

Draft Genome Sequences of Six Nontypeable *Haemophilus influenzae* Strains That Establish Bacteremia in the Infant Rat Model of Invasive Disease

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***Haemophilus influenzae* is an important cause of invasive disease. The infant rat is the accepted model of invasive *H. influenzae* disease. Here, we report the genome sequences of six nontypeable *H. influenzae* strains that establish bacteremia in the infant rat.**

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Haemophilus influenzae colonizes the human nasopharynx and is an opportunistic pathogen causing significant human disease. *H. influenzae* causes both mucosal and invasive infections, including otitis media, exacerbations of chronic obstructive pulmonary disease, and bacteremia (1–3). Vaccines directed at the *H. influenzae* type b capsule have significantly reduced invasive disease caused by such strains (3), and the majority of *H. influenzae* invasive disease is now caused by nontypeable strains (1). The infant rat is a widely used model of invasive *H. influenzae* disease (4, 5). *H. influenzae* type b strains readily establish bacteremia and meningitis in this model when infected via either the intraperitoneal or nasopharyngeal route (6). However, nontypeable *H. influenzae* (NTHi) strains less reliably establish and maintain bacteremia in this model. We have previously used the sequenced blood isolate R2866 in the rat model (7). An infective dose of 10⁵ CFU R2866 reproducibly establishes durable bacteremia. However, in our hands, other well-characterized NTHi strains isolated from the nasopharynx (strain 86-028NP) and middle ear (strain R2846) do not establish bacteremia, even at infective doses up to 10⁶ CFU. For ongoing studies on NTHi virulence we required additional strains able to establish bacteremia in the infant rat. We tested 14 NTHi isolates, all isolated from blood, CSF, or brain abscess, for their ability to establish bacteremia in the infant rat. Six strains were able to reproducibly establish bacteremia in the infant rat, while the rest were unable to do so regardless of dose.

We performed whole-genome sequencing on the six isolates that established bacteremia. NTHi samples were prepared using 50 ng of total genomic DNA according to Nextera DNA library kit protocols (Illumina, Inc). Samples were indexed according to standard protocols so that they could be pooled together and sequenced simultaneously in a single run on the Illumina MiSeq platform using paired-end 150-bp (strains HI1974, HI2114, and HI2116) or paired-end 250-bp (strains HI1980, HI1988, and HI2004) chemistry. Prior to sequencing, each library was run individually on the Agilent High Sensitivity DNA chip to confirm library quality and average insert size. Samples were pooled in equimolar amounts, and 8 pM of the pool was run on the sequencer. Per Illumina's recommendation, phiX control was spiked into the library pool prior to loading for quality control purposes. For the 300-cycle runs 5 to 10% phiX was used, and for the 500-cycle runs 1% phiX was used per Illumina protocols. A total of thirty to forty million reads were collected for each run. Raw sequence data were *de novo* assembled and aligned to a reference isolate using CLC Genomics Workbench (CLC Bio) to identify single-nucleotide polymorphisms as well as regions with insertions or deletions (indels). Sequencing results are summarized in Table 1. The sequences reported herein will facilitate further *in vivo* studies of invasive NTHi disease and virulence determinants.

TABLE 1 Summary of genome sequences for six invasive NTHi isolates

Strain name	Source	BioSample no.	GenBank accession no.	Genome coverage (×)	No. of contigs	G+C content (%)	Genome size (Mb)	No. of genes
HI1974	Blood	SAMN03702706	LFFT00000000	348	41	37.9	1.77	1,739
HI1980	Blood	SAMN03702713	LFFO00000000	1,088	20	37.8	1.76	1,712
HI1988	Blood	SAMN03702714	FFFN00000000	978	18	37.9	1.84	1,814
HI2004	Blood	SAMN03702711	LFQ00000000	1,052	21	37.9	1.77	1,736
HI2114	Blood	SAMN03702707	LFRR00000000	816	75	37.9	1.89	1,865
HI2116	Blood	SAMN03702708	LFSS00000000	387	34	38.0	1.83	1,793

Nucleotide sequence accession numbers. Genome sequences for the six strains described herein have been deposited in GenBank under the accession numbers listed in [Table 1](#).

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