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Clinical epidemiology and impact of *Haemophilus influenzae* airway infections in adults with cystic fibrosis

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Abstract

Background *Haemophilus influenzae* is prevalent within the airways of persons with cystic fibrosis (pwCF). *H. influenzae* is often associated with pulmonary exacerbations (PEx) in pediatric cohorts, but in adults, studies have yielded conflicting reports around the impact(s) on clinical outcomes such as lung function decline. Accordingly, we sought to discern the prevalence, natural history, and clinical impact of *H. influenzae* in adult pwCF.

Methods This single-centre retrospective cohort study reviewed all adult pwCF with *H. influenzae* sputum cultures between 2002 and 2016. From this cohort, persistently infected subjects (defined as: ≥2 samples with the same pulsotype and > 50% sputum culture-positive for *H. influenzae* in each year) were matched (1:2) to controls without *H. influenzae*. Demographic and clinical status (baseline health or during periods of PEx) were obtained at each visit that *H. influenzae* was cultured. Yearly biobank isolates were typed using pulsed-field gel electrophoresis (PFGE) to assess relatedness.

Results Over the study period, 30% ($n = 70/240$) of pwCF were culture positive for *H. influenzae*, of which 38 (54%) were culture-positive on multiple occasions and 12 (17%) had persistent infection. One hundred and thirty-seven isolates underwent PFGE, with 94 unique pulsotypes identified. Two (1.5%) were serotype f with the rest non-typeable (98.5%). *H. influenzae* isolation was associated with an increased risk of PEx (RR = 1.61 [1.14–2.27], $p = 0.006$), however, this association was lost when we excluded those who irregularly produced sputum (i.e. only during a PEx). Annual lung function decline did not differ across cohorts.

Conclusions Isolation of *H. influenzae* was common amongst adult pwCF but often transient. *H. influenzae* infection was not associated with acute PEx or chronic lung function decline.

Keywords *Haemophilus influenzae*, Pulmonary exacerbation, Natural history, Epidemiology

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Background

The airways of persons with cystic fibrosis (pwCF) harbor diverse bacterial communities [1, 2]. As lung function declines, community diversity decreases, largely due to an increase in the relative abundance of classical CF pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia* complex., and *Achromobacter* spp [1–3]. Chronic lung infection, punctuated by frequent pulmonary exacerbations (PEx), results in chronic inflammation, airways remodelling and ultimately leads to end-stage lung disease, manifesting in the need for lung transplantation or a case-fatality.

H. influenzae is a Gram-negative coccobacillus that exists in encapsulated (typeable A-F) and unencapsulated (non-typeable) forms (NTHi). In pwCF, *H. influenzae* is the second most prevalent species in the first five years of life with a peak prevalence of ~30% extending in to adolescence before declining to ~10% in adulthood [4]. Amongst *H. influenzae*, non-encapsulated strains have been more frequently identified in pwCF and are commonly associated with PEx in childhood [5]. Unique relative to other classical CF pathogens, strains of *H. influenzae* causing infections in pwCF are often transient, with persistent infection by a single strain reported only in a minority of pediatric cases [6]. In contrast, although it is widely accepted that presence of *H. influenzae* in pediatric pwCF is associated with adverse clinical outcomes [5, 7], the impact in adult populations is poorly understood [8].

Given the high turnover of *H. influenzae* strains within CF airways, studies examining the potential impact of early confirmed culture-directed therapy are lacking. The patterns of treatment (vs. observation) are currently practitioner-dependent and not well founded in any evidence-informed manner, representing a knowledge gap in this important clinical circumstance. In this study, we aimed to define the epidemiology and clinical impact of *H. influenzae* infections in adult pwCF. We hypothesized that prevalence of *H. influenzae* in an adult pwCF cohort would mirror that observed in pediatrics with predominance of non-typable strains. Furthermore, based off clinical experience, we hypothesized that the presence of *H. influenzae* (both encapsulated and non) in sputum did not associate with worsened clinical outcomes in adults.

