





Complete Genome Sequence of the Multidrug-Resistant Neonatal Meningitis *Escherichia coli* Serotype O75:H5:K1 Strain mcjchv-1 (NMEC-O75)

Daniel W. Nielsen,^a Nicole Ricker,^b Nicolle L. Barbieri,^c James L. Wynn,^d Oscar G. Gómez-Duarte,^e Junaid Iqbal,^e Lisa K. Nolan,^f Heather K. Allen,^b Catherine M. Logue^c

- ^aDepartment of Veterinary Microbiology and Preventive Medicine, Iowa State University College of Veterinary Medicine, Ames, Iowa, USA
- ^bFood Safety and Enteric Pathogens Research Unit, National Animal Disease Center, ARS-USDA, Ames, Iowa, USA
- ^cDepartment of Population Health, University of Georgia College of Veterinary Medicine, Athens, Georgia, USA
- ^dDepartment of Pediatrics and Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, Florida, USA
- eDivision of Pediatric Infectious Diseases, Department of Pediatrics, University at Buffalo, The State University of New York, Buffalo, New York, USA
- Department of Infectious Diseases, University of Georgia College of Veterinary Medicine, Athens, Georgia,

ABSTRACT Neonatal meningitis *Escherichia coli* (NMEC) is the second leading cause of neonatal bacterial meningitis worldwide. We report the genome sequence of the multidrug-resistant NMEC serotype O75:H5:K1 strain mcjchv-1, which resulted in an infant's death. The O75 serogroup is rare among NMEC isolates; therefore, this strain is considered an emergent pathogen.

A lthough neonatal meningitis *Escherichia coli* (NMEC) is the second leading cause of neonatal bacterial meningitis (NBM), after group B streptococci, NMEC is responsible for the greatest mortality in NBM cases (1–3). The NMEC serotype O75:H5:K1 strain mcjchv-1 (NMEC-O75) described here was isolated from a 30-day-old newborn with meningitis. The patient subsequently died of infection complications after 16 days of hospitalization despite appropriate antibiotic management (4). Although NMEC strains RS218 (5), CE10 (6), IHE3034 (7), NMEC O18 (8), and S88 (9) have been sequenced and are publicly available in the NCBI GenBank database, these isolates all belong to serogroups O7 and O18, and a depth of clinical information about the strains is not readily available. Here, we present the genome sequence of NMEC-O75, a clinical isolate with a previously published clinical history (4).

NMEC-O75 was grown on MacConkey agar and subsequently in Luria-Bertani broth at 37°C. Genomic DNA (gDNA) was extracted using the DNeasy blood and tissue Genomic-tip kit (Qiagen, Hilden, Germany) for Pacific Biosciences (PacBio, Menlo Park, CA) sequencing and the ChargeSwitch gDNA mini bacteria kit (Life Technologies, Carlsbad, CA) for Illumina sequencing. DNA yields were quantified using a Qubit fluorimeter double-stranded DNA (dsDNA) HS kit (Life Technologies). The Nextera Flex kit (Illumina, San Diego, CA) was used to prepare the genomic library for MiSeq sequencing, and the SMRTbell kit (Pacific Biosciences) was used to prepare the genomic library for PacBio sequencing with BluePippin (Sage Science, Beverly, MA) size selection to target a library size of 10 kb.

Genomic sequencing was performed on the PacBio (Menlo Park, CA) RS II and MiSeq (Illumina, San Diego, CA) instruments. Three single-molecule real-time

Received 27 July 2018 **Accepted** 21 August 2018 **Published** 13 September 2018

Citation Nielsen DW, Ricker N, Barbieri NL, Wynn JL, Gómez-Duarte OG, Iqbal J, Nolan LK, Allen HK, Logue CM. 2018. Complete genome sequence of the multidrug-resistant neonatal meningitis *Escherichia coli* serotype O75:H5:K1 strain mcjchv-1 (NMEC-O75). Microbiol Resour Announc 7:e01043-18. https://doi.org/10.1128/MRA.01043-18

Editor Julia Maresca, University of Delaware
Copyright © 2018 Nielsen et al. This is an
open-access article distributed under the terms
of the Creative Commons Attribution 4.0

International license.
Address correspondence to Catherine M.

Logue, Catherine.Logue@uga.edu.



(SMRT) cells were used for PacBio sequencing before the long reads were assembled with Canu version 1.5 (10). Next, the PacBio assembly was circularized in Geneious version 10.2 (11). Illumina reads that had been trimmed with Trimmomatic version 0.36 were used to further correct and polish the PacBio assembly with Pilon version 1.22 (Broad Institute) (12, 13). The trimmed Illumina reads were assembled with SPAdes (14), and potential contigs with plasmids were identified via the PlasmidFinder database (15) in ABRicate from polished PacBio and SPAdes assemblies (https://github.com/tseemann/abricate). tRNA-carrying and protein-encoding genes were assessed via Prokka version 1.13 (16). A complete workflow can be found at http://github.com/nielsend/genomeassembly.

