Burkholderia gladioli: Five year experience in a cystic fibrosis and lung transplantation center[☆]

Marcus P. Kennedy [a],*, Raymond D. Coakley [b], Scott H. Donaldson [b], Robert M. Aris [b], Kathy Hohneker [b], Joel P. Wedd [c], Michael R. Knowles [b], Peter H. Gilligan [c], James R. Yankaskas [b]

[a] *Department of Pulmonary Medicine, Unit 403, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA*
[b] *Department of Pulmonary and Critical Care Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7030, USA*
[c] *Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7030, USA*

Abstract

Background: The impact of infection with *Burkholderia gladioli* in cystic fibrosis, other chronic airway diseases and immunosuppressed patients is unknown.
Methods: A six-year retrospective review of all patients with *B. gladioli* infection was performed in a tertiary referral center with cystic fibrosis and lung transplantation programs. In addition, a targeted survey of all 251 lung transplant recipients was performed. Available *B. gladioli* isolates were analyzed via pulsed field gel electrophoresis.
Results: Thirty-five patients were culture positive for *B. gladioli,* including 33 CF patients. No bacteremia was identified. Isolates were available in 18 patients and all were genetically distinct. Two-thirds of these isolates were susceptible to usual anti-pseudomonal antibiotics. After acquisition, only 40% of CF patients were chronically infected (≥ 2 positive cultures separated by at least 6 months). Chronic infection was associated with resistance to ≥ 2 antibiotic groups on initial culture and failure of eradication after antibiotic therapy. The impact of acquisition of *B. gladioli* infection in chronic infection was variable. Three CF patients with chronic infection underwent lung transplantation. One post-transplant patient developed a *B. gladioli* mediastinal abscess, which was treated successfully.
Conclusions: The majority of patients' culture positive for *B. gladioli* at our center have CF. *B. gladioli* infection is often transient and is compatible with satisfactory post-lung transplantation outcomes.
© 2006 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

Keywords: Cystic fibrosis; *Burkholderia gladioli*; Lung transplantation; Bronchiectasis

1. Introduction

Members of the genus *Burkholderia* are metabolically versatile gram-negative organisms occupying diverse ecological niches including soil, plants and human respiratory tracts. The nomenclature of *Burkholderia gladioli*, which is distinct from the *Burkholderia cepacia* complex, has evolved from *Pseudomonas marginata* and *Pseudomonas gladioli* to its current designation. It was first recognized as a plant pathogen of Gladiolus and Iris species [1]. In 1989, *B. gladioli* was first described in the medical literature as a colonizing organism in 11 patients with cystic fibrosis (CF)-related lung disease (to date, the largest reported series), but the authors were unable to conclude whether colonization was associated with adverse outcomes [2]. Since then, case reports and small case series have highlighted morbidity associated with *B. gladioli* in a total of 13 patients. These included pulmonary disease related to *B. gladioli* in CF

patients (before [3–5] and after lung transplantation [6,7]), chronic granulomatous disease [8,9], and acquired immune deficiency syndrome [4]. Extra-pulmonary infection has been identified patients with CF [4–7], in immunocompromised hosts and patients with diabetes mellitus [4,10].

There is a paucity of information in the literature regarding prevalence of disease associated with *B. gladioli* in at-risk patient populations, including patients with CF and other chronic airway diseases, and patients who are immunosuppressed. However, the development of species-specific PCR testing now permits more accurate microbiological identification. This, in turn, creates an opportunity for improved epidemiological surveillance and more rigorous characterization of medical consequences of infection. [11]. In particular, although previous case reports and case series highlighted mortality and morbidity associated with *B. gladioli* infection after lung transplantation (suggesting infected patients may be unsuitable candidates for this procedure), these data are limited by their small sample size.

Because of these considerations, we examined the prevalence of *B. gladioli* and its disease associations at our institution over a six-year period, by performing a comprehensive review of all patients' culture positive for *B. gladioli* during this time.

