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Factors Influencing Acquisition of *Burkholderia cepacia* Complex Organisms in Patients with Cystic Fibrosis

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***Burkholderia cepacia* complex organisms are important transmissible pathogens found in cystic fibrosis (CF) patients. In recent years, the rates of cross-infection of epidemic strains have declined due to effective infection control efforts. However, cases of sporadic *B. cepacia* complex infection continue to occur in some centers. The acquisition pathways and clinical outcomes of sporadic *B. cepacia* complex infection are unclear. We sought to determine the patient clinical characteristics, outcomes, incidence, and genotypic relatedness for all cases of *B. cepacia* complex infection at two CF centers. We also sought to study the external conditions that influence the acquisition of infection. From 2001 to 2011, 67 individual organisms were cultured from the respiratory samples of 64 patients. Sixty-five percent of the patients were adults, in whom chronic infections were more common (68%) ($P = 0.006$). The incidence of *B. cepacia* complex infection increased by a mean of 12% (95% confidence interval [CI], 3 to 23%) per year. The rates of transplantation and death were similar in the incident cases who developed chronic infection compared to those in patients with chronic *Pseudomonas aeruginosa* infection. Multilocus sequence typing revealed 50 individual strains from 65 isolates. Overall, 85% of the patients were infected with unique strains, suggesting sporadic acquisition of infection. The yearly incidence of nonepidemic *B. cepacia* complex infection was positively correlated with the amount of rainfall in the two sites examined: subtropical Brisbane ($r = 0.65$, $P = 0.031$) and tropical Townsville ($r = 0.82$, $P = 0.002$). This study demonstrates that despite strict cohort segregation, new cases of unrelated *B. cepacia* complex infection continue to occur. These data also support an environmental origin of infection and suggest that climate conditions may be associated with the acquisition of *B. cepacia* complex infections.**

Burkholderia cepacia complex organisms are important respiratory pathogens in persons with cystic fibrosis (CF). Infection with *B. cepacia* complex organisms can result in increased mortality and morbidity, including "cepacia syndrome," and it has been associated with person-to-person transmission and poor outcomes following lung transplantation (1–5). Currently, the *B. cepacia* complex comprises 17 distinct species (6, 7). The recognition of transmissible strains led to improved microbiological screening protocols and the implementation of strict infection control policies that resulted in lower person-to-person transmission rates (8–10). While these policies have been successful in reducing cross-infections of epidemic strains, sporadic cases of *B. cepacia* complex infections continue to occur (11–14).

The routes of acquisition of sporadic *B. cepacia* complex infections in CF are not well understood, yet it is clear that environmental and clinical strains are closely related, with indistinguishable genotypes isolated from ecological niches and patients (15–17). Consequently, the environment is now recognized as a likely source of acquisition for sporadic strains, but relatively little is known about other factors that influence acquisition (14). Recent studies of *Burkholderia pseudomallei*, the etiological agent of melioidosis, have demonstrated that both the type of natural reservoir (e.g., soil) and other influencing factors (e.g., increased rainfall) play roles in acquisition (18, 19).

Our primary aim was to establish the incidence, prevalence, epidemiology, and outcomes of *B. cepacia* complex infection among persons with CF in Queensland, Australia, during an 11-year period. We also sought to assess the patient clinical characteristics at the time of infection. A secondary aim was to assess the impact of weather conditions on incidence rates of new cases of *B. cepacia* complex infection.

