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Epidemiology and natural history of *Pseudomonas aeruginosa* airway infections in non-cystic fibrosis bronchiectasis

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ABSTRACT The natural history and epidemiology of *Pseudomonas aeruginosa* infections in non-cystic fibrosis (non-CF) bronchiectasis is not well understood.

As such it was our intention to determine the evolution of airway infection and the transmission potential of *P. aeruginosa* in patients with non-CF bronchiectasis.

A longitudinal cohort study was conducted from 1986–2011 using a biobank of prospectively collected isolates from patients with non-CF bronchiectasis. Patients included were ⩾18 years old and had ⩾2 positive *P. aeruginosa* cultures over a minimum 6-month period. All isolates obtained at first and most recent clinical encounters, as well as during exacerbations, that were morphologically distinct on MacConkey agar were genotyped by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). A total of 203 isolates from 39 patients were analysed. These were compared to a large collection of globally epidemic and local CF strains, as well as non-CF isolates.

We identified four patterns of infection in non-CF bronchiectasis including: 1) persistence of a single strain (n=26; 67%); 2) strain displacement (n=8; 20%); 3) temporary disruption (n=3; 8%); and 4) chaotic airway infection (n=2; 5%). Patterns of infection were not significant predictors of rates of lung function decline or progression to end-stage disease and acquisition of new strains did not associate with the occurrence of exacerbations. Rarely, non-CF bronchiectasis strains with similar pulsotypes were observed in CF and non-CF controls, but no CF epidemic strains were observed. While rare shared strains were observed in non-CF bronchiectasis, whole-genome sequencing refuted patient–patient transmission.

We observed a higher incidence of strain-displacement in our patient cohort compared to those observed in CF studies, although this did not impact on outcomes.

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Introduction

Chronic airway infections are a hallmark of suppurative lung diseases, including non-CF bronchiectasis. Bronchiectasis is characterised by the abnormal dilation and thickening of the bronchial walls, enabling chronic airway infections, and manifests clinically with a persistent productive cough [1]. Affected individuals may additionally experience acute pulmonary exacerbations (PEx), which present with increased respiratory symptomology, worsened lung function and systemic symptoms [2].

The presence of *Pseudomonas aeruginosa* in the airways has been associated with accelerated decline in lung function, worsened quality of life and increased morbidity and mortality in non-CF bronchiectasis [3, 4]. Whilst both the evolution and patho-adaptation of *P. aeruginosa* infections have been extensively studied in cystic fibrosis (CF), our understanding of these processes lags in non-CF bronchiectasis [5, 6]. Despite notable advancements in non-CF bronchiectasis research, much of our current understanding of airway infections in non-CF bronchiectasis is extrapolated from CF. Specifically, the following observations have been made regarding *P. aeruginosa* infections in CF: 1) changes in clinical status such as PEx are not solely due to the acquisition of new infections but arise from perturbations affecting the original colonising strains [7]; 2) adult *P. aeruginosa* airway infections are generally stable with the exception of rare events of acquisition and eventual replacement or co-infection of epidemic (ePA) strains [7–10]; 3) transmission of *P. aeruginosa* infections is possible and may be associated with adverse clinical consequences [11–13].

Whilst recent studies [14, 15] have started to investigate these trends, continued research will contribute to a better understanding of non-CF bronchiectasis. Therefore, we set out to characterise the epidemiology, transmission characteristics and clinical outcomes of *P. aeruginosa* infection in non-CF bronchiectasis using a longitudinal collection of biobanked isolates. Given the similarities between CF and non-CF bronchiectasis lung disease, we hypothesised that similar trends in the epidemiology and outcomes of *P. aeruginosa* infections would be observed between both diseases.

Materials and methods

Patients and strains

Patients were identified from a retrospective review of the microbiologic records of the Calgary Bronchiectasis Clinic (CBC) Biobank (1980–2015). This biobank contains prospectively collected and inventoried bacterial pathogens recovered in real time from the sputum of non-CF bronchiectasis patients upon submission of a quantitative sputum culture as part of their routine care. From each sputum sample, plated on MacConkey agar, every individual morphotype of suspected *P. aeruginosa* was confirmed as *P. aeruginosa* using standard methodologies in real time. Morphotypes were defined according to *a priori* definitions (see supplemental table S1). Individual isolates were then subsequently transferred into skim milk stock and frozen at −80 °C and entered into the CBC Biobank. For inclusion in to our study, patients had a diagnosis of bronchiectasis with the exclusion of CF and two or more sputum samples within the biobank spanning ⩾6 months where *P. aeruginosa* was isolated. Bronchiectasis was confirmed radiographically [16]. From each patient, every individual *P. aeruginosa* morphotype from their first encounter (FE; the first isolate within the biobank), their most recent encounter (RE; the most recent isolate for active and inactive patients) and, where available, serial alternate year isolates and exacerbation encounters (EE; isolates identified at the time of PEx) were assessed.

