Identification and Characterization of “*Haemophilus quentini*” Strains Causing Invasive Disease in Ontario, Canada (2016 to 2018)

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ABSTRACT *Haemophilus influenzae* is a well-established human pathogen capable of causing a range of respiratory and invasive diseases. Since the 1970s, it has been observed that a nontypeable cryptic genospecies of *H. influenzae*, most often biotype IV, has been associated with the genitourinary tracts of females and with invasive neonatal infections. This distinct genospecies has been provisionally named “*Haemophilus quentini*.” Here, we report seven cases of invasive *H. quentini* disease in patients from Ontario, Canada, over a 2-year period. Significantly, while most reports of invasive disease with *H. quentini* to date have been in neonates, we observed five cases in adults (three in women of childbearing age and two in seniors) as well as two in neonates. Identification of *H. quentini* is challenging and was not possible for frontline laboratories, requiring work at the reference laboratory level. We describe in detail the biochemical results, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-Tof MS) results, and PCR results with several targets, including the 16S rRNA gene and multilocus sequence typing (MLST) genes, for the seven Ontario *H. quentini* isolates and several controls. Our data, combined with those of other publications, support the fact that *H. quentini* is distinct from *H. influenzae* and *Haemophilus haemolyticus*. This organism is recognized as a pathogen of neonates, but we hypothesize that it may be underrecognized as an important pathogen in adults as well, particularly pregnant women. By sharing the detailed descriptions of these isolates, we hope to enable other laboratories to better identify *H. quentini* so that the true prevalence of this organism and disease can be explored.

KEYWORDS invasive “*Haemophilus quentini*,” neonate, urogenital, *Haemophilus influenzae* cryptic genospecies, biotype IV, *Haemophilus haemolyticus*, nontypeable *Haemophilus*, multilocus sequence typing

The genus *Haemophilus* consists of several “blood-loving” species with various requirements for the X (hemin) and V (NAD) growth factors. *Haemophilus influenzae* is the most clinically significant and the best-studied species of the genus and causes a wide range of respiratory and invasive infections. There are six different capsular serovars of *H. influenzae*, designated “a” through “f,” as well as a nonencapsulated, or nontypeable (NT), variety, referred to as NTHi (nontypeable *H. influenzae*) (1, 2). Historically, *H. influenzae* serovar b (Hib) was the most common cause of childhood bacterial meningitis (3). In countries that introduced the Hib conjugate vaccine in the 1990s, invasive *H. influenzae* disease is now caused mainly by non-Hib serovars and NTHi (4, 5). Many recent studies have identified NTHi as an important emerging pathogen that is now responsible for the majority of invasive *H. influenzae* disease worldwide (5–7).

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However, some NTHi biotype IV strains, often referred to as "*H. influenzae* biotype IV" or "*H. influenzae* cryptic genospecies," and now provisionally named "*Haemophilus quentini*," constitute a distinct group of potential urogenital pathogens implicated in maternal and neonatal infections (8–11).

The recognition that some NTHi biotype IV isolates were commonly recovered from the genitourinary (GU) tract was first reported in Canada in the late 1970s (12, 13). Subsequently, many of these isolates were confirmed to be associated with urogenital and neonatal infections, and several groups postulated that these strains had a predilection for the female genital tract (8–11, 14–17). Using electrophoretic typing, Quentin et al. demonstrated in 1990 that a group of NTHi biotype IV isolates, predominantly from patients with obstetric infections or serious neonatal disease, was divergent enough from *H. influenzae sensu stricto* that it definitively represented a novel species of *Haemophilus* (10). These organisms were then called "*H. influenzae* cryptic genospecies" and, later, *Haemophilus quentini*, a name that has never been formally proposed (8–11). More recently, a number of reports have described *H. quentini* strains isolated from the urinary tracts of adult men and noted their association with urethritis in this group (16, 17). Several groups have explored the role of particular adhesins that may allow for enhanced adherence of *H. quentini* in the urogenital niche (14, 18–22).