MATERIALS AND METHODS

Patients, data, and isolate collection. All cases of *B. cepacia* complex, *B. pseudomallei*, and *Burkholderia gladioli* infections detected in CF patients attending The Prince Charles Hospital Adult CF Center and the Royal Children's Hospital Pediatric CF Center, Queensland, Australia, between January 2001 and December 2011 were included in the study. These hospitals are the major adult and pediatric CF centers for Queensland, providing care for almost 600 of the 758 CF patients in Queensland (20). The patient clinical details, including age, sex, copathogens, forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and body mass index (BMI) at the time of first infection, were recorded in patients who acquired *B. cepacia* complex infections during the study period. Age-adjusted pulmonary function prediction equations were used (21, 22), and BMI *z*-scores (for children) were calculated using United States National Center for Health Statistics and Centers for Disease Control normalized growth reference values in children. Chronic *B. cepacia* complex infec-

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tions were defined as those with >50% of samples collected over the following 12 months being culture positive, using a modification of the Leeds criteria (23). Transient infections were defined as those with ≤50% of samples collected over the following 12 months being culture positive and when no infection was identified during the preceding 12 months. The numbers of deaths and lung transplants following the acquisition of *B. cepacia* complex were recorded.

All isolates presumed to be *B. cepacia* complex by phenotype that were cultured from the respiratory tract of CF patients (sputum, bronchoalveolar lavage [BAL] fluid, and oropharyngeal swabs) were included. Culture methods were standardized between the study sites, and there were no changes in the culture protocols throughout the study period. The isolates were initially referred to a reference laboratory for phenotypic profiling and confirmatory *B. cepacia* complex-specific *recA* PCR as previously described (13, 24), and the confirmed isolates were forwarded to a research laboratory for species identification and genotyping using multilocus sequence typing (MLST). Molecular analyses of recurrent isolates were performed when the clinical laboratory observed changes in the phenotypic characteristics compared to earlier isolates from the same patient.

Species identification and genotyping. The MLST reagents, primers, and reaction conditions were identical to those described on the *B. cepacia* complex PubMLST website (see http://pubmlst.org/bcc/) (25). Thermal cycling was performed in a Bio-Rad MyCycler personal thermal cycler and cycle sequencing was undertaken by the Australian Genome Research Facility using the BigDye Terminator version 3.1 cycle sequencing kit and an ABI 3730xl genetic analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Sequence verification and editing were performed using Sequence Scanner version 1.0 (Applied Biosystems, Inc.) and Vector NTI Advance 11.0 (Invitrogen Australia Pty, Ltd.) software.

Species identification by *recA* gene sequence analysis and sequence type (ST) assignment was performed using the *B. cepacia* complex PubMLST database (see http://pubmlst.org/bcc/) (25). The extended MLST protocol was utilized for samples showing low-quality PCR amplicons using the standard MLST primer set (26). Species-level identification of isolates showing novel *recA* sequences was undertaken by constructing a phylogenetic tree of the concatenated nucleotide sequences from the seven MLST loci of established *B. cepacia* complex species (neighbor-joining method with distances calculated using the Juke and Cantor algorithm) using the MEGA software version 5.2, as previously described (6).

Isolates presumed to be *B. pseudomallei* and *B. gladioli* were confirmed using previously described 16S rRNA gene PCR assays (27, 28). Genotyping was performed on confirmed isolates using repetitive extragenic palindromic sequence-based PCR (rep-PCR) analysis (13).

Epidemiological analysis. Details of the person-to-person contact history for each infection, including overlapping same-center care (i.e., inpatient and outpatient care) and known social contacts, were provided by the CF center directors and nurse specialists.

Statistical analysis. Continuous variables were summarized as the mean and standard deviation (SD) and categorical variables as the frequency (in percentage). Patients with *B. cepacia* complex, defined as those who had culture-confirmed *B. cepacia* complex infection on at least one occasion, were compared to patients without *B. cepacia* complex infections using Student's *t* test. Using Fisher's exact test, the rates of mortality and transplantation were compared between adult patients who acquired a chronic *B. cepacia* complex infection and between patients with chronic *P. aeruginosa* infection, irrespective of the presence of copathogens. Using linear regression, we investigated the association between study year and disease prevalence for the whole cohort and the two CF centers individually. The association between the study year and disease incidence was investigated using Poisson regression with robust standard errors. Model assumptions were tested with the deviance goodness-of-fit statistic. A *P* value of <0.05 was used to define statistical significance.