Methods

Subject selection and sample collection

Adults attending the Calgary Adult CF Clinic (CACFC) from the onset of our Biobank in January 2002 to December 2016 were included in this study. Inclusion criteria included a confirmed diagnosis of CF, age ≥18 years, and ≥1 positive sputum culture for *H. influenzae*. Those who had received a lung-transplant during the period of time were censured at the time of transplant. At the clinic, sputum samples were collected at quarterly visits and during PEx as per clinic protocol. Collected samples are screened for pathogens using standard protocols [9] and entered into the CACFC Biobank. This Biobank includes >50,000 pathogens collected from >350 individuals as previously described and is relatively unique in that cultures are accompanied by quantitative data in the form of colony forming units (CFU) [10, 11]. This study received ethical approval through the University of Calgary (REB15–2744).

Those subjects identified with *H. influenzae* were then further stratified as having either: persistent (≥2 positive samples with the same pulsotype throughout the study and >50% sputum-culture positive for *H. influenzae* in each year) or a transient infection (≥1 positive sputum culture, but not meeting the criteria for a persistent infection). For each subject classified as having persistent *H. influenzae* infection, two control CF subjects without *H. influenzae* infection were randomly assigned, matched and compared for birth cohort, age (±2 years), and sex. Patient demographics were collected through chart review. Dynamic variables were recorded for each clinical encounter, including spirometry measurements (percent predicted forced expiratory volume in one second [ppFEV$_1$] and forced vital capacity [FVC]), weight and body mass index (BMI), medical therapies, use of supplemental oxygen, and enteral nutrition.

Clinical outcome definitions

The primary outcome of interest in this study was frequency of PEx, as defined from previous work from this cohort by Fuch's criteria [12]' [13]. To assess the clinical significance of *H. influenzae* infection, the frequency of PEx with *H. influenzae* isolation was compared with the frequency of PEx in the preceding and following clinic visits, whenever they occurred.

Haemophilus influenzae characterization

All clinical *H. influenzae* isolates (as well as co-infecting bacteria) obtained from pwCF since the start of the Biobank in 2002, were confirmed using standard methodologies for culture-based identification [9]. Antibacterial susceptibility testing was performed according to the most recently published Clinical & Laboratory Standards Institute (CLSI) guidelines at the time of isolate recovery, with additional testing done at the request of the treating physician [14]. Isolates were then frozen in glycerol stock and stored in the CACFC Biobank at -80 °C as previously described [10, 11]. From this Biobank, we followed *H. influenzae* isolates from each subject in the cohort, including the first (either at the start of the study or upon subjects entering the clinic) and last isolates (either the most temporally recent isolate from each patient or the last available isolate available prior to lung

transplantation, death, and/or transfer of care to another clinic) as well as serial annual isolates.

To assess for strain type and to establish clonality, strains underwent pulsed-field gel electrophoresis (PFGE) using established protocols [10]. Briefly, isolates were digested with 20U Sma1 (New England Biolabs) and run on 1% SeaKem Gold agarose at 6 V and an angle of 120°, with a 1 s initial switch and 20 s final switch, for 21 h at 10°C. Dendrograms were generated at 1.0% position tolerance using an unweighted pair-group method with arithmetic mean (UPGMA) and the Sørensen-Dice similarity coefficient and compared using BioNumerics, version 7.0 (Applied Maths, Austin, TX). Strains with banding patterns ≥ 80% identical (≤ 3 band differences) were considered related as per the Tenover criteria [15]. Finally, to evaluate if strains were typeable, individual *H. influenzae* isolates underwent polymerase chain reaction (PCR) amplification of the *bexB* gene, which is universally present in all encapsulated *H. influenzae* [16] and compared against a panel of positive and negative controls. Isolates with weak *bexB* bands were followed up with *bexA* PCR for confirmation and those positive for either *bexA or bexB* underwent Sanger sequencing to determine specific capsule types, as per Potts et al. [17].

Statistical analysis

Analyses were performed using STATA V13 (StataCorp, College Station, TX, USA). Symmetrical and asymmetrical variables were described as means with standard deviations (SD) and medians with interquartile ranges (IQR), respectively. Pairwise comparisons were conducted using one-way ANOVA (when comparing three groups of data) or Wilcoxon rank-sum test (for two groups of data). Fisher's exact test was used for proportions. Unadjusted risk ratios were calculated to determine the PEx risk. Mixed-effects linear regression analysis with an exchangeable variance-covariance structure was used to calculate the rate of lung function decline. A p-value ≤ 0.05 was considered significant.

