Epidemiology of Chronic Pseudomonas Lung Infections in Cystic Fibrosis Adolescents and Adults

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Abstract

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Rationale: Chronic *Pseudomonas aeruginosa* lung infection is common and associated with significant morbidity and mortality in cystic fibrosis. Whether recent advances in care have affected the frequency of this chronic infection is unknown.

Objectives: Determine if there was a significant change in the incidence of developing chronic *Pseudomonas aeruginosa* infection among adolescents and adults with cystic fibrosis between 2003 and 2012.

Methods: The retrospective cohort consisted of individuals with cystic fibrosis followed in the CF Foundation Patient Registry who were 13 years of age and older without chronic *Pseudomonas aeruginosa* at baseline. Multivariable regression models accounting for within

patient correlation were used to assess the change in incidence of developing chronic *Pseudomonas aeruginosa* infection between 2003 and 2012.

Measurements and Main Results: During the ten-year observation period, 15,504 individuals were followed for a median of 5 (IQR 2-9) years. The incidence of developing chronic *Pseudomonas aeruginosa* decreased from 14.3% in 2003 to 6.4% in 2012. After adjusting for potential confounding, there was a significant decrease in the relative risk of developing chronic *Pseudomonas aeruginosa* infection compared to 2003 (P value test of trend < 0.001). Relative to 2003, the risk of developing chronic *Pseudomonas aeruginosa* infection in 2012 was 0.33 (95% CI: 0.30-0.37).

Conclusions: Among individuals with cystic fibrosis, a significant decrease in the incidence of developing chronic *Pseudomonas aeruginosa* infection between 2003 and 2012 was observed. Whether this change in incidence results in changes in clinical outcomes warrants further exploration.

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INTRODUCTION

In cystic fibrosis (CF), inherited mutations within the cystic fibrosis transmembrane conductance regulator (CFTR) gene result in abnormal ion transport in the CFTR protein (1, 2). While abnormal function of this protein results in disease in multiple organ systems, the greatest impact on morbidity and mortality is in the lungs. Progressive respiratory failure is the leading cause of premature death in this population (3). Abnormal function of the CFTR protein impairs airway defense against infections resulting in chronic endobronchial infections (4, 5). Many bacterial pathogens are associated with worse pulmonary outcomes in CF, and co-infection is common.

Pseudomonas aeruginosa (Pa) is the most commonly cultured and virulent respiratory pathogen in adolescents and adults with CF (3). Pa is associated with a more rapid decline in lung function and worse survival (6–8). Over time, most individuals infected with Pa develop a chronic infection, and in the setting of chronic Pa infection, a mucoid phenotype can predominate. This form of infection can be challenging to eradicate due to formation of biofilms, development of antibiotic resistance and overall bacterial abundance (9–11). Chronic Pa infection results in worse lung function decline and survival even when compared to those not chronically infected (12, 13).

Advances in the diagnosis and management of CF have resulted in significant improvements in survival. In 2013, more than 50% of participants in the CF Foundation Patient Registry (CFFPR) were greater than the age of 18 (14). Between 2000 and 2010 mortality decreased by 1.8% per year, and if this observed rate continues, individuals born with CF in 2010 are predicted to live more than 50 years (15). In the older CF population little is known about the impact these advances in care have had on the rate of developing chronic Pa infections.

The objective of this study was to determine if there has been a significant change in the incidence of developing chronic Pa infection among adolescents and adults in the CFFPR over time. We hypothesize the incidence of chronic Pa infection has decreased among the older CF population compared to 2003.

METHODS:

STUDY POPULATION:

The CFFPR collects demographic and clinical data on individuals receiving care at a Foundation accredited CF care center in the United States. In 2012 more than 27,000 individuals provided data to the registry (16). We accessed data from the CFFPR from January 2001 to December 2012 for purposes of this study. All individuals greater than 12 years old were considered for inclusion. The population was dynamic in that individuals could enter the cohort throughout the study period if they reached age inclusion criteria and/or had a new diagnosis of CF after the age of 12 years. Alternatively, individuals could leave the population due to death, leaving the registry or meeting exclusion criteria. Individuals were excluded from further observation after meeting the definition of chronic Pa infection or following lung transplant. The University of Washington institutional review board approved this study.

STUDY DESIGN:

A retrospective cohort design was employed to study changes in the incidence of developing chronic Pa infection between 2003 and 2012. For this study, time based on calendar year was the primary predictor of interest. CFFPR data collected at clinical encounters was summarized quarterly for each calendar year. In quarters with more than one clinical encounter, the quarter was considered positive for Pa if any one clinical encounter had a Pa positive culture result. Baseline demographics and clinical variables were collected using data from the time of entry in the observed cohort. Both time independent and time dependent variables were used in

regression models as adjustment variables. Time independent variables included age at diagnosis, gender, CFTR functional class and baseline lung function. Time dependent covariates included age, CF-related diabetes (CFRD) and pancreatic insufficiency (PI). CFRD was defined as patients using insulin, and PI was defined by receipt of pancreatic enzyme replacement therapy. Age, age at diagnosis and baseline lung function were treated as continuous variables, and gender, CFRD and PI were classified as binary variables. CFTR functional groups were categorized as minimal (class I, II, and III), residual (class IV and V) and unclassified, as previously reported (17). Percent predicted forced expiratory volume in one second (FEV_1) at time of cohort entry was used to define baseline lung function.

The development of chronic Pa infection was the primary outcome of interest. Each patient entering the cohort at a given year (2003-2012) was assigned an initial state of Pa infection. A screening period using data from the previous eight consecutive quarters was used to define an individual's initial state of Pa infection. In the setting of an individual meeting age inclusion criteria, data from the previous eight quarters was used to define the initial state of Pa infection when available. A patient could enter the observation period as either never, intermittent or chronically infected with Pa. When culture data was available in three or more quarters within an eight-quarter period, chronic Pa infection was defined as having more than 50% positive for Pa. Chronic infection was considered an irreversible state of infection. Intermittent Pa infection was defined as having at least one quarter with a positive Pa culture but not meeting the criteria for chronic infection. If an individual never had a positive Pa culture they were defined as never infected. Patients defined as chronically infected during the screening period were excluded from the analysis cohort. Those patients who were never or intermittently Pa culture positive at entry were followed and their Pa status was assessed at each subsequent quarter during the observational period. The same definition used to define initial state of Pa infection was used during the observation period (Figure 1).

