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Clinical outcome of *Burkholderia cepacia* complex infection in cystic fibrosis adults☆

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Abstract

***Background*:** The *Burkholderia cepacia* complex (BCC) is one of the most important groups of organisms infecting cystic fibrosis (CF) patients. The aim of the study was to examine how infection with BCC affects clinical outcome. ***Methods*:** Nineteen CF adults infected with BCC and 19 controls infected with *Pseudomonas aeruginosa* were studied over a 4-year period. The best forced expiratory volume in 1 s (FEV_1) and body mass index (BMI) for each year were recorded and annual rate of decline calculated. ***Results*:** The BCC infected group displayed a significantly greater reduction of FEV_1 and BMI compared to the *P. aeruginosa* infected group ($p=0.001$ and $p=0.009$, respectively). Sixteen patients infected with a single *Burkholderia cenocepacia* strain had a significantly greater rate of FEV_1 decline compared to those infected with *Burkholderia multivorans* ($n=3$) or *P. aeruginosa* ($p=0.01$ and $p<0.0001$, respectively). The rate of BMI decline was significantly greater in patients infected with *B. cenocepacia* compared to those with *P. aeruginosa* ($p=0.007$), but not significantly different in those with *B. multivorans* ($p=0.29$). ***Conclusion*:** BCC infection is associated with an accelerated decline in pulmonary function and BMI. Infection with a single *B. cenocepacia* strain was associated with a more rapid decline in lung function than those infected with either *B. multivorans* or *P. aeruginosa*.

Keywords: Cystic fibrosis; Gram negative infection; Clinical outcome

1. Introduction

Burkholderia cepacia complex is a group of related species consisting of at least nine members. The organism was first isolated as a plant pathogen in onions in the late 1940s [1]. However, it was not until the mid 1980s that this gram negative bacillus was reported as an opportunistic pathogen in patients with cystic fibrosis (CF) [2,3]. Patients infected with *B. cepacia* complex show a significantly greater decline in pulmonary function compared to non-infected individuals [4] and consequently display increased morbidity and mortality [5,6]. There is strong evidence of patient-to-patient spread in CF populations both in and outside of hospital [7,8]. Furthermore, *B. cepacia* complex infection can lead to the development of 'cepacia syndrome', a rapidly fatal pneumonia with associated bacteraemia [2]. Treatment of infection is difficult as *B. cepacia* complex is inherently resistant to multiple antibiotics and so the use of antibiotic combinations as aggressive treatment is increasing [9].

The *B. cepacia* complex is made up of at least nine taxa, or genomovars. A genomovar is used to describe a species that is phylogenetically distinguishable but phenotypically indistinguishable from another. However, more recently several phenotypic distinguishing characteristics have been described for *B. cepacia* genomovars I, II, IV, V, VII–IX [10] and *B. cepacia* genomovar VI [11] which have allowed these to be formally named with approved binomial names.

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Table 1
Burkholderia cepacia complex: genomovar types and names

Genomovar type	Name
Genomovar I	*B. cepacia*
Genomovar II	*B. multivorans*
Genomovar III	*B. cenocepacia*
Genomovar IV	*B. stabilis*
Genomovar V	*B. vietnamiensis*
Genomovar VI	*B. dolosa*
Genomovar VII	*B. ambifaria*
Genomovar VIII	*B. anthina*
Genomovar IX	*B. pyrrocinia*

B. cepacia genomovar III has been recently named as *Burkholderia cenocepacia* and four subgroups have been identified (A to D) by *recA* sequence phylogeny and [12] (Table 1). With further study the number of genomovars may increase.

It is uncertain whether all genomovar types of *B. cepacia* complex that infect CF patients are clinically important. The aim of this study was to compare the clinical course of CF patients infected with *Burkholderia multivorans* (genomovar II), *B. cenocepacia* or *Pseudomonas aeruginosa*.

2. Materials and methods

Patients attending the adult CF centre in Belfast from 1996 to 2000, who were chronically infected with *B. cepacia* complex, were identified. All *B. cepacia* complex infected patients were infected for at least 6 years prior to the study period. This group of patients was matched for age (± 3 years) and lung function ($\pm 10\%$ predicted) with a control group of patients who were chronically infected with *P. aeruginosa.* Chronic infection was defined as three or more positive sputum cultures over a 6-month period. Sputum culture was performed on a 3-monthly basis throughout the study.