In the laboratory, it can be difficult to reliably distinguish NTHi from related species such as *Haemophilus haemolyticus* and *H. quentini* by use of standard or even newer methodologies (e.g., matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-ToF MS]). These three species differ from other *Haemophilus* species in their requirement for both X and V growth factors, but there is a high degree of similarity between them with respect to morphology and both phenotypic and genetic characteristics, as well as the possibility of interspecies recombination, which may further complicate laboratory identification (2, 23). Given that *H. haemolyticus* is typically considered a commensal organism with limited pathogenicity and is rarely implicated in invasive disease (2, 24, 25), while NTHi has emerged as a known significant pathogen and *H. quentini* is increasingly recognized as a neonatal, maternal, and urogenital pathogen (8, 9, 11, 14–17), accurate identification is important.

Several phenotypic and genetic methods have been put forward to assist in the differentiation of these species. Although *H. haemolyticus* was originally named for its hemolytic activity, nonhemolytic variants have been reported; this variability can lead to potential misidentifications as NTHi (24, 25). NTHi can be divided into eight biotypes based on biochemical reactions for urease, indole production, and ornithine decarboxylase activity (26). While *H. quentini* typically matches biotype IV (positive for urease and ornithine decarboxylase, negative for indole) (11; this study), and many *H. haemolyticus* strains have been found to belong to biotype III (positive for urease, negative for indole and ornithine decarboxylase), variable results have also been described (2, 27). Various genetic markers, including those encoding IgA1 protease (*iga*), [Cu, Zn] superoxide dismutase (*sodC*), protein D (*hpd*), fuculokinase (*fucK*), and outer membrane protein P6 (*ompP6*), have been evaluated for species differentiation, but no single target has proven 100% reliable (15, 27, 28). A combination of the methods described above and/or sequencing of the full-length or near-full-length 16S rRNA gene has shown the most promising results for accurate species identification (2, 29, 30). Quentin et al. in 1996 also identified a 242-bp fragment of the 16S rRNA gene that was amplified from *H. quentini*, but not from *H. haemolyticus* or *H. influenzae*, including *H. influenzae* biotype IV (15). Current commercial MALDI-ToF MS systems also have difficulty distinguishing between these organisms, principally due to the absence of *H. quentini* in the databases.

Here, we describe seven invasive clinical isolates of *H. quentini* (six from blood cultures and one from placenta) from Ontario, Canada. This work provides the molecular description of *H. quentini* as well as further evidence that this pathogen is distinct from *H. influenzae* and *H. haemolyticus*. We postulate that this pathogen may be underrecognized due to challenges with nomenclature and identification.

MATERIALS AND METHODS

Clinical characteristics. Between May 2016 and May 2018, Public Health Ontario (PHO), Toronto, Ontario, Canada, received isolates from seven individual cases of invasive infections caused by query *Haemophilus* species. These cultures (six blood culture isolates and one isolate cultured from a fetal placenta) were submitted by hospital laboratories in Ontario for identification. Patient profiles and initial organism descriptions are outlined in Table 1. (Additional clinical information and outcome data were not available for this work.) This investigation was approved by the PHO Research Office's Ethics Review Board, and a Privacy Impact Assessment was also completed.

Initial workup. Following routine PHO laboratory practices, the seven query *Haemophilus* species isolates were Gram stained; catalase and oxidase reactions were tested; and isolates were subcultured to chocolate agar plates. Isolates from fresh subcultures were identified by MALDI-ToF MS (Bruker Biotyper) using the on-plate formic acid extraction method, and biochemical profiles were produced for confirmation as well as for determination of the biotypes. When identification could not be confirmed, 16S rRNA gene PCR and gene sequencing were performed as described by Knox et al. in 1998, generating a 700-bp product for sequence analysis (31). Sequences were analyzed with BLASTn using the megablast algorithm, and the search was initially limited to sequences from type strains (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). If matches of $\geq 99\%$ were not found, the search was opened up to the general database. The interpretation criteria used were those outlined by the CLSI in guideline MM18 (32).

Serotyping was carried out using antisera from Remel Europe Ltd. The seven isolates were also forwarded to the National Microbiology Laboratory (NML) in Winnipeg, Manitoba, Canada, for confirmation of identification and further molecular testing as part of the national laboratory surveillance program.

MALDI-ToF MS library expansion. After the initial confirmation of the identification of isolate HQ1 as *H. quentini* (see below), this organism's spectra were added to the PHO in-house MALDI-ToF MS library as per manufacturer instructions (Bruker).