2. Methods

2.1. Patients and study design

A retrospective review was performed of all patients who had one or more positive *B. gladioli* cultures at UNC Hospitals between 1999 and 2006 was performed [12]. Patients were identified using an electronic survey of all microbiology cultures processed at the institution during the time period. The 1999 time point coincided with the introduction of a new institutional protocol for definitive speciation of all (CF and non-CF) *Burkholderia* isolates at the Cystic Fibrosis Foundation (CFF)-sponsored *B. cepacia* Reference Laboratory and Repository, University of Michigan, Ann Arbor, Michigan [11]. Thus, a careful record of all *Burkholderia* organisms, using state of the art culture and molecular techniques for speciation, was developed and maintained since this time. Because of the clinical importance of information pertaining to the course of infected patients after lung transplantation, a targeted survey of all 251 patients undergoing lung transplant since the initiation of our program in 1990 was also performed. This included a manual chart analysis of all patients transplanted prior to the commencement of electronic surveillance of microbiology results (1999). Additional patients with a culture yielding *B. gladioli* from this search were added to our analysis. Approval for this study was received from our institutional review board.

All available medical records of all cases that had confirmed positive cultures for *B. gladioli* were reviewed. Data regarding age, sex, ethnicity, comorbidities, pulmonary function tests (using Knudson %predicted formula), frequency of positive cultures, length of follow-up, sensitivity testing, other organisms cultured, and evidence of complications related to *B. gladioli* including hospital admission and death were recorded. The diagnosis of cystic fibrosis was confirmed by clinical phenotype and evidence of confirmatory tests (sweat chloride and/or cystic fibrosis transmembrane conductance regulator (CFTR) genotype).

2.2. Characterization of cystic fibrosis patients

The UNC Cystic Fibrosis Pulmonary Research and Treatment Center evaluated 769 CF patients in our pediatric and adult programs during this time period. A patient with CF was considered negative for *B. gladioli* infection if no culture
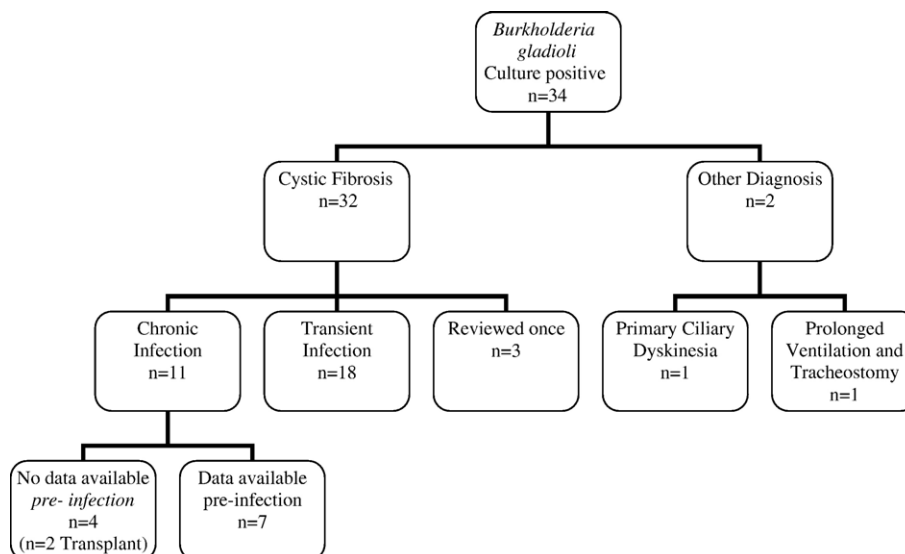

Fig. 1. Positive cultures for *B. gladioli*, 1999–2006. Excludes one cystic fibrosis that underwent lung transplant prior to 1999.

was positive for *B. gladioli* upon review of a database commenced in September 1999 (it is our institution's policy to culture airway secretions from cystic fibrosis patients at least 4 times a year).

CF patients who had positive cultures for *B. gladioli* were subdivided into two groups: chronic infection (≥2 positive cultures separated by at least 6 months) or transient infection (a single positive culture or >1 positive cultures for *B. gladioli* separated by less than six subsequent months and culture negative thereafter). Patients who were labeled as having chronic infection could have negative cultures for *B. gladioli* interspersed with positive cultures.

2.3. Pulmonary function analysis

To assess the impact of *B. gladioli* infection on pulmonary function, available spirometric values were reviewed for 5 years before and 5 years after acquisition of the organism in patients who were chronically infected. The best FEV1 (forced expiratory volume in one second) percent predicted for each quartile during this ten-year period was recorded. For each patient, the slope of the FEV1 plot before and after acquisition of *B. gladioli* was determined by linear regression.

