

An Invasive *Haemophilus haemolyticus* Isolate

Haemophilus haemolyticus is generally considered a human commensal and is found in the nasopharynx of a minority of individuals as well as in subgingival plaque (7). Most authors consider *H. haemolyticus* to be either nonpathogenic (2, 4, 7, 18) or a rare pathogen (1). The latter assessment appears to be based on two reports of endocarditis from the early part of the 20th century (3, 13); however, in neither case were requirements for the X and V factors reported, and thus, no species designation can be reliably made.

The only method of differentiation between *Haemophilus influenzae* and *H. haemolyticus* in clinical microbiology laboratories is based on the ability of *H. haemolyticus* to produce a zone of clear hemolysis on ovine, bovine, or equine blood agar plates (1, 6, 7, 9). However, the hemolytic activity may be lost on subculture (7), and it was recently shown that many isolates of *H. haemolyticus* are nonhemolytic even on primary isolation (11, 14).

Invasive *H. influenzae* disease is a reportable condition in Oklahoma, and all *H. influenzae* isolates from normally sterile sites are required to be submitted to the Oklahoma State Department of Health (OSDH) Public Health Laboratory (PHL) for confirmation and serotyping. For use in our ongoing studies of *H. influenzae* iron/heme acquisition, we obtained from the OSDH PHL all invasive *H. influenzae* isolates from 2003 and 2004. Seven of these isolates were subjected to multilocus sequence typing (MLST) as described by Meats et al. (12). We were consistently unable to amplify the gene fragment for the *fucK* gene from one strain, designated HI2028 and isolated from the blood of a 1-year-old male. Failure to amplify the *fucK* gene fragment from presumptive *H. influenzae* isolates has been considered an indicator of a misidentified strain (15). However, failure to detect the *fucK* gene cannot be considered conclusive since some strains of *H. influenzae* have recently been shown to lack the fucose operon (17, 19).

Based on the failure to amplify the *fucK* gene product, we considered that strain HI2028 may have been misidentified as *H. influenzae* and thus we proceeded to amplify 1,491 bp of the 16S rRNA gene from HI2028 using the primers UFPL and URPL (10). PCR amplicons were directly sequenced and yielded 1,448 bp of the 16S rRNA gene. Homology searches against the nucleotide collection using the MEGABLAST algorithm (<http://blast.ncbi.nlm.nih.gov>) with the determined 1,448 bp of the 16S rRNA gene from HI2028 indicated that the strain was *H. haemolyticus* but cannot be considered a definitive identification of the isolate as such.

A multilocus sequence analysis system based on 5 gene loci (*adk*, *pgi*, *recA*, *infB*, and 16S rRNA) has been described to separate *H. haemolyticus* from *H. influenzae* (11, 18). The sequences of these genes from HI2028 were determined and concatenated as described by McCrea et al. (11). The partial gene sequences from the 200 *Haemophilus* strains in the original study (11) were retrieved and concatenated. A bootstrap consensus tree using 1,000 replicates was constructed, rooted to *Escherichia coli*, using the neighbor-joining method in MEGA 5.05 (20). The strain HI2028 clustered with strains designated *H. haemolyticus* (data not shown). Additionally, the 5 gene sequences were retrieved for 14 available *H. influenzae* genomic sequences, for 5 recently published *H. haemolyticus* genomic sequences (5), and for the *H. haemolyticus* type strain ATCC 33390. Figure 1 shows strain HI2028