Results

Demographics of adult pwCF with *H. influenzae* sputum cultures

Between 2002 and 2016, *H. influenzae* was isolated from 71 of the 240 (29.6%) adult subjects who met inclusion and exclusion criteria (Fig. 1). The median age at the time of first isolation in this adult cohort was 23 years (interquartile range [IQR]: 19.8 to 30.1) with over half identified as female (n=37, 52.1%) (Table 1). One subject could not be matched to a control subject on the basis of extreme age and was excluded from further analysis. Amongst those with *H. influenzae* positive sputum, only 12 (17.1%) met criteria for persistent infection. Compared to those with transient infection and *H. influenzae* negative controls, those with persistent infection had no significant demographic differences. Persistently infected subjects were more likely to have a *S. aureus* co-infection at the time of initial *H. influenzae* isolation (RR=3.0 [1.4–6.5], p<0.001) and were less likely to have *P. aeruginosa* co-infection (RR=0.27 [0.1–0.73], p<0.001)

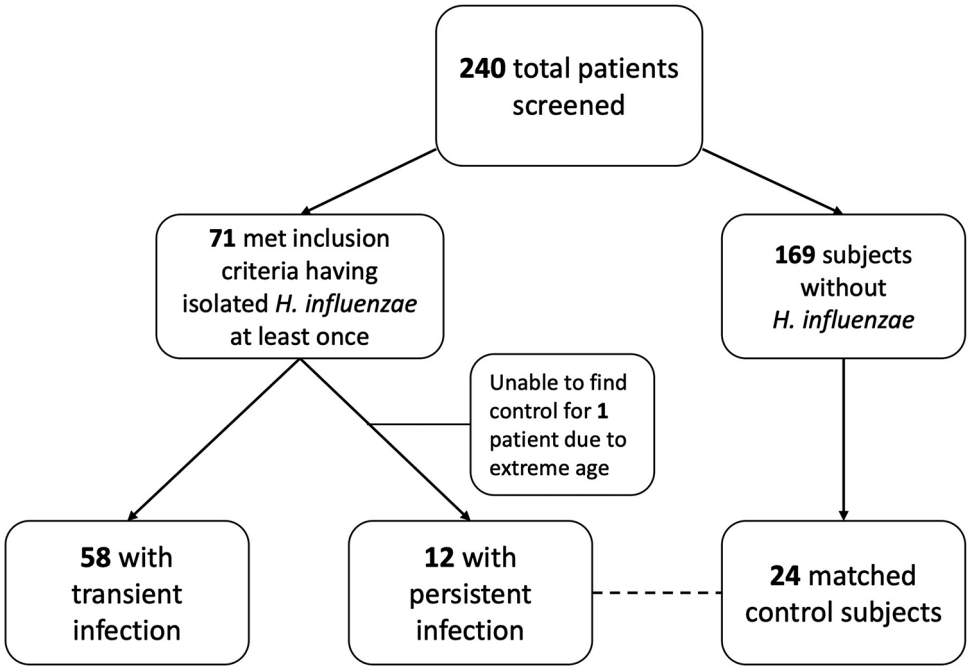

Fig. 1 Flowchart detailing division of patients into the three groups: transiently infected, persistently infected and control

Table 1 Cohort demographics at time of entry as a function of *H. influenzae* infections from 2002–2016. Bolded values indicate significant differences between groups.