Organisms of the *B. cepacia* complex were isolated from the patients' sputum by culture on MAST selective agar (MAST Diagnostics, Liverpool, UK). Isolates were initially analysed using the API 20NE phenotypic identification system (BioMèrieux, Marcy l'Etoile, France). Genomic DNA was prepared from all *B. cepacia* complex isolates as previously described [13] and their genomovar status and associated transmissibility/virulence markers determined by a combination of 16S/16S–23S [14], recA-based PCR [15], *B. cepacia* epidemic strain marker (BCESM) [16] and cable pilus molecular markers [17].

All *B. cenocepacia* isolates ($n = 16$) were characterised at the strain level by employing the random amplification of polymorphic DNA (RAPD) technique, with the BC270 primer (5′ -TGC GCG CGG G-3′), in accordance with the method of Mahenthiralingam et al. [18].

The best forced expiratory volume in 1 s (FEV_1) and body mass index (BMI) were recorded for each year. For deceased patients, information was obtained from 1996 to the time of death and the cause of death was noted. Independent *t*-tests were used to compare lung function and BMI between those with *B. cepacia* complex infection and those with *P. aeruginosa* infection at the start of the study. Further statistical analysis was determined by calculating the annual rate of change in FEV_1 and BMI for each group using regression analysis. The results were tested for linearity. Kruskal–Wallis analysis was used to analyse the results between those infected with *B. multivorans*, *B. cenocepacia* and *P. aeruginosa*, while unpaired *t*-tests were used to compare patients who had died during the study period and those still alive. Analysis of Variance (of residuals after removal of subject effects) and post-hoc multiple comparisons were also performed. The level of significance was set at probability (p) less than 5% ($p < 0.05$).

3. Results

Nineteen adult patients were chronically infected with *B. cepacia* complex from 1996 to 2000. Of these patients, 16 were infected with *B. cenocepacia* (IIIA), while three were infected with *B. multivorans*. All *B. cenocepacia* IIIA isolates were positive for the molecular markers, BCESM [16] and the cable pilus [17] as well as showing a typical Edinburgh–Toronto (ET12) strain PFGE banding profile [19] within a small collection of isolates chosen randomly. RAPD analysis of the 16 *B. cenocepacia* isolates demonstrated the presence of a single genotype. The group of *B. cepacia* complex patients was matched with 19 patients infected with *P. aeruginosa.* The mean (S.D.) age, male to female ratio, FEV_1 (% predicted) and BMI of each group in 1996 and 2000 is recorded in Table 2. All patients remained chronically infected with either *B. cepacia* complex or *P. aeruginosa* throughout the study period. The use of specific culture media for *B. cepacia* complex began in 1990 in Northern Ireland following an index case in 1989 [4]. There was no strain replacement in those infected with *B. cenocepacia*.

There was no significant difference in mean (S.D.) FEV_1 or BMI at the start of the study between those infected with

Table 2
Clinical details of patients in the study groups in 1996 and 2000 mean (S.D.)

Year	*B. cenocepacia*		B. multivorans		P. aeruginosa	
	1996	2000	1996	2000	1996	2000
Number	16	9	3	3	19	19
Sex	12M	5M	0M	0M	10M	10M
	4F	4F	3F	3F	9F	9F
Age	22.8	26	24.7	28.7	22.4	26.4
(years)	(4.7)	(4.8)	(7.5)	(7.5)	(5.7)	(5.7)
% FEV_1	64.4	61.4	84.8	82.0	72.7	76.1
	(25.3)	(28.8)	(11.4)	(7.4)	(25.0)	(24.4)
BMI	20.3	20.4	20.9	20.2	21.0	21.4
(kg/m^2)	(2.3)	(2.3)	(0.1)	(0.5)	(3.0)	(3.2)

B. cepacia complex [226 ml (± 98 ml) and 20.4 kg/m^2 (± 0.5 kg/m^2)] and those with *P. aeruginosa* infection [262 ml (± 88 ml) and 20.3 kg/m^2 (± 0.97 kg/m^2)], $p = 0.26$ and $p = 0.91$, respectively.

