



Complete Genome Sequences of Three *Ornithobacterium rhinotracheale* Strains from Avian Sources, Using Hybrid Nanopore-Illumina Assembly

 Amro Hashish,^{a,d} Timothy J. Johnson,^b Emily Smith,^b Dhiraj Chundru,^c Michele L. Williams,^c Nubia R. Macedo,^a Yuko Sato,^a Mostafa Ghanem,^c  Mohamed El-Gazzar^a

^aDepartment of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

^bDepartment of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA

^cDepartment of Veterinary Medicine, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia, USA

^dNational Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt

ABSTRACT *Ornithobacterium rhinotracheale* has been associated with respiratory disease in poultry, particularly turkeys, leading to significant economic losses. However, *O. rhinotracheale* is poorly studied, and a very limited number of complete genomes are available. Here, we report the complete genome sequences of three *O. rhinotracheale* strains, generated using a Nanopore-Illumina hybrid assembly approach.

Ornithobacterium rhinotracheale has emerged as an important bacterial pathogen for domestic poultry and wild birds (1–10); however, it is an understudied pathogen. Only seven complete genomes are available in GenBank (<https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/10734/>) to date (November 2022), three of which are from this study. Here, we report the complete genome sequences of three *O. rhinotracheale* strains, generated using a Nanopore-Illumina hybrid assembly approach (11). These complete genomes will facilitate better understanding of this pathogen.

Three *O. rhinotracheale* isolates were selected as a diverse group of isolates obtained from the laboratory of K. Nagaraja at the University of Minnesota. As described previously (12), bacterial reculture was performed on blood agar (with 5% sheep blood), and the cultures were incubated under microaerophilic conditions at 37°C for 48 h. Isolates were identified and confirmed as *O. rhinotracheale* using real-time PCR as described previously (13). DNA extraction was performed using a MagMAX pathogen RNA/DNA kit (Thermo Fisher Scientific, Waltham, MA, USA) on a Kingfisher Flex instrument (Thermo Fisher Scientific). Extracted DNA was used for sequencing library preparation with an Illumina DNA preparation-tagmentation kit with IDT for Illumina DNA/RNA unique dual (UD) indexes, set A (Illumina, USA). The sequencing was performed using the Illumina MiSeq system (v3 reagent kit, with 2 × 300-bp paired-end reads).

DNA extraction for Nanopore sequencing was performed with the Nanobind CBB Big DNA kit (Circulomics, Baltimore, MD, USA) using the Gram-negative bacteria high molecular weight DNA extraction protocol. Nanopore libraries were prepared by multiplexing DNA extracts from the three isolates using the SQK-LSK109 and EXP-NBD104 kits (Oxford Nanopore Technologies, UK) according to the manufacturer's protocol. Barcodes 1, 3, and 7 from the EXP-NBD104 kit were used.

Raw reads from Illumina sequencing were first trimmed for quality and sequencing adapters using Trimmomatic v0.33 (14) with default parameters. NanoFilt v2.8.0 (15) was used with default parameters to quality filter Nanopore reads and filter out sequences <1,000 bp in length. Hybrid assemblies were performed using Unicycler v0.5.0 (16) with default parameters. All genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.1 (17) as part of the RefSeq prokaryotic genome annotation

Editor Catherine Putonti, Loyola University Chicago

Copyright © 2023 Hashish et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Mohamed El-Gazzar, elgazzar@iastate.edu.

The authors declare no conflict of interest.