2.4. Microbiological studies

All airway specimens from CF patients are cultured on MacConkey agar, colistin-nalidixidic acid agar and BCSA (*B. cepacia* selective agar) [13]. Antibiotic susceptibility testing was performed with disc diffusion using *Pseudomonas aeruginosa* breakpoints [14]. The genotype of available *B. gladioli* isolates were analyzed by pulsed field gel electrophoresis (PFGE), using a protocol previously described [13]. Chromosomal digestion was performed using the restriction endonucleases XbaI and SpeI on all isolates. Genotype patterns were determined by visual inspection using standard methodology [15].

3. Results

Thirty-four patients had cultures growing *B. gladioli* between September 1999 and March 2006 (Fig. 1). Medical records were available on all patients, and all patients were included in this review. Thirty-two patients had CF (92%). The other two patients included a 28-year-old female with primary ciliary dyskinesia, who was transiently infected with *B. gladioli,* and a 63-year-old female with no prior pulmonary history who was being mechanically ventilated for complications of trauma. In both cases, the organism was pan-susceptible to usual anti-pseudomonal antibiotics tested. After surveying the medical records of all 251 patients undergoing lung transplantation at our center, we identified one additional patient with CF who was culture positive for *B. gladioli* prior to September 1999, giving a total of 35 patients in this series. All positive cultures were pulmonary in origin (sputum, bronchoalveolar lavage or tracheal

Table 1
Demographic features, genotype and concomitant positive cultures in 33 cystic fibrosis patients infected with *Burkholderia gladioli*

Demographic features	Total ($n=33$) [a]	Chronic infection [b] ($n=12$)	Transient infection ($n=18$)
Age (years) at *B. gladioli* acquisition (mean ±SD; range)	15.4±6 (6–33) $n=25$	13.5±5 (6–21)) $n=7$	16±8 (6–33) $n=18$
Male sex *n* (%)	23 (70)	7 (58)	13 (72)
Nonwhite race *n* (%)	3 (9)	0 (0)	2 (11)
Body mass index at *B. gladioli* acquisition (mean±SD)	20±4 $n=25$	20±2.6 $n=7$	19.7±5.5 $n=18$
Diabetes mellitus *n* (%)	5 (15)	0 (0)	5 (28)
Pancreatic sufficiency *n* (%)	3 (9)	2 (17)	0 (0)
Δ508/Δ508 homozygote *n* (%)	19 (58) $n=31$	8 (66) $n=11$	10 (56) $n=18$
FEV1 at *B. gladioli* acquisition mean %predicted±SD (range)	80±30 (11–125) $n=25$	82±19 (60–114) $n=7$	79±30 (11–125) $n=18$
B. gladioli resistance to =2 antibiotic groups *n* (%)	11 (33)	10 (83)	1 (6)
Other microbes			
Staphylococcus aureus n (%)	30 (91)	10 (83)	17 (94)
Pseudomonas aeruginosa n (%)	21 (64)	5 (42)	16 (89)
Burkholderia cepacia complex *n* (%)	4 (12)	2 (17)	2 (11)
Non-tuberculous mycobacteria *n* (%)	3 (9)	0 (0)	3 (17)

[a] Total = includes three CF patients cultured on only one occasion and one cystic fibrosis who underwent lung transplant prior to 1999.
[b] Includes three lung transplant recipients.

aspirate), and included one intrathoracic lymph node infection in a patient who underwent lung transplantation 3 months earlier (sampled via transbronchial needle aspirate and confirmed following surgical debridement). *B. gladioli* bacteremia or symptoms of septicemia were not identified. Yearly incidence rates of new infections did not change over the five-year time period. We did not identify any apparent clustering of cases (same clinic day appointment or simultaneous hospital admission), suggesting that patient-to-patient transmission was unlikely.

3.1. B. gladioli in cystic fibrosis (n = 33)

Between 1999 and 2006, 32 CF patients had positive sputum cultures for *B. gladioli*, giving a sputum-positive prevalence of 4.2% in all of our CF adult and pediatric populations. This included two patients who subsequently underwent lung transplantation. As stated earlier, one additional lung transplant patient had a culture that was positive for *B. gladioli* prior to September 1999 and has been included in the following analyses of 33 CF patients. Table 1 displays demographic features and clinical characteristics of all CF patients infected with *B. gladioli*. Four adult patients had identifiable regular outdoor occupational and/or recreational exposure (construction work, gardening and cross country running).

