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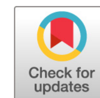

Prevalence and Outcomes of *Achromobacter* Species Infections in Adults with Cystic Fibrosis: a North American Cohort Study

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ABSTRACT *Achromobacter* species are increasingly being detected in cystic fibrosis (CF) patients, with an unclear epidemiology and impact. We studied a cohort of patients attending a Canadian adult CF clinic who had positive sputum cultures for *Achromobacter* species in the period from 1984 to 2013. Infection was categorized as transient or persistent (≥50% positive cultures for 1 year). Those with persistent infection were matched 2:1 with age-, sex-, and time-matched controls without a history of *Achromobacter* infection, and mixed-effects models were used to assess pulmonary exacerbation (PEx) frequency and lung function decline. Isolates from a biobank were retrospectively assessed, identified to the species level by *nrdA* sequencing, and genotyped using pulsed-field gel electrophoresis (PFGE). Thirty-four patients (11% of those in our clinic), with a median age of 24 years (interquartile range [IQR], 20.3 to 29.8 years), developed *Achromobacter* infection. Ten patients (29%) developed persistent infection. Persistence did not denote permanence, as most patients ultimately cleared infection, often after years. Patients were more likely to experience PEx at incident isolation than at prior or subsequent visits (odds ratio [OR], 2.7 [95% confidence interval {CI}, 1.2 to 6.7]; $P = 0.03$). Following persistent infection, there was no difference in annual lung function decline (−1.08% [95% CI, −2.73 to 0.57%] versus −2.74% [95% CI, −4.02 to 1.46%]; $P = 0.12$) or the odds of PEx (OR, 1.21 [95% CI, 0.45 to 3.28]; $P = 0.70$). Differential virulence among *Achromobacter* species was not observed, and no cases of transmission occurred. We demonstrated that incident *Achromobacter* infection was associated with a greater risk of PEx; however, neither transient nor chronic infection was associated with a worsened long-term prognosis. Large, multicenter studies are needed to clarify the clinical impact, natural history, and transmissibility of *Achromobacter*.

KEYWORDS *Achromobacter xylosoxidans*, infection transmission, emerging infections, epidemiology, eradication, infection control, inhaled corticosteroids, multilocus sequence typing, pulsed-field gel electrophoresis, whole-genome sequencing

Received 8 January 2017 **Returned for modification** 31 January 2017 **Accepted** 20 March 2017

Accepted manuscript posted online 26 April 2017

Citation Edwards BD, Greysson-Wong J, Somayaji R, Waddell B, Whelan FJ, Storey DG, Rabin HR, Surette MG, Parkins MD. 2017. Prevalence and outcomes of *Achromobacter* species infections in adults with cystic fibrosis: a North American cohort study. J Clin Microbiol 55:2074–2085. https://doi.org/10.1128/JCM.02556-16.

Editor Betty A. Forbes, Virginia Commonwealth University Medical Center

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Chronic inflammation and recurrent/chronic lung infection are the primary contributors to morbidity and mortality in cystic fibrosis (CF) patients (1). The significance of lower airway infection with classical CF pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae*, is well established. Recently, enhanced microbiologic techniques have identified increasing numbers of pathogens infecting the lower airways (2). One such example is *Achromobacter* spp., in particular *Achromobacter xylosoxidans*, which is now well recognized in CF populations (2).