Parameter (at study entry)	Group		
	Transient infection (*n* = 58)	**Persistent infection (*n* = 12)**	**Control (*n* = 24)**
Median age (years) (IQR)	22.5 (19.61–30.10)	27.64 (23.00-31.45)	28.10 (23.21–31.37)
Median age at diagnosis (years) (IQR)	0.81 (0.27–7.67)	1.38 (0.29–5.75)	1.38 (0.32–4.18)
No. males (%)	26 (44.83)	8 (66.67)	16 (66.67)
Median BMI (kg/m^2)(IQR)	20.51 (18.54–23.46)	22.13 (19.64–25.77)	21.44 (20.28–22.77)
Median FVC% (IQR)	91 (75–107)	87 (74.5–98)	88.5 (70–97.5)
Median FEV_1% (IQR)	73 (48–89)	52 (45–76.5)	64 (42–76.5)
Home oxygen (%)	4 (6.90)	1 (8.33)	4 (16.67)
Enteral nutrition (%)	4 (6.90)	2 (16.67)	1 (4.17)
Genotype (%)			
ΔF508 homozygous	29 (50)	6 (50)	10 (41.67)
ΔF508 heterozygous	19 (32.76)	3 (25)	9 (37.5)
Other	10 (17.24)	3 (25)	5 (20.83)
Subjects with comorbidity (%):			
Pancreatic insufficiency	45 (77.59)	10 (80.00)	20 (80.00)
CF-related diabetes	6 (10.34)	0 (0.00)	3 (12.5)
Osteopenia/osteoporosis	25 (43.10)	3 (25.00)	12 (50.00)
CF-related liver disease	8 (13.80)	2 (16.67)	6 (25.00)
Chronic therapies (%):			
Inhaled tobramycin	**6 (10.34)**	**0 (0.00)**	**7 (29.17)**
Azithromycin	**6 (10.34)**	**1 (8.33)**	**7 (29.17)**
Inhaled corticosteroid	24 (41.38)	5 (41.67)	9 (37.5)
SABA	20 (51.72)	4 (33.33)	19 (79.17)
LABA	33 (56.90)	9 (75.00)	17 (70.83)
DNase	18 (31.03)	3 (25.00)	12 (50.00)
PPI or H2 blocker	21 (36.21)	4 (33.33)	8 (33.33)
Rates of bacterial co-infections (%)			
S. aureus	**34 (60.71)**	**9 (75.00)**	**6 (25.00)**
P. aeruginosa	**22 (39.29)**	**3 (25.00)**	**22 (91.67)**

when compared to the control group. Consequently, higher baseline rates of chronic antibiotic use, including a higher proportion of pwCF using chronic azithromycin and inhaled tobramycin therapy, were observed in the control group. Across all three cohorts, only two subjects (2.9%), both from the persistent *H. influenzae* group, were started on cystic fibrosis transmembrane regulator (CFTR) modulator therapy (ivacaftor) during the study.

Natural history, epidemiology, characteristics, and co-infections with *H. influenzae*

We first sought to characterize the *H. influenzae* isolates by capsular identification, antimicrobial susceptibility, and co-infections at time of sputum culture. To further characterize *H. influenzae*, we sought to evaluate the prevalence of typeable strains across those with positive sputum cultures. One-hundred and thirty-five isolates (98.5%) were NTHi using *bexB* PCR and in silico serotyping after sequencing the PCR amplification product [11]. Four isolates were *bexB-positive* (3%), but only two were determined to be serotype f (1.5%), with the remaining two isolates missing capsule or serotype f backbone genes. While both serotype f isolates were related as determined by similar pulsotypes, they were isolated from different subjects (A059 and A274) years apart from each other. For subject A059, this was the only time they isolated *H. influenzae*, whereas A274 had another, non-typable isolate. Susceptibility of *H. influenzae* isolates to commonly used CF drugs were assessed as per CLSI guidelines. Antimicrobial resistance was generally low with resistance rates of 24.51% (62/253) for trimethoprim-sulfamethoxazole (TMP/SMX), 18.29% (45/246) for ampicillin, 17.24% (5/29) for cefuroxime, 8.11% (3/37) for clarithromycin, 0% (0/241) for ciprofloxacin, and 0% (0/11) for ceftriaxone.

To further discern co-infections at time of sputum culture, microbiology metadata was reviewed where available (303 of 306 isolates). In only a minority of cases (n=36, 13.8%) was *H. influenzae* the only organism cultured from sputum using standard laboratory growth techniques, whereas the remainder were polymicrobial with other classical CF pathogens. Methicillin-susceptible *S. aureus* (MSSA) co-culture in sputum was most common (n=202, 66.7%) followed by *P. aeruginosa*

(*n*=71, 23.4%), *B. cepacia* complex (*n*=21, 7.0%), methicillin-resistant *S. aureus* (MRSA) (*n*=12, 4.0%) and *S. maltophilia* (*n*=11, 3.6%). The median sputum bioburden, as measured by colony forming units (CFU/mL of sputum) of *H. influenzae* did not differ between those recovered in isolation or those from co-infections (10^6 polymicrobial vs. 10^6 monomicrobial, p=0.75). *P. aeruginosa* co-infection at time of initial *H. influenzae* infection was not associated with the subsequent development of persistent *H. influenzae* infection (RR 0.97 [0.58–1.62], p=1.00). Sputum burden (in CFU/mL) did not differ at first isolation of *H. influenzae* in those who had a transient infection compared to those with a persistent infection (10^6 transient vs. 10^7 persistent, p=0.11). There was also no difference in sputum burden (in CFU/mL) in those who cultured *H. influenzae* during a PEx compared to routine clinic visits (10^6 vs. 10^6, p=0.68).

