

# Evidence of transmission of *Burkholderia cepacia*, *Burkholderia multivorans* and *Burkholderia dolosa* among persons with cystic fibrosis

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## Abstract

Previous studies have identified specific *Burkholderia cepacia* complex strains that are common to multiple persons with cystic fibrosis (CF). Such so-called epidemic strains have an apparent enhanced capacity for inter-patient spread and reside primarily in *Burkholderia cenocepacia* (formerly *B. cepacia* complex genomovar III). We sought to identify strains from *B. cepacia* complex species other than *B. cenocepacia* that are similarly shared by multiple CF patients. We performed genotype analysis of 360 recent sputum culture isolates from 360 persons residing in 29 cities by using repetitive extragenic palindromic polymerase chain reaction (rep-PCR) and pulsed field gel electrophoresis. The results indicate that sharing of a common *Burkholderia multivorans* strain occurs relatively infrequently; however, several small clusters of patients infected with the same strain were identified. A cluster of seven patients infected with the same *B. cepacia* (genomovar I) strain was found. We also identified a large group of 28 patients receiving care in the same treatment center and infected with the same *Burkholderia dolosa* strain. These observations suggest that *B. cepacia* complex strains in species other than *B. cenocepacia* may be spread among CF patients.

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**Keywords:** *Burkholderia cepacia* complex; Bacterial genotyping; Cystic fibrosis; Transmission; Infection control

## 1. Introduction

Bacteria belonging to the *Burkholderia cepacia* complex are important pathogens in persons with cystic fibrosis (CF). Respiratory tract infection may persist for months or years or be associated with life-threatening acute illness [1]. Numerous studies have provided compelling evidence for transmission of ‘*B. cepacia*’ among CF patients. This observation, as well as the finding that most strains are inherently resistant to broad-spectrum antibiotics, has made prevention of infection a cornerstone of CF patient management [2]. Stringent infection control policies that segregate infected patients have decreased but not completely eliminated new infection. A better understanding of the epidemiology and ecology of these species is a pre-

requisite to optimizing infection control policies and is essential in efforts to elucidate the pathogenesis of human infection.

Recent taxonomic work has defined several phenotypically similar species (or genomovars) among bacteria previously identified merely as ‘*B. cepacia*’. The *B. cepacia* complex is currently comprised of nine species, *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina*, and *B. pyrrocinia* (representing genomovars I–IX, respectively) [3–6]. Among these, *B. multivorans* and *B. cenocepacia* account for the great majority of infection in CF [7–9].

Studies employing bacterial genotyping have identified distinct *B. cepacia* complex strains that are shared by multiple CF patients. Most of the ‘epidemic’ strains described to date are *B. cenocepacia*. Evidence of inter-patient spread of strains belonging to other *B. cepacia* complex species is more limited. In this study we examined a large collection of *B. cepacia* complex isolates recently recovered from US CF patients. Bacterial genotyping analysis was

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undertaken to identify strains common to multiple patients.

## 2. Materials and methods

### 2.1. Bacterial strains and study design

Bacterial isolates were recovered from sputum culture obtained between 1997 and 2003 during the course of routine health care of persons with CF attending treatment centers in the USA. Isolates were confirmed as *B. cepacia* complex and assigned to one of the nine species within this group by using species-specific 16S rDNA and *recA*-based polymerase chain reaction (PCR) assays previously described [10,11]. Only *B. cepacia* complex isolates belonging to species other than *B. cenocepacia* were included in the study. Study isolates were further limited to include only those from treatment centers wherein at least six patients were infected with the same *B. cepacia* complex species. In cases where more than one isolate was available from a given patient, only the first obtained was included in the study. Strains that were shared by two or more *non-sibling* patients attending the same treatment center were identified by genotyping analysis.