One patient died from respiratory failure while awaiting lung transplantation 1 year after 2 positive sputum cultures
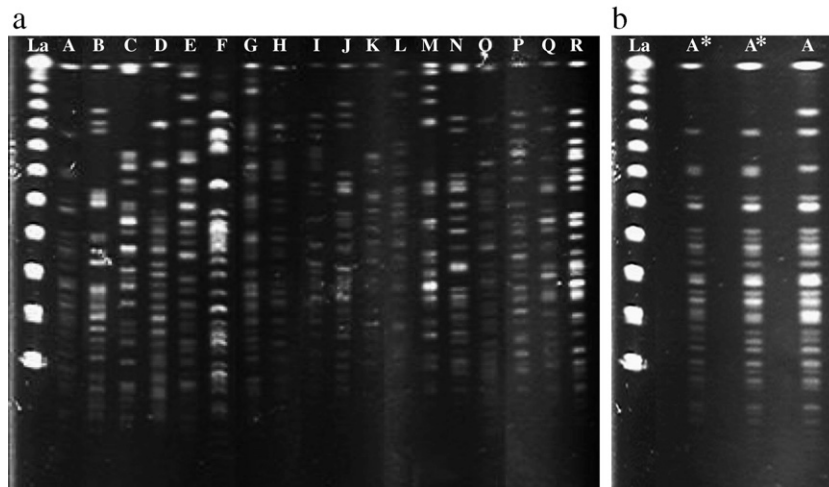
Fig. 2. Pulse gel electric field analysis of *B. gladioli* strains recovered from 18 patients. 2a: PFGE gel using the restriction endonuclease *Xba1* of *B. gladioli* strains recovered from 18 different patients demonstrates 18 different isolates (A–R). 2b: PFGE gel using endonuclease *XbaI* of sequential isolates (A) of *B. gladioli* recovered over a 4 month period from a single cystic fibrosis patient demonstrates that two of the three strains* were identical and all three were clonal based on having fewer than 4 band differences.

for *B. gladioli*, although his sputum was negative for *B. gladioli* at time of death. Sixteen percent of all adult CF patients whose cultures were positive for *B. gladioli* were referred for, or had undergone, lung transplantation.

Three patients were only seen once at the CF center and follow-up cultures were, therefore, available in 30 patients (Fig. 1). Mean follow up was 8 years (range 2–13). Eighteen patients (60%) were transiently infected ($n=16$ with one positive culture and $n=2$ with two positive cultures separated by less than 3 months) with *B. gladioli* and 12 patients (40%) were chronically infected (average number of positive cultures = 11; range 2–25). In 25 patients, clinical and microbiological data was available prior to acquisition of *B. gladioli* (18 transient infection and 7 with chronic infection). The average number of cultures before and after acquisition of *B. gladioli* in the transient infection group ($n=18$) was 13 (range 1–47) and 10 (range 1–23). Two patients with transient infection had only one negative culture available prior to acquisition of *B. gladioli* and one patient had only one negative culture available after infection with *B. gladioli*. The average number of cultures available prior to acquisition of *B. gladioli* in the chronically infected group ($n=7$) was 9 (1–17). One patient had only one culture available prior to chronic infection with *B. gladioli*. Mean age was $15.4+6$ (6–33) at time of initial positive culture. Mean FEV1 at time of acquisition of *B. gladioli* was $82\pm19\%$ in the chronically infected group and $79\pm30\%$ in the transiently infected group.

The impact of chronic *B. gladioli* infection on lung function in the group with clinical and microbiological data available before and after acquisition of the organism ($n=7$) was examined by plotting best FEV_1 values during each quartile for 5 years before and after acquisition and calculating linear slopes during each period. Because of the small number of patients in this group and the apparent heterogeneity in lung function trends, statistical testing was not performed. However, 3 patients appeared to have an accelerated decline in lung function after *B. gladioli* acquisition, whereas the slope of lung function change did not worsen, or improved, in 4 patients after acquisition.