The prevalence of shared strains of *P. aeruginosa* and the potential for cross-infection amongst non-CF bronchiectasis patients was assessed using a control cohort consisting of 812 isolates obtained from CF patients over the last 30 years, 22 isolates obtained from local environmental sources (both natural environments and hospital facilities) and 35 strains obtained from community-acquired blood stream infections (CA-BSI). Of the 812 CF-derived isolates, 65 representatives of globally distributed ePA strains known to commonly exist within patients with suppurative lung infections were used [9, 17].

Molecular typing

The pattern of *P. aeruginosa* infections, including whether patients were chronically infected with the same strain longitudinally or whether serial infections with different isolates occurred, was primarily assessed using a pulsed-field gel electrophoresis (PFGE) protocol described by Parkins *et al.* [9]. Strains conforming to the criteria of Tenover *et al.* [18] were considered related. PFGE profiles were compared using BioNumerics Version 7.0 (Applied Maths, Austin, TX, USA). The resulting dendrograms were generated at 2.0% position tolerance and 1.5% optimisation using the unweighted pair-group method with arithmetic mean (UPGMA) and the Sørensen–Dice similarity coefficient. Clusters of *P. aeruginosa* strains derived from three or more unrelated patients were *a priori* considered clonal [9].

Following PFGE, multilocus sequence typing (MLST) was used to confirm the relatedness of the FE and the RE isolates for each patient [19]. The resulting MLST profiles were then referenced to an online

P. aeruginosa MLST database (http://pubmlst.org/paeruginosa/) in order to identify allele and sequence types. Isolates were considered putatively clonally related if the MLST profiles had less than two loci altered but were assigned a novel sequence type if strains were one or more loci different than identified in the online database [9].

Isolates with similar MLST profiles from unrelated patients were sequenced using whole-genome sequencing (WGS) to investigate the potential for patient-to-patient transmission as described previously [20]. Contigs (sets of overlapping DNA segments that combined represent a consensus region of DNA) were assembled using the A5-MiSeq pipeline [21], annotated with PROKKA [22] and subjected to genome comparison using Roary [23].

Clinical information and definitions

In order to determine whether the different patterns of infection were associated with particular patient characteristics, acute PEx events, or the rate of lung function decline, a detailed chart review was performed [24]. Chronic *P. aeruginosa* colonisation at baseline was defined as per the Leeds criteria adapted from CF, whereby patients were defined as chronically infected if more than 50% of cultures obtained over the preceding year were positive for *P. aeruginosa* [25]. The aetiology of bronchiectasis was classified as: post-infectious, immunodeficiency, idiopathic and other. PEx events were clinician-defined based on documentation of increasing respiratory symptoms or radiographic changes resulting in new antimicrobial therapy. The pattern of infection was characterised using molecular typing and defined based on similarity of isolates. If all isolates were found to be clonally related then the patient was defined as a "stable" infection. If isolates collected from a patient were dissimilar, they were classified within the strain displacement category. Patients attending the CBC are seen on a consultation basis but continue to receive the bulk of their care from primary care physicians and local respirologists. With respect to infection prevention standards, we enforce strict cough and hand hygiene and actively discourage patient contact. Patients do not share clinic rooms, waiting rooms or inpatient rooms. However, we do not utilise universal contact precautions nor ask patients to wear surgical masks. The study was approved by the local ethics board (REB16-0035).

Statistical analysis

Non-normally distributed data were represented using median values with interquartile range (IQR). Categorical variables were analysed using the two-tailed Fisher's exact test. Continuous data were analysed using the two-tailed Mann–Whitney U-test. For our primary clinical outcome analysis, yearly lung function decline (% predicted forced expiratory volume in 1 s (FEV_1)) between chronic and strain-displacement groups was modelled through the use of generalised estimating equations (GEEs) with exchangeable correlation structures and robust standard errors. Analyses were conducted with STATA Version 13.1 (StataCorp, College Station, TX, USA) and Prism 5.0 (Graphpad Software, La Jolla, CA, USA).