Meteorological analysis. The association between the incidence of infection with nonepidemic *B. cepacia* complex strains and meteorological factors was investigated by both spatial and time series analyses. For the spatial analysis, patients with CF were divided into those residing north and south of the Tropic of Capricorn (23.5° south of the equator), and the incidence rates were compared in these patients over the 11 years of the study. To calculate the incidence rates, we divided the total number of incident cases by the total number patients at risk each year. For the time series analysis, we selected the two cities with the largest numbers of patients with CF: subtropical Brisbane and tropical Townsville. Yearly incidence rates (the number of incident cases that year divided by the number of patients at risk that year) were compared with yearly rainfall amount, mean dew point at 9 a.m., and the mean temperature at 9 a.m., using Spearman's correlation. For both analyses, patients at risk were defined as those patients having had two or more cultures that year. Correlations were assessed on a yearly time scale because of the difficulty in determining the exact date of *B. cepacia* complex acquisition. The meteorological data used in the analyses were taken from meteorological records from the Brisbane and Townsville airports, available from the Australian Government Bureau of Meteorology (see http://www.bom.gov.au/).

Ethics statement. The Prince Charles Hospital Ethics Committee approved the study (HREC/13/QPCH/51), and patient consent was not required for this analysis.

RESULTS

Patient characteristics. At least one *B. cepacia* complex, *B. pseudomallei*, or *B. gladioli* organism was isolated from the respiratory tracts of 73 patients over the study period (54 adults and 19 children). Sixty-five percent of patients with *B. cepacia* complex infection were >18 years of age, 59.4% were male, and 51.2% were homozygous for the pF508del mutation. Ninety-four percent of the *B. cepacia* complex isolates were cultured from expectorated sputum samples, with the remaining samples comprising two BAL fluid and one oropharyngeal swab in three pediatric patients and one isolate referred from a private laboratory. For the adult patients, the rate of sample collection in the *B. cepacia* complex cohort (5.1 mean samples/patient/year) was similar to that for the whole population (4.8 mean samples/patients/year). For all patients who acquired *B. cepacia* complex infection (including those in whom the infection was transient), 43% of the respiratory samples were positive after the initial detection, whereas 85% were positive for those who developed a chronic infection.

Clinical and microbiological characteristics of incident cases. Since 2001, there have been 64 patients with *B. cepacia* complex infection, including 48 (75%) who acquired *B. cepacia* complex infection during the study period. The clinical and microbiological characteristics of the new (incidence cases) group are presented in Table 1. The rates of codetection with *P. aeruginosa* at the adult and pediatric CF centers were 87% and 53%, respectively. Chronic infection was more common in adults (19/28 [68%]) than in children (4/17 [24%]) ($P = 0.006$). Those patients with chronic *B. cepacia* complex infection had poorer lung function at the time of first infection detection (mean [SD] FEV_1% predicted, 49.1 [20.0]; FVC% predicted, 70.0 [19.0]) compared with patients with transient infection (FEV_1% predicted, 77.5 [19.8] [$P < 0.001$]; FVC% predicted, 88.7 [15.0] [$P = 0.003$]).

Of the adult patients who acquired a *B. cepacia* complex infection during the study ($n = 30$), 23 developed a chronic infection. Five of these patients died (including one following lung transplantation) and three survived following transplantation. Compared with adults with chronic *P. aeruginosa* infection, the rates of transplantation, death, and death following transplantation were similar ($P = 0.75$, 0.09, and 0.57, respectively). Of the children,

TABLE 1 Clinical and microbiological characteristics of the study patients who acquired *Burkholderia cepacia* complex infections between 2001 and 2011