Reference identification and molecular methods. Serotyping was confirmed at the NML by slide agglutination using commercial antisera (Difco, Oakville, Ontario, Canada) and by PCR detection of serovar-specific genes and the capsule transport gene, *bexA* (33). Biotyping was performed as described previously (26), and growth factor requirements were determined by using X and V factor-impregnated disks (Oxoid, Nepean, Ontario, Canada). As controls, two invasive NTHi biotype IV isolates recovered in Ontario in 2017 (NTHi1 and NTHi2), a clinical reference strain of *H. quentini* from Hong Kong (HK-1), China, and the type strain of *H. haemolyticus* (ATCC 33390^T) were included in this investigation.

Antimicrobial susceptibility testing. β -Lactamase production was detected using DrySlide nitrocefin (BBL, Becton, Dickinson, Oakville, Ontario, Canada). Antibiotic susceptibility testing was carried out by the broth microdilution method using commercially available Sensititre plates (Oxoid, Nepean, Ontario, Canada) according to the manufacturer's instructions. MICs were interpreted according to CLSI guidelines (34) for the following antibiotics: amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, cefaclor, cefepime, cefixime, ceftriaxone, chloramphenicol, clarithromycin, imipenem, levofloxacin, meropenem, tetracycline, and trimethoprim-sulfamethoxazole. *H. influenzae* strains ATCC 49247 and ATCC 49766 were used for quality control of antimicrobial susceptibility testing.

MLST and sequencing of antigen genes. Multilocus sequence typing (MLST) was carried out according to established methods (35), and allele number assignment was done through the *H. influenzae* MLST website (<http://pubmlst.org/hinfluenzae>). Concatenation was done using sequences of six MLST alleles: *adk*, *atpG*, *frdB*, *mdh*, *pgi*, and *recA*. PCR and nucleotide sequencing of the *ompP6* (423 bp) (24), *hpd* (960 bp) (29), and *sodC* (300 bp) (36) genes and of the 16S rRNA gene (1,205 bp) (37), as well as PCR using primers that amplify a 242-bp region of the 16S rRNA gene unique to *H. quentini* (15), were performed as described previously. Software by DNASTAR Lasergene (Madison, WI, USA) was used for sequence assembly and alignment. Phylogenetic trees were constructed on the Phylogeny.fr platform using MUSCLE, gBlocks, PhyML, and TreeDyn for the alignment, curation, phylogeny, and tree-rendering steps, respectively (38–44). This study also made use of the NCBI BLAST website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

For comparison, the genome sequences of *H. quentini* strain MP1 (GenBank accession no. [NZ_MCI02000001.1](#)) and *H. haemolyticus* strain M19346 (GenBank accession no. [NZ_CP031243](#)) were retrieved from NCBI.

RESULTS

Initial description of isolates. All seven isolates received at PHO as suspected *Haemophilus* species (HQ1 to HQ7) were Gram-negative bacilli, were found to produce catalase and oxidase, and required X and V factors for growth. All seven isolates were nontypeable based on lack of agglutination with the six commercial antisera used for traditional serotyping of *H. influenzae* (Table 2). MALDI-ToF MS (Bruker Biotyper) identified the initial two isolates (HQ1 and HQ2) with confidence as *H. haemolyticus* (score, ≥ 2.0); however, they were ornithine decarboxylase positive, whereas *H. haemolyticus* is most often negative (45). Analysis of the partial 16S rRNA gene sequences of HQ1 and HQ2 resulted in low levels of homology (96%) to type strains of *H. haemolyticus* (CIP 103290; GenBank accession no. [NR_104930.1](#)), *H. influenzae* (NCTC8143; accession no. [LN831035.1](#)), and *H. aegyptius* (NCTC8502; accession no. [LS483429.1](#)) but

TABLE 1 Characteristics of seven invasive clinical isolates of suspected *Haemophilus quentini* from Ontario, Canada