We next sought to evaluate the natural history of carriage of *H. influenzae* across our cohorts. The 70 individuals with sputa positive for *H. influenzae* had a total of 519 culture-years. Forty-eight (68.6%) subjects had *H. influenzae* cultured on more than one occasion, and 12 (17.1%) of these met the criteria for persistent infection (Fig. 1). Sixty-five subjects (93%) had at least one isolate available in the Biobank for pulsotyping. Isolates from the remaining five subjects were either not found in the Biobank or there was insufficient material left to test. Subjects without pulsotyped isolates did not significantly differ on the basis of age, sex or lung function. Moreover, four of these subjects (80%) cultured *H. influenzae* on only one occasion. Of the 137 *H. influenzae* isolates, 94 unique pulsotypes were identified, unique from all other strains within our comprehensive Biobank (Supplemental Fig. 1). The median duration of time between culture dates of shared clones was 1.21 years (IQR 0.66 to 2.06). Thirty-five (54%) individuals had a single pulsotype, 23 (35%) two, 6 (9%) three, and 1 (2%) four recovered throughout the fourteen years of study. Amongst those with persistent infection by strain typing, 5 (42%) isolated a single pulsotype, 4 (33%) two, 2 (17%) three, and 1 (8%) had four different pulsotypes throughout the study (subject A367) (Fig. 2). Ultimately, most (*n*=63/70, 90%) subjects cleared their *H. influenzae* infection, as defined by culture negativity, with a median duration of sputum carriage of 2.78 years (IQR 1.30 to 4.48). The median number of cultures taken from each subject throughout the study was 20 (IQR 11–34), and each subject had a median of 12% of sputum cultures positive for *H. influenzae* (IQR 10 to 18), which differed significantly between those with transient and persistent infection (26.1% vs. 10.5%, p=0.009).

