ORIGINAL ARTICLE

Long-term clinical outcomes of 'Prairie Epidemic Strain' *Pseudomonas aeruginosa* infection in adults with cystic fibrosis

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ABSTRACT

Rationale Epidemic *Pseudomonas aeruginosa* (PA) plays an important role in cystic fibrosis (CF) lung disease. A novel strain, the 'Prairie Epidemic Strain' (PES), has been identified in up to 30% of patients in Prairie-based Canadian CF centres.

Objective To determine the incidence, prevalence and long-term clinical impact of PES infection.

Methods A cohort of adults with CF was followed from 1980 to 2014 where bacteria isolated from clinical encounters were prospectively collected. Strain typing was performed using pulse-field gel electrophoresis and multilocus sequence typing. Patients were divided into one of four cohorts: no PA, transient PA, chronic PA with unique strains and chronic PES. Proportional Cox hazard and linear mixed models were used to assess for CF-associated respiratory death or transplantation, and rates of $\%FEV_1$ and body mass index (BMI) decline.

Results 274 patients (51.7% male) were analysed: 44—no PA, 29—transient PA, 137—unique PA, 64—PES. A total of 92 patients (33.6%) died or underwent lung transplantation (2423.0 patient-years). PES infection was associated with greater risk of respiratory death or lung transplant compared with the no PA group (aHR, 3.94 (95% CI 1.18 to 13.1); p=0.03) and unique PA group (aHR, 1.75 (95% CI 1.05 to 2.92) p=0.03). Rate of lung function decline ($\%FEV_1$ predicted) was greatest in the PES group (1.73%/year (95% CI 1.63% to 1.82%); p<0.001). BMI improved over time but at an attenuated rate in the PES group (p=0.001).

Conclusions Infection with PES was associated with increased patient morbidity through three decades and manifested in an increased risk of respiratory death and/or lung transplantation.

Key messages

What is the key question?

- As the Prairie Epidemic Strain represents a prevalent transmissible strain of *P. aeruginosa* in Western North America, what is its clinical impact on morbidity and mortality in persons with cystic fibrosis (CF)?

What is the bottom line?

- The 'Prairie Epidemic Strain' of *P. aeruginosa* is only the second epidemic strain that is associated with increased risk of death or lung transplantation, and has increased morbidity in patients with CF.

Why read on?

- This cohort study represents the largest and longest study of a transmissible strain of *P. aeruginosa* to date, encompassing >2400 patient-years of follow-up, and links microbiological and clinical data over this period with clinical outcomes.

INTRODUCTION

Cystic fibrosis (CF) is a genetic disease characterised by chronic progressive airway infection, punctuated by acute exacerbations, ultimately leading to respiratory failure. *Pseudomonas aeruginosa (PA)* remains the archetypal pathogen and causes chronic airway infection in 60%–70% of adults with CF.[1–5] Following initial *P. aeruginosa* infection, the typical clinical course involves conversion to a mucoid phenotype, leading to increasing exacerbations, treatment burden and deteriorating clinical status.[6–8] While *P. aeruginosa* infection is associated with increased morbidity and mortality in CF regardless of lung function,[9 10] significant heterogeneity exists in the natural history of lung disease.

P. aeruginosa is commonly found in the natural and urban environmental niches, and thus patients with CF were thought to each harbour uniquely acquired strains.[11–15] However, in the last three decades, multiple genetically related strains of *P. aeruginosa* infecting unrelated patients with CF have been described, determined to be transmitted between patients, and are thusly termed epidemic or transmissible strains. While epidemic *P. aeruginosa* (ePA) strains have been extensively studied, much remains to be understood regarding acquisition and natural history of infection.[16 17] With the use of a comprehensive biobank (established in 1978), we identified a novel strain of *P. aeruginosa* (multilocus sequence typing (MLST) 192) infecting patients with CF attending our clinic.[18] Termed the 'Prairie Epidemic Strain' (PES), it had a stable prevalence rate through multiple adult cohorts over 25 years, resulted in chronic stable infections and had increased antimicrobial resistance compared with unique strains.[18] PES was found in 1/3 of the cohort and had a broad distribution in Western Canada.[19]