Strain no.	Isolation date	Specimen source	Patient sex	Patient age	Clinical information	Method(s) of bacterial identification by frontline laboratory	Organism identification by:	
							Frontline laboratory	Reference laboratory
HQ1	2016	Blood	Female	<1 wk	Neonate	API NH, Vitek 2 NH ID card (bioMérieux)	<i>H. influenzae</i>	<i>H. quentini</i>
HQ2	2016	Blood	Female	65–85 yr	Febrile	Vitek MS (bioMérieux)	<i>H. influenzae</i>	<i>H. quentini</i>
HQ3	2016	Fetal placenta	Female	25–45 yr	Labor	Vitek MS (bioMérieux), satellitism test ("Staph streak"), ALA porphyrin test ^a	<i>H. influenzae</i>	<i>H. quentini</i>
HQ4	2017	Blood	Male	<1 wk	Neonate	MALDI Biotyper (Bruker), satellitism test ("Staph streak")	<i>H. haemolyticus</i>	<i>H. quentini</i>
HQ5	2018	Blood	Female	25–45 yr	Dilation and curettage	Gram stain, oxidase, catalase	Gram-negative bacilli	<i>H. quentini</i>
HQ6	2018	Blood	Female	25–45 yr	N/A ^b	Vitek MS (bioMérieux)	<i>Haemophilus</i> species	<i>H. quentini</i>
HQ7	2017	Blood	Male	65–85 yr	Variceal bleed	Vitek MS (bioMérieux)	<i>H. influenzae</i>	<i>H. quentini</i> (atypical)

^aALA, δ -aminolevulinic acid.^bN/A, not available.

TABLE 2 Initial description of suspected *Haemophilus quentini* isolates and controls

Strain characteristic														MALDI-ToF MS result (score, >2.0)		16S rRNA gene sequence results ^a by use of the NCBI BLAST nr database for:					
Strain	Catalase	Oxidase	X factor	V factor	Indole	Nitrate	Urease	Ornithine decarboxylase	ONPG ^b	Porphyrin	Glucose	Sucrose	Lactose	Mannose	Xylose	Serovar	Biotype	Biotyper database	In-house database	Type strains	All strains
<i>H. quentini</i> (HK-1)	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ1	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ2	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ3	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c	96 (<i>H. haemolyticus</i> , <i>H. influenzae</i> , <i>H. aegyptius</i>) ^d	100 (<i>H. quentini</i> , <i>Haemophilus</i> species, <i>H. influenzae</i> cryptic genospecies) ^e
HQ4	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ5	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ6	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c		
HQ7	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	NT	III	<i>H. haemolyticus</i>	<i>H. quentini</i> ^c	96 (<i>H. haemolyticus</i> , <i>H. influenzae</i>) ^f	99 (<i>H. haemolyticus</i>) ^g
NTH1	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	NT	IV	<i>H. influenzae</i> ^c		98 (<i>H. influenzae</i>) ^h	99 (<i>H. influenzae</i>) ⁱ
NTH2	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	NT	IV	<i>H. influenzae</i> ^c		99 (<i>H. influenzae</i>) ^h	99 (<i>H. influenzae</i>) ⁱ
<i>H. haemolyticus</i> (ATCC 33390) ^j	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	NT	III	<i>H. haemolyticus</i> ^c		100 (<i>H. haemolyticus</i>) ^k	100 (<i>H. haemolyticus</i> , <i>Haemophilus</i> species) ^l

^aThe overall top hit(s) is shown for each strain. Each result is expressed as the percentage of homology (homologous species).^bONPG, o-nitrophenyl-β-D-galactopyranoside.^cOverall top hit by MALDI-ToF MS.^dThis result applies to strains HK-1 and HQ1 to HQ6. *H. haemolyticus* CIP 103290 (GenBank accession no. [NR_104930.1](#)), *H. influenzae* NCTC8143 (accession no. [LN831035.1](#)), and *H. aegyptius* NCTC8502 (accession no. [LS483429.1](#)).^eThis result applies to strains HK-1 and HQ1 to HQ6. *H. quentini* MCCM 02026 (GenBank accession no. [AF224307.1](#)) and S32F2 (accession no. [AY962106.1](#)); *Haemophilus* sp. strain 11PS (accession no. [EU909640](#)); *H. influenzae* cryptic genospecies, strain 2406 (accession no. [X87979.1](#)).^f*H. haemolyticus* CIP 103290 (GenBank accession no. [NR_104930](#)) and *H. influenzae* NCTC8143 (accession no. [LN831035](#)).^g*H. haemolyticus* M28486 (GenBank accession no. [CP031238](#)).^h*H. influenzae* NCTC8143 (GenBank accession no. [LN831035](#)).ⁱ*H. influenzae* NCTC11426 (GenBank accession no. [LS483411](#)) and KR494 (accession no. [CP005967](#)).^j*H. influenzae* NCTC8143 (GenBank accession no. [LN831035](#)) and seven other *H. influenzae* strains.^k*H. haemolyticus* CIP 103290 (GenBank accession no. [NR_104930](#)), P1-3 (accession no. [MK024214](#)), and P4-1 (accession no. [MK024224](#)).^l*H. haemolyticus* CIP 103290 (GenBank accession no. [NR_104930](#)), P1-3 (accession no. [MK024214](#)), and P4-1 (accession no. [MK024224](#)); *Haemophilus* sp. strain HK 1193 (accession no. [JF506654.1](#)).