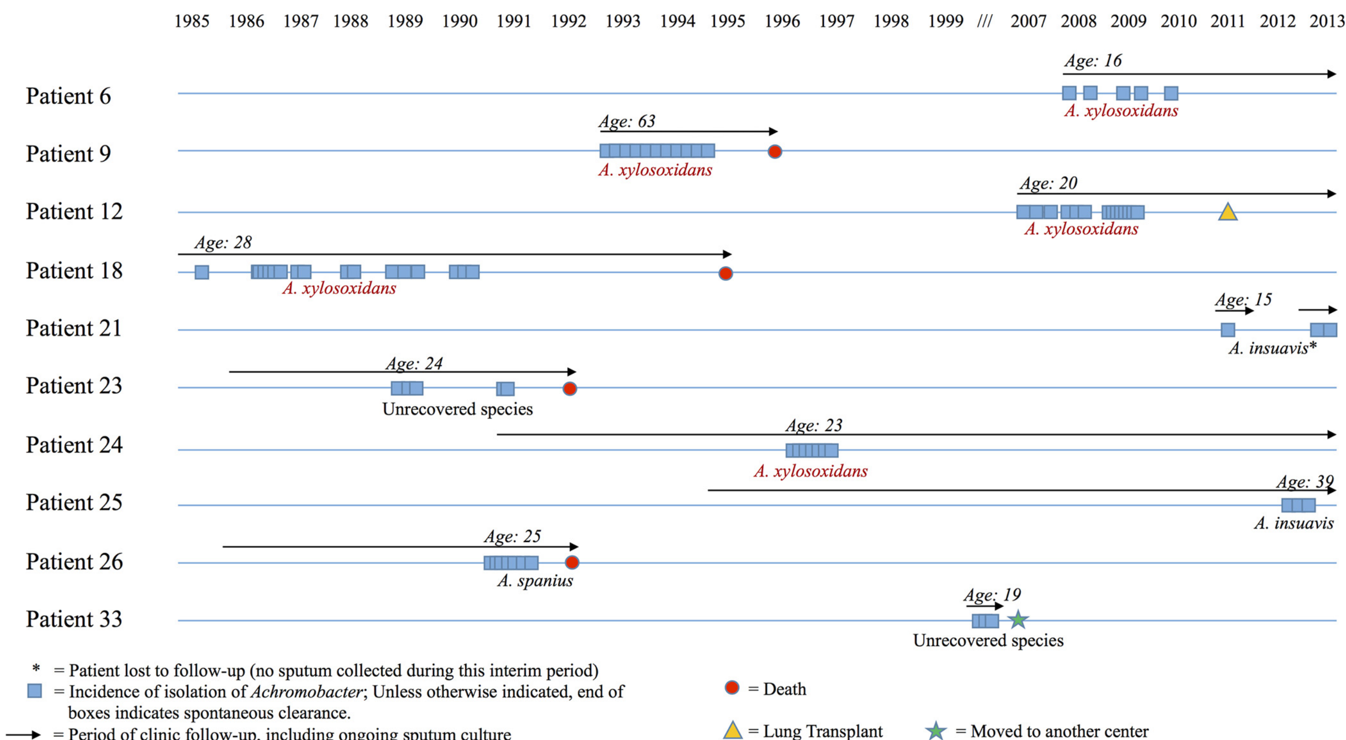

FIG 1 Natural history of chronic infection with *Achromobacter* species. The incidence of *Achromobacter* isolation by time is shown for patients with persistent infection. The age at incident *Achromobacter* infection is documented. Species were identified as *Achromobacter* spp. at initial culture and continued with this designation if the isolate was not recoverable (discarded or nonviable) from the biobank for species analysis.

Although early studies attributed all *Achromobacter* infections to *A. xylosoxidans*, a broad range of *Achromobacter* species exist in CF patients. Several species of *Achromobacter* have been identified (3), and the prevalences of *Achromobacter ruhlandii* (4) and *Achromobacter insuavis* (5, 6) have been observed to rival that of *A. xylosoxidans*.

Achromobacter spp. are aerobic, Gram-negative, catalase- and oxidase-positive, nonfermenting bacilli that are widely distributed in the environment (7). They are opportunistic pathogens, particularly in older patients, and have been recovered from blood, urine, the respiratory tract, and cerebrospinal fluid (CSF) (2, 8). They possess innate antimicrobial resistance, readily acquire adaptive resistance with antimicrobial exposure (9), and alter the expression of certain genes to promote chronic infection (10).

Whereas single-center European studies have reported prevalence rates of *Achromobacter* sp. infection in CF patients of 5 to 29% (7, 9, 11–14), national registries suggest rates of 4 to 7% (15, 16). The clinical impact of *Achromobacter* sp. respiratory infection remains unclear, as the evidence is confined to small cohort studies over short periods, with varied results (11, 12, 14, 17). Whereas some groups have identified clonality among isolates from patients with chronic *Achromobacter* sp. infections (18, 19), others have not (20, 21). Given the paucity of outcome data for North American CF cohorts, we sought to understand the natural history, impact, and epidemiology of *Achromobacter* sp. infections in a large Canadian CF center.

RESULTS

Population characteristics. Between 1984 and 2013, *Achromobacter* spp. were cultured from the sputa of 34 patients from our clinic of 306 CF patients (11%). Twelve patients had *Achromobacter* spp. cultured more than once, while 10 (29% of the cohort) met our predefined criteria for persistent infection (Fig. 1). Despite persistent infections ranging from 1 to 5 years, all but one patient eventually cleared *Achromobacter* spp., as determined by culture, for a period of at least 3 months before leaving the cohort (four

TABLE 1 Demographics of patients with *Achromobacter* sp. infection[a]