The NMEC-O75 genome consists of a single chromosome, one large plasmid, and four small plasmids. The chromosome consists of 4,939,457 bp, with 50.6% GC content. It encodes 91 tRNAs and contains 4,593 coding sequences. The large plasmid, pNMEC-O75A, is a hybrid IncFIA/IncFIB plasmid. It consists of 88,420 bp and 50.6% GC content and contains 97 coding sequences. The four small plasmids, pNMEC-O75B, pNMEC-O75C, pNMEC-O75D, and pNMEC-O75E, range from 1,983 to 6,465 bp and have 42.6 to 56.1% GC content. Genomic comparisons of NMEC-O75 with other NMEC genomes are ongoing.

Data availability. The chromosome and plasmids have been deposited in GenBank under the accession numbers CP030111, CP030112, CP030113, CP030114, CP030115, and CP030116.

ACKNOWLEDGMENTS

We thank David Alt and Darrell Bayles (National Animal Disease Center, Ames, IA) for their assistance in sequencing and bioinformatics expertise, respectively.

This work was supported by the Dean's Office at Iowa State University's College of Veterinary Medicine and NIH grant K08GM106143.

The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

- Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, Boileau P. 2011. Neonatal bacterial meningitis: 444 cases in 7 years. Pediatr Infect Dis J 30:212–217. https://doi.org/10.1097/INF.0b013e 3181fab1e7.
- Croxen MA, Finlay BB. 2010. Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Micro 8:26–38. https://doi.org/10.1038/nrmicro 2265
- Logue CM, Doetkott C, Mangiamele P, Wannemuehler YM, Johnson TJ, Tivendale KA, Li G, Sherwood JS, Nolan LK. 2012. Genotypic and phenotypic traits that distinguish neonatal meningitis-associated *Escherichia* coli from fecal E. coli isolates of healthy human hosts. Appl Environ Microbiol 78:5824–5830. https://doi.org/10.1128/AEM.07869-11.
- Iqbal J, Dufendach KR, Wellons JC, Kuba MG, Nickols HH, Gómez-Duarte OG, Wynn JL. 2016. Lethal neonatal meningoencephalitis caused by multi-drug resistant, highly virulent *Escherichia coli*. Infect Dis 48: 461–466. https://doi.org/10.3109/23744235.2016.1144142.
- Wijetunge DSS, Karunathilake KHEM, Chaudhari A, Katani R, Dudley EG, Kapur V, DebRoy C, Kariyawasam S. 2014. Complete nucleotide sequence of pRS218, a large virulence plasmid, that augments pathogenic potential of meningitis-associated *Escherichia coli* strain RS218. BMC Microbiol 14:203. https://doi.org/10.1186/s12866-014-0203-9.
- Lu S, Zhang X, Zhu Y, Kim KS, Yang J, Jin Q. 2011. Complete genome sequence of the neonatal-meningitis-associated *Escherichia coli* strain CE10. J Bacteriol 193:7005. https://doi.org/10.1128/JB.06284-11.
- 7. Moriel DG, Bertoldi I, Spagnuolo A, Marchi S, Rosini R, Nesta B, Pastorello I, Corea VAM, Torricelli G, Cartocci E, Savino S, Scarselli M, Dobrindt U, Hacker J, Tettelin H, Tallon LJ, Sullivan S, Wieler LH, Ewers C, Pickard D, Dougan G, Fontana MR, Rappuoli R, Pizza M, Serino L. 2010. Identification of protective and broadly conserved vaccine antigens from the genome of extraintestinal pathogenic Escherichia

- coli. Proc Natl Acad Sci U S A 107:9072–9077. https://doi.org/10.1073/pnas.0915077107.
- Nicholson BA, Wannemuehler YM, Logue CM, Li G, Nolan LK. 2016. Complete genome sequence of the neonatal meningitis-causing *Escherichia coli* strain NMEC O18. Genome Announc 4:e01239-16. https://doi.org/10.1128/genomeA.01239-16.
- Peigne C, Bidet P, Mahjoub-Messai F, Plainvert C, Barbe V, Medigue C, Frapy E, Nassif X, Denamur E, Bingen E, Bonacorsi S. 2009. The plasmid of *Escherichia coli* strain S88 (O45:K1:H7) that causes neonatal meningitis is closely related to avian pathogenic *E. coli* plasmids and is associated with high-level bacteremia in a neonatal rat meningitis model. Infect Immun 77:2272–2284. https://doi.org/10.1128/IAI.01333-08.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017.
 Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/ 10.1093/bioinformatics/bts199.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal .pone.0112963.
- 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.

Volume 7 Issue 10 e01043-18



- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- 15. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa
- L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

Volume 7 Issue 10 e01043-18 mra.asm.org **3**