100% homology to the non-type strain deposits of *H. quentini* (strains MCCM 02026 [accession no. [AF224307.1](#)] and S32F2 [accession no. [AY962106.1](#)]) as well as “*H. influenzae* cryptic genospecies” (strain 2406; accession no. [X87979.1](#)) (Table 2). Therefore, the presumptive identification of HQ1 and HQ2 was *H. quentini*. After the addition of *H. quentini* spectra (HQ1) to the in-house MALDI-ToF MS spectral library, the subsequent isolates (HQ3, HQ4, HQ6, and HQ7) produced scores of ≥ 2.0 for both *H. quentini* and *H. haemolyticus*, with *H. quentini* always the top hit; HQ5 produced a score of ≥ 2.0 only for *H. quentini* (Table 2). To ensure accuracy, the isolates were referred to the NML for confirmation.

Confirmation of identification. All basic characteristics of the seven isolates initially described by PHO were confirmed by the NML. Additionally, *bexA*-specific PCR confirmed the absence of the capsule transport gene in all the *H. quentini* isolates. All seven isolates were also PCR positive for *hpd*, *ompP6*, *sodC*, and the *H. quentini*-specific region of the 16S rRNA gene (Table 3). Six isolates (HQ1 to HQ6) were confirmed to be biotype IV and fermented glucose and xylose but not sucrose, lactose, or mannose. The seventh isolate (HQ7) was biotype III and fermented glucose and mannose but not sucrose, lactose, or xylose (Table 2).

Antibiotic susceptibility testing. Although antibiotic resistance in *H. quentini* is not well documented and likely not a common occurrence, a single case with a β -lactamase-positive strain was reported in China; this strain was also resistant to tetracycline and nalidixic acid and showed reduced susceptibility to levofloxacin (46). None of the seven isolates investigated here produced β -lactamase, and no resistance to any of the antibiotics tested was observed.

MLST. MLST found that the *fucK* gene was absent in the seven isolates HQ1 to HQ7, as well as in *H. quentini* isolate HK-1 and *H. haemolyticus* ATCC 33390^T (Table 3). Nucleotide sequences for the following genes were compared: *hpd*, *ompP6*, *sodC*, the 16S rRNA gene, and six concatenated MLST genes: *adk*, *atpG*, *frdB*, *mdh*, *pgi*, and *recA* (combined, 2,712 bp). HQ1 to HQ6 were found to have identical gene sequences for *hpd*, which were also shown to be identical to those of both HK-1 and MP1, while HQ7 differed by 21 single nucleotide polymorphisms (SNPs) (97.8% similarity). HQ1 to HQ6, HK-1, and MP1 all had identical *ompP6* sequences; however, HQ7 differed by six SNPs (98.6% similarity). Based on the *sodC* gene, HQ1 to HQ6 were split into two groups: HQ1, HQ2, and HQ5 were identical as a group, while HQ3, HQ4, and HQ6 were identical as the second group. The two groups differed by two SNPs (99.3% similarity). The *sodC* sequence for HK-1 was identical to those for HQ1, HQ2, and HQ5, but the *sodC* sequence for MP1 was found to be unique and differed from those of the two groups by two and four SNPs, respectively. HQ7 differed from HQ1, HQ2, HQ5, and HK-1 by 12 SNPs (96% similarity), from HQ3, HQ4, and HQ6 by 10 SNPs (96.7% similarity), and from MP1 by 14 SNPs (95.3% similarity). The same groupings were observed for the near-full-length 16S rRNA gene sequences: these sequences were identical in HQ1, HQ2, HQ5, and HK-1 and differed from those of HQ3, HQ4, and HQ6 by one SNP (99.9% similarity). The 16S rRNA gene sequence for MP1 differed from those of the two groups by one (99.9% similarity) and two (99.8% similarity) SNPs, respectively. HQ7 differed from HQ1, HQ2, HQ5, and HK-1 by nine SNPs (99.3% similarity), from HQ3, HQ4, and HQ6 by 10 SNPs (99.2% similarity), and from MP1 by 10 SNPs (99.2% similarity). The same groupings were observed when the six MLST genes were concatenated, but in this case, MP1 was not unique and shared 100% similarity with HQ1, HQ2, HQ5, and HK-1. The two groups differed by five SNPs (99.8% similarity); SNPs were located in the *adk* (one SNP), *frdB* (one SNP), and *mdh* (three SNPs) genes. HQ7 differed from HQ1, HQ2, HQ5, HK-1, and MP1 by 114 SNPs (95.8% similarity) and from HQ3, HQ4, and HQ6 by 119 SNPs (95.6% similarity), with SNPs observed in all six MLST genes.