Documentation of antibiotic therapy directed at *B. gladioli* shortly after acquisition was noted for all chronically infected patients and for 14/18 transiently infected patients (in 4 patients it was uncertain from clinical records whether they received antibiotic therapy or not). Antibiotic therapies varied in class, route, time and number (one or two antibiotic classes). Of note, 10/11 initial *B. gladioli* isolates from those patients who developed chronic infection were resistant to >2 antibiotic classes, whereas the majority (17/18) of initial isolates from transiently infected patients were susceptible to usual anti-pseudomonal antibiotics. Resistance was more prevalent to 3rd generation cephalosporins (28%) and quinolones (20%).

Table 1 highlights concomitant positive cultures for *Staphylococcus aureus*, *P. aeruginosa*, *B. cepacia* complex and non-tuberculous mycobacteria. There was a high prevalence (91%) of chronic infection with *S. aureus*. 7 of 12 (58%) with chronic *B. gladioli* infection had no other gram-negative pathogens isolated after acquisition. In the remaining 5 patients, other gram-negative organisms were less abundant and infrequently isolated. In contrast, there was a higher and more typical prevalence (89%) of *P. aeruginosa* in the group transiently infected with *B. gladioli*.

B. cepacia complex species were identified in 4 patients (3 with *B. cenocepacia* (Genomovars III); and one with *B. multivorans* (Genomovars II)). In 3 patients, the organism was identified once prior to *B. gladioli* and therefore did not persist. One of these patients was subsequently chronically infected with *B. gladioli*. In the other patient, continuous growth of *B. gladioli* was replaced by *B. cenocepacia*.

3.2. Lung transplant patients (n = 3)

Three lung transplant recipients were infected with *B. gladioli* (one prior to September 1999). The average age at transplant was 39 years. The culture positive prevalence of *B. gladioli* in our lung transplant population was 1.2%. All lung transplant recipients infected with this organism had continuously positive cultures before lung transplantation and at least one positive culture afterward. The two patients transplanted since 1999 were treated with intravenous antibiotic therapy directed against *B. gladioli* for 3 months after transplantation, in compliance with our institution's protocol for patients infected with other *Burkholderia* species. All patients were treated with standard immunosuppressant regimens, including prednisone, cyclosporine, and azathioprine. One patient, now 11 years post-transplant was culture positive for *B. gladioli* for the first month post transplant, but BAL cultures have been negative since. Respiratory specimens from the second patient grew *B. gladioli* (as the predominant gram-negative organism) for at least 7 years pre-transplant. The patient is now 3 years post-transplant with recurrence of infection and in-hospital treatment on two occasions. The third patient, with pre-transplant microbiology predominated by *B. gladioli* (resistant to trimethoprim–sulfamethoxazole and ceftazidime), developed a post-transplant febrile mediastinal lymph node infection with abscess formation 3 months post-transplant. Needle aspirate culture was positive for *B. gladioli,* which was successfully treated with combined medical and surgical management. Cultures were negative for *B. gladioli* 2 years later.

3.3. Pulsed gel field electrophoresis (n = 18)

PGFE was performed on 20 isolates from 18 patients including 17 CF patients (*n* = 9 continuous (*n* = 2 post-transplant) and *n* = 8 transient culture positive) and one PCD patient (Fig. 2a). Strains from each patient were genetically distinct. One patient had 3 strains available for PGFE analysis (Fig. 2b). Two of the three strains were identical and all three were clonal based on having fewer than 4 band differences.

4. Discussion

We undertook this review of *B. gladioli* infection, the first retrospective analysis of the incidence and clinical relevance of this organism in a large population of at risk patients, to address the clinical significance of this organism. Advances in the differentiation of *B. gladioli* from other *Burkholderia* species and other gram-negative organisms over the last decade present the opportunity for more accurate characterization of epidemiology and pathology of *B. gladioli* infection. One prior report of six "*B. gladioli* infected" cystic fibrosis patients from a single center suggested cross infectivity in the absence of appropriate infection control

precautions, but post-publication analysis correctly identified this isolate as *B. cenocepacia* [16,17]. Thus, accurate differentiation from other *Burkholderia* species now permits assimilation of more accurate information with important implications for patient care.

An intrathoracic site characterized the infection in all our patients with one patient exhibiting a mediastinal lymph node infection. This is consistent with previous published literature indicating that the majority of positive cultures are from the airway [2–10].

