Microbiology Resource Announcements

| Clinical Microbiology | Announcement

Complete genome sequences of two verona integron-encoded metallo-ß-lactamase (VIM)-producing enterobacterales isolated from dogs in the United States

Dhruv Desai,[1] **Stephen D. Cole**[1]

AUTHOR AFFILIATION See affiliation list on p. 2.

ABSTRACT This announcement reports the complete genome sequences, created by combined Illumina and Oxford Nanopore sequencing, of two carbapenemase-producing Enterobacterales (*Escherichia coli* [LaAc-1–20] and *Enterobacter hormaechei*, strain 19632–21) isolated from dogs in the United States. Both isolates harbor a blaVIM-4 gene found on a 47 kb plasmid and on the bacterial chromosome, respectively.

KEYWORDS carbapenemase, Dogs, veterinary microbiology, carbapenem-resistance, whole genome sequencing

Carbapenemase-producing Enterobacterales (CPE) pose a significant risk because of their broad resistance to antimicrobial drugs (1). Verona integron-mediated metallo-ß-lactamases (VIM) are particularly significant because they are not affected by inhibitor combinations (2–4). The emergence of CPE among pets is concerning for veterinary and public health (5, 6). We performed whole genome sequencing (WGS) of two bla_{VIM}-harboring Enterobacterales isolates from canine specimens submitted to a diagnostic laboratory as part of routine clinical care, which is exempt from IACUC approval. *Escherichia coli* (LaAC-1–20) was isolated from the feces of an 8-year-old female dog with a history of a CPE urinary tract infection using a chromogenic agar medium (ChromID Carba Agar, bioMerieux, Marcy L'Etoile, FR) and *Enterobacter hormaechei* (19632–21) isolated from a 10-year-old male dog with a blood stream infection via blood culture (Bactec Aerobic, Becton-Dickinson, Franklin Lakes, NJ) with subculture to tryptic soy agar with 5% sheep blood (TSAB) (Remel, Lenexa, KS). Antimicrobial susceptibility testing was performed on the Vitek 2 (AST-GN98 card) according to the manufacturer and interpreted according to Clinical and Laboratory Standards Institute (7). Isolates were phenotypically confirmed to produce a carbapenemase (8).

Bacteria were cultivated on under ambient conditions at 35°C. DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Hilden, NJ) with the gram-negative bacteria protocol. Hybrid WGS was performed at a fee-for-service laboratory (SeqCenter, Pittsburgh, PA). Short-read sequencing was performed on libraries generated using Illumina DNA Prep Kit and IDT 10 bp UDI indices on the Illumina NextSeq 2000 platform generating 2 × 151 bp reads. Total reads were 5,998,832 (LaAc-1–20) and 6,116,380 (19632–21). For long read, libraries were prepared using the Oxford Nanopore Technologies (ONT) Genomic DNA by Ligation Kit (SQK-LSK109) according to the manufacturer's protocols without shearing or size-selecting. Nanopore R9 flow cells (R9.4.1) were used with the MinION platform. The number of reads was 930,801 and 235,955 for LaAC-20–1 and 19632–21, respectively, with the high-accuracy basecalling mode of Guppy 5.0.16. Quality control and adaptor trimming were conducted using bcl-convert for Illumina and Porechop (0.2.3_seqan2.1.1) for ONT sequences. Unicycler (v.0.5.0) software was used to perform a circular hybrid assembly with ends determined and trimmed by

Editor André O. Hudson, Rochester Institute of Technology, Rochester, New York, USA

Address correspondence to Stephen D. Cole, scole@vet.upenn.edu.

The authors declare no conflict of interest.

Received 13 August 2024
Accepted 13 January 2025
Published 5 February 2025

Copyright © 2025 Desai and Cole. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

TABLE 1 Genome characteristics of the isolates included in this announcement.

Isolate	LaAc-1–20	19632–21
Bacterial species	*Escherichia coli*	*Enterobacter hormaechei*
Chromosome size (bp)	4,819,506	4,834,862
Number of genes	5,077	4,899
Protein coding sequences	4,708	4,785
Number of plasmids	11	5
Range of plasmid sizes (bp)	2,064–114,629	2,279–135,804
GC content (%)	50.68	55.14

miniasm on long and anchor reads and rotated around *dnaA* by Racon. QUAST (v.5.1.0) was used for assembly statistics (N50 = 4,819,506, N50 = 4,834,862, respectively) and annotation performed by Prokka (v.1.14.5) (9–11) in circular notation mode. Bacterial species identification was confirmed via Kmerfinder (v.1.2) (12). Default parameters were used unless otherwise noted.