Parameter	Value for group				P value[d]	
	Total (n = 34)	Transient infection (n = 24)	Persistent infection (n = 10)	Controls (n = 18)	Transient infection vs persistent infection	Persistent infection vs controls
Median age (IQR)	24 (20.3–29.8)	25 (21.0–30.3)	24 (19.3–27.3)	22 (18.0–25.8)	0.80	0.45
No. (%) of males	15 (44.1)	9 (37.5)	6 (60.0)	11 (61.1)	0.23	0.95
Median BMI (IQR)	20 (18.3–21.4)	20 (18.4–21.1)	19 (18.2–21.8)	21 (19.2–23.1)	0.76	0.50
No. (%) of patients with pancreatic insufficiency	29 (85.3)	20 (83.3)	9 (90.0)	13 (72.2)	0.62	0.27
Median FVC% (IQR)	73 (56.3–95.8)	69 (56.8–102.3)	75 (54.8–85.5)	86 (64.8–104.8)	0.25	0.11
Median FEV_1% (IQR)	51 (35.5–81.3)	47 (34.5–87.3)	57 (38.3–75.5)	66 (43.3–88.5)	0.63	0.22
No. (%) of patients with home O_2	4 (11.8)	3 (12.5)	1 (10.0)	0 (0.0)	0.84	0.17
No. (%) of patients who received:						
Inhaled tobramycin[b]	7 (20.6)	5 (20.8)	2 (20.0)	4 (22.2)	0.96	0.89
Azithromycin[b]	4 (11.8)	3 (12.5)	1 (10.0)	4 (22.2)	0.84	0.42
Inhaled corticosteroid	8 (23.5)	3 (12.5)	5 (50.0)	3 (16.7)	**0.02**	0.06
No. (%) of patients with chronic coinfection						
P. aeruginosa	28 (82.4)	20 (83.3)	8 (80.0)	12 (66.6)	0.82	0.45
S. aureus	25 (73.5)	17 (70.8)	8 (80.0)	7 (38.8)	0.58	**0.04**
No. (%) of patients with comorbidity[c]						
CFRD	5 (14.7)	3 (12.5)	2 (20.0)	3 (16.7)	0.57	0.83
Sinus disease	10 (29.4)	6 (25.0)	4 (40.0)	10 (55.6)	0.38	0.43
Bone disease	9 (26.5)	7 (29.2)	2 (20.0)	4 (22.2)	0.58	0.89
CFLD	8 (23.5)	6 (25.0)	2 (20.0)	2 (11.1)	0.75	0.52
DIOS	3 (8.8)	3 (12.5)	0 (0.0)	2 (11.1)	0.24	0.27

[a]Baseline data are from the 2 years prior to incident *Achromobacter* culture. Note that the "persistent" cohort included a 10th patient who was unable to be compared to control patients for clinical outcomes.
[b]Azithromycin or tobramycin use at time of *Achromobacter* sp. culture or control study entry.
[c]CFRD, CF-related diabetes; CFLD, CF liver disease; DIOS, distal intestinal obstruction syndrome.
[d]Values in bold indicate those which were statistically significant.

died, one moved to another center, and five continue to be monitored). Patients had a median of nine negative sputum cultures collected following clearance of *Achromobacter*. One patient had ongoing infection at study completion, for a period lasting 3 years.

Fifteen patients of the total cohort (44%) were male. The median age at first culture was 24 years (interquartile range [IQR], 20.3 to 29.8 years) (Table 1). The median forced expiratory volume in 1 s (FEV_1) predicted at study entry (2 years prior to the first *Achromobacter* culture) was 51% (IQR, 35.5 to 81.3%), and the forced vital capacity (FVC) predicted was 73% (IQR, 56.3 to 95.8%). Most patients (88%) were not on supplemental home oxygen. The median body mass index (BMI) of the cohort was 19.8 kg/m^2 (IQR, 18.3 to 21.4 kg/m^2). Eleven patients (32%) were on chronic antibiotics (seven on inhaled tobramycin and four on oral azithromycin) at the time of *Achromobacter* culture. The median number of visits in the 2 years prior to incident isolation was 8 (IQR, 5 to 12), and that in the 2 years following was 10 (IQR, 7 to 14). The median number of sputum samples collected in the 2 years following "transient" infection was 6 (IQR, 4 to 10). In patients with persistent *Achromobacter* infection (during which they were defined as being "persistent"), the percentage of positive sputum cultures was 79% (IQR, 70 to 88%). Thirty patients (88%) were chronically coinfected with *P. aeruginosa*, while 26/34 (76%) patients had coinfection with *S. aureus*.

Pulmonary exacerbation (PEx) at the first isolation of *Achromobacter* occurred in 14 patients (42%; 95% confidence interval [CI], 25 to 58%). Relative to the visits immediately before and after, patients were more likely to experience PEx at incident culture, i.e., 14/33 (42%) visits versus 14/66 (21%) visits (odds ratio [OR], 2.7 [95% CI, 1.1 to 6.7]; $P = 0.03$). Of these exacerbations at first isolation, 4 (29%) were severe, requiring intravenous therapies and/or hospitalization.

The mean age at first isolation was not significantly different between those who experienced exacerbation (23.8 years) and those who did not (28.2 years) (difference, 4.4 years; 95% CI, −2.1 to 10.9 years). There was also no difference in baseline FEV_1 (as recorded at the visit prior to first isolation) in those with exacerbation (FEV_1, 57.5%) and those without exacerbation (FEV_1, 50.9%) (difference, −6.6%; 95% CI, −28.5 to 15.3%). Further, patients on chronic antibacterials were not more likely to experience exacerbation than those not on chronic antibacterials (4/14 patients [28.6%] versus 5/20 patients [25%]) (difference, 3.57%; 95% CI, −24% to 33%). An increasing bioburden as measured in CFU per milliliter of sputum did not predict exacerbation risk (data not shown). The 10 patients who eventually developed persistent infection were no more likely to experience PEx at the time of incident *Achromobacter* infection than the patients with transient infections (4/10 patients [40%] versus 10/24 patients [42%]; risk ratio [RR], 1.0 [95% CI, 0.3 to 2.8]).