Received 6 October 2022

Accepted 10 January 2023

Published 31 January 2023

TABLE 1 Sequencing, annotation, and typing results for the sequenced strains

Parameter ^a	Data for <i>O. rhinotracheale</i> strain:		
	A171	K223	97
Raw sequence reads			
Illumina paired-end read length (nucleotides)	300	300	300
No. of Illumina reads used	99,581	130,665	50,152
Avg Illumina coverage (×)	381	484	62
No. of Nanopore reads	27,383	21,287	6,593
Avg Nanopore coverage (×)	97.08	75.18	97.12
Nanopore read N_{50} (bp)	15,541	17,237	2,188
Assembly statistics for closed genomes			
No. of contigs	1	2	1
Total chromosome length (bp)	2,409,995	2,407,355	2,418,866
Plasmid ^b	Absent	Present	Absent
Total plasmid length (bp)	NA	14,785	NA
GC content (%)	37.45	37.37	37.43
Hybrid assembly N_{50} (bp)	2,409,995	2,407,355	2,418,866
Annotation results			
No. of CDSs (protein)	2,237	2,289	2,247
No. of noncoding RNAs	3	3	3
No. of tRNAs	43	43	43
No. of rRNAs	9	9	9
No. of CDSs (without protein)	26	29	29
No. of CRISPR arrays	1	1	1
No. of hypothetical proteins	541	563	568
No. of proteins with functional assignments	1,697	1,726	1,679
Serotype from agar gel precipitation test			
	A	K	Unknown
ST from <i>O. rhinotracheale</i> MLST ^c			
	ST-1	NA (too divergent to conclude ST)	ST-1
ANIb (%) vs <i>O. rhinotracheale</i> type strain DSM 15997 (accession no. CP003283.1) ^d			
	99.9	89.49	99.86
GenBank accession no.			
BioSample accession no.	SAMN27124716	SAMN27139539	SAMN27150225
BioProject accession no.	PRJNA821850	PRJNA821858	PRJNA821870
Complete genome assembly accession no.			
Chromosome	CP094844	CP094846	CP094845
Plasmid		CP094847	

^a CDS, coding sequence; ST, sequence type; MLST, multilocus sequence typing; ANI, average nucleotide identity; NA, not applicable.

^b The presence or absence of plasmids in the genome sequences was determined using the Plasmid database (19) via PLSDB (<https://ccb-microbe.cs.uni-saarland.de/plsdb>).

^c MLST was performed using the *O. rhinotracheale* MLST scheme (20) available via PubMLST (21) (<https://pubmlst.org/organisms/ornithobacterium-rhinotracheale>).

^d ANIb are ANI values based on BLAST+ calculated via the JSpeciesWS online service (22) (<https://jspecies.ribohost.com/jspeciesws/#home>).

project (BioProject accession number [PRJNA224116](#)). Reads generated with each sequencing platform, assembly statistics, annotation of the closed genomes, sequence typing, average nucleotide identity (ANI) values, and GenBank accession numbers are presented in Table 1. The hybrid assembly resulted in the formation of complete, closed circular genomes for the three isolates. However, the number of contigs for K223 is two due to the presence of a plasmid, which resulted in one closed contig for the chromosome and one closed contig for the plasmid.

It is noteworthy (as shown in Table 1) that the ANI values for two genomes (A171 and 97) versus the *O. rhinotracheale* type strain DSM 15997 (accession number [CP003283.1](#))

were above the species cutoff value of 95 to 96%, while the ANI value for genome K223 was below the species cutoff value (89.49%). These findings support the suggestion by Alispahic et al. (18) that some *O. rhinotracheale* serotypes belong to a different bacterial species.

Data availability. The genomes are available in GenBank under the accession numbers shown in Table 1.

ACKNOWLEDGMENTS

This project was supported by the Agriculture and Food Research Initiative (competitive grant 2015-68004-23131) from the U.S. Department of Agriculture, National Institute of Food and Agriculture. Bioinformatic analyses were supported with tools available from the Minnesota Supercomputing Institute of the University of Minnesota.

We thank the Iowa State University Veterinary Diagnostic Laboratory faculty and staff for assistance with sample processing and diagnostic testing.