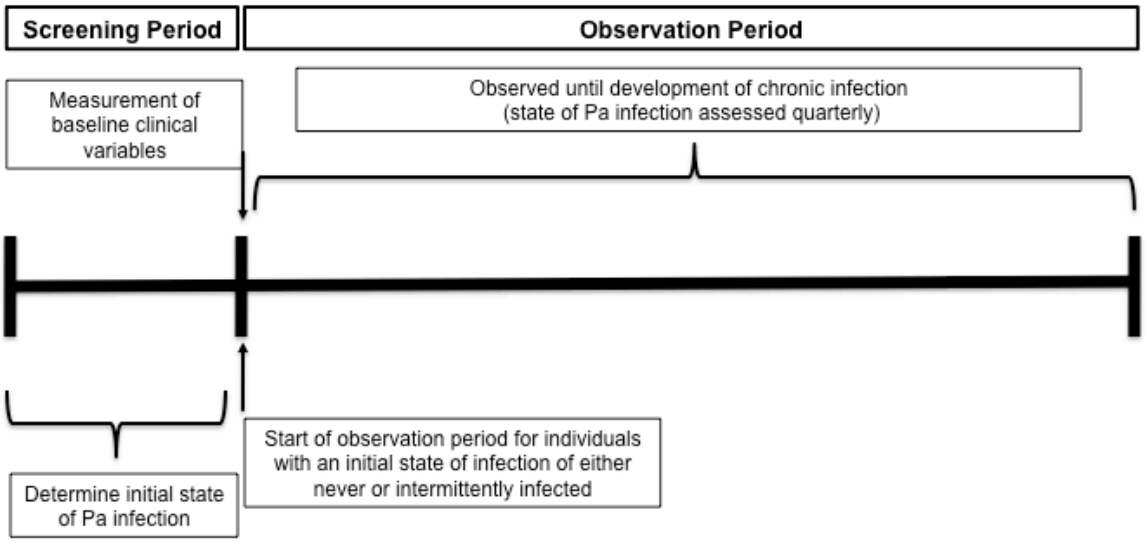

Figure 1: Study Design.

ANALYSIS:

Descriptive statistics were used to describe the cohort data. Continuous variables were reported as medians with inter quartile ranges. Binary and categorical variables were reported as proportions. The incidence of chronic infection was summarized by year. Generalized estimating equations (GEE) analysis with independent working correlation structure and robust variance estimation were used to account for repeated measures within a longitudinal dataset (18). A multivariable GEE regression model including a priori adjustment variables and using Poisson distribution and log link was used to calculate the relative risk of developing chronic infection by calendar year. As a sensitivity analysis, a multivariable GEE regression model was used to calculate the relative risk of developing chronic Pa infection in a cohort restricted to individuals who aged into the cohort at 13 years old. A two-tailed P-value, not adjusted for multiple comparisons, less than 0.05 was considered statistically significant.

RESULTS:

OVERVIEW OF COHORT:

During the ten year observation, 15,504 individuals 13 years of age and older were followed for development of chronic Pa infection. The median period of observation was five years (IQR 2-9) years. Baseline demographics for this cohort are summarized in Table 1. Compared to the 9,192 individuals excluded from the observation group due to meeting the definition of chronic Pa during the screening period, individuals free of chronic Pa infection at the start of the observation period were younger in age, had better lung function and a greater proportion had residual CFTR function. Changes within the cohort between 2003 and 2012 are summarized in Table E1. Among individuals free of chronic Pa infection, those with minimal CFTR function remained relatively stable between 2003 and 2012 whereas the proportion with residual CFTR functional increased from 15.5% in 2003 to 23.9% in 2012. The proportion of individuals in the cohort with other or unknown CFTR functional classes decreased from 19.9% in 2002 to 6.2% in 2012. The average FEV_1 improved from 73.2% in 2003 to 81.5% in 2012 among the observation group. Those with CFRD increased from 10.5% to 19.6% between 2003 and 2012.

Table 1. Comparison of baseline cohort demographics between individuals followed during the observation period with an initial state of Pa infection of either never or intermittent and individuals excluded from the observation group with chronic Pa infection at baseline.

	Free of chronic infection N =15,504	Chronic at baseline N = 9,192
Median age, years (IQR)	15.6 (13.6-24.5)	20.0 (13.0-29.0)
Median age of diagnosis, years (IQR)	0.86 (0.21-7.17)	0.59 (0.17-3.39)
Gender, Female N (%)	7096 (45.8%)	4588 (49.9%)
Race, Caucasian N (%)	14513 (93.6%)	8784 (95.6%)
Ethnicity, Hispanic N (%)	843 (5.4%)	487 (5.3%)
f508del status		
Homozygous, N (%)	6166 (39.8%)	4207 (50.8%)
Heterozygous, N (%)	5876 (37.9%)	3133 (37.8%)
Other/Unknown, N (%)	3462 (22.3%)	1635 (10.3%)
CFTR Functional Classification [a]		
Minimal, N (%)	9301 (60.0%)	6207 (67.5%)
Residual, N (%)	1964 (12.7%)	418 (4.6%)
Other/Unknown, N (%)	4239 (27.3%)	2567 (27.9%)
FEV_1, % predicted, Mean (SD)	81.9% (24.1)	67.8% (25.7)
Co-morbidities;		
CF-related diabetes [c], N (%)	1165 (10.2%)	1741 (19.3%)
Pancreatic insufficiency [d], N (%)	10018 (92.8%)	8227 (89.5%)
Microbiology [e]		
Pseudomonas aeruginosa, N (%)	4392 (41.9%)	7229 (91.3%)
Mucoid Pseudomonas phenotype, N (%)	2474 (56.3%)	5797 (80.2%)
Staphylococcus aureus, N (%)	7455 (71.2%)	4207 (53.0%)
MSSA, N (%)	6264 (84.0%)	3199 (76.0%)
MRSA, N (%)	1947 (26.1%)	1422 (33.8%)
Haemophilus Influenzae, N (%)	1656 (15.8%)	592 (7.5%)
Burkholderia species, N (%)	422 (4.0%)	237 (3.0%)
Alcaligenes xylosoxidans, N (%)	747 (7.1%)	562 (7.1%)
Stenotrophomonas, N (%)	1628 (15.5%)	859 (10.8%)