Results

Patient demographics

The inclusion criteria were met by 39 patients with non-CF bronchiectasis (table 1). The aetiology of non-CF bronchiectasis was primarily post-infective (51%) and idiopathic (44%), although there were single cases of immunodeficiency (common variable immunodeficiency) and toxic inhalation. Median age at enrolment was 58 (IQR 23–81). Baseline % predicted FEV_1 at cohort inclusion was 53% (IQR 39.8–73%) and % predicted forced vital capacity (FVC) was 67% (IQR 51–83%). Eleven patients (28%) had a history of lung resection prior to enrolment, with one patient (2.6%) receiving a lung transplant during his time at the non-CF bronchiectasis clinic. One patient died during the study and 43% of patients eventually required long-term oxygen supplementation. Thirty-one patients (79%) had chronic infection at the FE [25]. Notably, chronic infection at baseline did not differ between the strain-displacement and stable groups ($p=1.00$), nor did length of microbiological follow-up.

Natural history of P. aeruginosa *in non-CF bronchiectasis and clinical outcomes*

A total of 203 isolates (range: two to eight per patient), representing eight distinct morphotypes out of a possible 34 which were defined *a priori* (see supplementary table S1 and figure S1), were genotyped and patterns of colonisation were distinguished based on the PFGE profiles for each patient. In only one instance were multiple morphotypes apparent at a single time point. The median duration between the earliest and most recent isolates was 3.2 years (IQR 1.1–8.3) and ranged from 0.5–21 years. If all isolates characterised for each patient over the study period were clonally related, the patient was defined to have a "stable" infection. Conversely, if the initial isolate was no longer observed but a new isolate was, the intermediate isolates for each patient were used to determine the overall history of infection. Using this classification, we observed four patterns of *P. aeruginosa* colonisation: 1) absolute persistence of the

TABLE 1 Baseline patient demographics

Selected patient demographics	Stable (n=26)	Strain displacement (n=13)
Age at enrolment years	66.7 (47.9, 72.7)	61.46 (41, 71)
Duration of follow-up years	2.8 (1, 7)	4.8 (1, 10.4)
FEV_1 % predicted	51 (39, 65)	58 (40.5, 78.5)
Respiratory comorbidities		
Sinusitis	11 (42)	6 (46)
Reactive airway disease	7 (27)	3 (23)
COPD	8 (31)	1 (8)
Aetiology		
Idiopathic	13 (50)	4 (31)
Post-infective	13 (50)	7 (54)
Immunodeficiency		1 (8)
Other		1 (8)
Recorded antibiotics used		
Inhaled tobramycin	6 (23)	3 (23)
Azithromycin	6 (23)	1 (8)
Ciprofloxacin	3 (12)	3 (23)
Additional therapies		
Inhaled β_2-agonist	23 (88)	10 (77)
Inhaled CS	16 (62)	5 (38)
Ipratropium bromide	2 (8)	6 (46)
Systemic CS	5 (19)	2 (15)
Spiriva	6 (23)	2 (15)
Long-term oxygen therapy	12 (46)	5 (38)

Data are presented as n (%) or median (interquartile range). FEV_1: forced expiratory volume in 1 s; COPD: chronic obstructive pulmonary disease; CS: corticosteroid.

original infecting strain without invasion by an extraneous isolate (n=26; 67%); 2) strain displacement where the original strain was no longer observed (n=8; 20%); 3) temporary acquisition of a new strain type eventually reverting back to the original strain (n=3; 8%); and 4) chaotic airways colonisation where new strains were continually identified (n=2; 5%) (figure 1). To assess if unstable airways infections associated with a different aetiology and subsequent clinical course, those with stable infections were compared to those where more than one strain type was identified (combining the three other groups) (figure 2).

