

Complete Genome Sequence of Encapsulated *Haemophilus influenzae* Type f KR494, an Invasive Isolate That Caused Necrotizing Myositis

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***Haemophilus influenzae* serotype f (Hif) is an etiologic agent of bacterial invasive disease. Here, we report the first annotated genome sequence of the Hif strain KR494, which was isolated from a patient suffering from sepsis and necrotizing myositis. The genome sequence will increase the understanding of Hif pathogenesis.**

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Haemophilus influenzae is a pathogen restricted to humans, and it resides in the upper respiratory tract, causing airway infections and, occasionally, invasive disease. Most systemic *Haemophilus* infections are associated with encapsulated strains, and *H. influenzae* serotype b (Hib) has been the most invasive serotype. However, since the introduction of a vaccine against Hib in the 1990s, serotype f (Hif) is now the most common invasive encapsulated *Haemophilus* species (1, 2). Hence, the clinical importance of Hif is emerging (3–6). The genomes of Hib strain 10810, *H. influenzae* serotype d (Hid) strains Rd Kw20 and Aw, and nontypeable *H. influenzae* (NTHi) strains were previously sequenced and annotated (7, 8). Here, we report the first fully annotated genome sequence of a Hif strain (KR494). Hif KR494 was recently isolated and caused necrotizing myositis in a previously healthy 70-year-old male (4). Hif KR494 possesses several virulence traits, as the isolate is serum resistant and adheres to and readily invades epithelial cells (4).

The draft genome sequence of Hif KR494 was obtained using Illumina 100-bp paired-end technology (HiSeq 2000; Illumina, CA). The total 8,888,894 reads (~400× coverage) were assembled using SOAPdenovo 1.05 (9), resulting in 272 contigs that were further combined into 12 scaffolds (frame sequence). Closely related reference sequences (Hib 10810 [GenBank accession no. NC_016809] and Hid Rd Kw20 [GenBank accession no. NC_000907]) were used to complete the assembly through the alignment between the reference and frame sequences. PCR gap closure was performed for highly complex regions. Gap filling and single-base proofreading of the assembly results were performed by SOAPaligner/soap2 (9). Finally, a complete genome sequence map containing 1,856,176 bp was achieved. The *de novo* gene prediction was conducted with Glimmer 3.02 (10). Functional annotation was accomplished by BLAST analysis of the predicted genes with the Kyoto Encyclopedia of Genes and Genomes (11), Clusters of Orthologous Groups (12), Swiss-Prot, TrEMBL (13), NCBI (14), and Gene Ontology (15) databases. The rRNA, tRNA, and small RNA (sRNA) genes were predicted using RNAmmer (16), tRNAscan (17), and Rfam (18), respectively.

The Hif KR494 genome consists of only chromosomal DNA without any plasmids. The average G+C content of the genome is 38.05% (38.68% in the genes and 33.18% in the intergenic regions). The genome is composed of 1,742 putative protein-coding genes with an average length of 913 bp. The Hif genome contains 6 rRNA operons and 58 tRNAs. The majority of the annotated genes are involved in amino acid metabolism (9.6%) and protein translation (9.0%). Genes were found for most of the major known *Haemophilus*-related virulence factors, i.e., *Haemophilus* adhesion and penetration protein (Hap), opacity-associated protein A (OopA), IgA1 protease, *Haemophilus* surface fibril (Hsf), proteins D, E, and F, and genotype IIIb fimbriae (19–24). The gene locus *hmmw1A1B*, encoding high-molecular-weight adhesion protein, and common *Haemophilus* antibiotic resistance genes (*bla*, *cat*, and *tet*) were absent. More detailed characterizations of the KR494 genome, including pangenomic analysis with other *H. influenzae* serotypes and the identification of Hif exclusive genomic features, are currently in progress.

Nucleotide sequence accession number. The complete genome sequence of *H. influenzae* KR494 has been deposited in GenBank under the accession no. CP005967.

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REFERENCES

1. Agrawal A, Murphy TF. 2011. *Haemophilus influenzae* infections in the *H. influenzae* type b conjugate vaccine era. *J. Clin. Microbiol.* 49:3728–3732.
2. Ladhani SN, Collins S, Vickers A, Litt DJ, Crawford C, Ramsay ME, Slack MP. 2012. Invasive *Haemophilus influenzae* serotype e and f disease, England and Wales. *Emerg. Infect. Dis.* 18:725–732.
3. Hagiya H, Murase T, Naito H, Hagioka S, Morimoto N. 2012. Severe soft tissue infection of the lower extremity caused by *Haemophilus influ-*

