093/nar/gkab1107>
11. Florensa AF, Kaas RS, Clausen P, Aytan-Aktug D, Aarestrup FM. 2022. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microb Genom* 8:000748. <https://doi.org/10.1099/mgen.0.000748>
12. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, Phillippy AM. 2016. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol* 17:132. <https://doi.org/10.1186/s13059-016-0997-x>
13. O'Kane PM, Connerton IF. 2017. Characterisation of aerotolerant forms of a robust chicken colonizing *Campylobacter coli*. *Front Microbiol* 8:513. <https://doi.org/10.3389/fmicb.2017.00513>
14. Lin J, Michel LO, Zhang Q. 2002. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother* 46:2124–2131. <https://doi.org/10.1128/AAC.46.7.2124-2131.2002>
15. Marotta F, Garofolo G, di Marcantonio L, Di Serafino G, Neri D, Romantini R, Sacchini L, Alessiani A, Di Donato G, Nuvoloni R, Janowicz A, Di Giannatale E. 2019. Antimicrobial resistance genotypes and phenotypes of *Campylobacter jejuni* isolated in Italy from humans, birds from wild and urban habitats, and poultry. *PLoS One* 14:e0223804. <https://doi.org/10.1371/journal.pone.0223804>
16. Shrestha RD, Agunos A, Gow SP, Varga C. 2023. Assessing antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* and its association with antimicrobial use in Canadian turkey flocks. *Epidemiol Infect* 151:e152. <https://doi.org/10.1017/S0950268823001462>
17. European Centre for Disease Prevention and Control. 2024. Campylobacteriosis. In Annual epidemiological report for 2022. ECDC, Stockholm. https://www.ecdc.europa.eu/sites/default/files/documents/CAMP_AER_2022_final.pdf.
18. Han J, Wang Y, Sahin O, Shen Z, Guo B, Shen J, Zhang Q. 2012. A fluoroquinolone resistance associated mutation in *gyrA* affects DNA supercoiling in *Campylobacter jejuni*. *Front Cell Infect Microbiol* 2:21. <https://doi.org/10.3389/fcimb.2012.00021>
19. Hull DM, Harrell E, van Vliet AHM, Correa M, Thakur S. 2021. Antimicrobial resistance and interspecies gene transfer in *Campylobacter coli* and *Campylobacter jejuni* isolated from food animals, poultry processing, and retail meat in North Carolina, 2018–2019. *PLoS One* 16:e0246571. <https://doi.org/10.1371/journal.pone.0246571>