Epidemiology of CF patients with persistent infection. Nine of the 10 patients with persistent infection were matched to age- and cohort-matched patients (we were unable to obtain suitable control patients against the 10th patient, a 64-year-old female). Patient characteristics were not different between those with persistent infection and age-matched controls. The control group trended toward improved pulmonary and nutritional outcomes (Table 1). Medications did not significantly differ in the control populations, apart from inhaled corticosteroids (ICS), which were prescribed more commonly in those who developed persistent *Achromobacter* sp. infection. In a comparison of the persistent and matched cohorts for baseline bacterial coinfection, 8/10 patients (80.0%) versus 12/18 patients (66.6%) ($P = 0.45$) were coinfected with *P. aeruginosa*, while 8/10 patients (80.0%) versus 7/18 patients (38.8%) ($P = 0.04$) were coinfected with *S. aureus*.

Association of *Achromobacter* sp. infections with long-term outcomes in CF patients. In all patients who experienced *Achromobacter* infection, there was no significant difference in the rate of annual lung function decline (as measured by the FEV_1 percent predicted) preceding compared to following *Achromobacter* infection (−0.79%/year [95% CI, −1.60% to 0.01%/year] versus −0.22%/year [95% CI, −1.01% to 0.57%/year]). Similarly, no significant difference in the odds of PEx was noted in the pre- and postinfection periods (OR, 0.74 [95% CI, 0.50 to 1.11]; $P = 0.15$).

There was no difference in annual lung function decline in those transiently versus persistently infected with *Achromobacter* (0.59% [95% CI, −0.73% to 1.91%] versus −0.39% [95% CI, −1.44% to 0.67%]; $P = 0.26$). The odds of experiencing a PEx following infection in these cohorts were not significantly different (OR, 1.02 [95% CI, 0.42 to 2.46]; $P = 0.96$).

Comparing persistently infected patients to matched controls demonstrated no difference in annual lung function decline (−1.08% [95% CI, −2.73% to 0.57%] versus −2.74% [95% CI, −4.02% to −1.46%]; $P = 0.12$). Further, there was no significantly increased risk in PEx occurrence in the persistently infected cohort relative to matched controls (OR, 1.21 [95% CI, 0.45 to 3.28]; $P = 0.70$).

Microbiological characteristics of *Achromobacter*. Within our biobank, 115 *Achromobacter* isolates were found, spanning 29 years. We sought only incident isolates, the last available isolate for each patient in the biobank, and isolates at 2-year intervals for individuals with prolonged carriage. Some isolates were either not found or not recovered from the frozen state despite multiple attempts. We were able to characterize 31 available isolates (19 incident, 3 intermediate, and 9 follow-up isolates) from 18/34 patients. Whereas isolates were exclusively identified as *A. xylosoxidans* by the clinical microbiology laboratory in real time, retrospective analysis of samples from the biobank revealed a broader distribution of isolates, as follows: *A. xylosoxidans*, 9 isolates (50%); *A. insuavis*, 5 isolates (28%); *Achromobacter dolens*, 2 isolates (11%); *Achromobacter spanius*, 1 isolate (6%); and *A. ruhlandii*, 1 isolate (6%). Species establishing persistent infections were as follows: *A. xylosoxidans*, 5/8 isolates (62.5%); *A. insuavis*, 2/8 isolates (25%); and *A. spanius*, 1/8 isolates (12.5%). *A. xylosoxidans* was not more likely