The genetic relationships of HQ1 to HQ7 with *H. quentini* clinical isolates from Hong Kong (HK-1) and the United Kingdom (MP1), *H. haemolyticus* reference strains (ATCC 33390^T and M19346), nontypeable *H. influenzae* biotype IV clinical isolates (NTHi1 and NTHi2), and an *H. influenzae* reference strain (Rd KW20) are depicted in Fig. 1A to E.

TABLE 3 PCR and MLST results^a

Strain	PCR result for the following gene target:				H. influenzae MLST allele no. ^b						
	hpd	ompP6	sodC	H. quentini-specific 16S rRNA gene	adk	atpG	frdB	fucK	mdh	pgi	recA
HQ1	+	+	+	+	8	27	27	No product	37	15	24
HQ2	+	+	+	+	8	27	27	No product	37	15	24
HQ3	+	+	+	+	8 (99.8% match)	27	27 (99.8% match)	No product	37 (99.3% match)	15	24
HQ4	+	+	+	+	8 (99.8% match)	27	27 (99.8% match)	No product	37 (99.3% match)	15	24
HQ5	+	+	+	+	8	27	27	No product	37	15	24
HQ6	+	+	+	+	8 (99.8% match)	27	27 (99.8% match)	No product	37 (99.3% match)	15	24
HQ7	+	+	+	+	81 (99.8% match)	63	86 (98.2% match)	No product	63 (98.0% match)	84	60
NTH1	+	+	-	-	11	43	35	11	51	31	18
NTH2	+	+	-	-	30	2	15	7	22	38	3
H. quentini HK-1	+	+	+	+	8	27	27	No product	37	15	24
H. haemolyticus ATCC 33390 ^T	+	+	+	-	80	66	83	No product	126	101	74

^aPCR and MLST descriptions of seven invasive clinical isolates of suspected *Haemophilus quentini* from Ontario, Canada (HQ1 to HQ7), two isolates of invasive nontypeable *H. influenzae* biotype IV from Ontario, Canada (NTH1 and NTH2), a clinical blood isolate of *H. quentini* from Hong Kong, China (HK-1), and the type strain of *H. haemolyticus* (ATCC 33390^T).

^bThe percentage in parentheses refers to the percent match of the query to the nucleotide sequences of the indicated allele number.

FIG 1 Phylogenetic trees constructed on the phylogeny.fr platform showing the relatedness of seven invasive *Haemophilus quentini* clinical isolates from Ontario, Canada (HQ1 to HQ7), clinical *H. quentini* isolates from Hong Kong (HK-1) and the United Kingdom (MP1), *H. haemolyticus* reference (M19346) and type (ATCC 33390^T) strains, two nontypeable *H. influenzae* biotype IV clinical isolates from Ontario, Canada (NTHi1, NTHi2), and an *H. influenzae* reference strain (Rd KW20) based on *hpd* (A), *ompP6* (B), *sodC* (C), the 16S rRNA gene (D), and concatenated sequences of six MLST genes (*adk*, *atpG*, *frdB*, *mdh*, *pgi*, and *recA*) (E). NTHi1, NTHi2, and Rd KW20 were negative for *sodC*.