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ePA are notorious for multidrug resistance potential and their association with adverse clinical outcomes. Transmissible strains have been associated with an accelerated rate of decline in pulmonary function to respiratory failure,[20] increased risk of death or lung transplantation,[21] increased frequency of pulmonary exacerbations[22 23] and greater antibiotic treatment burden[24] with toxicities.[25] Accordingly, identification of transmissible strains has impacted clinical management and infection control policies on a global scale. However, these studies have been limited to short-term follow-up owing to their prospective nature. Our group, however, is ideally positioned to study the longitudinal clinical impact of ePA. Given the high prevalence of PES, persistence through time and widespread endemicity in Western North America, we sought to assess the clinical impact of the PES strain on adults with CF.

MATERIALS AND METHODS

Clinic population

The Calgary Adult Cystic Fibrosis Clinic (CACFC) provides care for all adult patients in Southern Alberta. Upon enrolment, patients consent to the routine submission and banking of bacterial isolates at each encounter. All sputum-derived pathogens are stored at −80°C with approval from the Conjoint Health Research Ethics Board (REB E23087).

Patient and bacterial strain selection

A longitudinal historical cohort study was conducted with adult patients with CF attending CACFC between 1 January 1980 and 30 December 2014. Patients were included if they were ≥18 years, had a confirmed CF clinical diagnosis including sweat chloride values ≥60 mmol/L and mutation analysis (provided the patient survived beyond 1993)[26] and ≥3 months of follow-up (clinical+microbiological information). Data were recorded until 30 December 2014, the designated end point, or until censoring (ie, death, transplant, transfer). Baseline data including sex, genotype, CF-comorbidities, spirometry and body mass index (BMI), and longitudinal outcome data including dates and cause of death, and/or transplant, spirometry, BMI, sputum culture and exacerbation data, were collected through detailed chart and database review.

Patients were categorised by infecting *P. aeruginosa* strains as follows: (i) no history of infection, (ii) history of transient *P. aeruginosa* (unique or PES strain) infection where the strain was cleared spontaneously or with eradication treatments, (iii) chronic infection with unique strains and (iv) chronic infection with PES (including those patients who experienced strain displacement of their unique strains by PES as it occurred early within 2 years following clinic entry).[18] Chronic infection was defined per Leeds criteria.[27] For patients who developed chronic *P. aeruginosa* infection during or immediately prior to the study period, >50% positivity of a minimum of three cultures in a 12-month period was required. Stability of infection using previously established methods was assessed through sputum analysis at two time points: the first encounter (FE) and the most recent encounter (RE; prior to death/transplant/transfer or last clinic visit); annual sputum samples were screened for (i) patients when discordant strains were identified in order to determine when the change occurred and (ii) randomly in a subset of patients to ensure stability of infecting strains. From each relevant time point, up to five *P. aeruginosa* isolates from the CACFC biobank were typed, representing distinct morphotypes identified on MacConkey agar in real time, when samples were submitted as part of standard care.[18]

Molecular typing

Pulsed-field gel electrophoresis (PFGE) was used as the primary screening procedure for each isolate and compared against 40 representatives of known epidemic strains as previously described.[18] Isolates matching known ePA strains or those infecting ≥3 unrelated individuals were considered clonal a priori. Patients with any isolate with discordant PFGE profiles and select random isolates (quality assurance) underwent MLST using previously established methods to ensure accuracy of strain typing.[28–31] Following this, patients with discordant PES isolates after the initial encounter were considered a priori to have a 'superinfection' wherein they underwent strain displacement. A random sample of isolates from patients with PES and unique *P. aeruginosa* strains identified by PFGE underwent confirmatory testing with either whole genome sequencing (WGS),[32 33] MLST[30] or a qualitative PES-specific PCR assay.[34]

Statistical analysis

Symmetrical and asymmetrical variables were reported as means with SDs and medians with IQRs, respectively. Continuous variables and proportions were compared between groups using t-tests and χ^2 tests, respectively. For all long-term outcomes, multivariable models were used with baseline and time-varying data by adding in variables with $p<0.15$ in univariate analysis, or those with a strong a priori hypothesis (ie, sex, age) in a stepwise manner.[35–38] For death and transplant outcomes, Kaplan-Meier survival analysis was used to conduct unadjusted between-group comparisons. Cox proportional hazard models using follow-up time were used to determine HRs for time to respiratory death or lung transplantation. To assess for left truncation bias in the Cox model, a second analysis using calendar time was conducted. Lung function (%FEV_1) and BMI decline were modelled using a mixed effects model with random slopes and intercepts. Other secondary outcomes were assessed using linear generalised estimating equations. Study outcomes were established prior to data collection and analysis. Analyses were two-sided with α-significance level of 0.05 using STATA V.13.1 software (College Station, Texas, USA).