The data were shown to be linear with FEV_1 and BMI changing steadily with time. The *B. cepacia* complex infected cohort displayed a significantly greater reduction in FEV_1 and BMI compared to the *P. aeruginosa* infected group ($p = 0.001$ and $p = 0.009$, respectively). Comparison of the rates of decline of FEV_1 between those infected with *B. cenocepacia*, *B. multivorans* and *P. aeruginosa* showed an overall significant difference, $p = 0.0005$. Subsequent post-hoc multiple comparisons showed that the median (interquartile range) rate of decline of FEV_1 (ml/year) in those infected with *B. cenocepacia* [− 140 ml/year (− 280 to − 100 ml/year)] was significantly greater compared to those infected with *B. multivorans* [6 ml/year (− 110 to 32 ml/year)] or *P. aeruginosa* infection [− 32 ml/year (− 87 to 21 ml/year)] ($p = 0.01$ and $p < 0.0001$, respectively) (Fig. 1). The rate of FEV_1 decline between those with *B. multivorans* and *P. aeruginosa* was not significantly different ($p = 0.88$).

The overall comparison of the annual rate of BMI decline between the three groups was significantly different, $p = 0.027$. However, post-hoc multiple comparisons showed no difference in the rate of decline between those infected with *B. multivorans* [0.1 kg/m^2/year (− 0.05 to 0.25 kg/m^2/year)] and *B. cenocepacia* [− 0.3 kg/m^2/year (− 0.6 to 0.7 kg/m^2/year)] or between those with *B. multivorans* and *P. aeruginosa* [0.2 kg/m^2/year (− 0.04 to 0.4 kg/m^2/year)] ($p = 0.29$ and $p = 0.77$, respectively). However, patients infected with *B. cenocepacia* had a significantly greater annual rate of BMI decline compared to those infected with *P. aeruginosa* ($p = 0.007$) (Fig. 2).

Further analysis using Univariate Analysis of Variance (removing subject effects and not imposing a linear effect) showed a time by group interaction for rate of FEV_1 decline between the three groups, $p = 0.004$. There was a trend towards a year by group effect for the rate of BMI decline between the groups, $p = 0.38$ but this was not statistically significant.

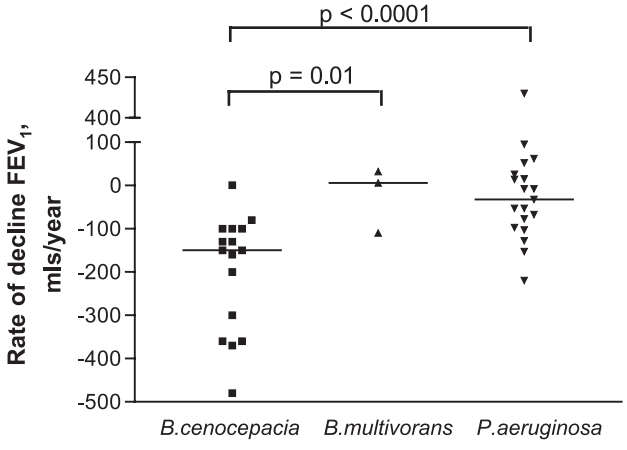

Fig. 1. Comparison of the rate of FEV_1 decline between the study groups. ■ *B. cenocepacia*, ▲ *B. multivorans*, ▼ *P. aeruginosa*.

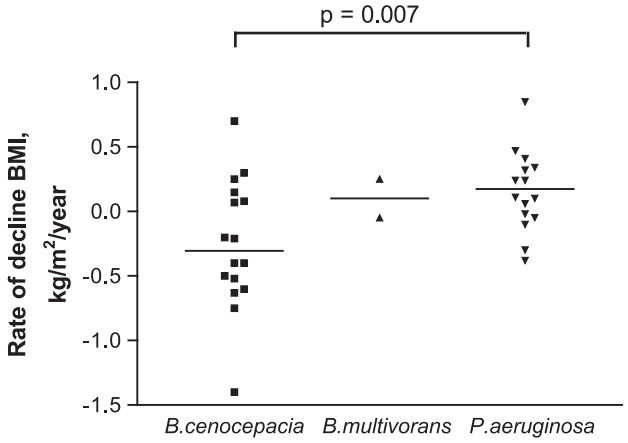

Fig. 2. Comparison of the rate of BMI decline between the study groups. ■ *B. cenocepacia*, ▲ *B. multivorans*, ▼ *P. aeruginosa*.