[a] Minimal: Both alleles containing mutations resulting in minimal CFTR function (Class 1, 2, or 3); Residual: at least one allele containing mutation resulting in partial CFTR function (Class 4 or 5); Unclassified: at least one allele with unknown CFTR function and if other allele function known, mutation resulting in minimal CFTR function. [c] CF-related diabetes: use of insulin; [d] Pancreatic insufficiency: use of pancreatic enzymes; [e] S. aureus: Staphylococcus aureus, MRSA: Methicillin-resistant Staphylococcus aureus, MSSA: Methicillin-resistant Staphylococcus aureus, H. influenza: Haemophilus influenzae, Burkholderia: Burkholderia species, Achromobacter: Alcaligenes xylosoxidans, Stenotrophomonas: Stenotrophomonas Maltophilia

PSEUDOMONAS AERUGINOSA INCIDENCE:

The incidence of developing chronic Pa infection among the cohort was 14.3% (N = 1347) in 2003 and decreased to 6.4% (N = 522) in 2012 (Figure 2). The decrease in incidence of developing chronic Pa infection was comparable among age groups (13-18, 19-35, >35) and

CFTR functional groups (I-III, IV-V, other) (Figure E1 and E2). There was a significant decrease in the incidence of developing chronic Pa infection throughout the observation period (P value test of trend <0.001). Compared to 2003, the relative risk of developing chronic Pa infection in 2012 was 0.45 (95% CI: 0.41-0.49) with the intervening years also having a significant decrease in incidence (Table 2). After adjusting for age, age at diagnosis, gender, CFTR functional class, baseline FEV_1, CF-related diabetes and pancreatic insufficiency, the decrease in relative risk of developing chronic Pa infection remained significant (P value test of trend < 0.001) (Table 2 and Figure 3).

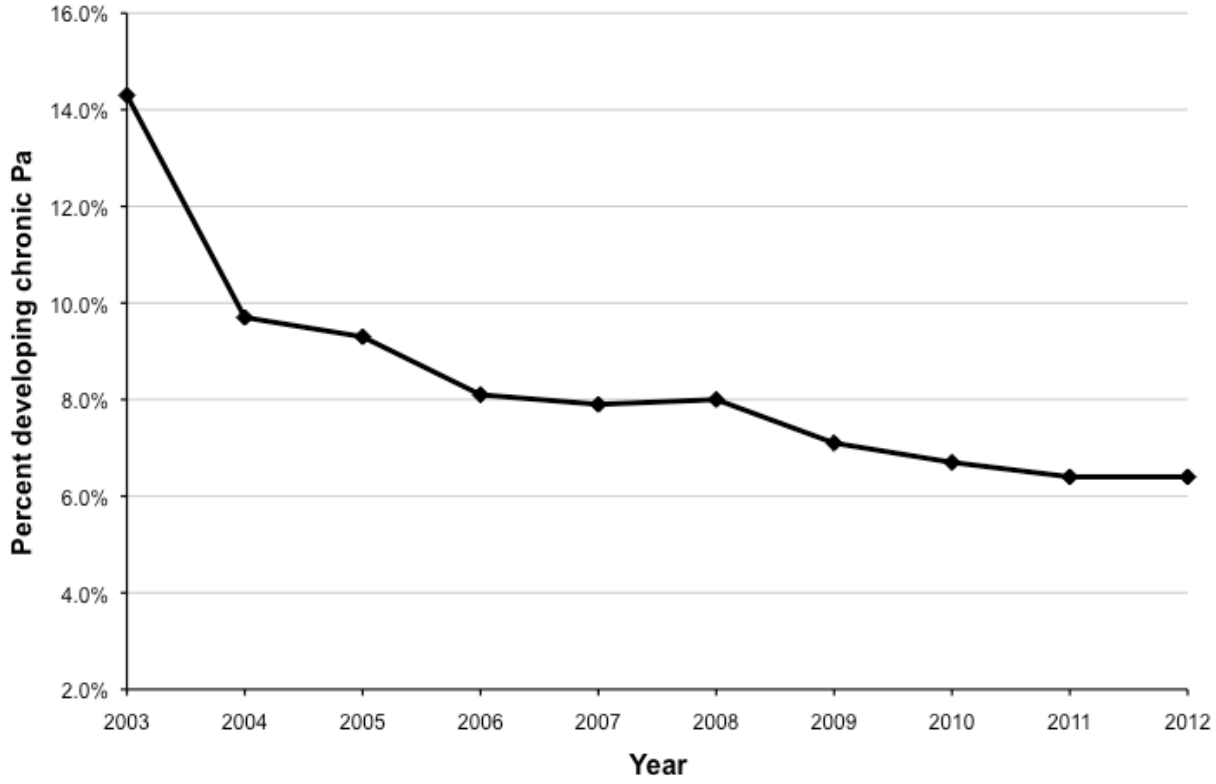

Figure 2. Annual incidence of developing chronic Pa infection.

Table 2. Unadjusted and adjusted RR of developing chronic Pseudomonas aeruginosa infection when compared to 2003.