RESULTS

Patient population

A total of 306 patients were screened (data collection+sputum typing if available), and 274 (51.7% male) were included in the analysis (table 1). Patients were excluded if they lacked ≥3 months of clinical details, had not contributed to the biobank or had chronic infection with epidemic strains of *P. aeruginosa* (ePA) other than PES. Of those excluded, 20 patients had no sputum for typing, 5 patients had an untypeable strain and 7 patients had other transmissible strains (two—Type B, five—Liverpool epidemic strain (LES).[21] Those with established infections with other transmissible *P. aeruginosa* strains had transferred to the CACFC at a median age of 24.6 years from CF centres in Eastern Canada. There were no significant baseline demographic differences with regard to age, sex, BMI and lung function of the excluded group compared with the included cohort (table 1) ($p>0.05$).

The mean (SD) study entry age was 23.1 (8.2) for the total cohort. A total of 1308 *P. aeruginosa* isolates were typed by PFGE, and of these, an additional 108 underwent MLST and 86 whole genome sequencing[32 33] (MG Surette, Professor, McMaster University, personal communication, 2016). The transient *P. aeruginosa* group comprised of 27 patients with unique strains and 2 patients with the PES strain. A small

Table 1 Baseline characteristics of the study cohort

Parameters	No PA n=44	Transient PA n=29	Chronic PA (unique) n=137	Chronic PA (PES) n=64
Male sex, No. (%)	26 (59.0%)	18 (62.1%)	65 (47.4%)	34 (53.1%)
Age, median (IQR), years	18.7 (18.3–26.6)	18.8 (18.4–22.1)	20.6 (18.3–27.9)	18.5 (18.3–21.9)
Height, mean (SD), cm	170.5 (8.7)	169.7 (9.5)	166.8 (8.6)	169.6 (7.6)
Weight, mean (SD), kg	63.26 (12.8)	62.00 (10.6)	58.02 (11.0)	58.48 (9.5)
Body mass index (BMI)*, mean (SD)	21.65 (3.6)	21.45 (2.8)	20.73 (3.1)	20.26 (2.6)
FEV_1, mean (SD)				
Volume, L	2.95 (1.1)	3.21 (1.3)	2.43 (1.0)	2.41 (1.0)
Predicted, %	78.95% (26.1)	84.61% (27.1)	68.75% (24.9)	63.61% (23.8)
FVC, mean (SD)				
Volume, L	4.1 (1.2)	4.31 (1.3)	3.58 (1.1)	3.61 (1.1)
Predicted, %	96.1% (24.4)	100.6% (21.7)	87.6 (24.7)	83.5 (21.6)
Genotype, No. (%)				
DeltaF508 homozygous	22 (50.0%)	16 (55.2%)	58 (42.3%)	33 (51.6%)
DeltaF508 heterozygous	15 (34.1%)	11 (37.9%)	47 (34.3%)	11 (17.2%)
Other	7 (15.91%)	2 (6.90%)	16 (11.7%)	13 (20.3%)
Unknown†	0	0	16 (11.7%)	7 (10.9%)
Co-pathogens‡, No. (%)				
Burkholderia cepacia complex	5 (11.4%)	3 (10.3%)	11 (8.0%)	6 (9.4%)
Methicillin-resistant *S. aureus*	2 (4.5%)	3 (10.3%)	4 (2.9%)	2 (3.1%)
Mycobacterium abscessus	5 (11.4%)	4 (13.8%)	2 (1.5%)	1 (1.6%)
Comorbidities§, No. (%)				
Pancreatic insufficiency	35 (79.54%)	22 (75.86%)	120 (87.59%)	62 (96.88%)
CF-related diabetes	2 (4.55%)	2 (6.90%)	6 (4.38%)	4 (6.25%)