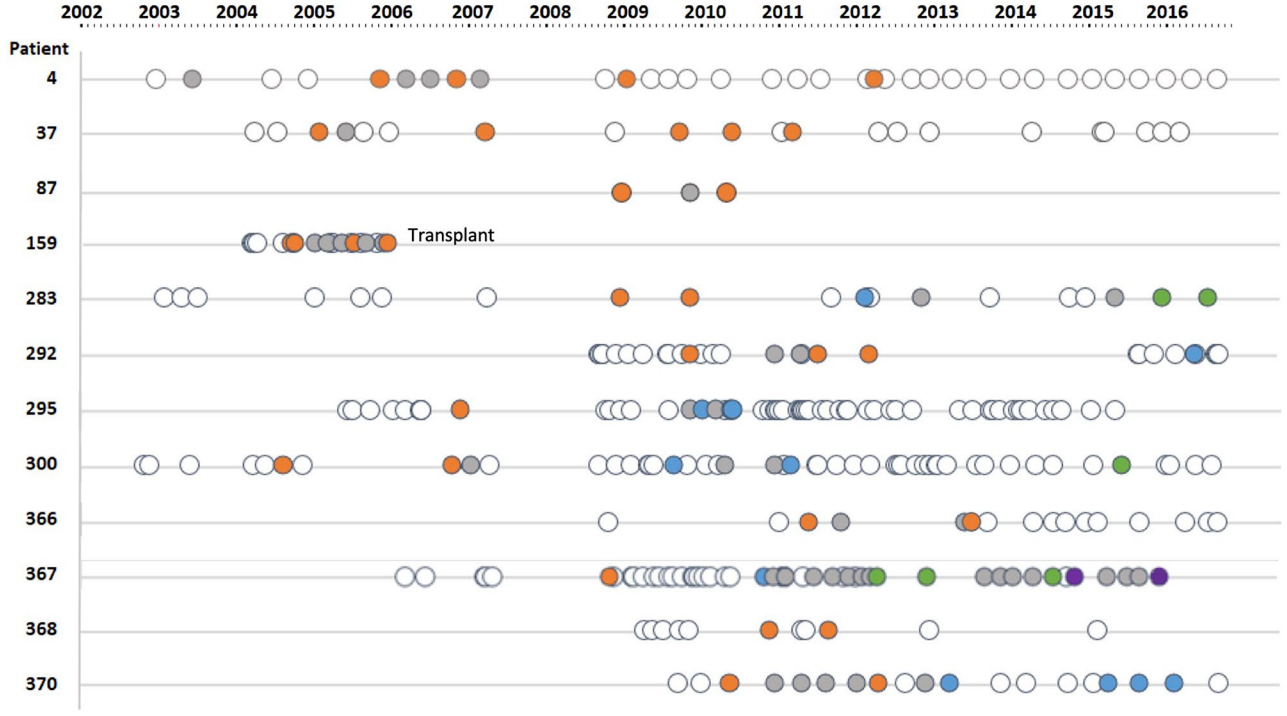

Fig. 2 Natural history of *H. influenzae* in expectorated sputum in those with persistent infection. Different colours were used to designate a change in sequential pulsotypes for each pwCF and the same colour does not indicate a shared pulsotype across pwCF. Grey indicates the sample contained *H. influenzae*, but it was not typed. White indicates that the subject had a sputum sample that did not contain *H. influenzae*

Association of *H. influenzae* sputum positivity and clinical outcomes

We next evaluated whether *H. influenzae* presence was associated with adverse clinical outcomes including PEx events and lung function decline. No increase in the frequency of PEx was observed at the time of incident *H. influenzae* infection (removing subjects with *H. influenzae* prior to study enrolment) compared to the clinic visits immediately preceding and following incident infection (14/51 PEx during infection vs. 17/102 PEx when uninfected; RR=1.65 [0.88–3.07], p=0.11). However, when all *H. influenzae* infections (i.e., total prevalence) were assessed, the PEx frequency was greater during periods when *H. influenzae* was cultured from sputum as compared to periods where *H. influenzae* was not isolated (50/281 PEx during infection vs. 62/562 PEx when uninfected; RR=1.61 [1.14–2.27], p=0.006). The risk was similar when we only included those who had infections with incident pulsotypes, thought to represent new infections (RR=1.62 [1.12–2.35], p=0.01). However, when only individuals who were regular sputum-producers (defined as those with sputum produced in the preceding and following 6 months) were analyzed, there was no difference in PEx frequency in the *H. influenzae* sputum positive cohort as compared to controls (RR=1.29 [0.82-2.00], p=0.17). Again, the risk was similar in regular sputum-producers with incident pulsotypes (RR=1.37 [0.87–2.16], p=0.18). Subjects with transient *H. influenzae* had a trend towards a higher risk of PEx at the time of incident *H. influenzae* infection as those with persistent *H. influenzae* infections, but this was not significant (33/147 PEx for transient vs. 15/113 PEx for persistent, RR=1.69 [0.97–2.96], p=0.06).

There were no significant differences in lung function decline ($ppFEV_1$ predicted) when comparing the two years prior to initial *H. influenzae* infection to the following two years (-2.44% [95% CI: -4.14% to -0.74%] vs. -1.50% [95% CI: -2.71% to -0.29%], p=0.32, respectively). Moreover, those with transient *H. influenzae* infections had a similar annual lung function decline as subjects with persistent infections (-1.47% [95% CI: -1.98% to −0.96%] vs. -1.16% [95% CI: -1.60% to -0.72%], p=0.61). Looking at all three cohorts, we see a similar rate of $ppFEV_1$ decline between the groups (control: -1.31% [95% CI: -1.71% to -0.90%], transient: -1.47 [95% CI: -1.98% to -0.97%] and persistent: -1.16% [95% CI: -1.60% to -0.72%], p=0.58). Finally, when assessing chronic co-infections, we see a similar decline in subjects with and without *P. aeruginosa* (-1.60% [95% CI: -5.44–0%] vs. -1.2% [95% CI: -4.68–1.82%], p=0.72) but *S. aureus* was associated with an accelerated decline (-2.25% [95% CI: -6.52–0%] vs. 0.88% [95% CI: -3.41–3.85%], p=0.008).