During the study, seven patients infected with *B. cenocepacia* died, of which three were due to 'cepacia syndrome'. There were no deaths in the *B. multivorans* group. In the total population of patients ($n = 50$) infected with *P. aeruginosa* in 1996 in the CF centre, four (8%) died during the study period. This was significantly less than the seven (36.8%) patients infected with *B. cepacia* complex who died during the study period (χ^2 8.6, $p = 0.03$). The mean (S.E.) time from the start of the study period to time of death was 3.4 (0.2) years in the *B. cepacia* group. The mean (S.D.) rates of decline of FEV_1 and BMI during the 2 years prior to death were significantly greater in the seven patients who died during the study period [− 286 (± 142.6) ml/year and − 0.5 (± 0.5) kg/m^2/year] in comparison to the 12 patients infected with *B. cepacia* complex who were still alive [− 103 (± 89.8) ml/year and − 0.03 (± 0.4) kg/m^2/year] ($p = 0.003$ and 0.04, respectively).

4. Discussion

Previous studies have found that CF patients infected with *B. cepacia* complex have a greater deterioration in lung function, require more frequent antibiotic therapy and also display increased mortality compared to patients with *P. aeruginosa* [4,8,20,21]. This study provides further evidence that infection with *B. cepacia* complex is associated with an increase in patient morbidity and mortality compared to infection with *P. aeruginosa*. Most studies have shown that in pulmonary infection with *B. cepacia* complex outcome varies considerably from a rapid fatal decline in lung function associated with bacteraemia to an acceleration of pulmonary decline to chronic asymptomatic infection [2,21,22], suggesting that pathogenicity within the *B. cepacia* complex varies. Our data further suggests that the

clinical course of adult CF patients infected with the *B. cepacia* complex may also depend on the nature of the sub species responsible for infection. Within our study population, we found that patients chronically infected with a single strain (IIIA, ET12) of *B. cenocepacia* displayed a significantly greater reduction in FEV_1 compared to patients with *B. multivorans* infection. There is a significant Beta error present due to the small number of *B. multivorans* patients, but interquartile ranges are given to demonstrate the spread of values. It is not known whether there are any differences in clinical outcome for the four subgroups of *B. cenocepacia* infection.

We could not detect any difference in BMI decline between the genomovar species. Our data suggests that the clinical course in patients infected with *B. multivorans* maybe comparable to that seen with *P. aeruginosa* rather than *B. cenocepacia*.

These results are consistent with the study of Mahenthiralingam et al. [23], who showed that *B. cenocepacia* was associated with 'cepacia syndrome' and a high mortality in CF patients compared to those infected with *B. multivorans*. Studies have demonstrated a reduction in survival following pulmonary transplantation in CF patients infected with *B. cepacia* complex compared to those either without *B. cepacia* complex infection or those with panresistant *P. aeruginosa* infection [24,25]. In addition, CF patients undergoing pulmonary transplantation had a worse outcome when infected with *B. cenocepacia* prior to surgery in comparison to patients with pre-transplant *B. multivorans* or *B.vietnamiensis* infections [26].

In regard to the pathogenicity of *B. multivorans*, a recent study by Schwab et al. [27] demonstrated that a *B. multivorans* strain (J-1) was able to penetrate well-differentiated airway epithelial cell cultures, a phenomenon thought to result in bacteraemia and 'cepacia syndrome'. Whiteford et al. [28] reported deaths associated with an outbreak of *B. cepacia* complex in patients in a Glasgow clinic. The strain responsible for this has subsequently been identified as *B. multivorans* [29]. Mahenthiralingam et al. [23] showed that phylogenetic analysis of the *recA* gene of the strain of *B. multivorans* infecting a Vancouver population was genetically different from the index *B. multivorans* strain in the Glasgow epidemic. So, there is increasing evidence for various strains of *B. multivorans* with different pathogenicity and clinical outcomes [20].

It is uncertain if infection with *B. cepacia* complex is the cause of a more rapid pulmonary decline in CF patients or if it is merely a marker of disease severity in those with more severe underlying disease. Case control studies have shown that in patients with CF, *B. cepacia* complex infection is an independent negative prognostic indicator [20]. Tablan et al. [6] had closely matched parameters characterising disease severity yet reported early excess mortality in patients infected with *B. cepacia* complex. McCloskey et al. [4] studied the same patient group as in our study and showed that there was no difference in pulmonary function between patients at the time of acquisition of *B. cepacia* complex compared to *P. aeruginosa*, but did demonstrate that lung function declined more rapidly in the *B. cepacia* complex group. At the time of the study by McCloskey et al., differentiation of genomovars could not be performed so the *B. cepacia* complex infected group was studied as a whole. It has been demonstrated that patients infected with *B. cepacia* complex had a higher frequency of intravenous antibiotic therapy and higher long-term mortality when compared to patients without *B. cepacia* complex infection [22].