REFERENCES

- Vandamme P, Segers P, Vancanneyt M, van Hove K, Mutters R, Hommez J, Dewhirst F, Paster B, Kersters K, Falsen E. 1994. *Ornithobacterium rhinotracheale* gen. nov., sp. nov., isolated from the avian respiratory tract. *Int J Syst Bacteriol* 44:24–37. <https://doi.org/10.1099/00207713-44-1-24>.
- Clark S, Ahlmeyer V. 2018. Current health and industry issues facing the turkey industry. In *The Proceeding of Annual meeting of the United States Animal Health Association 2018*. United States Animal Health Association, St. Joseph, MO.
- Al-Hasan BA, Alhatami AO, Abdulwahab HM, Bustani GS, Wahab Alkuwaity EA. 2011. The first isolation and detection of *Ornithobacterium rhinotracheale* from swollen head syndrome-infected broiler flocks in Iraq. *Vet World* 14:2346–2355. <https://doi.org/10.14202/vetworld.2021.2346-2355>.
- Canal CW, Leão JA, Rocha SLS, Macagnan M, Lima-Rosa CAV, Oliveira SD, Back A. 2005. Isolation and characterization of *Ornithobacterium rhinotracheale* from chickens in Brazil. *Res Vet Sci* 78:225–230. <https://doi.org/10.1016/j.rvsc.2004.10.003>.
- Chávez M, León YM, Ugalde YS, Cárdenas NB, López AM, Rivero EL, Redondo AV. 2011. Evidência sorológica, em Cuba, da circulação de *Ornithobacterium rhinotracheale* em galinhas poedeiras com síndrome respiratória crônica. In *XXII Congresso Latino-Americano de Avicultura*. Latin America Association of Poultry. <https://pt.engormix.com/avicultura/artigos/ornitobacterium-rhinotracheale-galinhas-poedeiras-t37346.htm>.
- Churria CDG, Sansalone P, Machuca M, Vigo G, Sguazza G, Origlia J, Píscopo M, Loyola MH, Petruccelli M. 2012. Tracheitis in a broiler chicken flock caused by dual infection with *Cryptosporidium* spp. (Apicomplexa: Cryptosporiidae) and non-hemolytic *Ornithobacterium rhinotracheale*. *Braz J Vet Pathol* 5:89–93.
- Ha HJ, Christensen N, Humphrey S, Haydon T, Bernardi G, Rawdon T. 2016. The first detection of *Ornithobacterium rhinotracheale* in New Zealand. *Avian Dis* 60:856–859. <https://doi.org/10.1637/11457-062116-Case>.
- Nisar M, Thieme S, Hafez HM, Senties-Cúe G, Chin RP, Muhammad SP, Aboubakr H, Goyal SM, Nagaraja KV. 2020. Genetic diversity of *Ornithobacterium rhinotracheale* isolated from chickens and turkeys in the United States. *Avian Dis* 64:324–329. <https://doi.org/10.1637/aviandiseases-D-20-0007>.
- Umali DV, Shirota K, Sasai K, Katoh H. 2018. Characterization of *Ornithobacterium rhinotracheale* from commercial layer chickens in eastern Japan. *Poult Sci* 97:24–29. <https://doi.org/10.3382/ps/pex254>.
- Van Veen L, Van Empel P, Fabri T. 2000. *Ornithobacterium rhinotracheale*, a primary pathogen in broilers. *Avian Dis* 44:896–900. <https://doi.org/10.2307/1593063>.
- Goldstein S, Beka L, Graf J, Klassen JL. 2019. Evaluation of strategies for the assembly of diverse bacterial genomes using MinION long-read sequencing. *BMC Genomics* 20:23. <https://doi.org/10.1186/s12864-018-5381-7>.
- Hashish A, Sinha A, Sato Y, Macedo NR, El-Gazzar M. 2022. Development and validation of a new TaqMan real-time PCR for the detection of *Ornithobacterium rhinotracheale*. *Microorganisms* 10:341. <https://doi.org/10.3390/microorganisms10020341>.
- Hashish A, Sinha A, Sato Y, Macedo NR, El-Gazzar M. 2022. Correction: Hashish et al. Development and validation of a new TaqMan real-time PCR for the detection of *Ornithobacterium rhinotracheale*. *Microorganisms* 2022, 10, 341. <https://doi.org/10.3390/microorganisms10050917>.
- 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>.
- De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics* 34:2666–2669. <https://doi.org/10.1093/bioinformatics/bty149>.
- 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>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Alispahic M, Endler L, Hess M, Hess C. 2021. *Ornithobacterium rhinotracheale*: MALDI-TOF MS and whole genome sequencing confirm that serotypes K, L and M deviate from well-known reference strains and numerous field isolates. *Microorganisms* 9:1006. <https://doi.org/10.3390/microorganisms9051006>.
- Schmartz GP, Hartung A, Hirsch P, Kern F, Fehlmann T, Müller R, Keller A. 2022. PLSDB: advancing a comprehensive database of bacterial plasmids. *Nucleic Acids Res* 50:D273–D278. <https://doi.org/10.1093/nar/gkab1111>.
- Thieme S, Mühldorfer K, Lüscho D, Hafez HM. 2016. Molecular characterization of the recently emerged poultry pathogen *Ornithobacterium rhinotracheale* by multilocus sequence typing. *PLoS One* 11:e0148158. <https://doi.org/10.1371/journal.pone.0148158>.
- Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 3:124. <https://doi.org/10.12688/wellcomeopenres.14826.1>.
- Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.