An important epidemiological result of this study was that all isolates available for testing were genotypically distinct. These results suggest no incidents of cross infection or geographical clustering in our cohort. This is consistent with our experience with a larger cohort of CF patient infected with *B. multivorans* where evidence for person-to-person spread based on the presence of the same genotype was unusual [14]. These data indicate that environmental acquisition rather than person-to-person spread is typical for these two organisms. In contrast, person-to-person spread is well documented for two other members of the *B. cepacia* complex in the absence of appropriate infection control precautions, *B. cenocepacia* and *B. dolosa* (Genomovars VI) [18,19].

Although two patients did not have CF, all patients had an identifiable co-morbid condition that plausibly predisposed them to *B. gladioli* infection. *CFTR* mutation distribution in the CF patients was in proportion with expected distribution for our predominately Caucasian population [20]. In the prior published case reports and series [2–10], 17/24 patients (71%) had cystic fibrosis. The fact that the majority of patients testing positive for *B. gladioli* at our center had CF likely reflects our active cystic fibrosis and lung transplant programs and special culture techniques used to culture and identify this organism. There is no national CFF data for this organism. We have not identified any prior estimate of the prevalence of *B. gladioli* in cystic fibrosis in the literature, although one publication, reporting on the incidence of *Burkholderia* strain replacement in 379 patients recovered from 112 CF treatment centers, suggested a sputum positive prevalence of approximately 10:1 for *B. cepacia* complex species relative to *B. gladioli* in CF [21].

Increased morbidity and mortality have clearly been associated with *B. cepacia* complex (especially *B. cenocepacia*) [17,21,22] and *B. dolosa* [19] infections in CF. Further, epidemic spread, incidents of bacteremia and sepsis, and poorer lung transplant outcomes are associated with these organisms [18,19,22–31]. However, the impact of *B. gladioli* on CF outcomes is less clear [2–7]. Our results suggest significantly different outcomes with *B. gladioli*, especially when contrasted against *B. cenocepacia*. First, the majority of patients who contract *B. gladioli* manifest transient infection, which in our case was associated with the use of antibiotics against isolates that were not as antibiotic resistant based on in vitro susceptibility results. Second, bacteremia, or a "cepacia-like syndrome", was not identified in our cohort, although *B. gladioli* bacteremia and

diffuse infection with extrathoracic abscess formation has been highlighted in prior CF patients pre and post-transplant [4–6].

We approached the important question of whether culture positivity for *B gladioli* results in more severe pulmonary disease in CF by analyzing lung function (FEV1) trends before and after acquisition of the organism. However, because of the small size of this group with adequate clinical and microbiologic data, we are unable to make any firm conclusions. Some patients appeared to deteriorate from a pulmonary perspective after *B. gladioli* infection, including three patients in whom the predominant gram-negative organism was *B. gladioli* and who required lung transplantation. Continued observation of this cohort of patients may in time clarify morbidity and mortality related to *B. gladioli* infection.

Our analyses suggest that chronic *B. gladioli* infection is associated with lower rates of chronic *P. aeruginosa* infection suggesting that *B. gladioli* is capable of becoming the predominant gram-negative pathogen. In contrast to *P. aeruginosa*, chronic infection with *S. aureus* (91%) was higher than expected (18–26% in CF adults, 30% in all CF patients [22,32]). It will be interesting to determine whether the strong association between *B. gladioli* and *S. aureus* infection that we observed is validated by review of new cases and data from other CF centers. If real, it may provide insights into the biology of bacterial communities in the CF lung.

Pan-susceptibility to usual anti-pseudomonal antibiotics in two thirds of isolates is also in contrast with prior *B. gladioli* [7–9] case reports and series, nor is it consistent with the *B. cepacia* complex literature (invariably aminoglycoside resistant) [7,22,24,26]. However, resistance to 2 or more antibiotic families was identified in patients who were continuously culture positive patients (10/11) and in those who ultimately required lung transplantation (3/3), suggesting that antibiotic resistance is associated with chronic infection.