Not only is there clinical variation between patients infected with *B. cenocepacia*, *B. multivorans* and *P. aeruginosa* but there may be a subgroup of those infected with *B. cenocepacia* with a worse prognosis. During this study, 7 out of 16 patients infected with *B. cenocepacia* (IIIA, ET12) died. This subgroup of patients displayed accelerated rates of FEV_1 and BMI decline when compared to those still alive, confirming that spirometry and nutritional status are important prognostic indicators of survival. In 50% of patients who died, the cause of death was 'cepacia syndrome'. Our results demonstrate considerable variation in clinical outcome for patients infected with *B. cenocepacia* (IIIA, ET12). It is not clearly understood why certain patients with *B. cenocepacia* infection display a general decline in lung function, while other patients develop 'cepacia syndrome'. Differences in virulence may be a possible explanation for individuals infected with distinct strains. Organisms of the *B. cepacia* complex produce a variety of classical virulence determinants including proteases, haemolysins, lipase and endotoxin [30]. The cable pilus gene [31] and BCESM [16] are transmissibility markers that have been identified that may increase virulence among certain strain types. Cable pili are expressed by the infamous ET12 strain (one of the 'hypertransmissible' or 'epidemic' strains) [32], while BCESM has been identified in several epidemic stains of *B. cepacia* complex but is not present in non-epidemic strains [16].

There is potential for significant variation in the genetic content between strains due to the large genome size of *B. cepacia* complex bacteria (2–4 replicons) which may alter virulence [33]. But patients infected with the same strain can display considerable variation in clinical outcome, indicating that the host response may also be important. Mannose-binding lectin (MBL) is essential in innate immunity, especially in protecting against bacterial and viral infections during infancy, before the adaptive immune system is established [34]. Garred et al. [34] demonstrated that *B. cepacia* complex infection was significantly more frequent in carriers of MBL-variant alleles than in homozygotes.

Prevention of *B. cepacia* complex infection may be the most important clinical issue due to the high level of resistance to antimicrobial drugs and the increase in morbidity and mortality associated with infection. The most effective strategy to date has been segregation of patients based on the presence or absence of infection. In the Adult

CF Centre in Belfast, we further segregate patients with *B. cenocepacia* from those with other *B. cepacia* genomovar infections, such as *B. multivorans*. Recent studies have found that virulent and transmissible strains of *B. cenocepacia* can replace other genomovar infections, such as *B. multivorans* [23], so segregation is extremely important. In our unit, there have been two new cases of *B. cepacia* complex infection within the past 7 years since adopting this policy.

In addition to the segregation policies, it is vitally important to carry out frequent sputum cultures to detect *B. cepacia* complex infection as early as possible and hence reduce the risk of cross infection. Reliable sputum culture and unequivocal identification of *B. cepacia* complex does require experience and expertise. Due to their complex taxonomy, confirmation that isolates are organisms of the *B. cepacia* complex can be problematic when using commercially available phenotypic identification kits [35,36]. As such methods do not allow genomovar differentiation, a significant number of PCR-based methods have been described to aid laboratory diagnosis of *B. cepacia* complex isolates. Furthermore, molecular-based tests have also been applied directly to CF sputum in an attempt to facilitate rapid as well as early detection and identification of these pathogens [14].

The power of this study is limited by the size of our *B. multivorans* population, with one patient having insufficient data for BMI analysis. Further studies involving larger numbers of patients need to be conducted in order to confirm these findings. Despite these limitations, a number of conclusions can be drawn. Genomovar status appears to be clinically important. Although an association has previously been demonstrated between infection with *B. cepacia* complex and an accelerated decline in pulmonary function, this data suggests a more rapid decline in lung function in patients infected with *B. cenocepacia* (IIIA, ET12) compared to those infected with *B. multivorans* or *P. aeruginosa*. These findings emphasise the importance of genomovar identification methods becoming integrated into routine diagnostic laboratories and the necessity for strict infection control policies.

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