	Unadjusted		Adjusted [a]	
Year	RR [b]	(95% CI)	RR [c]	(95% CI)
2003	-	-	-	-
2004	0.68	(0.63-0.74)	0.6997	(0.65-0.76)
2005	0.65	(0.60-0.70)	0.6213	(0.57-0.68)
2006	0.56	(0.52-0.62)	0.4936	(0.45-0.54)
2007	0.56	(0.51-0.61)	0.483	(0.44-0.53)
2008	0.56	(0.51-0.61)	0.4756	(0.43-0.52)
2009	0.5	(0.45-0.55)	0.3993	(0.36-0.44)
2010	0.47	(0.43-0.51)	0.3919	(0.35-0.44)
2011	0.45	(0.41-0.50)	0.3677	(0.33-0.41)
2012	0.45	(0.41-0.49)	0.3322	(0.30-0.37)

[a] Adjusted for: age, age at diagnosis, gender, CFTR functional class, baseline FEV_1, CF-related diabetes and pancreatic insufficiency; [b] Trend test $P < 0.001$; [c] Trend test $P < 0.001$

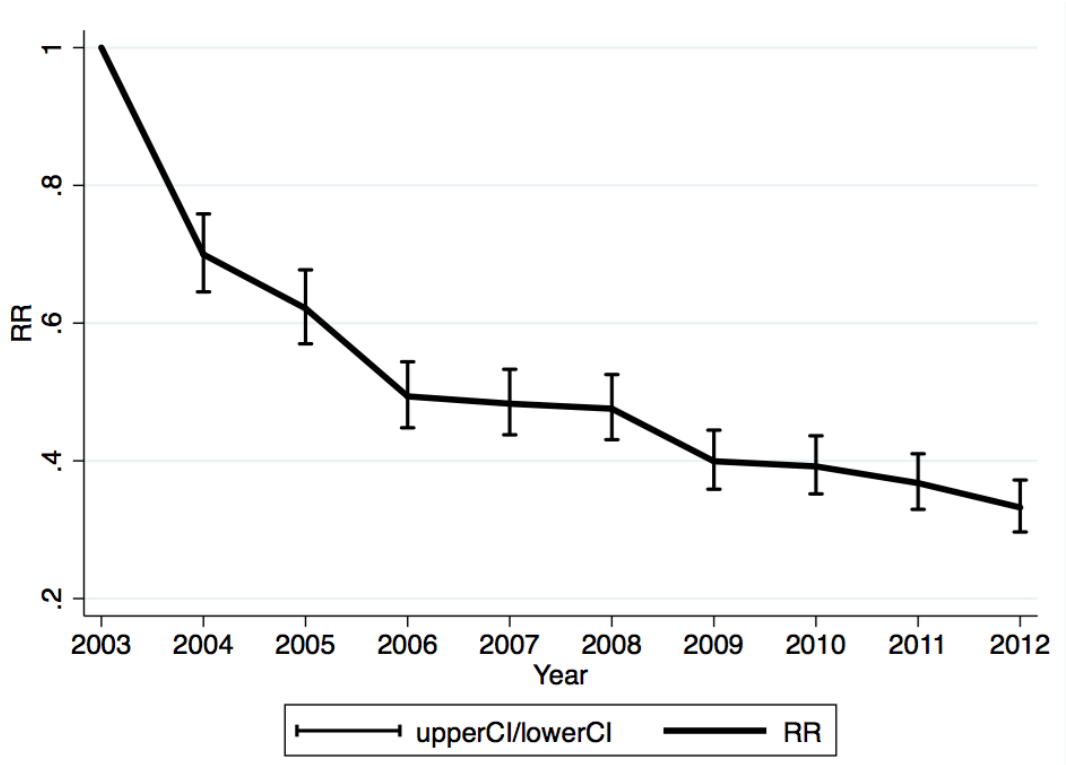

Figure 3. Risk of chronic *P. aeruginosa* by year relative to 2003 adjusting for age, age at diagnosis, gender, CFTR functional class, baseline FEV1, CF-Related Diabetes, Pancreatic Insufficiency.

As a sensitivity analysis, the change in incidence of developing chronic Pa during the observation period was compared only among individuals who aged into the cohort at 13 years old. Over the 10-year observation period, there was a significant decrease in the incidence of developing chronic Pa infection (P value test of trend <0.001) (Figure E3).

DISCUSSION:

Respiratory failure is the most common cause of death among individuals with CF, and chronic respiratory infections play a central role in development of lung disease (19). Among a large cohort of adolescents and adults with CF, we found a significant decrease in the incidence of developing chronic Pa infection between 2003 and 2012. The observed decrease in incidence of chronic Pa infection remained significant after adjusting for potential confounding variables including age, age at diagnosis, gender, CFTR functional class, baseline FEV_1, CF-related diabetes and pancreatic insufficiency.

Recent observational studies suggest the prevalence of Pa infection is decreasing in both adolescents and adults (14, 20). The effect of Pa infection on progression of lung disease is in part dependent on the type of Pa infection. Both chronic Pa infection and the mucoid phenotype of Pa are associated with increased morbidity and mortality when compared to those without Pa infection or non-chronic, non-mucoid Pa infections (12). This study is one of the first in recent times to look at changes in the incidence of chronic Pa infection in this population. In a prior study using a small cohort of adults from a single center, we found a trend toward decreasing prevalence of chronic Pa infection; however this trend did not reach our predetermined significance level (21). Other prior studies have also demonstrated a fall in the frequency of chronic Pa infection over time (22, 23). Between 1974 and 1995, Frederikson et al. found a decrease in the prevalence and incidence of chronic Pa infection in a cohort CF individuals (22). Lee et al. found a significant decrease in the prevalence of chronic Pa infection between 1990 and 2000 (23). Lee et al. did not investigate the change in incidence of

developing chronic Pa infection. In addition to using a significantly larger cohort, this study differs from prior studies in several ways. First, this study looked specifically at adolescents and adults. Second, our observation period occurred in the post-inhaled antibiotic era: a time period where multiple randomized studies documented the benefit of inhaled antibiotic for both clearance of early Pa infection (24, 25) and improvement in outcomes in those chronically infected with Pa (26). Third, we chose an analysis approach that accounted for within patient correlation and adjusted for potential confounding variables. Not accounting for within patient correlation may spuriously lead to low P values, and not accounting for confounding may result in significant associations between variables that are driven by a third unaccounted for variable. Lastly, chronic Pa infection in the airways is challenging to clear, and many believe it is not possible to eradicate Pa from the airways (27). Knowing this, we chose to measure the incidence of developing chronic infection, as it may be a more sensitive measurement of change compared to prevalence.