- enzae* (serotype f, biotype II) in an adult patient. *Intern. Med.* 51: 1783–1787.
4. Resman F, Svensjö T, Ünal C, Cronqvist J, Brorson H, Odenholt I, Riesbeck K. 2011. Necrotizing myositis and septic shock caused by *Haemophilus influenzae* type f in a previously healthy man diagnosed with an IgG3 and a mannose-binding lectin deficiency. *Scand. J. Infect. Dis.* 43: 972–976.
 5. Suarez CJ, Glover WA, Cowan J, Smith A, Clarridge JE III. 2013. Mycotic aneurysm of the abdominal aorta caused by *Haemophilus influenzae* type f. *J. Med. Microbiol.* 62:658–660.
 6. Ronit A, Berg RM, Bruunsgaard H, Plovsing RR. 2013. *Haemophilus influenzae* type f meningitis in a previously healthy boy. *BMJ Case Rep.* pii:bcr2013008854. doi:10.1136/bcr-2013-008854.
 7. Hogg JS, Hu FZ, Janto B, Boissy R, Hayes J, Keefe R, Post JC, Ehrlich GD. 2007. Characterization and modeling of the *Haemophilus influenzae* core and supragenomes based on the complete genomic sequences of Rd and 12 clinical nontypeable strains. *Genome Biol.* 8:R103. doi:10.1186/gb-2007-8-6-r103.
 8. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM, McKenney K, Sutton G, FitzHugh W, Fields C, Gocyne JD, Scott J, Shirley R, Liu L, Glodek A, Kelley JM, Weidman JF, Phillips CA, Spriggs T, Hedblom E, Cotton MD, Utterback TR, Hanna MC, Nguyen DT, Saudek DM, Brandon RC, Fine LD, Fritchman JL, Fuhrmann JL, Geoghagen NSM, Gnehm CL, McDonald LA, Small KV, Fraser CM, Smith HO, Venter JC. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 269:496–512.
 9. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. *GigaScience.* 1:18. doi:10.1186/2047-217X-1-18.
 10. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
 11. Kanehisa M, Goto S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* 28:27–30.
 12. Tatusov RL, Koonin EV, Lipman DJ. 1997. A genomic perspective on protein families. *Science* 278:631–637.
 13. Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M. 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Res.* 31:365–370.
 14. Maglott D, Ostell J, Pruitt KD, Tatusova T. 2011. Entrez gene: gene-centered information at NCBI. *Nucleic Acids Res.* 39:D52–D57. doi:10.1093/nar/gkq1237.
 15. Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, Eilbeck K, Lewis S, Marshall B, Mungall C, Richter J, Rubin GM, Blake JA, Bult C, Dolan M, Drabkin H, Eppig JT, Hill DP, Ni L, Ringwald M, Balakrishnan R, Cherry JM, Christie KR, Costanzo MC, Dwight SS, Engel S, Fisk DG, Hirschman JE, Hong EL, Nash RS, Sethuraman A, Theesfeld CL, Botstein D, Dolinski K, Feierbach B, Berardini T, Mundodi S, Rhee SY, Apweiler R, Barrell D, Camon E, Dimmer E, Lee V, Chisholm R, Gaudet P, Kibbe W, Kishore R, Schwarz EM, Sternberg P, Gwinn M, et al. 2004. The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res.* 32:D258–D261. doi:10.1093/nar/gkh036.
 16. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.
 17. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25: 955–964.
 18. Burge SW, Daub J, Eberhardt R, Tate J, Barquist L, Nawrocki EP, Eddy SR, Gardner PP, Bateman A. 2013. Rfam 11.0: 10 years of RNA families. *Nucleic Acids Res.* 41:D226–D232. doi:10.1093/nar/gks1005.
 19. Mhlanga-Mutangadura T, Morlin G, Smith AL, Eisenstark A, Golomb M. 1998. Evolution of the major pilus gene cluster of *Haemophilus influenzae*. *J. Bacteriol.* 180:4693–4703.
 20. Rodriguez CA, Avadhanula V, Buscher A, Smith AL, St. Geme JW, III, Adderson EE. 2003. Prevalence and distribution of adhesins in invasive non-type b encapsulated *Haemophilus influenzae*. *Infect. Immun.* 71: 1635–1642.
 21. Watson ME, Jr, Nelson KL, Nguyen V, Burnham CA, Clarridge JE, Qin X, Smith AL. 2013. Adhesin genes and serum resistance in *Haemophilus influenzae* type f isolates. *J. Med. Microbiol.* 62:514–524.
 22. Su YC, Jalalvand F, Mörgelin M, Blom AM, Singh B, Riesbeck K. 2013. *Haemophilus influenzae* acquires vitronectin via the ubiquitous protein F to subvert host innate immunity. *Mol. Microbiol.* 87:1245–1266.
 23. Hallström T, Blom AM, Zipfel PF, Riesbeck K. 2009. Nontypeable *Haemophilus influenzae* protein E binds vitronectin and is important for serum resistance. *J. Immunol.* 183:2593–2601.
 24. Foxwell AR, Kyd JM, Cripps AW. 1998. Nontypeable *Haemophilus influenzae*: pathogenesis and prevention. *Microbiol. Mol. Biol. Rev.* 62: 294–308.