### 2.2. Genotyping analyses

Genotyping was performed using rep-PCR with a BOX A1R primer (BOX-PCR) as previously described [12]. A subset of isolates (as detailed in Section 3) was further assessed by macrorestriction digestion of chromosomal DNA and pulsed field gel electrophoresis (PFGE), also as described previously [12]. For both analyses, densitometric analysis, normalization and interpolation of the resulting patterns was performed using Quantity One 4.1 and Molecular Analyst Fingerprinting Plus software (Bio-Rad). Similarity matrices were calculated using Pearson's product moment correlation coefficient. Cluster analyses of similarity matrices were performed by the unweighted pair group method with arithmetic averages (UPGMA). For BOX-PCR, a similarity coefficient cutoff of 85% was used to define isolates of the same strain. PFGE patterns were analyzed according to published interpretive criteria [13].

## 3. Results

From a database of 1356 CF patients infected with *B. cepacia* complex and receiving care in 172 US cities, 360 isolates from 360 patients in 29 treatment centers were analyzed based on the study inclusion criteria. Groups of six or more patients attending the same center and infected with *B. multivorans*, *B. cepacia*, or *B. dolosa* were identified. We found no treatment centers in which at

least six non-sibling patients were infected with *B. stabilis*, *B. vietnamiensis*, *B. ambifaria*, *B. anthina* or *B. pyrrocinia*.

### 3.1. *B. multivorans* clusters

There were 26 treatment centers wherein at least six non-sibling patients were infected with *B. multivorans*. A total of 319 isolates from as many patients were genotyped. In 14 of the 26 centers we identified at least two non-sibling patients infected with the same strain (Table 1). Although pairs of patients harboring the same strain were most common, we also identified several sets of three and four patients with a shared strain. In center E, the same strain infected six of 17 *B. multivorans*-infected patients. These isolates clustered by BOX-PCR with a similarity coefficient of 94% (Fig. 1A); this strain has been designated strain OHBM. In center N, five of eight *B. multivorans*-infected patients shared a common strain, with isolates clustering by BOX-PCR with a similarity coefficient of 95% (Fig. 1A); this strain was designated strain TUL2. All isolates from both clusters were further analyzed by PFGE, which showed that isolates within each group differed by no more than two bands (data not shown).

Table 1  
Clusters of patients infected with the same *B. multivorans* strain

Treatment center	Number of patients <sup>a</sup>	Clusters <sup>b</sup>				
		Two	Three	Four	Five	Six
A	41	1	2	2	–	–
B	38	6	1	–	–	–
C	22	1	–	–	–	–
D	18	1	–	–	–	–
E	17	2	1	–	–	1
F	15	–	–	–	–	–
G	15	1	–	–	–	–
H	13	–	–	–	–	–
I	13	–	–	–	–	–
J	11	2	–	–	–	–
K	11	–	–	–	–	–
L	11	1	–	–	–	–
M	9	–	–	–	–	–
N	8	–	–	–	1	–
O	8	1	–	–	–	–
P	7	–	–	–	–	–
Q	7	–	–	1	–	–
R	7	–	–	–	–	–
S	7	–	–	–	–	–
T	6	1	–	–	–	–
U	6	–	–	–	–	–
V	6	–	–	–	–	–
W	6	–	–	–	–	–
X	6	1	–	–	–	–
Y	6	–	–	–	–	–
Z	6	1	–	–	–	–

<sup>a</sup>One isolate from each patient was analyzed.

<sup>b</sup>Number of clusters of two, three, four, five or six patients infected with a common strain.

