



Draft Genome Sequences for a Diverse Set of Isolates from 10 *Neisseria* Species

Megan Nichols,^a Nadav Topaz,^b Xiong Wang,^{a,c} Xin Wang,^b Dave Boxrud^a

^aMinnesota Department of Health Public Health Laboratory, St. Paul, Minnesota, USA

^bMeningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

^cDepartment of Veterinary Biomedical Sciences, University of Minnesota, St. Paul, Minnesota, USA

ABSTRACT Neisseria is a diverse genus that includes commensal and pathogenic species that pose a public health threat. While the pathogenic species have been studied extensively, many of the commensals have limited genomic information available. Here, we present draft genome sequences for a diverse set of 37 isolates from 10 *Neisseria* species.

Neisseria is a broad and genetically diverse genus consisting of Gram-negative betaproteobacteria that make up a large part of the normal flora in humans and animals, colonizing the mucosal membranes of the upper respiratory, gastrointestinal, and genitourinary tracts (1). The genus is largely composed of commensal species but also includes several rare zoonotic pathogens, as well as two important human pathogens, *N. meningitidis* and *N. gonorrhoeae*. These two species are of considerable significance due to their ability to cause serious illness in humans, such as meningitis, sepsis, and gonococcal infections (2). Additionally, several rare zoonotic pathogens, such as *N. animaloris*, have been associated with human wound infections from dog and cat bites (1). While the pathogenic species have been studied extensively and have a large collection of available genomic data, the commensal species have been studied far less extensively, and many only have limited available genomic information. To supplement the existing *Neisseria* genomic collection, we present genomic data for isolates from 10 *Neisseria* species: *N. animaloris*, *N. bergeri*, *N. cinerea*, *N. elongata*, *N. mucosa*, *N. polysaccharea*, *N. sicca*, *N. subflava*, *N. wadsworthii*, and *N. weaveri*.

Bacteria were isolated from clinical specimens collected in Minnesota from 2004 to 2015 and cultivated on tryptic soy blood agar. The cultures were incubated for 24 to 48 hours at 35°C and 5% CO₂. Bacterial DNA was extracted using the QIAamp DNA blood minikit on the Qiagen QIAcube, according to the manufacturer's guidelines, and DNA concentrations were quantitated using the Qubit double-stranded-DNA (dsDNA) high-sensitivity (HS) assay kit (Thermo Fisher Scientific). Samples were prepared for whole-genome sequencing according to the Nextera XT DNA library preparation protocol and manufacturer's (Illumina) guidelines. Barcoded libraries were then pooled and loaded onto the Illumina MiSeq platform using 500-cycle V2 chemistries for multiplexed 250-bp paired-end sequencing. The Illumina reads were then trimmed using cutadapt 1.8 (3) and assembled using SPAdes 3.10.1 (4).

In both clinical and research settings, it is essential to accurately identify the pathogenic *Neisseria* species in order to effectively provide treatment. However, *Neisseria* spp. have historically been characterized by conventional identification methods, which has left them prone to species misidentification, as not all members of the genus are easily and clearly distinguishable by their phenotypic and biochemical properties alone (5). The high rate of horizontal gene transfer that occurs within and between

Received 16 April 2018 Accepted 17 April 2018 Published 17 May 2018

Citation Nichols M, Topaz N, Wang X, Wang X, Boxrud D. 2018. Draft genome sequences for a diverse set of isolates from 10 *Neisseria* species. Genome Announc 6:e00409-18. https://doi .org/10.1128/genomeA.00409-18.

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Dave Boxrud, dave.boxrud@state.mn.us.

TABLE 1 Metadata and GenBank accession numbers for draft genome assemblies reported in this study
--