	Results for patients at the CF center for:	
Patient characteristics[a]	Adults ($n = 30$)	Pediatrics ($n = 18$)[b]
Age (mean [SD]) (yr)	26.6 (9.1)	8.9 (4.8)
Sex (no. [%] males)	23 (77)	8 (44)
CFTR genotype (no. [%])		
p.delF508 homozygous	11 (37)	9 (50)
Chronic infection (no. [%])[c]	19 (68)[d]	4 (24)
Died (no. [%])	5 (17)	2 (12)[e]
Lung transplantation (no. [%])	4 (13)	1 (6)
Died after transplant	1 (3)	0 (0)
BMI (mean [SD])	21.5 (5.7)	−0.46 (−0.11)
FEV_1 (mean [SD]) (% predicted)	52.4 (22.7)	81.5 (17.2)[f]
FVC (mean [SD]) (% predicted)	71.7 (21.9)	89.6 (15.5)[f]
Copathogens (no. [%])		
Pseudomonas aeruginosa	26 (87)	9 (53)
MSSA	19 (63)	6 (35)
Aspergillus spp.	13 (43)	4 (23)

[a] CFTR, cystic fibrosis transmembrane conductance regulator; BMI, body mass index (values are BMI *z*-scores for patients age ≤18 years); FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MSSA, methicillin-susceptible *Staphylococcus aureus*.

[b] Data available for only 17 pediatric patients.

[c] Chronic infection was defined by the Leeds criteria (23).

[d] Data available for only 28 patients due to limited duration of follow-up.

[e] One of the children died at age 20 years in adult care from acute myeloid leukemia.

[f] Lung function data not included for 5 children due to age of acquisition.

two patients died and one survived following lung transplantation.

Outcomes of *Burkholderia cenocepacia* Australian epidemic strain (ST-39) infections. During the 11-year study, eight of the nine cases (including five patients who had the Australian epidemic strain at the start of the study period) infected with the Australian epidemic strain died (all were adults with chronic infections). In contrast, only six of the other 24 adult patients with chronic *B. cepacia* complex infection (i.e., those who did not have Australian epidemic strain infection) died ($P = 0.002$).

Mixed *Burkholderia* spp., *B. pseudomallei*, and *B. gladioli* infections. While the majority of patients (95%) were infected with one *B. cepacia* complex species, there were three patients who acquired a second *B. cepacia* complex species during the study period. In two cases, these infections occurred simultaneously, while in the third patient, the isolations were separated by 9 years. In addition to those patients with only *B. cepacia* complex infection, 13 patients each had *B. pseudomallei* and/or *B. gladioli* infection. These included four patients who were infected with one *B. cepacia* complex species and either *B. pseudomallei* ($n = 2$) or *B. gladioli* ($n = 2$) during the study. Another nine patients had either *B. pseudomallei* ($n = 4$), *B. gladioli* ($n = 4$) or both ($n = 1$) without *B. cepacia* complex infection. All cases of mixed *Burkholderia* spp. infection were observed in adults.

Prevalence and incidence. The prevalence and incidence of *B. cepacia* complex infections between January 2001 and December 2011 are presented in Table 2. The prevalence was stable during the study period for all patients. The mean increase in cases each study year was 0.20 (95% confidence interval [CI], −0.03 to 0.43) ($P = 0.09$); for adults, the mean increase was −0.17 (95% CI, −0.46 to 0.11) ($P = 0.20$), and for children, it was 0.27 (95% CI, −0.04 to 0.79; $P = 0.07$). The incidence increased during the study period by an average of 12% per year (incidence rate ratio [IRR], 1.12 [95% CI, 1.03 to 1.23]) ($P = 0.009$). The increase in incidence was significant in adults (IRR, 1.18 [95% CI, 1.08 to 1.29]) ($P < 0.001$) but not children (IRR, 1.05 [95% CI, 0.91 to 1.21]) ($P = 0.54$).