The published opinions regarding appropriateness of lung transplantation in CF patients colonized with *B. gladioli* are mixed [7,33]. The importance of accurate speciation of *Burkholderia* isolates is vital with regard to transplantation, since many centers do not offer the procedure to patients with *Burkholderia* species. We identified a prevalence of 1.2% in our pre-transplant population. Although one patient developed mediastinal infection, requiring surgical debridement and prolonged antibiotic therapy, he ultimately had a favorable clinical course. On review of prior case reports and case series, we identified 4 lung transplant patients with cystic fibrosis and *B. gladioli* infection. One patient with disseminated disease prior to transplant, involving multiple subcutaneous abscesses, died in the peri-transplant period [5]. Another patient developed pulmonary, pleural space and chest wall infection and died 6 months post transplant due to lung rejection [6], while the remaining two patients developed post transplant wound infections which were successfully treated medically [7]. On balance, therefore, we feel that pre-transplant infection with *B. gladioli* should not contraindicate lung transplantation in CF. However; we

suggest that caution is warranted, comorbid status should be carefully assessed and close follow-up performed with awareness of chest wall, pleural space and mediastinal infection. The use of prolonged postoperative intravenous antibiotic therapy may be beneficial, given the relatively benign course in 2 of our 3 patients.

In summary, with advancements in microbiological testing, we have an opportunity to accurately define disease related to individual *Burkholderia* species including *B. gladioli*. In this retrospective chart review, we have identified that the predominant source of *B. gladioli* isolates in our institution are from CF patient pulmonary secretions. Importantly, in our patient population there have been no episodes of spread between patients, based on PFGE results. Further, in contrast to *B. cenocepacia* and *B. dolosa*, infection may be transient, especially when an antibiotic susceptible strain is isolated. However, antibiotic resistant strains did tend to be chronic, with 3 patients ultimately requiring lung transplantation. We do not feel that infection with *B. gladioli* is a contraindication to lung transplantation but feel caution is warranted and close observation is advised. The ability to accurately identify and follow this cohort over a prolonged time-period offers the opportunity to further investigate the impact of *B. gladioli* on pulmonary disease in cystic fibrosis.

Acknowledgements

References

[1] Palleroni NJ, Ballard RW, Ralston E, Doudoroff M. Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia, P. marginata, P. alliicola* and *P. caryophylli*. J Gen Microbiol 1970;60:199–214.

[2] Christenson JC, Welch DF, Mukwaya G, Muszynski MJ, Weaver RE, Brenner DJ. Recovery of *Pseudomonas gladioli* from respiratory tract specimens of patients with cystic fibrosis. J Clin Microbiol 1989;27: 270–3.

[3] Barker PM, Wood RE, Gilligan PH. Lung infection with *Burkholderia gladioli* in a child with cystic fibrosis: acute clinical and spirometric deterioration. Pediatr Pulmonol 1997;23:123–5.

[4] Graves M, Robin T, Chipman AM, Wong J, Khashe S, Janda JM. Four additional cases of *Burkholderia gladioli* infection with microbiological correlates and review. Clin Infect Dis 1997;25:838–42.

[5] Jones AM, Stanbridge TN, Isalska BJ, Dodd ME, Webb AK. *Burkholderia gladioli*: recurrent abscesses in a patient with cystic fibrosis. J Infect 2001;42:69–71.

[6] Khan SU, Gordon SM, Stillwell PC, Kirby TJ, Arroliga AC. Empyema and bloodstream infection caused by *Burkholderia gladioli* in a patient with cystic fibrosis after lung transplantation. Pediatr Infect Dis J 1996;15(6):37–9.

[7] Kanj SS, Tapson V, Davis RD, Madden J, Browning I. Infections in patients with cystic fibrosis following lung transplantation. Chest 1997;112:924–30.

[8] Ross JP, Holland SM, Gill VJ, DeCarlo ES, Gallin JI. Severe *Burkholderia* (*Pseudomonas*) *gladioli* infection in chronic granulomatous disease: report of two successfully treated cases. Clin Infect Dis 1995;21:1291–3.

[9] Boyanton Jr BL, Noroski LM, Reddy H, Dishop MK, Hicks MJ, Versalovic J, et al. *Burkholderia gladioli* osteomyelitis in association with chronic granulomatous disease: case report and review. Pediatr Infect Dis J 2005;24:837–9.

[10] Ritterband D, Shah M, Cohen K, Lawrence J, Seedor J. *Burkholderia gladioli* keratitis associated with consecutive recurrent endophthalmitis. Cornea 2002;21:602–3.

[11] Whitby PW, Pope LC, Carter KB, LiPuma JJ, Stull TL. Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. J Clin Microbiol 2000;38:282–5.