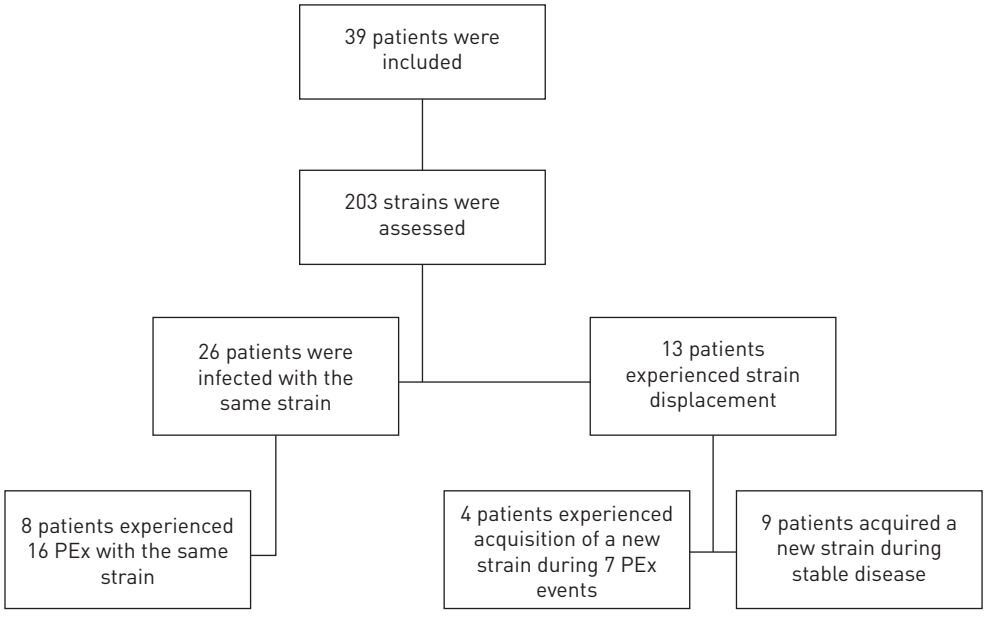

FIGURE 1 Flow chart of study design including the total number of non-cystic fibrosis bronchiectasis patients enrolled. PEx: pulmonary exacerbation.