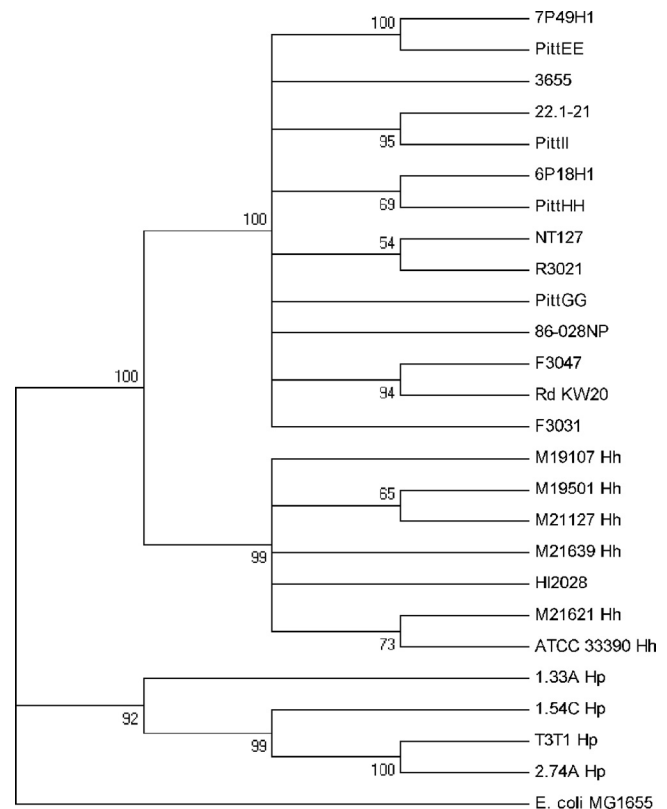


FIG 1 Neighbor-joining dendrogram of *Haemophilus* strains. The tree, rooted to *E. coli* strain MG1655, is based on concatenated *adk*, *pgi*, *recA*, *infB*, and 16S rRNA gene sequences, with bootstrap values of greater than 50% of 1,000 bootstraps indicated. Strains are genome-sequenced *H. influenzae* strains unless indicated otherwise. Hh, *H. haemolyticus* strains, including the type strain ATCC 33390 and five recently sequenced isolates (5); Hp, *H. parainfluenzae* strains. HI2028 is the strain identified in this report.

clustering with the *H. haemolyticus* strains in a neighbor-joining tree generated as described above. Additional characteristics of HI2028 are shown in Table 1.

In summary, we have identified a strain of nonhemolytic *H. haemolyticus*, originally designated *H. influenzae*, from a patient with bacteremia. In reporting the *H. haemolyticus* genomic sequence, Jordan et al. stated that several presumptive *H. influenzae* isolates from invasive disease had been confirmed as *H. haemolyticus*, although no data were presented (5). Our finding supports the assertion of Jordan et al. and suggests that *H. haemolyticus* may occasionally be found among presumed *H. influenzae* clinical isolates and should be considered a more frequent cause of invasive disease than currently thought. Such

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TABLE 1 Characteristics of strain HI2028 in comparison to *H. haemolyticus*, *H. influenzae*, and the Biotype IV cryptic genospecies of *H. influenzae*

Characteristic	HI2028	<i>H. haemolyticus</i>	<i>H. influenzae</i>	Biotype IV cryptic genospecies ^a
X factor required	+	+	+	+
V factor required	+	+	+	+
H ₂ S production ^b	+	+	V	V
Indole production ^b	+	V ^c	V	–
Urease activity ^b	+	+	V	+
Ornithine ^b decarboxylase activity	–	–	V	+
Hemolysis ^d	–	V	–	–
Protein P6 amino acids at residues 33, 42, 59, 61, and 152 ^e	G, S, N, E, A	G, S, N, E, A	A, A, D, T, A	G, S, N, E, S

^a The cryptic Biotype IV genospecies causes urogenital and neonatal infections (16) and is closely related to *H. haemolyticus* (14).

^b Phenotype was determined as described by Kilian (6).

^c V, results are variable among strains (7).

^d Lack of hemolysis with strain HI2028 was determined on 5% defibrinated horse blood using BBL haemophilus identification quad plates (BD).

^e The amino acids at specific residues within the P6 protein have been used to differentiate *H. influenzae* and *H. haemolyticus* (14).

misidentification of *H. haemolyticus* strains as *H. influenzae* may potentially lead to errors in national burden of invasive *H. influenzae* disease estimates as is the case with other clinical microbiology laboratory errors (8).

Nucleotide sequence accession numbers. The partial gene sequences for *adk*, *pgi*, *recA*, *infB*, and the 16S rRNA from HI2028 have been deposited in GeneBank with the respective accession numbers JQ254883 to JQ254887. The GeneBank accession number for the gene encoding the P6 protein of HI2028 is JQ247079.

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