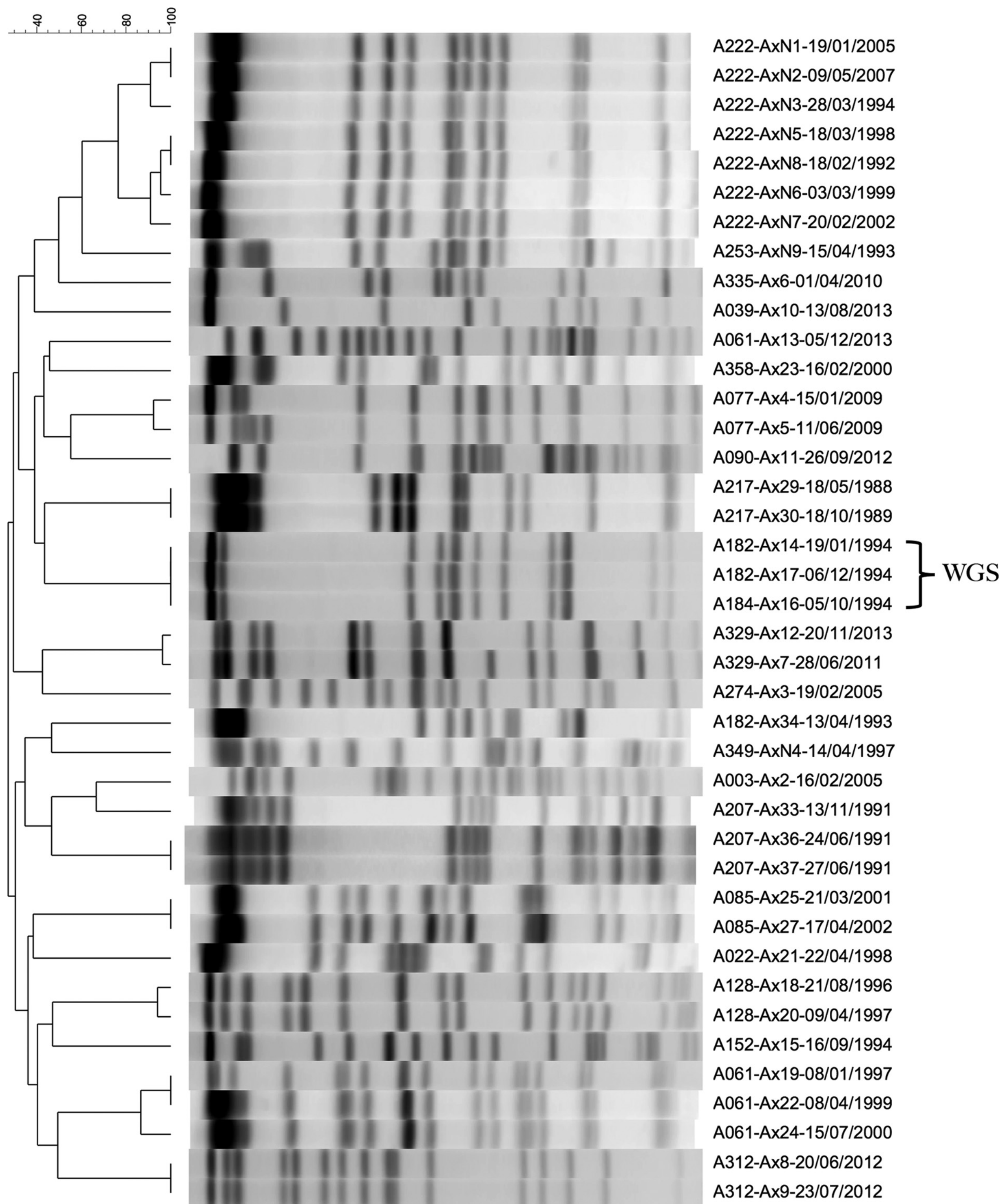

FIG 2 Dendrogram for pulsed-field gel electrophoresis of *Achromobacter* isolates recovered from the Calgary Adult CF Clinic biobank and assessed for natural history and epidemiology. Designations show patient-strain-date (day/month/year).

to culminate in persistent infection than in transient infection (5/8 versus 4/10 isolates; RR, 1.6 [95% CI, 0.6 to 4.0]; $P = 0.63$).

Pulsed-field gel electrophoresis (PFGE) was used to genotype the 31 available strains. A total of 89% (16/18) of patients were initially identified to be infected by unique *Achromobacter* sp. pulsotypes (Fig. 2). The results suggested one possible shared strain between two patients in 1994 (a strain chronically infecting a 64-year-old

female was transiently identified in a 31-year-old female). Clonality between the isolates was confirmed by whole-genome sequencing (WGS) (the isolates differed by 15 single nucleotide polymorphisms [SNPs]) (10, 22; data not shown). Upon confirmation testing of other pathogens within the suspect sputum sample, we identified that in addition to the *A. xylosoxidans* strain of patient A182 being identified in the sputum of patient A184, the *P. aeruginosa* PFGE pulsotypes of these patients were discordant (each representing nonclonal strains), such that patient A182's isolate at that particular time point showed patient A184's chronic pulsotype, and *vice versa* (data not shown). A review of clinical records over the prior 2 years identified no period of $<$48 h in which the patients shared the same space, other than the one clinic appointment during which the sputum in question was collected. Rather than transmission of infection, this was thought to represent mislabeling of sputum prior to submission to the clinical microbiology laboratory. Patients with persistent infections demonstrated stable pulsotypes over time, save for one patient (A061) whose sputum grew a second unique strain 16 years after an initial transient infection.

There was no difference in biofilm biomass between isolates causing persistent versus transient infections ($P = 0.89$) (see Table S2 in the supplemental material). We also compared the biofilm production capacity of recovered *Achromobacter* species. *A. xylosoxidans* isolates produced significantly more biofilm than all included non-*A. xylosoxidans* isolates ($P = 0.0001$) as well as just those present at incident infection ($P = 0.0070$).

***Achromobacter* clearance.** As afforded by a multidecade observational cohort study, we assessed the durability of the "persistent" definition defined *a priori* based on those available in the literature. Over a median 3.1 (IQR, 2.6 to 6.15) years of microbiologic follow-up after incident infection, only one patient remained infected with *Achromobacter*. Three persistently infected patients were treated with oral antimicrobials that the *Achromobacter* isolate was sensitive to at the time of final culture (trimethoprim-sulfamethoxazole for PEx with known *S. aureus/Achromobacter*, ciprofloxacin for PEx with known *P. aeruginosa/Achromobacter*, and doxycycline/colistin for attempted *Achromobacter* eradication), and they cleared the infection following therapy completion. The remaining 60% (6/10 patients) of patients appeared to spontaneously clear infection without any antimicrobial treatment. Before the time of clearance, two of these patients developed infection with a new pathogen (*S. aureus* or *Burkholderia cepacia* complex) that may have assumed a dominant pathogenic role.

DISCUSSION

Achromobacter species derived from the sputa of CF patients are garnering increasing attention. Indeed, improvements in diagnostic techniques enabling the correct distinction of these species from other CF pathogens, such as *P. aeruginosa*, and selective antimicrobial pressures may have contributed to their initial recognition (19, 23), although their rates did not appear to be changing over the last decade (7). Our study is the first to report epidemiological and clinical outcome data for CF patients infected with *Achromobacter* species in a North American cohort.