*BMI is calculated as weight in kilograms divided by height in metres squared.
†Categorised as unknown genotype if neither allele was known.
‡Co-pathogens were denoted as present if patient had ≥1 sputum isolate at any time during the study period, and do not represent chronic infections nor do they represent baseline status.
§Comorbidities at enrolment.
CF, cystic fibrosis; PA, *Pseudomonas aeruginosa*; PES, Prairie Epidemic Strain.

number of patients with chronic *P. aeruginosa* had mixed infection with more than one strain of *P. aeruginosa*, and were categorised as unique if both strains were unique, and as LES if infected with a unique and LES strain (two patients); no patients had mixed infection with PES as a constituent. Patients with chronic PES had a median of 4 isolates typed (IQR 3–7, up to 32), and those with chronic unique PA had a median of 3 (IQR 2–4, up to 11) typed.

Assessment of prevalence rates of PES infection relative to all patients with chronic *P. aeruginosa* infection through three decades of patients enrolling at the CACFC demonstrated rates of 37.5% prior to 1985 (3/8 patients), 20.5% for 1986–1990 (8/39 patients), 32.2% for 1990–1995 (19/59 patients), 35.9% for 1996–2000 (14/39 patients), 23.4% for 2000–2005 (13/55 patients) and 8.51% for 2005 onwards (8/94 patients). Rates were similar with the exception of the earliest cohort compared with the cohort 2005 onwards (37.5% (95% CI 3.9% to 71.0%) vs 8.5% (95%CI 2.9% to 14.2%), p=0.01) and were concordant with our prior findings.[18] The mean duration of follow-up for patients in each group was: (i) no *P. aeruginosa*—5.61 years; (ii) transient infection—10.86 years; (iii) unique chronic *P. aeruginosa*—9.22 years and (iv) PES—12.33 years. The unique chronic *P. aeruginosa* and PES group had a higher proportion of females, and lower baseline weight, BMI and lung function compared with the group with no *P. aeruginosa* infection.

Incident infection

A total of 10 patients (60.0% male, mean age 27.4 (SD 4.9) years) developed new PES infections in the study period. Eight PES infections occurred in patients with chronic *P. aeruginosa* infection through 'superinfection', and two occurred in patients without a history of chronic *P.* aeruginosa. These infections occurred evenly through the study period, occurred within 2 years of entry into the CACFC, and most recently in 2013. In those experiencing strain displacement, we did not discover the PES strain on testing of any of the early isolates by PFGE or PCR.[34] Of the two patients with PES infection with the absence of antecedent chronic *P. aeruginosa* infection, one underwent transplantation within 3 months of the event, and the second cleared the infection after eradication therapy. The incidence rate of new PES infection was 4.2 per 1000 person-years (95% CI 1.6 to 6.9 per 1000 person-years). Mean $\%FEV_1$ decline was greater following PES infection (−2.20% (−1.83% to −2.65%) vs −1.85% (−1.31% to −2.42%) per year), but did not achieve statistical significance. Conversely, BMI had a declining trend in this group following PES infection (−0.18/year (−0.01 to −0.33), p=0.04). In patients with incident PES infections, eight died (pretransplant respiratory death) or underwent lung transplantation, resulting in an incidence rate of 1.5 events/10 person-years.

CLINICAL OUTCOMES

Primary outcome

Of 274 patients, a total of 92 patients (33.6%) died or underwent lung transplantation over a 35-year period (2423.0 total patient-years). Death or transplantation occurred in six patients (13.6%) with no history of *P. aeruginosa* infection, four patients (13.8%) with history of transient *P. aeruginosa* infection, 44 with (32.1%) unique chronic *P. aeruginosa* infection, and 38