[12] Kennedy MP, Coakley RD, Donaldson SH, Aris RM, Hohneker K, Olson E, et al. *Burkholderia gladioli*: five year experience in a cystic fibrosis referral and lung transplantation center [abstract]. Chest 2005;124(4):S152.

[13] Heath DG, Hohneker K, Carriker C, Smith K, Routh J, LiPuma JJ, et al. Six-year analysis of *Burkholderia cepacia* complex isolates among cystic fibrosis patients at a referral center for lung transplantation. J Clin Microbiol 2002;40: 1188–93.

[14] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Information Supplement, vol. 25; 2005. p. 40–2. M100-S15.

[15] Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, et al. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J Clin Microbiol 1994;32:407–15.

[16] Wilsher ML, Kolbe J, Morris AJ, Welch DF. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. Am J Respir Crit Care Med 1997;155:1436–40.

[17] Clode FE, Metherell LA, Pitt TL. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. Am J Respir Crit Care Med 1999;160:374–5.

[18] Aris RM, Routh JC, LiPuma JJ, Heath DG, Gilligan PH. Lung transplantation for cystic fibrosis patients with *Burkholderia cepacia* complex. Survival linked to genomovar type. Am J Respir Crit Care Med 2001;164:2102–6.

[19] Kalish LA, Waltz DA, Dovey M, Potter-Bynoe G, McAdam AJ, Lipuma JJ, et al. Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. Am J Respir Crit Care Med 2006;173(4):421–5.

[20] Knowles MR, Friedman KJ, Silverman LM. Genetics, diagnosis and clinical phenotype. In: Yankaskas JR, Knowles MR, editors. Cystic Fibrosis in Adults. Philadelphia PA: Lippincott-Raven Publishers; 1999. p. 27–42.

[21] Bernhardt SA, Spilker T, Coffey T, LiPuma JJ. *Burkholderia cepacia* complex in cystic fibrosis: frequency of strain replacement during chronic infection. Clin Infect Dis 2003;37:780–5.

[22] Tablan OC, Chorba TL, Schidlow DV, White JW, Hardy KA, Gilligan PH, et al. *Pseudomonas cepacia* colonization in patients with cystic fibrosis: risk factors and clinical outcome. J Pediatr 1985;107:382–7.

[23] Lewin LO, Byard PJ, Davis PB. Effect of *Pseudomonas cepacia* colonization on survival and pulmonary function of cystic fibrosis patients. J Clin Epidemiol 1990;43:125–31.

[24] Gilligan PH. Micorbiology of cystic fibrosis lung disease. In: Yankaskas JR, Knowles MR, editors. Cystic Fibrosis in Adults. Philadelphia PA: Lippincott-Raven Publishers; 1999. p. 93–114.

[25] Mahenthiralingam E, Baldwin A, Vandamme P. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. J Med Microbiol 2002;51:533–8.

[26] Isles A, Maclusky I, Corey M, Gold R, Prober C, Fleming P, et al. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J Pediatr 1984;104:206–10.

[27] Mahenthiralingam E, Vandamme P, Campbell ME, Henry DA, Gravelle AM, Wong LT, et al. Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. Clin Infect Dis 2001;33:1469–75.

[28] Johnson WM, Tyler SD, Rozee KR. Linkage analysis of geographic and clinical clusters in *Pseudomonas cepacia* infections by multilocus enzyme electrophoresis and ribotyping. J Clin Microbiol 1994;32: 924–30.

[29] De Soyza A, Morris K, McDowell A, Doherty C, Archer L, Perry J, et al. *Burkholderia cepacia* complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. Lancet 2001;358:1780–1.

[30] Jones AM, Dodd ME, Govan JR, Barcus V, Doherty CJ, Morris J, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. Thorax 2004;59:948–51.

[31] Blackburn L, Brownlee K, Conway S, Denton M. 'Cepacia syndrome' with *Burkholderia multivorans*, 9 years after initial colonization. J Cyst Fibros 2004;3:133–4.

[32] Knowles MR, Gilligan PH, Boucher RC. Cystic fibrosis. In: Mandell GL, Douglas RG, Bennett JE, editors. Principles and Practice of Infectious Diseases. 5th edition. New York NY: Churchill Livingstone; 2004. p. 767–72.

[33] Khan SU, Arroglia AC, Gordon SM. Significance of airway colonization by *Burkholderia gladioli* in lung transplant candidates. Chest 1998;114:658.