The observed decrease in incidence may be a result of several potential changes during the observation period. As noted above, the period of time in which the incidence of chronic Pa infection was measured occurred in the post-inhaled antibiotic era. Inhaled anti-pseudomonal antibiotics can decrease rate of decline in lung function and decrease density of Pa in respiratory culture (26). In the setting of newly acquired Pa infection, inhaled anti-pseudomonal antibiotics can eradicate Pa from the subsequent respiratory culture (24, 25, 28). With eradication, individuals have delayed reacquisition of Pa (29, 30), better lung function (31) and less frequent pulmonary exacerbations (32, 33). Studies involving early eradication have shown delayed development of chronic Pa infection. In a recent retrospective cohort study published by Mayer-Hamblett et al., those who remained free of Pa for more than 12 months after eradication therapy had a significantly reduced risk of developing chronic Pa infection (HR: 0.26; 95% CI: 0.17-0.40) (30). A majority of the Pa eradication studies utilized the pediatric population. A recent small study found eradication of newly acquired Pa to be successful in the

adult population (34). In our study, a significant decrease in the incidence of developing chronic Pa occurred during the observation period for both the 18-35 and greater than 35 age groups (Figure E1).

Another potential explanation for the decrease in incidence of developing chronic Pa infection over the observation period is the change in the cohort. Within this dynamic cohort the proportion of subjects with CFTR functional class IV and V increased over the 10-year observation period. Advances in diagnosing CF have resulted in more individuals with CF being diagnosed as adults, and fewer are homozygous for the f508del mutation (35). Greater residual CFTR function may result in improved immunity with respect to clearing respiratory pathogens. A recent study using data from the G551D Observational cohort by Heltshe et al. found significantly reduced odds of isolating Pa from the airways after starting ivacaftor when compared to culture data prior to ivacaftor (OR: 0.65; $P < 0.001$) (36). A 23% reduction in mucoid Pa was also noted. Augmentation of CFTR function with ivacaftor was hypothesized as a potential explanation for the changes observed. When the change in incidence of developing chronic Pa infection was assessed among CFTR functional groups, we found similar significant decreases in incidence over the 10-year observation period arguing against changes within the cohort being the primary reason for a decrease in overall incidence (Figure E1). Lung function (FEV_1) also improved during the observation period in this cohort. As lung function declines, the odds of chronic Pa infection increases (13). This improvement in lung function may also explain the observed decrease in development of chronic Pa infection. Lastly the dramatic drop in incidence of developing chronic Pa infection between 2003 and 2004 may have been a result of using a left censored cohort with individuals either aging into the cohort at 13 or entering at a later age following a new diagnosis. When available, data from two years prior to meeting entry criteria for the observation group was used to assign initial state of Pa infection. If the frequency of respiratory cultures is low, using two years to define initial state of Pa infection may not be enough time to accurately classify an individual's state of Pa infection. As a sensitivity analysis,

we used a cohort restricted to individuals who age into the cohort at age 13. The probability of having chronic infection increases with age so this group is less likely to have chronic Pa infection upon entry to the observation period. Additionally, this age group tended to have more frequent respiratory cultures when compared to older adults. The initial drop in incidence was no longer observed in this group, but the significant decrease in incidence of developing chronic infection over the observation period persisted.

There are several limitations to consider with this study. There is no universally agreed upon definition of chronic Pa infection. Numerous different definitions have been used when studying chronic Pa infection (13, 37, 38). Most recent studies use the Leeds criteria or a modified form. Under the Leeds criteria, chronic Pa infection is defined as having a respiratory culture positive for Pa in more than 50% of the months in which a sample is collected during a 12 month period (13). With this definition, respiratory a culture was recommended at least every three months. In the cohort used to validate the Leeds criteria, the average number of months in a 12-month period in which culture data was available was 8.9. In the CFFPR the median number of cultures ranged between 2 (IQR: 1-3) and 3 (IQR: 2-4) for individuals 13 years and older during the study period (Table E2). Application of Leeds criteria to our cohort might result in significant misclassification. We chose a less stringent definition for chronic Pa infection knowing the frequency of culturing in our cohort was low, and while our definition may have resulted in misclassifying individuals, this form of misclassification can bias results toward the null. Development of the mucoid Pa phenotype correlates with development of chronic infection (39). We plan to validate our definition of chronic infection by comparing the incidence of developing chronic Pa infection with the incidence of developing mucoid Pa.

With the definition of chronic Pa being dependent on the number of cultures collected within a given time period, detection bias may occur since individuals with worse lung function tend to have more frequent encounters in which a respiratory culture can be obtained. To minimize this potential bias culture data was summarized quarterly. The number of quarters per

year with respiratory culture data also increased during the observation period (Table E2). With the definition of chronic Pa infection being dependent on the number of quarters with culture data this may have resulted in a higher incidence of developing chronic Pa in the later years of the observation period, which would have biased the results toward the null.

Lastly, after meeting criteria for chronic Pa infection individuals were no longer included in subsequent calculations of annual incidence, and while new subjects entered the observation group annually, a significant number of the subjects remained in the observation group for an extended period of time without developing chronic infection. Those individuals may represent a unique population with innate characteristics protecting them from developing chronic infections. Over time this unique population may begin to make up a larger proportion of the observation group due to study design reasons explained above resulting in a lower incidence that is not reflective of true interventions occurring during the observation period. As a sensitivity analysis to determine if this select population could explain the observed drop in incidence of developing chronic infection, we first looked at only those patients who aged into the cohort when they turned 13 years of age. We continued to see the trends observed in the larger cohort (Figure E3). To further explore the role this change in cohort may be contributing to changes in the incidence of chronic Pa infection, we plan to censor individuals at 4 years of observation. By censoring, the group at risk of developing chronic Pa infection is less likely to become enriched with unique phenotypes at lower risk of developing chronic infections irrespective of trends in the care of CF patients.

In summary, using analytical methods to account for within patient correlation and influences of potential confounding we observed a significant decrease in the annual incidence of developing chronic Pa infection between 2003 and 2012. Our study is one of first in the post inhaled antibiotic era to look at changes in incidence among adolescents and adults with CF. Additional studies are needed to validate our new definition for chronic Pa infection. Whether

these changes in chronic Pa infection result is a significant change in clinical outcomes warrants further investigation as well.