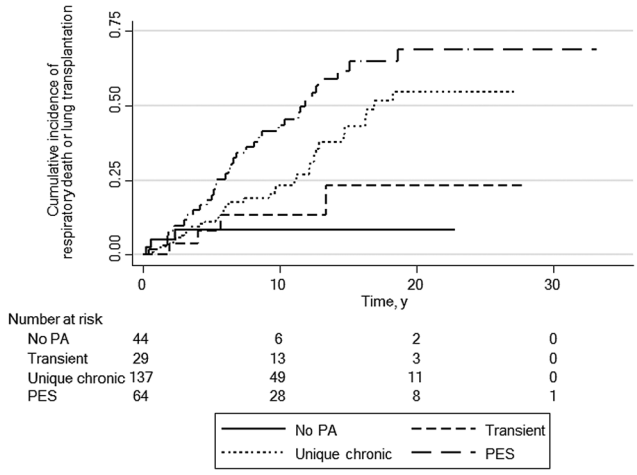

Figure 1 Comparison of time to respiratory death or lung transplantation by *P. aeruginosa* status. Infection with PES was associated with a greater risk of respiratory death or lung transplantation compared with patients without *P. aeruginosa* infection (unadjusted HR, 4.16 (95% CI 1.28 to 13.6) p=0.02; adjusted HR, 3.94 (95%CI 1.18 to 13.1) p=0.03) or those with chronic infection with unique strains (unadjusted HR, 1.77 (95% CI 1.11 to 2.82) p=0.02; adjusted HR, 1.75 (95% CI 1.05 to 2.92) p=0.03). Patients were followed from the time of clinic enrolment (median age 19.5) to the time of death or censoring; they were censored if they died of non-respiratory causes, or were lost to follow-up. PA, *Pseudomonas aeruginosa*; PES, Prairie Epidemic Strain; Respiratory death—refers to all deaths that were due to cystic fibrosis associated pulmonary disease and occurred prior to transplant (data reviewed and assessed by two independent reviewers (RS and MDP).

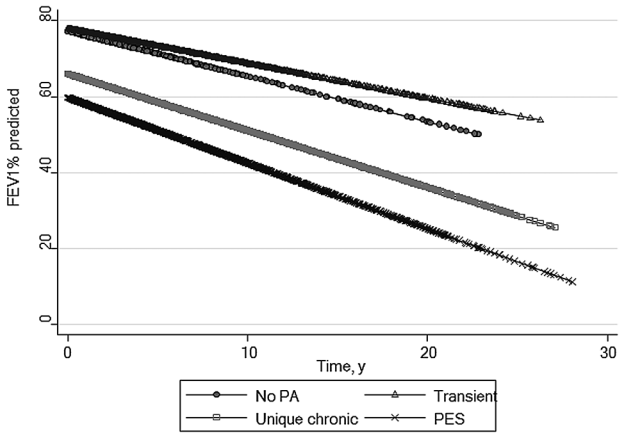

Figure 2 Annual mean pulmonary function decline (%FEV_1) over time by *P. aeruginosa* status. The figure depicts the mean annual rate of lung function decline in each patient group, and the lines were generated from the fitted slopes and intercepts generated from the mixed effects models. Mean annual decline in %FEV_1 predicted was 1.73% (95% CI 1.63% to 1.82%) in patients with chronic PES infection, 1.49% (95% CI 1.42% to 1.56%) in patients with chronic *P. aeruginosa* infection with unique strains, 0.92% (95% CI 0.78% to 1.05%) in patients with history of transient *P. aeruginosa* infection and 1.18% (0.96% to 1.4%) in patients with no *P. aeruginosa* history. For the %FEV_1 comparisons, the PES group compared with the patient groups with no *P. aeruginosa* infection or those with infection with unique strains had a p value of <0.001. PA, *Pseudomonas aeruginosa;* PES, Prairie Epidemic Strain.

(59.4%) in the PES group. Of the 61 deaths, 32 (52.5%) occurred prior to transplant due to end-stage lung disease based on detailed review of chart and database records including autopsy reports by two assessors (RS and MDP). The remainder of the deaths were following lung transplant (19), or due to other causes (2—cancer, 1—suicide, 1—overdose, 7—unknown).