Isolates HQ1 to HQ6 were either identical or very closely related to known *H. quentini* isolates HK-1 and MP1 and did not cluster with any of the *H. influenzae* or *H. haemolyticus* isolates examined in this study. HQ7 either appeared to occupy an intermediate position between *H. quentini* and *H. haemolyticus* (based on *hpd* [Fig. 1A] and the 16S rRNA gene [Fig. 1D]), shared similarity with *H. haemolyticus* (based on *ompP6* [Fig. 1B] and concatenated MLST gene sequences [Fig. 1E]), or appeared to be unique (based on

TABLE 4 Comparison of nucleotide sequences of the *hpd*, *ompP6*, *sodC*, and 16S rRNA genes and of concatenated MLST genes of seven invasive clinical isolates of suspected *Haemophilus quentini* from Ontario, Canada, with corresponding sequences obtained from GenBank for *H. quentini* strain MP1, *H. haemolyticus* strain M19236, and *H. influenzae* strain Rd KW20

Strain no.	Gene	% similarity with the following strain (GenBank accession no.):		
		<i>H. quentini</i> MP1 (NZ_MCI102000001)	<i>H. haemolyticus</i> M19346 (NZ_CP031243)	<i>H. influenzae</i> Rd KW20 (NC_000907)
HQ1, HQ2, HQ5	<i>hpd</i>	100	98.3	91.9
	<i>ompP6</i>	100	98.8	94.3
	<i>sodC</i>	99.3	96.0	N/A ^a
	16S rRNA	99.9	97.7	95.8
	Concatenated MLST	100	96.5	94.8
HQ3, HQ4, HQ6	<i>hpd</i>	100	98.3	91.9
	<i>ompP6</i>	100	98.8	94.3
	<i>sodC</i>	98.7	96.7	N/A
	16S rRNA	99.8	97.6	95.7
	Concatenated MLST	99.8	96.5	94.9
HQ7	<i>hpd</i>	97.8	97.9	91.8
	<i>ompP6</i>	98.6	99.8	94.6
	<i>sodC</i>	95.3	97.3	N/A
	16S rRNA	99.2	97.8	95.7
	Concatenated MLST	95.8	96.2	94.7

^aN/A, not applicable. Rd KW20 is negative for *sodC*.

sodC [Fig. 1C]). The percentages of similarities of HQ1 to HQ7 with *H. quentini* MP1, *H. haemolyticus* M19346, and *H. influenzae* Rd KW20 are shown in Table 4.

Comparison of previously published whole-genome sequence data for *H. quentini* strain MP1, collected in the United Kingdom, with previously published whole-genome sequences for HQ1 and HQ2 found that these three strains had identical concatenated MLST gene sequences (23, 47). The current study confirmed the finding that HQ1 and HQ2 were identical and identified additional clinical isolates collected in Canada (HQ5), Hong Kong (HK-1), and the United Kingdom (MP1) with the same concatenated MLST gene sequences. However, MP1 differed from the other *H. quentini* isolates HQ1 to HQ6 and HK-1 by slight variations in its *sodC* (two to four SNPs) and 16S rRNA (one to two SNPs) genes. This study also identified another group of identical clinical *H. quentini* isolates (HQ3, HQ4, and HQ6) that differed from the group of HQ1, HQ2, HQ5, and HK-1 by only five SNPs in their concatenated MLST sequences (Fig. 1E). It is well known that MLST sequence types differ widely among NTHi strains (4); however, only slight variations were observed among the *H. quentini* isolates from Canada, Hong Kong, and the United Kingdom, suggesting that *H. quentini* may be a monophyletic group.