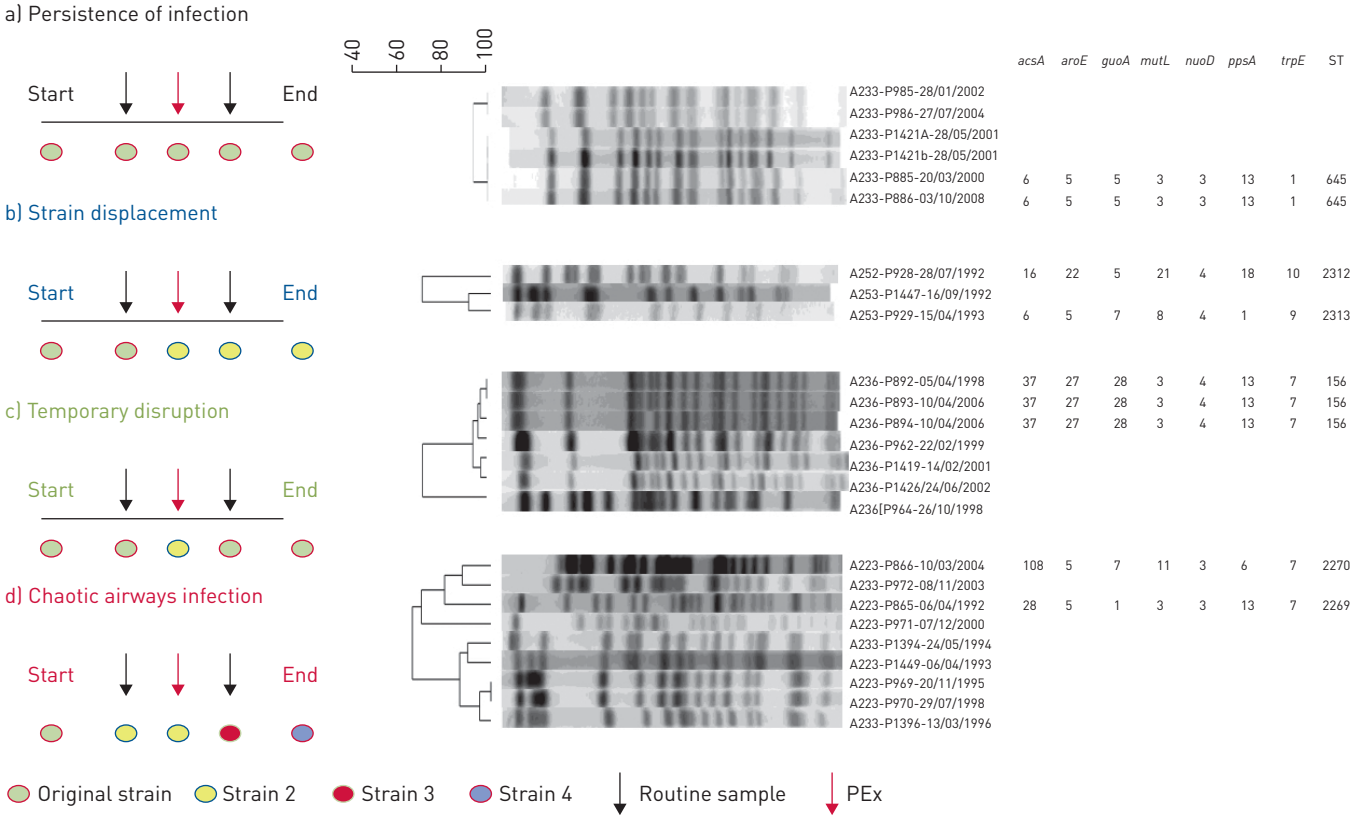

FIGURE 2 Displaying the diversity in the natural history of infections of *Pseudomonas aeruginosa* in patients with non-cystic fibrosis bronchiectasis including: a) patients with stable infection by a single strain of *P. aeruginosa* (persistence of infection; n=26); b) strain displacement (n=8); c) temporary disruption (n=3); d) chaotic airways infection (n=2). The dendrograms were generated at 2.0% position tolerance and 1.5% optimisation using the unweighted pair-group method with arithmetic mean (UPGMA) and the Sørensen–Dice similarity coefficient. Isolates were named following the format "A(patient)-P(isolate)-(date isolated)". ST: sequence typing; PEx: pulmonary exacerbation.

Patterns of infection analysis revealed no association with patient demographics, including aetiology of non-CF bronchiectasis, comorbidities, sex, age and duration of culture follow-up. Antibiotic therapies received during the study, including inhaled tobramycin and azithromycin, had no association with stable or unstable infections. Similarly, the rate of FEV_1 decline between patients with stable strains −0.7% per year (95% CI −1 to −0.5%) and those with strain displacement +0.2% per year (95% CI −0.2 to −0.6%) was not found to be different (p=0.37). Additionally, 12 patients experienced 23 PEx events during clinic visits, of which 16 events (70%) were associated with the same chronically colonising strain being isolated (p=0.01) (figure 2). Four of the 12 patients experienced seven PEx events (30%) which were associated with a new strain, different from the chronically infecting isolate. Of the seven PEx events associated with different strains, four were transient infections, one persisted after strain-displacement and two were identified in individuals with chaotic airways infection where they were subsequently displaced by a further new strain.

Prevalence of clonal isolates and patient-to-patient transmission in non-CF bronchiectasis

Overall, no *P. aeruginosa* clone within our non-CF bronchiectasis cohort was identified to be disproportionally prevalent. While four pairs of patients shared strains, this did not fit our definition for clonality (MLST types=156, 2314, 2293, 2294) (figure 3). Of these patients, one pair of patients with sequence type (ST)=156 had attended the clinic within the same year but never during the same clinic day. The remaining pairs of patients had at least ⩾1 year between the collection of isolates (ST=2314, 2293, 2294). Whole-genome analysis of these strains showed that isolates from the same patient exhibited a greater degree of similar gene content, suggesting independent acquisition in each patient as opposed to transmission (figure 4).

A comparison of the 203 non-CF bronchiectasis derived *P. aeruginosa* isolates revealed no known representatives from our regional and global ePA collection. While PFGE patterns between AUST-3 and five non-CF bronchiectasis isolates were observed to be 89% similar, discrepancies in three MLST alleles