Chronic PES infection was associated with a significantly greater risk of CF-associated respiratory death or lung transplantation compared with patients with no *P. aeruginosa* infection (unadjusted HR, 4.16 (95% CI 1.28 to 13.6) p=0.02; adjusted HR, 3.94 (95% CI 1.18 to 13.1) p=0.03) or those with chronic infection with unique *P. aeruginosa* strains (unadjusted HR, 1.77 (95% CI 1.11 to 2.82) p=0.02; adjusted HR, 1.75 (95% CI 1.05 to 2.92) p=0.03; figure 1). Chronic infection with unique *P. aeruginosa* strains was not significantly associated with greater risk of respiratory death or lung transplant compared with patients with no history of *P. aeruginosa* infection (unadjusted HR, 2.33 (95% CI 0.72 to 7.58) p=0.16; adjusted HR, 2.25 (95% CI 0.69 to 7.37) p=0.18). Genotype (deltaF508 homozygous HR 1.89 (95% CI 1.04 to 3.39)), % FEV_1 predicted (HR 0.98 (95% CI 0.98 to 0.99)) and BMI (1.09 (95% CI 0.98 to 1.21)) were included in the adjusted models. When calendar time was used in the Cox model, the adjusted HR for PES infection was similar to that in the primary analysis (HR 4.11 (95% CI 1.41 to 11.98) p=0.01). When a sensitivity analysis was performed by including the unknown deaths into the Cox model, chronic PES infection was still associated with the risk of respiratory death and transplant compared with the patients with no *P. aeruginosa* history, although to a lesser degree (adjusted HR, 2.95 (95% CI 1.02 to 8.54) p=0.046).

Secondary outcomes

Over the course of the 35-year study period, the annual mean rate of decline of patients with chronic PES infection (1.73% (95% CI 1.63% to 1.82%)) was significantly greater than those chronically infected with unique strains (1.49% (95% CI 1.42% to 1.56%) p<0.001) and those with no *P. aeruginosa* infection (1.18%, (95% CI 0.96% to 1.40%) p<0.001) (figure 2). Patients infected with PES had a lower BMI than those without *P. aeruginosa* at study entry, and slightly lower than those infected with unique strains (table 1). BMI increased in all groups over time, but the rates of mean BMI change were significantly less in both the PES (0.10/year, 95% CI 0.07 to 0.13; p=0.001) and unique chronic *P. aeruginosa* groups (0.10/year, 95% CI 0.08 to 0.13; p=0.001) compared with the group without *P. aeruginosa* infection (0.23/year, 95% CI 0.16 to 0.30) (figure 3).

Pulmonary exacerbations were measured by home, hospital and total IV antibiotic days in the study period. Although the mean number of IV antibiotic days/year was greatest in the PES group in all categories, it did not achieve statistical significance relative to the other groups. Patients with chronic unique *P. aeruginosa* or PES infection had a significantly greater mean annual number of hospital admissions, admitted days in hospital and clinic assessments compared with patients without *P. aeruginosa* infection (table 2). However, no significant differences were noted between the chronic *P. aeruginosa* (unique and PES) infection patient groups with regard to these outcomes.

DISCUSSION

Although PES is a newly described transmissible *P. aeruginosa* strain, it has infected patients with CF for more than three decades, and is likely pervasive in Western Canada with potential extension to the proximal Northwest USA.[18] We therefore

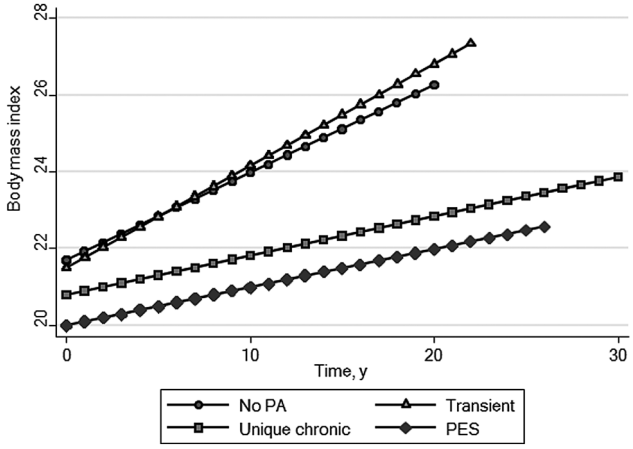

Figure 3 Annual mean body mass index (BMI) change over time by *P. aeruginosa* status. The figure depicts the mean annual change in BMI in each patient group. The lines were generated by fitted slopes and intercepts generated from the mixed effects models. For the BMI comparisons, the p value for the groups with chronic *P. aeruginosa* infection (PES and unique strains) compared with the group without *P. aeruginosa* infection (0.23/year, 95% CI 0.16 to 0.30) was 0.001; PES group (0.10/year, 95% CI 0.07 to 0.13) compared with those with chronic infection with unique *P. aeruginosa* strains (0.10/year, 95% CI 0.08 to 0.13) had a p value of 0.89. BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2). PA, *Pseudomonas aeruginosa*; PES, Prairie Epidemic Strain.