DISCUSSION

Since the initial report in the 1970s, there has been growing recognition that some NTHi biotype IV strains, also known as the cryptic genospecies of *Haemophilus* or, most recently, *H. quentini*, constitute a distinct genospecies, separate from *H. influenzae* and *H. haemolyticus* (10, 16, 23, 48). This pathogen has been found to colonize the urogenital tract and to cause urinary, maternal, and neonatal infections, yet reports are still relatively rare (8–11, 16, 17, 30, 49, 50). Recently, there was a report of this organism causing a disseminated infection in an immunocompromised adult male (51). Those authors also described the challenges of identifying this organism and indicated that in their experience, unsurprisingly, *H. quentini* cross-reacted with *H. influenzae* on the BioFire FilmArray panels they used; they performed 16S rRNA gene PCR and sequencing to confirm the identification (51). The significance of this pathogen may be underestimated due in part to difficulties with identification, as well as confusion with the changing nomenclature.

In the present study, among the six cases of invasive disease caused by typical *H. quentini* (biotype IV), five strains were isolated from blood cultures, and one was cultured from a fetal placenta. Of these six cases, there were two neonatal cases (one

male and one female), in which the organisms were isolated from blood cultures. The remaining four cases occurred in adult women, three of childbearing age (two cases were associated with pregnancy, and in one case, this information was unknown). The remaining adult female patient was an elderly woman with an *H. quentini* bloodstream infection (previously reported by Eshaghi et al. in 2016 [47]). The final case involved an atypical isolate of *H. quentini* (biotype III) that was isolated from the blood culture of an elderly male patient with a variceal bleed. This isolate did not share high sequence homology in a number of target protein genes (*hpd*, *ompP6*, *sodC*) and MLST genes with the six typical *H. quentini* isolates recovered in Ontario, Canada, in the same time frame. These cases represent a departure from what has been described previously in the literature with a significant number of invasive infections in adults.

16S rRNA gene sequence analysis is an excellent option to aid in the differentiation of these closely related organisms; however, this method is not available in many frontline clinical laboratories (2, 15, 30). With the shift to the use of MALDI-ToF MS systems as the primary means of bacterial identification for many clinical laboratories, it is noteworthy that the commercially available databases do not include *H. quentini*. In our experience with the Bruker MALDI Biotyper, even with the in-house addition of *H. quentini* spectra, differentiation between this organism and its close relatives is not straightforward; multiple species have confidence scores of ≥ 2.0 when *H. quentini* is run. Of note, in our evaluation to date, the in-house *H. quentini* entry always yields the top score when *H. quentini* isolates are run, including even the reference strain HK-1 and the atypical biotype III isolate HQ7. None of the *H. influenzae* or *H. haemolyticus* strains run to date, not even those of NTHi biotype IV, have resulted in a match to our in-house *H. quentini* entry (Table 2). Combined, these results indicate that a match to the in-house *H. quentini* spectra, though not yet definitive, at least alerts users that *H. quentini* is a possible and perhaps likely identification. These results thus demonstrate that with the addition of appropriate spectra, MALDI-ToF MS holds promise as a convenient means of distinguishing between these closely related species.

In summary, the nontypeable nature of isolates HQ1 to HQ6 and their biotype IV result, combined with the presence of *sodC* and the *H. quentini*-specific 16S rRNA gene, the absence of *fucK*, and the near-full-length 16S rRNA gene sequence results, led to the definitive identification of *H. quentini*. HQ7 was initially identified as *H. quentini* due to the presence of the *sodC* gene and the *H. quentini*-specific 16S rRNA gene region, as well as its near-full-length rRNA gene sequence, which matched 99.2% to that of the known *H. quentini* strain MP1 (GenBank accession number [NZ_MCI102000001](#)). Further genetic analysis showed that HQ7 might be a hybrid strain involving multiple gene recombinations with *H. haemolyticus*. These findings confirm previous reports on the challenges in distinguishing between NTHi, *H. haemolyticus*, and *H. quentini* (23, 52). This study adds to the scant reports of invasive disease cases due to *H. quentini* in adults and represents the first report characterizing multiple invasive *H. quentini* case isolates obtained within a single area over a short period. In addition, this study highlights the importance of reliable methods to distinguish between related species of *Haemophilus* for accurate identification of disease-causing strains in our surveillance system. Our observations lead us to believe that *H. quentini* may be underrecognized as an important pathogen in adults, particularly pregnant women, in addition to neonates. By sharing the detailed descriptions of these Ontario isolates as well as our experience in identifying this pathogen, we hope to enable other laboratories to better identify *H. quentini* so that the true prevalence of this organism and associated diseases can be explored.

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