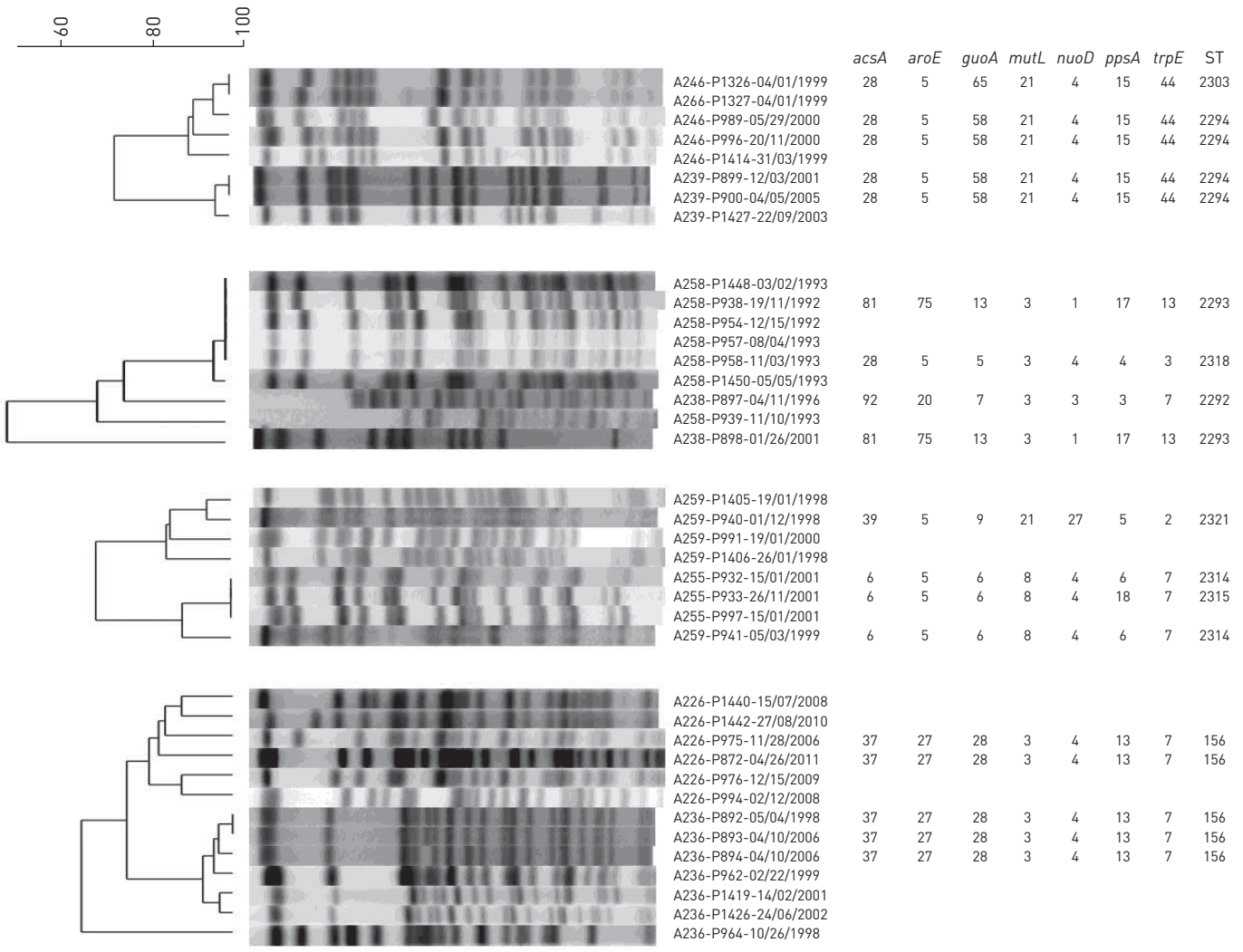

FIGURE 3 Cluster diagram for four patient pairs with genotyped strains sharing the same sequence typing (ST). The dendrograms were generated at 2.0% position tolerance and 1.5% optimisation using the unweighted pair-group method with arithmetic mean (UPGMA) and the Sørensen–Dice similarity coefficient. Isolates were named following the format "A(patient)-P(isolate)-(date isolated)".

were found and did not meet our definition for clonally related strains. However, similarities in the PFGE profiles between 19 non-CF bronchiectasis *P. aeruginosa* isolates from nine patients with 31 CF-derived *P. aeruginosa* isolates from 14 patients were observed. Four non-CF bronchiectasis isolates were observed to share 96% similar pulsotypes with one CA-BSI derived *P. aeruginosa* isolate. Furthermore, five non-CF bronchiectasis isolates were found to share 89% similar PFGE profiles with one environmental isolate. MLST data from non-(non-CF bronchiectasis) isolates was not available. Interestingly, the MLST profiles of the non-CF bronchiectasis derived *P. aeruginosa* isolates obtained in our study included eight MLST strain types (102, 175, 291, 390, 507, 645, 847 and 1752) than had previously been identified throughout regions of Europe [26]. One strain in particular (MLST type=175) was previously isolated in Canada, the UK, Poland, Spain and France from sputum, environmental sources, bronchial lavages, urinary-tract infections and soft-tissue infections.

Comparison of PFGE and MLST as P. aeruginosa *screening strategies*

While all isolates underwent PFGE as the primary screening modality, the FE and the RE for each patient were characterised using MLST for 33 patients (85%). Despite repeated attempts, we were unable to fully characterise the MLST profiles of the remaining six patients. Five of these patients had only the FE fully typed by MLST and one patient had neither the RE nor the FE fully characterised. In four of these six patients, PFGE profiles were found to be the same between the FE and the RE. When confirming *P. aeruginosa* infection patterns, we found that the narratives obtained using PFGE were concordant with MLST in 27 out of 31 patients (87%). Differences between the MLST and PFGE findings arose where