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APPENDIX

SUPPLEMENTARY TABLES

Table E1. Changes in cohort over observation period.

	2003 (N = 9418)	2004 (N = 8668)	2005 (N = 8431)	2006 (N = 8223)	2007 (N = 8142)
Median age, years (IQR)	21 (16-30)	20 (15-29)	19 (13-28)	18 (13-27)	17 (13-26)
Diagnosis, median years (IQR)	1.4 (0.3 to 12.7)	1.5 (0.3 to 13.1)	1.5 (0.3 to 13.0)	1.6 (0.3 to 13.0)	1.6 (0.3 to 12.9)
Gender, Female, N (%)	4201 (44.6%)	3852 (44.4%)	3751 (44.5%)	3681 (44.8%)	3669 (45.1%)
Race, Caucasian N (%)	8886 (94.4%)	8163 (94.2%)	7924 (94.0%)	7694 (93.6%)	7612 (93.5%)
Ethnicity, Hispanic N (%)	373 (4.0%)	380 (4.4%)	385 (4.6%)	393 (4.8%)	410 (5.0%)
f508del Status					
Homozygous, N (%)	3420 (36.3%)	3093 (35.7%)	2973 (35.3%)	2853 (34.7%)	2841 (34.9%)
Heterozygous, N (%)	3644 (38.7%)	3370 (38.9%)	3313 (39.3%)	3265 (39.7%)	3250 (39.9%)
Other/Unknown, N (%)	2354 (25.0%)	2205 (25.4%)	2145 (25.4%)	2105 (25.6%)	2051 (25.2%)
CFTR Mutation Classification					
Minimal, N (%)	5147 (54.7%)	4625 (53.4%)	4474 (53.1%)	4301 (52.3%)	4283 (52.6%)
Residual, N (%)	1464 (15.5%)	1416 (16.3%)	1404 (16.7%)	1389 (16.9%)	1388 (17.1%)
Other, N (%)	1878 (19.9%)	1788 (20.6%)	1779 (21.1%)	1818 (22.1%)	1815 (22.3%)
Unknown, N (%)	929 (9.9%)	839 (9.7%)	774 (9.2%	715 (8.7%)	656 (8.1%
FEV_1, % predicted, Mean (SD)	73.2% (24.8)	74.5% (24.5)	75.7% (24.1)	77.0% (24.0)	77.9% (24.2)
CF-related diabetes [c], N (%)	959 (10.5%)	933 (11.2%)	964 (11.8%)	1053 (13.2%)	1124 (14.2%)
Pancreatic insufficiency [d], N (%)	6146 (78.2%)	5772 (78.6%)	5789 (79.3%)	5786 (80.0%)	5896 (80.9%)
Cultures, Median (IQR)	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)	2 (1 to 4)
Microbiology [e]					
Pseudomonas, N (%)	2880 (52.8%)	2257 (45.8%)	2109 (43.5%)	1855 (38.4%)	1854 (36.9%)
Mucoid, N (%)	1932 (67.1%)	1406 (62.3%)	1207 (57.2%)	1022 (55.1%)	1008 (54.4%)
S. aureus, N (%)	3347 (61.4%)	3287 (66.7%)	3311 (68.3%)	3392 (70.3%)	3600 (71.7%)
MSSA, N (%)	2908 (86.9%)	2796 (85.1%)	2754 (83.2%)	2816 (83.0%)	2935 (81.5%)
MRSA, N (%)	644 (19.2%)	717 (21.8%)	830 (25.1%)	874 (25.8%)	1041 (28.9%)
H. influenza N, %	705 (12.9%)	653 (13.2%)	674 (13.9%)	674 (14.0%)	704 (14.0%)
Burkholderia, N (%)	305 (5.6%)	266 (5.4%)	277 (5.7%)	264 (5.5%)	279 (5.6%)
Alcaligenes, N (%)	364 (6.7%)	344 (7.0%)	346 (7.1%)	392 (8.1%)	377 (7.5%)
Stenotrophomonas, N (%)	694 (12.7%)	688 (14.0%)	700 (14.4%)	749 (15.5%)	772 (15.4%)
Incidence of Chronic Pa	1347 (14.3%)	842 (9.7%)	781 (9.3%)	664 (8.1%)	646 (7.9%)

Table E1. (continued)