Table 2 Pulmonary exacerbations, clinic assessments and hospital admissions over time

PA infection status (mean (SE)), p value*	No PA n=44	Transient PA n=29	Chronic PA (unique) n=137	Chronic PA (PES) n=64
PEx—IV antibiotic use, days/year				
Total IV antibiotics	1.98 (1.46) ref	1.23 (0.60) p=0.56	1.59 (0.36) p=0.76	2.88 (0.72) p=0.54
Home IV antibiotics	0.08 (0.05) ref	0.08 (0.04) p=1.00	0.095 (0.02) p=0.80	0.23 (0.07) p=0.08
Inpatient IV antibiotics	1.51 (1.19) ref	0.75 (0.39) p=0.55	1.18 (0.25) p=0.79	2.27 (0.68) p=0.57
Clinic days/year	3.45 (0.30) ref	3.73 (0.31) p=0.49	4.47 (0.18) p=0.003	4.37 (0.19) p=0.008
Hospital admissions/year	0.28 (0.07) ref	0.28 (0.10) p=0.97	0.48 (0.05) p=0.03	0.53 (0.08) p=0.02
Hospital days/year	2.98 (0.93) ref	1.86 (1.62) p=0.36	5.97 (1.66) p=0.047	6.80 (1.91) p=0.03

*p Values calculated in comparison with the reference group (no PA group)—1.00.
IV, intravenous; PA, *P. aeruginosa*; PES, Prairie Epidemic Strain; PEx, pulmonary exacerbation.

sought to determine if PES was associated with adverse outcomes in adults with CF in the pretransplant period.

Transmissible *P. aeruginosa* strains in CF are common, and a number have been well described in Europe, Australia and North America.[39 17 40–42] Perhaps, the most notorious epidemic strain, LES, has been identified in >15% of patients with CF in the UK.[43] Aaron *et al*[21] conducted a prospective study in Ontario, Canada to assess the prevalence and outcomes of transmissible *P. aeruginosa* strains and demonstrated that 15% of patients were infected with LES. The patients with CF infected with LES had an increased rate of death or lung transplantation compared with those infected with unique *P. aeruginosa* strains, but had a similar rate of pulmonary function decline. This contrasts the work of Jones *et al*, where an increased rate of lung function decline was observed in patients with LES relative to those with chronic unique infections, but not a disproportional rate of progression to end-stage lung disease.[24] In our study cohort, we observed an association for the first time with an epidemic strain of *P. aeruginosa*, with both increased rates of lung function decline and progression to end-stage lung disease.

Herein, we determined *P. aeruginosa* strain type predominantly by PFGE owing to the reduced costs, efficiency and established local expertise. Any patient with more than one strain type was assessed for an alternate strain type confirmation using WGS and MLST. Further, we confirmed our findings with random audits using WGS, MLST and a qualitative PCR assay developed specifically to identify PES.[44] No cases of misidentification were observed in our audits, and the large number of screened isolates by PFGE for each patient makes it exceedingly unlikely that patients were misclassified.

When clinical outcomes were assessed as a function of *P. aeruginosa* infection, we observed a significantly greater risk of pretransplant CF respiratory death or lung transplantation in the PES group. PES infection resulted in a greater rate of pulmonary function decline, and likely led to the increased risk of lung transplantation seen in this group. Interestingly, the BMI of patients increased over the study period in all patient groups, but the mean BMI was lowest in the PES group at baseline and increased at a lesser rate as compared with patients without *P. aeruginosa* infection. This improvement in nutritional status has been well described in more recent cohorts,[45 46] and likely follows recognition of early work on the importance of nutrition in slowing progression of chronic lung disease.[47 48] PES was overall associated with lower baseline status, and worsened clinical outcomes in the pretransplant period. Conversely, in a recently completed study assessing the post-transplant outcomes of PES in CF, although patients were likely to be younger at the time of transplant, no significant differences were noted in survival, or secondary outcomes including acute cellular rejection, or development of bronchiolitis obliterans syndrome.[49]