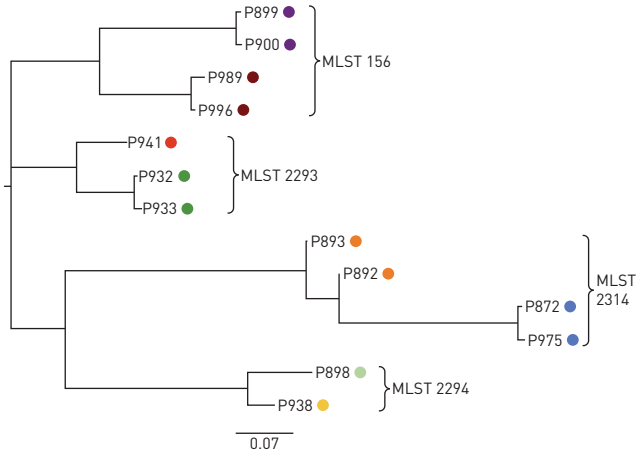

FIGURE 4 Clonal strains of *Pseudomonas aeruginosa* were identified from independent patient-pairs using multilocus sequence typing (MLST). Individual strains are coloured by patient. Strain relatedness was assessed using whole-genome sequencing and phylogeny generated from a matrix with the presence and absence of core and accessory genes using Roary [23].

serial strains were considered clonally-related by PFGE but were assigned novel strain types due to differences at one or more MLST loci, with the FE and the RE isolates from two patients having discordant MLST profiles due to differences in three loci.

Discussion

Our longitudinal study of non-CF bronchiectasis patients has observed several distinctions in the epidemiology of *P. aeruginosa* infections as compared to CF. Whereas *P. aeruginosa* infections in CF adults are known to be relatively stable, strain displacement with unique strains was observed commonly in non-CF bronchiectasis [7, 9]. This may be more similar to early airways infections in younger CF cohorts where *P. aeruginosa* strain turnover appears more common until a dominant strain emerges [27]. Furthermore, when new strains were observed, they were not necessarily associated with PEx events, in a similar fashion to what has been previously observed in CF [7]. Whereas cross-infection of *P. aeruginosa* has been observed in CF, we did not find any evidence of cross-infection amongst non-CF bronchiectasis patients despite observing patient-pairs sharing clonal strains [8, 9]. These trends may be due to differences in other components of the respiratory microbiota, mucus physiology and potential for pathogenic exposure of non-CF bronchiectasis patients [28].

Studies surrounding the epidemiology of infection in non-CF bronchiectasis airways are recent and ongoing. De Soyza *et al.* [29] recently assessed *P. aeruginosa* obtained from 40 non-CF bronchiectasis adults between 2008 and 2011 to better understand the potential for cross-infection. In a similar fashion they noted that the majority of patients were stably infected by unique strains of *P. aeruginosa* with only one potential cross-infection event identified. Likewise, Mitchelmore *et al.* [15] observed that non-CF bronchiectasis patients commonly harboured unique strains of *P. aeruginosa* and, in a similar fashion to our own study, noted the occasional presence of shared isolates between patients with CF and those with non-CF bronchiectasis, which likely represent environmentally prevalent strains. Furthermore, as all patients within our study had at least two longitudinal isolates obtained over a greater duration of follow-up, we were able to observe a higher rate of emergence of new/novel strains. These mixed infections observed in our cohort have similarly been observed in recent work by Hilliam *et al.* [14], which may indicate co-infection of non-CF bronchiectasis patients. In their study, co-infection by multi-lineage strains of *P. aeruginosa* and genomic diversification was observed in seven of 24 non-CF bronchiectasis patients when multiple isolates from a single time point were assessed. The diversification and adaptation of *P. aeruginosa* to the lung environment was observed through the acquisition of loss-of-function mutations within virulence determinants, which has been suggested to play a role in the adaptations in both CF and non-CF bronchiectasis [30–33].

Given the ubiquity of *P. aeruginosa* in the natural environment and abundant data from more recent works [34] showing shared clonal but unrelated connected strains, we believe that the four patient-pairs with shared strains observed in our study are not due to cross-infection. We demonstrated through WGS that isolates differed much more between patients than within patients, suggesting independent environmental acquisition [17, 34, 35]. Our findings agree with recent evidence positing the likelihood of independent acquisition of isolates from the environment [14, 15]. However, these findings do not exclude the potential for cross-infection in non-CF bronchiectasis, as a genetically-related strain has been identified using WGS from three unrelated patients sharing the same waiting area and lung function room at a UK centre [15]. However, in our case, no environmental source of cross-infection was found. Given that the

majority of patients acquiring *P. aeruginosa* are doing so from environmental sources, we suggest that tightening already stringent infection control standards for non-CF bronchiectasis patients in our clinic (specifically as they pertain to *P. aeruginosa*) is unwarranted.