Table 1 outlines the genome characteristics. Genome coverage for LaAc-20–1 and 19632–21 was 160× and 173×, respectively. Both isolates were high-risk carbapenem-resistant lineages (ST167 and ST78) by *in silico* multilocus sequence typing (v.2.0) (13–15). The $bla_{\text{VIM-4}}$ gene was present on the chromosome 19632–21 but was found on a 47 kb plasmid of LaAc-20–1.

ACKNOWLEDGMENTS

The authors would like to thank Daniel Snyder, Melissa Fritz, and Julia Leister for technical and bioinformatic support. Dr. Cody Mannino and Dr. Neil Mittelman for isolate submission and clinical data. We would also like to express our gratitude to Dr. Laura Goodman and Dr. Kevin Cummings for analysis advice.

AUTHOR AFFILIATION

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA

AUTHOR ORCIDs

Stephen D. Cole http://orcid.org/0000-0002-2681-0082

AUTHOR CONTRIBUTIONS

Stephen D. Cole, Conceptualization, Data curation, Formal analysis, Writing – review and editing.

DATA AVAILABILITY

Data have been deposited in GenBank under the BioProject accession no. PRJNA1144140. The genomes at JBGEDN010000000 and JBGEDO000000000. The 47 kb plasmid of LaAc-20-1 is at JBGEDN010000004.1. Raw sequence reads are at SRX25596228 and SRX25596226 for LaAc-20-1 SRX25596229 and SRX25596227 for 19632-21.

REFERENCES

1. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF. 2018. Carbapenemase-producing organisms: a global scourge. Clin Infect Dis 66:1290–1297. https://doi.org/10.1093/cid/cix893
2. Gupta N, Limbago BM, Patel JB, Kallen AJ. 2011. Carbapenem-resistant enterobacteriaceae: epidemiology and prevention. Clin Infect Dis 53:60–67. https://doi.org/10.1093/cid/cir202
3. Codjoe FS, Donkor ES. 2017. Carbapenem resistance: a review. Med Sci (Basel) 6. https://doi.org/10.3390/medsci6010001
4. Lee Y-L, Chen H-M, Hii I-M, Hsueh P-R. 2022. Carbapenemase-producing enterobacterales infections: recent advances in diagnosis and treatment. Int J Antimicrob Agents 59:106528. https://doi.org/10.1016/j.ijantimicag.2022.106528
5. Sellera FP, Da Silva L, Lincopan N. 2021. Rapid spread of critical priority carbapenemase-producing pathogens in companion animals: a one health challenge for a post-pandemic world. J Antimicrob Chemother 76:2225–2229. https://doi.org/10.1093/jac/dkab169

6. Dietrich J, LeCuyer TE, Hendrix GK, Burbick CR, Jacob ME, Byrne BA, Olsen K, Mitchell M, Ceric O, Lin R, Joneson J, Lintner M, Fox A, McClendon D, Alexander T, Joyce K, Byrd M, Clinton J, Snipes K, Peak L, Cole SD. 2024. Prevalence and molecular epidemiology of carbapenemase-producing enterobacterales isolated from dog and cat faeces submitted to veterinary laboratories in the USA. Zoonoses Public Health 71:538–548. https://doi.org/10.1111/zph.13144
7. CLSI. 2024. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. In CLSI supplement VET01S, 7th ed. Clinical and Laboratory Standards Institute.
8. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB, Jenkins SG, Limbago BM, Das S. 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among enterobacteriaceae. J Clin Microbiol 55:2321–2333. https://doi.org/10.1128/JCM.00193-17
9. 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
10. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153
11. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595
12. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup FM. 2014. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. J Clin Microbiol 52:139–146. https://doi.org/10.1128/JCM.02452-13
13. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11
14. Gomez-Simmonds A, Annavajhala MK, Wang Z, Macesic N, Hu Y, Giddins MJ, O'Malley A, Toussaint NC, Whittier S, Torres VJ, Uhlemann A-C. 2018. Genomic and geographic context for the evolution of high-risk carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78 . MBio 9. https://doi.org/10.1128/mBio.00542-18
15. Pan S, Liu S, Tai S, Yu J, Yuan E, Duan Y. 2023. Genomic analysis of an *Escherichia coli* sequence type 167 isolate harboring a multidrug-resistant conjugative plasmid, suggesting the potential transmission of the type strains from animals to humans. Infect Drug Resist 16:5077–5084. https://doi.org/10.2147/IDR.S420635