### 3.2. *B. cepacia* clusters

Two centers were identified that each cared for at least six patients infected with *B. cepacia*. Among the eight patients in the first center were three patients infected

with a common strain. The second center also cared for eight *B. cepacia*-infected patients. Seven of these eight patients harbored the same strain. Isolates from these seven patients clustered with a similarity coefficient of 88% (Fig. 1B). PFGE analysis showed that all seven isolates had

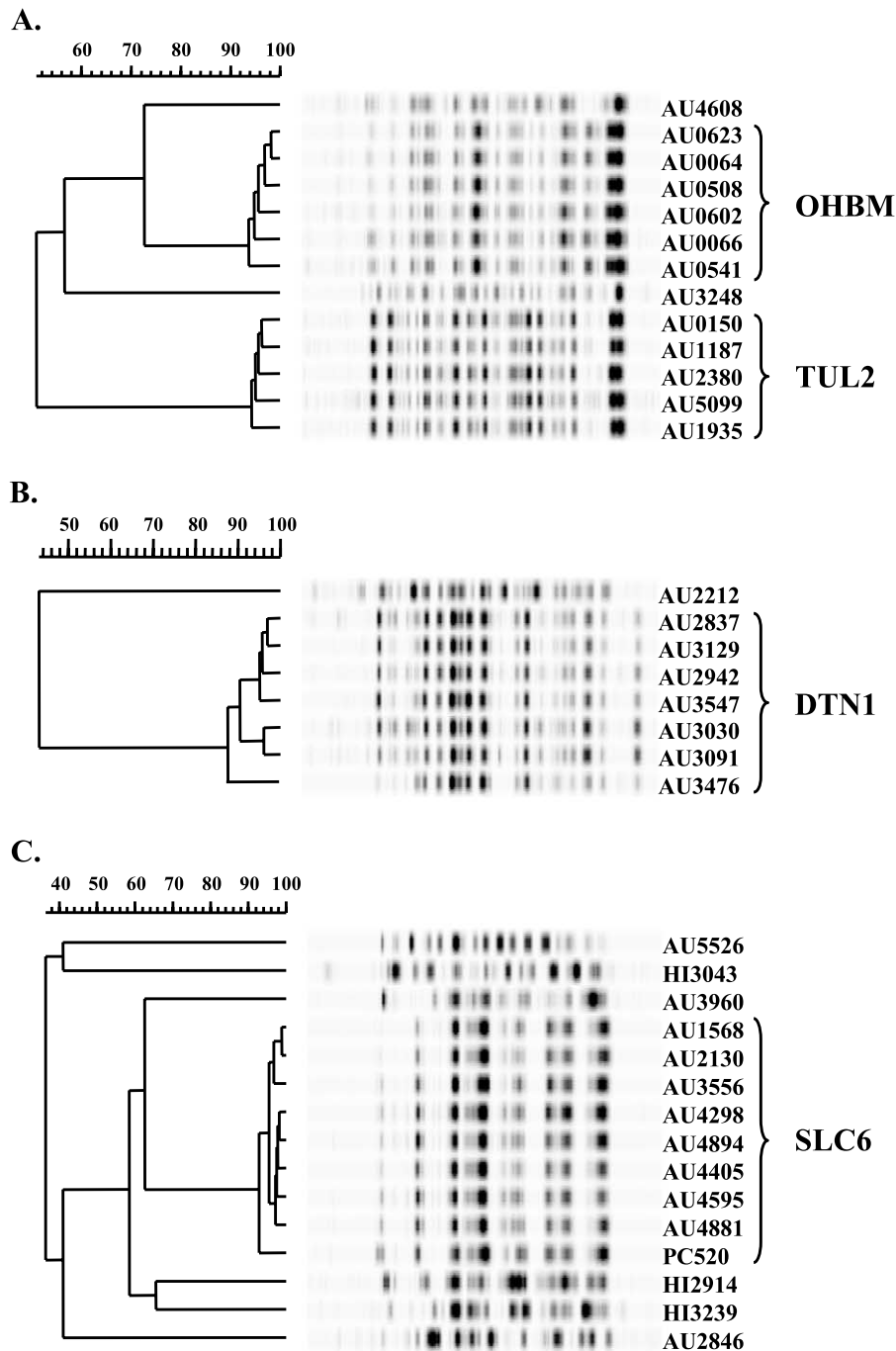


Fig. 1. Dendrogram derived from the UPGMA linkage of Pearson's product moment coefficients between BOX-PCR patterns. A: *B. multivorans* isolates from center E showing strain OHBM, and center N showing strain TUL2. Isolates AU4608 and AU3248, from centers E and N, respectively, are different strain types included for comparison. B: *B. cepacia* (genomovar I) isolates from eight patients attending the same treatment center; seven of eight are strain DTN1. C: *B. dolosa* isolates. Isolates with AU prefix clustering as strain SLC6 are from patients attending the same treatment center; 20 additional patients from this center were infected with this strain as well (not shown). Isolate PC520 was involved in an episode of person-to-person transmission of '*Pseudomonas cepacia*' reported in 1990 [24]. The remaining six isolates shown are from CF patients receiving care in other US treatment centers (isolates AU5526, AU3960 and AU2846) or in three European countries (isolates HI2914, HI3239 and HI3043).

identical banding patterns (data not shown). This strain has been designated DTN1.

### 3.3. *B. dolosa* cluster

Only one treatment center cared for six or more patients infected with *B. dolosa*. In this single center, 28 patients infected with *B. dolosa* received care. Genotyping analysis revealed that all 28 patients were infected with the same strain. These 28 isolates clustered by BOX-PCR with a similarity coefficient of 91% (Fig. 1C). PFGE analysis demonstrated that all 28 isolates had banding profiles that did not differ by more than two bands. This strain has been designated SLC6.

## 4. Discussion

Previous studies employing bacterial genotyping analyses have identified *B. cepacia* complex strains that are shared by multiple CF patients, implying acquisition from a common source or inter-patient transmission. Few such strains, however, have been described in detail. Strain ET12 is common among CF patients in eastern Canada and the UK [14,15], while strain PHDC and the ‘Midwest clone’ each infect multiple patients in the USA [16,17]. These three strains, as well as several other ‘epidemic’ strains reported by Mahenthiralingam et al. [18], reside in *B. cenocepacia*.