While no clonal strains were identified in our study, we identified a strain (ST=175) with a widespread distribution within hospital centres in Canada, the UK and Central Europe [26]. As this strain is associated with multidrug resistance and severe infections, continued surveillance of ST175 will be important for its management and prevention of its transmission [36–38]. Furthermore, we did not identify any ePA amongst attendees at our regional non-CF bronchiectasis center, despite a high prevalence of ePA (~39%) amongst CF adults attending our regional CF clinic (mere feet away but generally seen on alternate days) [9, 24]. Given that individuals with CF and non-CF bronchiectasis experience similar structural airways disease and the same predilection for lower airways infection, and given that the viability of *P. aeruginosa* generated through cough aerosol is independent of disease aetiology [39], we believe it is the high levels of social interconnectivity of historical CF cohorts that manifests in markedly different *P. aeruginosa* epidemiology in patients with non-CF bronchiectasis [12].

Studies comparing PFGE and MLST as screening techniques have inferred distinct advantages associated with each technique, with MLST suggested as offering higher predictive power with unique isolates and PFGE as being more sensitive to detecting clonality [40]. In our study, we observed several situations where longitudinal isolates of *P. aeruginosa* from individual patients were found to have discrepant MLST profiles, with two or more alleles differing between the RE and the FE, resulting in the designation of "distinct, novel strain-type" despite a conserved pulsotype. In these cases, while clonality was inferred using PFGE, the extended duration between testing of the isolates may allow for random mutations in the housekeeping genes over time, resulting in the designation of a new strain. Overall, we found that the use of MLST and PFGE were complimentary and that the use of both techniques may offer the highest predictive resolution of clonality.

This study has a number of limitations. Most importantly, this was a multi-decade retrospective study of prospectively collected samples. Clinical data was derived from a referral centre and therefore does not include all relevant samples nor clinical data and, given the time span of the study, there is certainly potential for effect modification by time due to changing standards of care. Whilst all samples are derived from a single centre, our clinic is similar to other referral bronchiectasis clinics and is, to our knowledge, the only clinic that prospectively collects and stores pathogens derived from both CF and non-CF bronchiectasis patients. The small sample size of our study and the slow rate of lung function decline limit the ability to identify potential relationships between patterns of infection and clinical progression. We also acknowledge the limitation of evaluating *P. aeruginosa* from a minimum of two time points per patient, which may not represent the evolution of *P. aeruginosa* infection in all cases observed. To address this, we randomly genotyped a further 63 isolates using PFGE (initially 140 isolates) from various non-CF bronchiectasis patients who experienced chronic infection or strain-displacement at various time points. Despite screening more isolates the pattern of infection for each patient remained consistent, suggesting sampling bias has not influenced our findings. While every individual morphotype that was identified (on MacConkey agar) in real-time underwent retrospective genotyping at each time point in our cohort, these results may have been insensitive to detecting multi-lineage simultaneous infections which have been detected in a recent work [27]. However, this would require that multi-lineage infections have the exact same morphotype, something that has not been previously reported. Future prospective studies should consider sampling multiple examples of each identified morphotype, something not possible in a study drawing from a biobank such as ours.

Taking advantage of a regional biobank has presented our study with a unique opportunity to follow the history of *P. aeruginosa* infections in non-CF bronchiectasis, in some cases over 20 years. While a majority of non-CF bronchiectasis patients experience persistent colonisation by an individual *P. aeruginosa* strain, a higher than expected number of individuals were observed who experienced strain displacement over the course of their disease. Clinical progression was independent of the natural history of *P. aeruginosa* infection and strain displacement was not linked with episodes of PEx. We did not observe the existence of any novel non-CF bronchiectasis ePA or known CF ePA in non-CF bronchiectasis. Furthermore, the sharing of strains amongst CF and non-CF bronchiectasis cases was noted in only a few instances, which we suspect represents environmentally ubiquitous isolates.

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Author contributions: T.E. Woo was responsible for collecting strains and served as the lead on the project. T.E. Woo, J.C. Bowron, M.G. Surette, B. Waddell and J. Duong performed strain typing. R. Lim, C.H. Mody and T.E. Woo were responsible for patient data collection. T.E. Woo, M.G. Surette and R. Somayaji performed statistical analyses. The

manuscript was prepared by T.E. Woo but was revised by all authors. The Calgary Non-CF Bronchiectasis Biobank is maintained by H.R. Rabin and M.D. Parkins. D.G. Storey and M.D. Parkins envisioned the project together and serve as guarantors of the work.

Conflict of interest: R. Somayaji has received research fellowship funding from Cystic Fibrosis Canada, Alberta Innovates and the Canadian Institutes of Health Research, and grants from Cystic Fibrosis Canada, the Canadian Institute of Health Research and the Royal College of Physicians and Surgeons outside the submitted work. M.D. Parkins has received research funding from Cystic Fibrosis Canada, the Canadian Institute of Health Research, the Lung Association of Alberta and the Northwest Territories, Gilead Sciences, The University of Calgary and Calgary Laboratory Services.

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