The cumulative prevalence of *Achromobacter* isolation of 11% in our center is comparable to those previously reported for small centers, which ranged from 5 to 29% (11, 12), although this spans 3 decades, making the incidence of infection very low. While others have reported that older patients and patients with greater lung disease burden appear to be predisposed to infection with *Achromobacter* species (11, 18), we did not observe any correlation between age, nutritional status, CF comorbidities, baseline lung status, or home oxygen use and incident infection.

Rates of chronic infection have typically ranged from 3 to 12% of incident *Achromobacter* infections (5, 24, 25), again with the risk factors of older age and the burden of lung disease (11, 26). Coinfection with *P. aeruginosa* (2, 17, 19, 27) is common due to increased exposure to antibiotics for chronic infection (26). The rate of persistent infection in our population was high, at 29%, likely due to a less stringent definition (25). We found no baseline characteristics, including age and lower FEV_1 scores, which were predictive of strain persistence. No microbial factors influenced the risk of

progression to persistence. The significance of the larger proportion of persistently infected patients with chronic *S. aureus* infection, which was also observed previously (26), is unclear. Uniquely, we observed that ICS were associated with the risk of persistence, in contrast to transient infections or controls. Indeed, the use of ICS has been identified as a detrimental factor in other microbiological outcomes, including risk of infection with specific pathogens (*Aspergillus fumigatus*) (28), time to PEx (29), and reduced bacterial killing during PEx (30). This is potentially concerning given the overused status of ICS in CF patients (31).

Studies have attributed persistent *Achromobacter* infections predominantly to *A. xylosoxidans* (2, 11, 12, 18), with a few exceptions, including *A. insuavis* and *A. dolens* (5, 6). We now know that this may represent a broader species distribution. Nonetheless, our results reiterate these findings, with *A. xylosoxidans* accounting for 62% of persistent infections and, additionally, with a persistent infection by *A. spanius*, which has not previously been reported. The infrequent recovery of *A. ruhlandii* in our center differs from previous data (4, 32, 33) and may reflect variance in the environmental distribution of species in North America.

The virulence factors enabling certain *Achromobacter* species to persist in airways are undetermined. Biofilm production in *A. xylosoxidans* was previously found to be significantly associated with CF patient infections (9). It may also facilitate horizontal gene transfer between bacteria, promoting the spread of antimicrobial resistance (9, 34), which may provide a differential adaptive stability in the environment and airways to establish chronic infection (5, 24, 35). We found that *A. xylosoxidans* demonstrated increased biofilm biomass production compared to that of other species (*A. insuavis* and *A. dolens*). There was no evidence that this promoted persistence. We had a limited number of non-*A. xylosoxidans* species with which to compare biofilm production levels. Given the now understood diverse spectrum of *Achromobacter* species distribution, future studies should focus on differential behaviors, including biofilm production, in these species.

Ours represents the longest assessment of the epidemiology and impact of *Achromobacter* sp. infections. This afforded the observation that a large number of patients with persistent infection may eventually clear the *Achromobacter* infection, which was not previously apparent. The reason for spontaneous clearance of infection after prolonged carriage is not immediately clear and may simply reflect the natural pattern of infection for *Achromobacter*, the organism being overtaken by another pathogen, or immune clearance by the patient. Regardless, this highlights how the natural history of novel infections in CF airways may differ from that of *P. aeruginosa*, suggesting a need for unique terminology to account for these fundamental differences (7, 36). Additionally, it demonstrates the difficulty of short-term studies and the value of long-term follow-up.

Despite the increasingly frequent isolation of *Achromobacter* from CF patients, its pathogenicity is unclear (11, 12, 26), though the growing body of evidence seems to support the significance of its isolation (17, 37, 38). Although PEx in CF patients are common, we hypothesized a discernible clinical impact with subsequent lung function decline after *Achromobacter* detection. We observed that the first isolation of *Achromobacter* was associated with PEx and that patients were almost three times more likely to experience exacerbation than at the visit before or after incident isolation, which has not previously been noted. Factors predicting who might experience exacerbation at first isolation, including age, lung function, or bioburden, were not obvious in this small study. Patients on chronic suppressive antimicrobial therapy were just as likely to experience exacerbation at the first isolation with *Achromobacter*. We did not assess viral pathogens or nonpathogenic contributors to exacerbation risk (environment, pollution, or therapy compliance); furthermore, there may be alternative, patient-specific factors that are not yet clear but that increase the risk of exacerbation.

Predicting the clinical course following persistent *Achromobacter* isolation is challenging. Studies of small European and South American cohorts have found no evidence of decline in FEV_1 (11, 12, 39), while others dispute this (14, 17, 37). An

increased need for antimicrobials (11) and hospitalization (26) have also been reported. We found no evidence of clinical decline (lung function loss or risk of PEx) following infection, nor was there a significant change in nutritional status (BMI) over the course of follow-up, similar to the results of a previous study (12). It may be valuable to follow-up with patients for a longer duration to see if such correlations become evident. Treating patients who showed exacerbation at first isolation with oral antibiotics did not reduce the subsequent PEx frequency over the next 2 years.

