Draft Genome Sequence of an Isolate of Nontypeable *Haemophilus influenzae* from an Acute Exacerbation of Chronic Obstructive Pulmonary Disease in Tasmania

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**ABSTRACT** Nontypeable *Haemophilus influenzae* (NTHi) is an important cause of human illness, including pneumonia and acute exacerbations of chronic obstructive pulmonary disease (COPD). We report here the draft genome of an isolate of NTHi collected from the sputum of a patient presenting with COPD in Tasmania, Australia.

Chronic obstructive pulmonary disease (COPD) is a serious, progressive condition characterized by a persistent reduction in lung airflow (1). It has emerged as the third leading cause of mortality, claiming more than 3 million lives worldwide in 2016 (2). In Australia, it is estimated that COPD affects 1.45 million people (3). COPD is an important disease in the state of Tasmania, where higher rates of smoking are observed with respect to the national rate (4). Nontypeable *Haemophilus influenzae* (NTHi) is a key pathogen that colonizes damaged airways in COPD patients and causes acute exacerbations that contribute to morbidity and mortality (5–7). Here, we present the draft assembled genome sequence of an NTHi strain isolated from a case of COPD in Tasmania.

NTHi strain RHH-38 was isolated in 2018 at the Royal Hobart Hospital in Tasmania from the sputum of a COPD patient presenting with an acute exacerbation. The sputum specimen was homogenized and cultured on chocolate blood agar plates at 35°C in a CO₂ atmosphere followed by storage at 2 to 8°C. Bacterial identification was performed using a Bruker matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometer. A single colony of *Haemophilus influenzae* was suspended in 200 μl phosphate-buffered saline (PBS), and then genomic DNA was extracted using the DNeasy blood and tissue kit (catalog number 69504; Qiagen, USA), and then genomic DNA was further purified using the High Pure PCR template preparation kit (catalog number 11796828001; Roche, Germany). DNA library preparation was carried out using a Nextera XT DNA library preparation kit (catalog number FC-131-1024; Illumina, USA) as described previously (8, 9). Sequencing was performed using an Illumina MiSeq platform with 150-bp paired-end sequencing. In total, 1,161,034 paired-end reads were generated, representing an average read depth of 88.83-fold. Reads were trimmed of adapters using Trimmomatic (10), and de novo assembly of reads was performed with SPAdes v3.12.0 (11). All parameters were set to default except for the size of k-mers, which were manually set to 21, 33, 43, 53, 63, and 75. This resulted in the generation of...
and a 1,914,787-bp draft genome consisting of 68 contigs (\(\geq 200\) bp) that covered 86.7% of the reference *H. influenzae* 86-028NP genome (12). The \(N_{50}\) value was 66,703 bp, and the overall GC content was 38.1%. The genome assembly quality, including completeness with respect to the reference genome, was determined using the QUAST quality assessment tool (13).

In *silico* MLST analysis, performed by submission of the draft genome to the *H. influenzae* multilocus sequence typing (MLST) website (https://pubmlst.org/hinfluenzae/) (14), assigned RH-38 to sequence type 422 (ST422) based on seven housekeeping genes. The draft genome was annotated using RASTtk (15–17), which identified a total of 2,019 genes consisting of 1,960 coding sequences and 59 RNA genes. Default parameters were used for all software unless otherwise specified.

In conclusion, this study presents the published genome sequence assembly of an NTHi isolate from a case of COPD in Tasmania. The application of genome sequencing has the potential to provide insights into recurrent exacerbations of COPD due to NTHi and the ability to distinguish between relapse and reinfection.

This work was conducted in accordance with ethics approval number H0016214 from the Tasmanian Health and Medical Human Research Ethics Committee.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JAAECN0000000000. The version described in this paper is version JAAECN0100000000. The associated BioProject and BioSample accession numbers are PRJNA603840 and SAMN13942196, respectively.

**ACKNOWLEDGMENT**

R. KC is the recipient of a Health Tasmania Graduate Research Scholarship from the University of Tasmania.

**REFERENCES**

1. Petskon EL, Hnizdo E, Attfield M. 2007. Definition of COPD GOLD stage I. Thorax 62:1107–1108.
2. World Health Organization. 2017. Disease burden and mortality estimates: specific-cases mortality, 2000–2016. World Health Organization, Geneva, Switzerland. http://www.who.int/healthinfo/global_burden_disease/estimates/en/.
3. Toelle BG, Xuan W, Bird TE, Abramson MJ, Atkinson DN, Burton DL, James AL, Jenkins CR, Johns DP, Maguire GP, Musk AW, Walters EH, Wood-Baker R, Hunter ML, Graham BJ, Southwell PJ, Vollmer WM, Buist AS, Marks GB. 2013. Respiratory symptoms and illness in older Australians: the Burden of Obstructive Lung Disease (BOLD) study. Med J Aust 198:144–148. https://doi.org/10.5694/mja11.11640.
4. Australian Bureau of Statistics. 2019. Higher rates of chronic health conditions in Tasmania. Australian Bureau of Statistics. https://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.5.55.001-2017-18-Media%20Release-Higher%20rates%20of%20chronic%20health%20conditions%20in%20Tasmania%20(Media%20Release)--10015.
5. Sethi S, Murphy TF. 2001. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. Clin Microbiol Rev 14:336–363. https://doi.org/10.1128/CMR.14.2.336-363.2001.
6. Sethi S, Evans N, Grant BJ, Murphy TF. 2002. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 347:465–471. https://doi.org/10.1056/NEJMoa012561.
7. Hillas G, Perlikos F, Tzanakis N. 2016. Acute exacerbation of COPD: is it the “stroke of the lungs”? Int J Chron Obstruct Pulmon Dis 11: 1579–1586. https://doi.org/10.2147/COPD.S106160.
8. Gautam SS, Kc R, Leong KW, Mac Aogáin M, O’Toole RF. 2019. A step-by-step beginner’s protocol for whole genome sequencing of human bacterial pathogens. J Biol Methods 6:e110. https://doi.org/10.14400/jbm.2019.276.
9. Gautam SS, Mac Aogáin M, Cooley LA, Haug G, Fyfe JA, Globan M, O’Toole RF. 2018. Molecular epidemiology of tuberculosis in Tasmania and genomic characterization of its first known multidrug-resistant case. PLoS One 13:e0192351. https://doi.org/10.1371/journal.pone.0192351.
10. 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.
11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulasov AS, Lesin VM, Klenk EN, Pham S, Prilipetsky E, Pyshkin 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.
12. Harrison A, Dyer DW, Gillaspay A, Ray WC, Mungur R, Carson MB, Zhong H, Gipson J, Gipson M, Johnson LS, Lewis L, Bakaletz LQ, Munson RS, Jr. 2005. Genomic sequence of an otitis media isolate of nontypeable Haemophilus influenzae: comparative study with *H. influenzae* serotype d, strain KW20. J Bacteriol 187:4627–4636. https://doi.org/10.1128/JB.187.13.4627-4636.2005.
13. 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.
14. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BigGdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124. https://doi.org/10.12688/wellcomeopenres.14826.1.
15. Aziz RK, Bartels D, Best AA, DeLongh M, Disz T, Edwards RA, Formisano K, Gerdes S, Glass EM, Kulab M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Pascian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
16. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek RA, McNeil LK, Paarmann D, Pascian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
17. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek RA, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
18. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.