In contrast, subjects with transient *H. influenzae* infections had a higher annual hospitalization rate in the two years following infection when compared to those persistently infected (0.50/year for transient [CI: 0.32–0.69] vs. 0.16 times/year for persistent [CI: 0.04–0.28] p=0.008). There was no significant difference in the mean annual number of outpatients intravenous antibiotic days (2.00 days/year for transient [CI: 0.20–3.80] vs. 0.32 days/year for persistent [CI: 0.13–0.76], p=0.11). When compared to controls, those persistently infected had no difference in hospitalizations (0.16 times/year for persistent [CI: 0.04–0.28] vs. 0.36 times/year for control [CI: 0.17–0.56] p=0.120) and rather, control subjects had more outpatient intravenous antibiotic days (0.32 days/year for persistent [CI: 0.13–0.76] vs. 1.91 [CI:0.47–3.34], p=0.047).

Discussion

As a consequence of *H. influenzae* predominantly being considered a pathogen of the pediatric population, the epidemiology and clinical significance of *H. influenzae* is poorly understood in adult pwCF. Moreover, the incident recovery *of H. influenzae* from adult pwCF often leads to therapeutic uncertainty for clinicians. Our study is the first to specifically dissect the natural history and consequences of *H. influenzae* infection in adult pwCF. Understanding of the natural history of any infection is key as it allows for prognostication and can aid in treatment decision-making. In our study, demographics were similar across between the control group, those transiently infected, and those persistently infected with *H. influenzae*. While registry data has suggested the prevalence of *H. influenzae* infection to be around ~10% in adult pwCF [4], we observed that nearly one-third of pwCF had *H. influenzae* isolated at least once when followed longitudinally, with 17% meeting criteria for persistent infection. Our findings in adults mirror similar pediatric cohort studies that have identified a 1-year incidence of 20% and 5-year prevalence of 23% of *H. influenzae* positivity [18, 19]. Although direct comparisons are limited, our study suggests prevalence of *H. influenzae* across adult pwCF may be underreported.

The diversity of pulsotypes observed in our cohort expands on other studies that have evaluated strain dynamics amongst cohorts of pwCF [11, 19, 20], and is aligned with an earlier study by our group that assessed genetic diversity using whole genome sequencing where no evidence of infection transmission was identified [11]. After NTHi, serotype f was the most common serotype identified in our cohort. A finding in keeping with Canada's changing *H. influenzae* epidemiology, where is has emerged to the most prevalent encapsulated serotype [21, 22]. Furthermore, longitudinal analysis of chronically infected patients demonstrated intermittent detection of specific pulsotypes in between *H. influenzae*-negative sputum samples. Taken together, these findings suggest a reservoir source of *H. influenzae* that can re-infect and

colonize the lung over time. Nasopharyngeal colonization by *H. influenzae*, one of the most prevalent bacteria in the sinuses of pwCF [23, 24], may be a reservoir for such recurrent infections. Further characterization using paired sputum and sinus samples from the same subject would be of interest to evaluate commonality of *H. influenzae* strains within individuals.

The clinical relevance of *H. influenzae* in a sputum culture is unclear, particularly in contrast to other classical pathogens such as *P. aeruginosa* [8]. This knowledge gap is important as clinicians need to differentiate between cases that represent colonization compared to those that may require antibacterial therapy. Over the course of our study, isolation of *H. influenzae* from sputum was associated with an increased risk of PEx, however, this association was absent in those with regular sputum production. In addition, PEx risk did not increase with the first *H. influenzae* infection, even when we controlled for new/incident pulsotypes. Taken together, this suggests that *H. influenzae* recovered only at time of PEx is more likely to come from limited sputum producers and may not accurately reflect the lower respiratory tract colonization. To support this, lung function decline in the control group was similar to that of the transiently infected or persistently infected subjects (-1.31% vs. -1.47% vs. -1.16% FEV_1/year), and while we did find a possible association of *H. influenzae* with PEx, this did not translate across clinical outcomes of interest. An exception is that the transiently infected cohort had a higher hospitalization frequency, though this could be explained by confounders (i.e., higher rates of *P. aeruginosa* co-infections) and requires further exploration.