For the other secondary outcomes, both chronically infected *P. aeruginosa* patient groups had a greater number of hospital admissions, admitted days and clinic assessments annually. We observed a trend in the PES group relative to the other groups in increased need for IV antibiotics, though it did not reach significance. This is in contrast to other works with the likes of Manchester epidemic strain, Australian-2 and LES strains where increased treatment requirements were clearly identified.[21 39–41] Additionally, treating clinicians were unaware of *P. aeruginosa* status owing to the retrospective nature of the analysis, and although similar to Australian CF physicians,[40] this was in contrast to UK studies. Consequently, knowledge of infection status may have lowered their threshold to treat due to concerns of the potential deleterious outcomes of ePA strains.

We observed a declining prevalence of PES infection in sequential cohorts of young adults transitioning to our adult CF clinic, concomitant with changes in CF attitudes and policies regarding patient interaction and infection control. Notably, incident infection with ePA was still uncommon in historical cohorts—even prior to the introduction of policies to reduce social interaction among patients. Aaron *et al* observed an incidence rate for ePA infection of 7.0 per 1000 person-years, which was similar to our findings with the PES strain (4.2 per 1000 person-years). However, environmental sampling did not demonstrate any transmissible *P. aeruginosa* strains, which is similar to the findings in other studies, and highlights the need

for increased understanding of transmission events. This contrasts data which suggest that *P. aeruginosa* aerosol generation through cough is common, highlighting the complexity of infection acquisition.[50–52] Indeed, in a recent study by our group, we observed a significant association of transmissible PA acquisition with past social behaviours, highlighting further that periods of social contact may pose substantial risk.[53 54]

The primary strength of this study is the large sample size and extended duration of follow-up of 35 years, and approximately 2400 patient-years. This study was powered to examine relevant long-term clinical outcomes including death, transplant and lung function decline. Additionally, as we typed >1300 strains and each patient had early and late sputum isolates tested, new infection or strain replacement was readily identified. Although we had a larger number of primary outcome events of death or transplantation relative to prior works[21 24] (92 events), this was still relatively low as was reflected by the wide CIs. As patients with chronic *P. aeruginosa* infection (unique and PES) had lower baseline values of lung function or nutritional status, the precise casual mechanisms in relation to *P. aeruginosa* acquisition could not be determined with the study period and design. Our study primarily assessed prevalent cases of *P. aeruginosa*, thus limiting our ability to draw causal inferences relating to PES infection. Other limitations included the retrospective study design, effect modification by time, selection bias (ie, sampling of morphotypes), information bias (ie, missing data, loss to follow-up) and confounding not accounted for in the analysis. Regardless, our study was the first to assess clinical outcomes associated with a transmissible *P. aeruginosa* strain over multiple decades of patient follow-up time. Moving forward, collaborative studies with other centres will enable us to improve our understanding of the natural history, and transmission characteristics of PES, and more broadly, of transmissible *P. aeruginosa* strains in CF.

CONCLUSION

Our study demonstrates that patients with chronic PES infection, as an example of an epidemic strain of *P. aeruginosa*, have a greater risk of CF-associated respiratory death or transplant, as well as an accelerated rate of $\%FEV_1$ decline with progression to end-stage lung disease. However, the incidence of PES infections in adults with CF remained low even in times of encouraged socialisation. Active surveillance of paediatric and adult patients with CF is recommended for early identification and management of ePA infection given its associated deleterious effects on the CF population.

Contributors All authors meet the criteria for authorship as required by Thorax submission guidelines. RS and JCL were primarily responsible for data collection and analysis. BW, CDS and SP were responsible for bacterial strain typing and analysis. HRR and MDP were responsible for collection and maintenance of the CACFC biobank and clinical care records and documentation. RS was responsible for the creation of the manuscript. MDP, MGS and HRR were responsible for the project's inception and supervision. All authors contributed to the development of the final manuscript. MDP serves as guarantor of the work.

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