Species identification and diversity. Sixty-seven *B. cepacia* complex organisms were cultured from the 64 patients, of which 65 isolates were available for *recA* gene sequence analysis and MLST. Sequence analysis of the *recA* gene for these isolates revealed 36 distinct alleles. The species diversity of the *Burkholderia* species isolates are presented in Table 3. Fifty-four isolates were identified to the species level using the *B. cepacia* complex PubMLST database, and eight of the 11 isolates containing novel *recA* gene sequences were reliably identified by phylogenetic sequence analysis of established *B. cepacia* complex species. The most fre-

TABLE 2 Annual prevalence and incidence of infections with nonepidemic strains of *B. cepacia* complex

	Infection data by location								
	Adult CF center[a]			Pediatric CF center[b]			Combined		
Yr	No. of patients	Prevalence (%)	Incidence (%)	No. of patients	Prevalence (%)	Incidence (%)	No. of patients	Prevalence (%)	Incidence (%)
2001	152	10.5	0.7	241	0.4	0.4	393	4.3	0.5
2002	176	9.7	1.1	242	0.4	0.0	418	4.3	0.5
2003	194	8.2	0.5	246	0.4	0.0	440	3.9	0.2
2004	217	7.4	0.5	280	1.1	0.7	497	3.8	0.6
2005	216	6.9	0.5	278	1.1	0.7	494	3.6	0.6
2006	215	4.7	0.5	284	2.5	2.1	499	3.4	1.4
2007	235	5.5	1.3	303	1.3	0.3	538	3.2	0.7
2008	242	6.2	2.1	323	1.5	0.6	565	3.5	1.2
2009	248	6.0	2.0	331	1.8	0.6	579	3.6	1.2
2010	265	5.3	1.9	328	1.5	0.6	593	3.2	1.2
2011	272	5.9	1.1	318	0.6	0.0	590	3.1	0.5

[a] The incidence rate ratio for *B. cepacia* complex cases at the adult CF center was 1.18 (95% CI, 1.08 to 1.29) ($P < 0.001$).

[b] The incidence rate ratio for *B. cepacia* complex cases at the pediatric CF center was 1.05 (95% CI, 0.91 to 1.21) ($P = 0.54$).

TABLE 3 *Burkholderia* species diversity among the study patients

Burkholderia sp. and/or *recA* lineage	No. (%) of isolates in: Total[a]	Adult CF center	Pediatric CF center
Burkholderia cepacia complex	65 (100.0)	48 (73.8)	17 (26.2)
B. cenocepacia recA lineage B	17 (26.2)	11 (22.9)	6 (35.3)
B. multivorans	17 (26.2)	13 (27.1)	4 (23.5)
B. cenocepacia recA lineage A	11 (16.9)	10 (20.8)	1 (5.9)
B. cepacia	8 (12.3)	5 (10.4)	3 (17.6)
B. vietnamiensis	3 (4.6)	3 (6.3)	
B. ambifaria	1 (1.5)	1 (2.1)	
B. arboris	1 (1.5)	1 (2.1)	
B. contaminans	1 (1.5)		1 (5.9)
B. diffusa	1 (1.5)	1 (2.1)	
B. latens	1 (1.5)		1 (5.9)
B. pyrrocinia	1 (1.5)		1 (5.9)
Indeterminant	3 (4.6)	3 (6.3)	
B. gladioli	7 (100.0)	6 (85.7)	1 (14.3)
B. pseudomallei	7 (100.0)	7 (100.0)	

[a] Eight patients were infected with two different *Burkholderia* species during the study period.

TABLE 4 Correlation of yearly incidence rates of nonepidemic strains of *B. cepacia* complex infection in Brisbane and Townsville with local weather conditions, 2001 to 2011

Meteorological variable	Values for: Brisbane Correlation	Brisbane *P*	Townsville Correlation	Townsville *P*
Rainfall	0.65	0.031	0.82	0.002
Dew point	0.47	0.147	0.25	0.453
Temperature	0.04	0.911	−0.51	0.113

quently encountered *B. cepacia* complex species included *B. cenocepacia*, *Burkholderia multivorans*, and *B. cepacia*.