lsolate name	GenBank accession no.	Species	Collection yr	Isolation source	N ₅₀ (bp)	Avg coverage (×)
C2015003240	POYC0000000	N. animaloris	2015	Swab	307,764	24
C2014021188	POYB0000000	N. subflava	2014	Bronchial wash	343,438	22
C2014019557	POYA0000000	N. cinerea	2014	Urine	252,188	12
C2014013859	POXZ0000000	N. cinerea	2014	Blood	366,008	18
C2014003241	POXY0000000	N. elongata	2014	Abdominal wound	110,032	20
C2014002478	POXX0000000	N. sicca	2014	Eye	51,187	17
C2013024221	POXW0000000	N. cinerea	2013	Blood	207,528	14
C2013018262	POXV0000000	N. elongata	2013	Endotracheal tube	92,876	10
C2013013825	POXU0000000	N. weaveri	2013	Leg wound	207,454	18
C2013011231	POXT0000000	N. polysaccharea	2013	Throat swab	131,026	23
C2013010062	POXS0000000	N. elongata	2013	Endotracheal tube	98,407	35
C2012029644	POXR0000000	N. animaloris	2012	Swab	684,286	23
C2012028592	POXQ0000000	N. cinerea	2012	Sputum	285,815	31
C2012011976	POXP0000000	N. subflava	2012	Sputum	1,167,507	42
C2011033015	POXO0000000	N. subflava	2011	Blood	231,464	26
C2011020199	POXN0000000	N. subflava	2011	Sputum	264,440	17
C2011020198	POXM0000000	N. subflava	2011	Sputum	342,535	47
C2011009653	POXL0000000	N. subflava	2011	Urine	217,621	16
C2011004960	POXK0000000	N. subflava	2011	Sputum	378,795	19
C2011003085	POXJ0000000	N. elongata	2011	Swab	116,473	32
C2010015191	POXI0000000	N. weaveri	2010	Hand	290,757	62
C2010010207	POXH0000000	N. elongata	2010	Peritoneal fluid	123,763	19
C2010005502	POXG0000000	N. sicca	2010	Sputum	553,284	23
C2009035459	POXF0000000	N. weaveri	2009	Leg tissue	203,342	67
C2009028987	POXE0000000	N. weaveri	2009	Wound	165,690	31
C2009010520	POXD0000000	N. subflava	2009	Blood	380,740	49
C2008002238	POXC0000000	N. subflava	2008	Knee	222,323	35
C2008001664	POXB0000000	N. subflava	2008	Jejunostomy	131,757	65
C2008000421	POXA0000000	N. wadsworthii	2008	Finger	83,929	25
C2008000329	POWZ0000000	N. bergeri	2008	Meninges	135,935	27
C2008000328	POWY0000000	N. bergeri	2008	Lung	124,886	36
C2008000159	POWX0000000	N. mucosa	2008	CSF ^a	123,178	31
C2007003584	POWW0000000	N. sicca	2007	Blood	211,701	35
C2007002879	POWV0000000	N. subflava	2007	Sputum	209,740	36
C2006001571	PQVC0000000	N. sicca	2006	Blood	62,634	27
C2005001510	POWU0000000	N. subflava	2005	Sinus	295,777	29
C2004002444	POWT0000000	N. mucosa	2004	Eye	46,652	17

^aCSF, cerebrospinal fluid.

species due to the cocolonization of pathogens and commensals in the body (2) has also contributed to species misidentification and taxonomic confusion. Alternatively, the use of ribosomal multilocus sequence typing (rMLST) has been shown to be effective at delineating between species (6) and has led to the discussion of species reclassification within the genus. Of particular interest is the proposed reclassification of the most diverse variant of *N. polysaccharea* as a separate species, with the suggested name *N. bergeri* (3). The genomic sequences for isolates from 10 *Neisseria* species in this study will provide data for future studies examining species delineation among *Neisseria* spp., especially for those that previously had limited available genomic information.

Accession number(s). The draft genome sequences have been deposited in GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

The Minnesota Department of Health received support by appointment to the Research Participation Program at the Center for Food Safety and Applied Nutrition, U.S. Food & Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food & Drug Administration. This study was also supported by the CDC Advanced Molecular Detection Initiative (AMD-76).

REFERENCES

- Liu G, Tang CM, Exley RM. 2015. Non-pathogenic *Neisseria*: members of an abundant, multi-habitat, diverse genus. Microbiology 161:1297–1312. https://doi.org/10.1099/mic.0.000086.
- Marri PR, Paniscus M, Weyand NJ, Rendón MA, Calton CM, Hernández DR, Higashi DL, Sodergren E, Weinstock GM, Rounsley SD, So M. 2010. Genome sequencing reveals widespread virulence gene exchange among human *Neisseria* species. PLoS One 5:e11835. https://doi.org/10.1371/ journal.pone.0011835.
- 3. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12.
- 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.

- Bennett JS, Jolley KA, Earle SG, Corton C, Bentley SD, Parkhill J, Maiden MCJ. 2012. A genomic approach to bacterial taxonomy: an examination and proposed reclassification of species within the genus *Neisseria*. Microbiology 158:1570–1580. https://doi.org/10.1099/mic.0.056077-0.
- 6. Bennett JS, Watkins ER, Jolley KA, Harrison OB, Maiden MCJ. 2014. Identifying *Neisseria* species by use of the 50S ribosomal protein L6 (*rplF*) gene. J Clin Microbiol 52:1375–1381. https://doi.org/10.1128/ JCM.03529-13.