Given the negative impact associated with chronic *P. aeruginosa* infection, the practice of early eradication has become applied universally, with multiple regimens being compared for efficacy (40). Due to concerns about the adverse impact and antibiotic resistance of *Achromobacter* spp., some groups, understandably, have adopted a similar practice (41). In the present study, we did not note that changing therapy at the time of incident *Achromobacter* culture reduced the risk of progression to persistent infection. Given the global importance of these organisms, it seems that a multicenter study similar to STAR-2 (studying methicillin-resistant *S. aureus* [MRSA] eradication versus placebo) is in order (42).

The transmissibility of *Achromobacter* is controversial. Two small single-center studies found that all patients were infected with unique *Achromobacter* strains (20, 21). Conversely, several studies (both single and multicenter) have suggested that shared strains do exist (2, 12, 18, 19, 24, 25, 27, 33, 43–48), with common strains accounting for 5 to 50% of total *Achromobacter* infections. Additionally, chronic infection was most commonly due to persistence of the original infecting strain (14, 19, 45). Extreme variations in the prevalence of shared clones of other organisms known to be transmissible (e.g., *P. aeruginosa*) exist in different clinics, reflecting some combination of organism fitness, patient population, and historical infection control practices. In our center, where an epidemic *P. aeruginosa* strain accounts for >1/3 of all chronic infections, we confirmed that no true shared infections occurred (49). Uniquely relative to other studies of infection transmission in CF patients, we sought to confirm potential patient-patient spread not only through WGS but also by assessing other organisms in the same sputum. In doing so, we refuted a potential case of infection transmission and identified an important step relevant to future studies of infection transmission in CF patients. Specimen mislabeling is among the most common preanalytic errors identified in clinical laboratories and is a particular risk in environments, such as CF clinics, where the same sample type is collected from multiple patients (50). Our data support the argument that transmission of *Achromobacter* among CF patients appears to be an uncommon event.

A primary strength of our study is that we are one of the first to describe the epidemiology and outcomes of *Achromobacter* infection, including by species, but several limitations must be considered. These include the retrospective study design, selection bias, and information bias, including misclassification of infection status and missing data (including the inability to determine species type for 41% of our cohort). We also must be cognizant of the potential bias of effect modification and the difference in CF management given the length of time spanned by our study. Patients had serial cultures enabling us to study incident cases of *Achromobacter* infection, but it is possible that some were prevalent cases based on sampling frequency. It is possible that despite serial cultures, the association of increased risk of exacerbation occurrence with the first isolation of *Achromobacter* was coincidental and that incident infection occurred prior to exacerbation. Our ability to assess clinical outcomes was also limited by the study sample size, and we suggest that multicenter and/or registry-based studies of acute and chronic *Achromobacter* sp. infections in North American cohorts are warranted. Furthermore, given the lag time after samples were collected, a proportion of isolates were not recoverable from our biobank, thereby limiting our ability to determine species and to assess biofilm formation. In selecting controls without predetermined limitations (i.e., only those with chronic *P. aeruginosa* infection), we were unable to compare the relative pathogenic potentials of *Achromobacter* spp. versus other organisms. Such a consideration may be valuable in future studies drawing

from a larger control patient population. Changes in PEx frequency or lung function decline may have been evident in a larger cohort or with longer follow-up. Finally, there is currently no uniform definition of "chronic" or "persistent" *Achromobacter* infection. While our definition is similar to those applied previously to chronic *Pseudomonas* infection (51, 52) and there is value in developing uniform definitions for epidemiologic purposes, we have demonstrated that a definition derived through a detailed understanding of organism-specific natural history acquired from long-term studies supersedes uniform standardized periods, as other organisms may lack the long-term tenacity of *P. aeruginosa*.

Conclusions. In this retrospective cohort study spanning a period of almost 30 years, we studied, for the first time, the epidemiology, clinical impact, and transmissibility of *Achromobacter* infections in CF patients in a North American population. The prevalence and species distribution of *Achromobacter* in this cohort were similar to those in other small center studies. Baseline characteristics of patients persistently infected with *Achromobacter*, including age, lung function, antibiotic use, and CF comorbidities, were not different. There was no evidence of lung function decline or risk of PEx following persistent infection with *Achromobacter*. Notably, patients were at increased risk of experiencing PEx at the time of first isolation of *Achromobacter*. We found that patients with persistent infection maintained the same strain for prolonged periods, with no evidence of transmission.

MATERIALS AND METHODS

Population. The Calgary Adult CF Clinic monitors all patients with CF residing in Southern Alberta, Canada. Upon clinic enrollment, patients provide consent for prospective collection, storage, and analysis of respiratory secretions and sputum-derived organisms. Patient follow-up is intended to be quarterly. Patients were included if *Achromobacter* spp. were cultured from routine assessments of sputum from January 1984 to December 2013. Patients were classified as having transient infection (defined as having $\geq$1 positive culture but not meeting the definition for persistent infection) or persistent infection (defined as having $\geq$50% of all cultures in a 12-month period that grew *Achromobacter* [with $\geq$3 cultures collected]). For patients who were persistently infected, we collected data on control CF patients (age [$\pm$2 years], birth cohort, and sex matched at a 2:1 ratio) without a history of *Achromobacter* sputum infection.