Multilocus sequence typing and rep-PCR genotyping. All of the 65 *B. cepacia* complex isolates were typeable by MLST, revealing 50 individual STs (see Dataset S1 in the supplemental material). Seventy-two percent ($n = 36$) of the STs were novel. Overall, there was a high degree of genotype diversity, with 85% ($n = 40$) of patients infected with unique STs, while only seven STs represented genotypes found in clusters of two or more patients (see Dataset S1 in the supplemental material). Nine adults, including four incident cases (during the period 2001 to 2004), were infected with the *B. cenocepacia* Australian epidemic strain (ST-39) (13). *B. cepacia* strain ST-675 was common to three patients, and STs 181, 434, 632, 673, and 703 were each detected in clusters of two patients. rep-PCR analysis of the 14 *B. pseudomallei* and *B. gladioli* isolates revealed unique genotypes in all instances (data not shown).

Epidemiological analyses. Of the nine adults with the *B. cenocepacia* Australian epidemic strain (ST-39), four acquired the infection during the study period and had documented having close social contact outside the hospital setting. Since 2004, there have been no new cases of this strain. In all other instances of *B. cepacia* (ST-675), *B. multivorans* (ST-181), and *B. cenocepacia* (STs 434, 632, 673, and 703) shared strain infections, there was no evidence of personal contact between patients either at the CF center or outside the hospital.

Meteorological analyses. The rate of nonepidemic *B. cepacia* complex infection was significantly higher in patients with CF residing north of the Tropic of Capricorn than in those residing south of the Tropic of Capricorn (IRR, 2.61; 95% CI, 1.03 to 6.11). In the time series analysis, the amount of rainfall was positively correlated with the yearly incidence of infection in both Brisbane and Townsville (Table 4).

DISCUSSION

This study shows that accurate identification of *B. cepacia* complex organisms coupled with the implementation of strict patient segregation (27, 29) have reduced the spread of epidemic strains. In contrast to earlier reports (13, 30), only four instances of likely cross-infection resulting in the acquisition of the *B. cenocepacia* Australian epidemic strain were identified, and there was evidence of close personal contact outside the hospital between these patients. However, our study has also confirmed that sporadic cases of *B. cepacia* complex infection continue to occur and new cases are occurring more frequently. These cases also demonstrated genotypic diversity and novel STs. Together, these data suggest that the environment is the likely source of acquisition.

Several studies have provided evidence of environmental isolates recovered from various natural, agricultural, pharmaceutical, and industrial sources that are indistinguishable from those isolated from persons with CF (14, 15, 17). The changes in species diversity in several CF centers following the introduction of strict infection controls may also be attributable to the acquisition of environmental *B. cepacia* complex infection (11, 12). Notably, we also predominantly encountered nonepidemic *B. cenocepacia*, *B. multivorans*, and *B. cepacia* isolates, along with a broad range of recently described species, including *Burkholderia arboris*, *Burkholderia contaminans*, *Burkholderia diffusa*, and *Burkholderia latens* (6, 7).

In our earlier work (13), we speculated that increased rates of *B. cepacia* infections in CF patients living in subtropical regions of Australia may be attributable to particular environmental niches for specific *Burkholderia* species. Alternatively, differences in climatic or other environmental conditions, as we have observed with *P. aeruginosa* (31, 32), might play a role in variations in the prevalences of environmental opportunistic pathogens in CF. During periods of monsoonal rainfall in tropical areas, the incidence of *B. pseudomallei* infections is known to increase significantly (19). We report that the yearly incidence of nonepidemic *B. cepacia* complex infection was significantly correlated with higher rainfall amount in the two sites studied (subtropical Brisbane and tropical Townsville). In addition, we found that the incidence rate over the 11 years of the study was higher in patients from tropical regions north of the Tropic of Capricorn. This suggests that the acquisition of *B. cepacia* complex infection may be linked with increased rainfall. Environmental surveys to determine bacterial persistence and the load over time and during different weather conditions may further explain the changes in infection rates over time.