Across our historical cohort of pwCF, infection by *H. influenzae* occurred more frequently amongst those without chronic *P. aeruginosa* infection. The antagonistic relationship between *H. influenzae* and *P. aeruginosa* is well established in non-CF bronchiectasis and may relate to direct or indirect competition [25, 26]. In the era of highly effective modulator therapy (HEMT) and known microbiome restructuring post-elexacaftor-ivacaftor-tezacaftor therapy, prevalence rates of *H. influenzae* may increase owing to the declining rates of *P. aeruginosa* chronic infection [27–29]. This may be further influenced by the increased risk of infection in those not taking nebulized antibacterials and chronic azithromycin – a trend expected to increase in conventional CF cohorts on the basis of increasing modulator use and decreasing chronic *P. aeruginosa* infection rates [30, 31]. In the future, a study examining the role of *H. influenzae* with a concurrent evaluation of the CF lung microbiota could help elucidate further clinical relevance on perturbation of the community.

We acknowledge several limitations to our study. First, our study focused on differential clinical outcomes and was unable to infer causation due to its retrospective cohort design and the limitations inherent in any study of this nature. In addition, incidence of persistent infection could have been underrepresented as 19 (27.1%) of the 70 subjects had previously cultured *H. influenzae* at time of study enrollment, indicating prior infection. We could not further discern these infection dynamics as these were often performed at a pediatric facility where sputum pathogens are not routinely stored and thus unavailable for analysis and/or pulsotyping. Furthermore, by assessing a subset of our total samples (annual isolates), some cases of persistence may not have been included. The single-centre nature of our study limited generalizability to other patient populations and there may be selective bias with sampling sputa at both regular intervals and with exacerbations. Finally, our study predominately predates the advent of highly effective CFTR modulator therapy. Recent work has suggested a relative restructuring of the CF lung microbiome following elexacaftor-ivacaftor-tezacaftor therapy, suggesting prevalence of *H. influenzae* may be altered in future adult pwCF cohorts [29].

Conclusion

In summary, our study found *H. influenzae*, predominantly NTHi, in as many as 30% of individuals when followed longitudinally. The vast majority of infections were transient, and even if a subject carried the same strain for a prolonged period, it would eventually be eliminated, manifesting in many serial infections with different strains. No adverse impact associated with infection was observed on short-term or long-term outcomes. Taken together, *H. influenzae* may become more relevant in the future understanding of the adult CF lung milieu due to the increased utilization of modulator therapy and anticipated recapitulation of milder lung disease.

Abbreviations

BMI	Body Mass Index
CF	Cystic Fibrosis
CLSI	Clinical and Laboratory Standards Institute
DNase	Inhaled Dornase Alfa
FEV1	Forced Expiratory Volume in First Second
FVC	Forced Vital Capacity
H2	Histamine H2 Antagonist
IQR	Interquartile Range
LABA	Long-Acting Beta Agonist
MRSA	Methicillin-Resistant Staphylococcus aureus
MSSA	Methicillin-Susceptible Staphylococcus aureus
NTHi	Non-typable Haemophilus influenzae
PFGE	Pulsed Field Gel Electrophoresis
PEx	Pulmonary Exacerbation
PPI	Proton Pump Inhibitor
pwCF	Persons with Cystic Fibrosis
RA	Relative Abundance
SABA	Short-Acting Beta Agonist
TMP/SMX	Trimethoprim/Sulfamethoxazole

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10050-7.

Supplementary Material 1

Supplementary Material 2

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Author contributions
BRW and NA were responsible for sample collection. NA was primarily responsible for DNA extraction and microbiome analysis. Sample analysis and statistical analyses were performed by BRW, NA, BJW, HRR, MGS, CST and MGS. MDP, CST and NA extracted and analyzed clinical data. BRW wrote the initial draft of the manuscript, and all authors contributed to its revision. MDP is the guarantor of this work. All authors read and approved the final manuscript.

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Data availability
All data from the manuscript are included herein.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the Declaration of Helsinki and was approved by the University of Calgary Conjoint Regional Health Ethics Board with the following reference numbers: REB15–0854 and REB15–2744. Patients were recruited from the Calgary Adult CF Clinic and all patients provide written informed consent for the collection and storage of specimens and subsequent analysis. All samples were de-identified. Patients have contributed samples to this regional ethics board (REB) collection from 1998-present (REB15–0854) and provide consent for ongoing research purposes (REB15–2744).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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