Clinical data. We collected data through chart review for 2 years preceding and following the initial *Achromobacter* sp. culture. For the CF controls, we collected data for two consecutive years matched to those of our test patients. For all patients, baseline demographic data (age, sex, BMI, and CF mutations), medications, pulmonary function as measured by forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) at each visit, comorbid sputum pathogens, exacerbations, and medical comorbidities were recorded.

The primary outcome of interest was the proportion of patients with pulmonary exacerbation (PEx) at the time of first isolation of *Achromobacter* relative to those for the visits immediately preceding and following isolation. A PEx was defined as symptoms consistent with acute infection for which new oral or parenteral antimicrobial therapy was started by the CF physician in either the clinic or hospital setting (2, 53). Prior work from our group demonstrated concordance between clinically diagnosed PEx and Fuchs criteria (30). Next, we assessed the impacts of transient and persistent *Achromobacter* infections, including (i) rate and risk factors for progression to persistent *Achromobacter* infection, (ii) differential decline in FEV_1 in patients following *Achromobacter* infection, and (iii) the risk of PEx after initial *Achromobacter* infection. Although we collected clinical data for 2 years pre- and postinfection, we monitored patients longer to assess the pattern of infection. This study was approved by the conjoint health research ethics board at the University of Calgary (approvals REB-15-0854 and REB-15-2744).

Characterization of *Achromobacter* species. All *Achromobacter* species were identified as part of routine care by use of standard methodologies (54). In real time, isolates were frozen in skim milk and stored at −80°C. From our prospectively maintained biobank, we retrospectively confirmed the genus identification and characterized viable isolates. All first and last available isolates, as well as isolates at 2-year intervals (where available), were retrospectively assessed. PCR sequencing of the 16S rRNA gene was used to confirm that the isolates were from the genus *Achromobacter*, using single-colony preparations and primers 8F and 926R and running the results through NCBI's GenBank. Isolates with >99% sequence identity to *Achromobacter* spp. were considered to be *Achromobacter*. Species identification was determined by *nrdA* locus sequencing, one of the multilocus sequence typing (MLST) loci for *Achromobacter* spp. (18, 54).

To investigate the presence of clonality, strains underwent pulsed-field gel electrophoresis (PFGE) according to established protocols adapted from the work of Parkins et al. (36). SpeI (New England BioLabs)-digested samples were run in 1% SeaKem Gold agarose. Dendrograms were generated with a 1.0% position tolerance, using the unweighted-pair group method using average linkages (UPGMA) and the Sørensen-Dice similarity coefficient. Strains with banding patterns that were $\geq$80% identical ($\leq$3 band differences) were considered related, conforming to the Tenover criteria (55). To investigate if

strains with the same PFGE pulsotype were acquired independently from the natural environment or related more directly via patient-patient spread, isolates underwent whole-genome sequencing (WGS) and single nucleotide polymorphism (SNP) analysis (56). If a suspected case of transmission was identified, we sought to perform genotyping on other relevant organisms from the same sputum sample to ensure that a true event had occurred.

To assess if biofilm formation played a role in *Achromobacter* sp. airway persistence, a modification of the protocol of Tomlin et al. (57) was performed. Isolates were grown overnight in tryptic soy broth, normalized to an optical density at 600 nm (OD_{600}) of 0.01, and plated in Nunclon Delta Surface 96-well plates with Nunc-Immuno TSP lids with pins (Thermo Scientific, Kamstrupvej, Roskilde, Denmark). Plates were then incubated at 37°C overnight on a rocker table. The biofilms that formed on lid pins were stained with 0.1% crystal violet, subsequently washed with water, and destained with 95% ethanol. Finally, a plate reader was used to quantify crystal violet staining at 550 nm.

Statistical analysis. Symmetrical and asymmetrical variables were described as means with standard deviations (SD) and medians with interquartile ranges (IQR), respectively. Pairwise comparisons were conducted using the Wilcoxon rank sum test for continuous variables and the Fisher exact test for proportions. Unadjusted risk ratios were calculated to determine the PEx risk at initial acquisition compared to that at preceding or subsequent clinical encounters. Mixed-effects linear regression models with an exchangeable correlation structure were conducted to assess the rate of lung function decline. Mixed-effects logistic regression models with a Poisson distribution were constructed to assess the odds of PEx. The mixed-effects models were utilized to compare pre- and post-*Achromobacter* infection variables within patients, between the transient and persistent groups, and between patients with persistent infection and matched controls. All hypotheses were evaluated with a two-sided α value of 0.05, and analyses were conducted with STATA V14.2 (StataCorp, College Station, TX).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.02556-16.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

B.D.E., J.G.-W., R.S., B.W., F.J.W., and D.G.S. have no conflicts to report. M.D.P., H.R.R., and M.G.S. have received research support from Gilead Sciences. M.D.P. and H.R.R. have performed advisory board work for Gilead, Novartis, Roche, and Vertex. No conflicts are relevant to the work discussed herein.

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