Earlier studies demonstrated that *B. cepacia* complex infection is associated with increased morbidity and mortality for patients with CF (2, 8). However, most of these studies have focused on clinical outcomes in patients with epidemic and virulent genotypes, such as the *B. cenocepacia* ET-12 strain (33). Our results show that patients with chronic infections had lower lung function than patients with transient infections. Furthermore, adult patients had lower lung function and were more likely to have chronic *B. cepacia* complex infection and other airway CF pathogens identified than children. When patients with chronic *B. cepacia* complex were compared to patients with chronic *P. aeruginosa*, irrespective of other copathogens, we observed no increase in mortality or requirement for lung transplantation. We did, however, find increased mortality in patients attending the adult center with the *B. cenocepacia* Australian epidemic strain (ST-39) compared to those with nonepidemic *B. cepacia* complex infection.

Other studies have also suggested that the clinical outcomes for patients infected with *B. cepacia* complex are species dependent and that the host-pathogen interaction is also likely to play a role in virulence and clinical impacts (14, 33, 34). Jones and colleagues (33) reported excess mortality and accelerated clinical decline in patients with the ET-12 strain, whereas our study, which was dominated by sporadic *B. cepacia* complex infection, did not find increased rates of mortality or the need for lung transplantation. We have not performed further clinical outcome analyses at this stage, as we had a small number of patients who developed chronic infection; however, this will be important to perform in the future (i.e., with larger numbers of patients), as nonepidemic *B. cepacia* complex infection may not have the same impact on clinical outcomes as epidemic strains, which are less prevalent in most CF centers in the current era. It is also known that person-to-person transmission is strain dependent and that some strains are more adapted to causing human infection than others (12). While it appears that the major epidemic strains belonging to *B. cenocepacia* (including ET-12, PHDC, and ST-39) are more transmissible than other strains, outbreaks of other species have the potential to occur (12, 14).

Several studies have demonstrated that the majority of patients who acquire *B. cepacia* complex infection develop chronic infections (35–37). A Portuguese study reported that pediatric patients with CF had more frequent chronic infections than transient infections (35). Our finding differed, and we report here that infections in younger patients were more likely to be transient, and when transient, they occurred in patients with better lung function. An eradication program for pediatric patients with newly acquired *B. cepacia* complex infection may be a significant factor for this difference, and there is some support in the literature for this idea (37). However, spontaneous clearance may also occur, as we have demonstrated in patients with methicillin-resistant *Staphylococcus aureus* infection (38).

When indistinguishable genotypes of bacterial pathogens are isolated from patients with CF, it is suggested that health care-associated transmission or exposure to common environmental strains was the route of acquisition. Our recent MLST studies of *P. aeruginosa* in Australia confirm the presence of numerous shared strains, many of which are widespread in other local and international ecological settings, thus increasing the potential for infection in the susceptible host, e.g., the CF lung (31, 39). In the current study, we also observed several small clusters of patients with indistinguishable *B. cepacia*, *B. multivorans*, and *B. cenocepacia* STs, which were not associated with person-to-person transmission. Furthermore, four of the strains detected during this study were recently encountered among nonepidemic clinical isolates from New Zealand, including the shared strain ST-181, which was isolated from two unrelated patients with CF (40).

Our data show that *B. gladioli* accounted for <10% of all *Burkholderia* species infections across the study period. In contrast, data from North America indicate that *B. gladioli* is the third most common *Burkholderia* species encountered in patients with CF (41). All but one patient with *B. gladioli* lived in subtropical areas at the time of acquisition, while, consistent with our earlier observations, most patients with *B. pseudomallei* infection lived in tropical regions (28). Genotypic analysis of these species confirmed unique genotypes among all patients, suggesting independent acquisition of infection.

In conclusion, despite strict cohort segregation and infection control policies, new cases of *B. cepacia* complex infection continue to occur. These cases in recent years have been dominated by unique strains and are likely to be acquired from the environment. We have demonstrated several lines of evidence to support this, including (i) a lack of epidemiological linkage between patients with the same strain, (ii) the potential linkage with changes in rainfall, and (iii) a high number of nonepidemic, unrelated, and novel strains.

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