	2008 (N = 8060)	2009 (N = 8008)	2010 (N = 8016)	2011 (N = 8083)	2012 (N = 8145)	p-Value
Median age, years (IQR)	15 (13-25)	14 (13-24)	13 (13-22)	13 (13-21)	13 (13-20)	<0.0001
Diagnosis, median years (IQR)	1.6 (0.3-12.4)	1.5 (0.3-11.9)	1.4 (0.2-11.0)	1.3 (0.2-10.2)	1.2 (0.2-9.6)	<0.0001
Gender, Female, N (%)	3651 (45.3%)	3606 (45.0%)	3616 (45.1%)	3640 (45.0%)	3671 (45.1%)	0.9767
Race, Caucasian N (%)	7518 (93.3%)	7444 (93.0%)	7438 (92.8%)	7484 (92.6%)	7530 (92.5%)	0.0153
Ethnicity, Hispanic N (%)	425 (5.3%)	433 (5.4%)	452 (5.6%)	478 (5.9%)	497 (6.1%)	<0.0001
f508del Status						
Homozygous, N (%)	2847 (35.3%)	2806 (35.0%)	2816 (35.1%)	2840 (35.1%)	2876 (35.3%)	0.9499
Heterozygous, N (%)	3205 (39.8%)	3170 (39.6%)	3184 (39.7%)	3213 (39.8%)	3224 (39.6%)	
Other/Unknown, N (%)	2008 (24.9%)	2032 (25.4%)	2016 (25.2%)	2030 (25.1%)	2045 (25.1%)	
CFTR Mutation Classification						
Minimal, N (%)	4266 (52.9%)	4214 (52.6%)	4254 (53.1%)	4295 (53.1%)	4356 (53.5%)	<0.0001
Residual, N (%)	1369 (17.0%)	1368 (17.1%)	1345 (16.8%)	1343 (16.6%)	1338 (16.4%)	
Other, N (%)	1804 (22.4%)	1833 (22.9%)	1874 (23.4%)	1919 (23.7%)	1944 (23.9%)	
Unknown, N (%)	621 (7.7%)	593 (7.4%)	543 (6.8%)	526 (6.5%)	507 (6.2%)	
FEV_1, % predicted, Mean (SD)	78.5% (24.2)	79.7% (23.7)	80.6% (23.6)	80.8% (23.5)	81.5% (23.2)	<0.0001
CF-related diabetes [c], N (%)	1211 (15.5%)	1266 (16.3%)	1327 (17.0%)	1451 (18.4%)	1562 (19.6%)	<0.0001
Pancreatic insufficiency [d], N (%)	6017 (82.2%)	6099 (83.1%)	6241 (83.6%)	6428 (84.2%)	6623 (84.9%)	<0.0001
Cultures, Median (IQR)	3 (1-4)	3 (1-4)	3 (2-4)	3 (2-4)	3 (2-4)	<0.0001
Microbiology [e]						
Pseudomonas, N (%)	1748 (34.4%)	1661 (31.9%)	1635 (30.5%)	1630 (28.8%)	1580 (26.6%)	<0.0001
Mucoid, N (%)	943 (54.0%)	812 (48.9%)	751 (45.9%)	717 (44.0%)	702 (44.4%)	<0.0001
S. aureus, N (%)	3715 (73.1%)	3906 (75.0%)	4081 (76.0%)	4333 (76.7%)	4565 (17.0%)	<0.0001
MSSA, N (%)	3006 (80.9%)	3130 (80.1%)	3264 (80.0%)	3419 (78.9%)	3633 (79.6%)	<0.0001
MRSA, N (%)	1149 (30.9%)	1253 (32.1%)	1446 (35.4%)	1548 (35.7%)	1652 (36.2%)	<0.0001
H. influenza N, %	741 (14.6%)	761 (14.6%)	811 (15.1%)	857 (15.2%)	827 (13.9%)	0.0117
Burkholderia, N (%)	250 (4.9%)	248 (4.8%)	236 (4.4%)	261 (4.6%)	261 (4.4%)	0.0016
Achromobacter, N (%)	419 (8.3%)	413 (7.9%)	465 (8.7%)	478 (8.5%)	502 (8.5%)	0.0002
Stenotrophomonas, N (%)	757 (14.9%)	846 (16.2%)	926 (17.3%)	999 (17.7%)	1001 (16.9%)	<0.0001
Incidence of Chronic Pa	647 (8.0%)	569 (7.1%)	536 (6.7%)	521 (6.5%)	522 (6.4%)	<0.0001

[a] Minimal: Both alleles containing mutations resulting in minimal CFTR function (Class 1, 2, or 3); Residual: at least one allele containing mutation resulting in partial CFTR function (Class 4 or 5); Unclassified: at least one allele with unknown CFTR function and if other allele function known, mutation resulting in minimal CFTR function. [c] CF-related diabetes: use of insulin; [d] Pancreatic insufficiency: use of pancreatic enzymes; [e] S. aureus: Staphylococcus aureus, MRSA: Methicillin-resistant Staphylococcus aureus, MSSA: Methicillin-resistant Staphylococcus aureus, H. influenza: Haemophilus influenzae, Burkholderia: Burkholderia species, Achromobacter: Alcaligenes xylosoxidans, Stenotrophomonas: Stenotrophomonas Maltophilia

Table E2. By year proportion of quarters with respiratory culture data

Year	No Quarters N (%)	1 Quarter N (%)	2 Quarters N (%)	3 Quarters N (%)	4 Quarters N (%)
2003	3960 (42.1%)	2317 (24.6%)	1614 (17.1%)	998 (10.6%)	529 (5.6%)
2004	3738 (43.1%)	2008 (23.2%)	1393 (16.1%)	923 (10.7%)	606 (7.0%)
2005	3579 (42.5%)	1795 (21.3%)	1336 (15.9%)	1009 (12.0%)	712 (8.5%)
2006	3398 (41.3%)	1565 (19.0%)	1298 (15.8%)	1134 (13.8%)	828 (10.1%)
2007	3118 (38.3%)	1492 (18.3%)	1277 (15.7%)	1248 (15.3%)	1007 (12.4%)
2008	2982 (37.0%)	1271 (15.8%)	1293 (16.0%)	1355 (16.8%)	1159 (14.4%)
2009	2798 (34.9%)	1188 (14.8%)	1281 (16.0%)	1431 (17.9%)	1310 (16.4%)
2010	2648 (33.0%)	1195 (14.8%)	1233 (15.4%)	1528 (19.1%)	1412 (17.6%)
2011	2432 (30.1%)	1146 (14.2%)	1270 (15.7%)	1596 (19.8%)	1639 (20.3%)
2012	2213 (27.2%)	1156 (14.2%)	1217 (14.9%)	1773 (21.8%)	1786 (21.9%)

SUPPLEMENTARY FIGURES

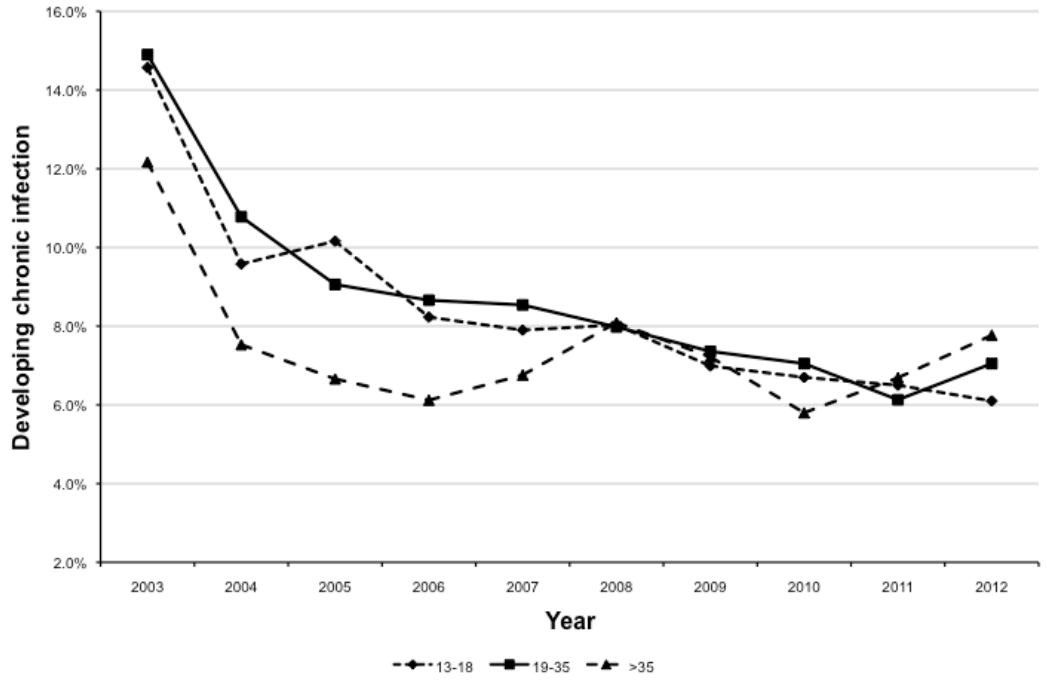

Figure E1. Incidence of developing chronic Pa by age group

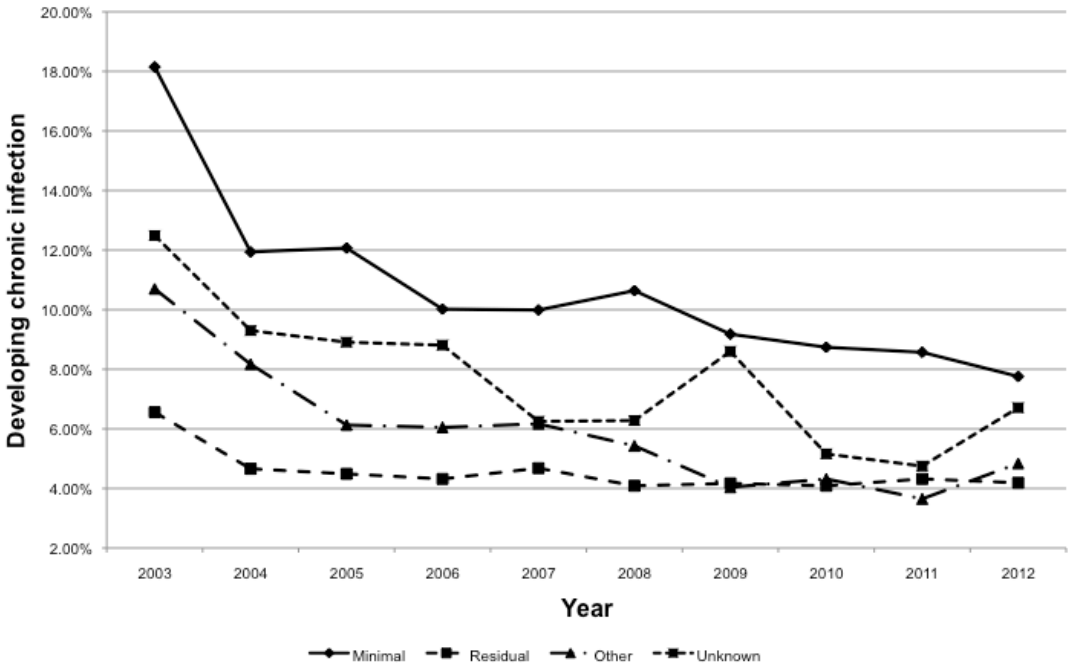

Figure E2. Incidence of developing chronic Pa by CFTR functional group. Minimal (CFTR functional class I-III), Residual (CFTR functional class IV-V)

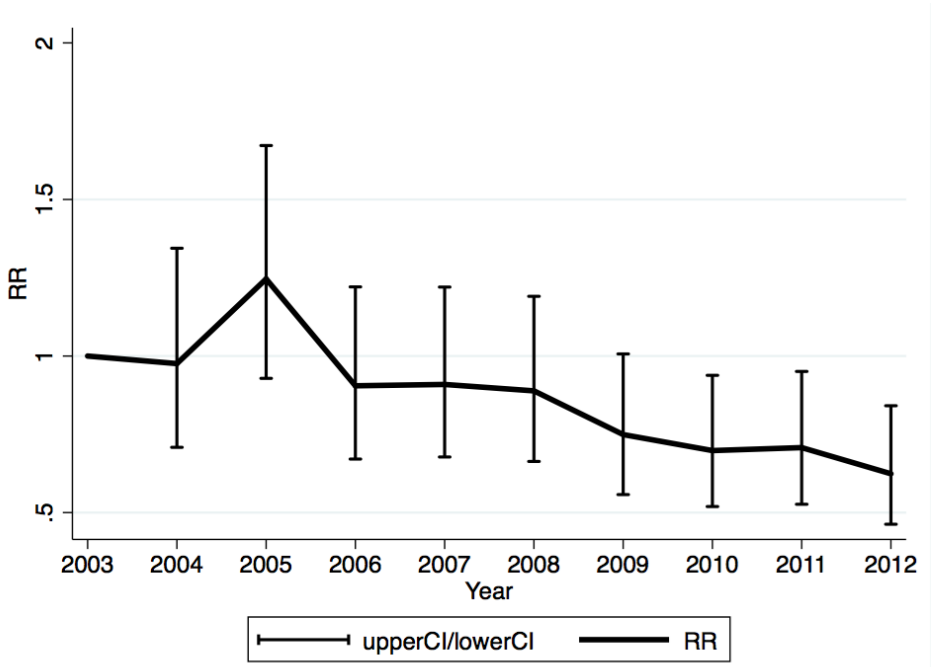

Figure E3. Risk of chronic *P. aeruginosa* among individuals entering the cohort at 13 years-old by year relative to 2003 adjusting for age, age at diagnosis, gender, CFTR functional class, baseline FEV1, CF-Related Diabetes, Pancreatic Insufficiency