Evidence of shared strains from among the remaining *B. cepacia* complex species is limited. *B. multivorans* is the only other species reported to date as being implicated in possible inter-patient transmission. Segonds et al. [19] reported two strains of *B. multivorans* that were common among French CF patients. The strain involved in an outbreak among pediatric CF patients in Glasgow described by Whiteford et al. [20] as well as the strain common among four adult CF patients in Cardiff reported by Millar-Jones et al. [21] were also subsequently found to be *B. multivorans* [22,23]. In contrast, Mahenthiralingam et al. [18] found very little evidence of inter-patient spread of *B. multivorans* in Vancouver, BC, during a 17-year period.

Among the 360 patients assessed in the study reported here, most (319 patients) were infected with *B. multivorans*, which accounts for approximately 38% of *B. cepacia* complex-infected CF patients in the USA [7]. Although shared *B. multivorans* strains were found in more than half (14 of 26) of the treatment centers investigated, most were found only in pairs of non-sibling patients. A few clusters of three and four patients each were identified, but among this large sampling of patients and treatment centers we found only a single group of five and another group of six patients infected with a common strain. These results indicate that despite its relatively common occurrence in CF, *B. multivorans* is only infrequently shared among patients.

These data suggest that most infections result from acquisition of distinct strains from independent sites.

Infections with *B. cepacia* (genomovar I) and *B. dolosa* (genomovar VI) occur much less frequently in CF [7]. Only two centers were identified that cared for six or more *B. cepacia*-infected patients, but in both we found shared strains. More interesting was the single center that cares for multiple patients infected with *B. dolosa*. Although this species accounts for only approximately 3% of *B. cepacia* complex infection in US CF patients (unpublished data), it is responsible for 35% of infection in this center. Because genotyping analysis using both BOX-PCR and PFGE demonstrated that all *B. dolosa*-infected patients in this center carried the same strain (designated SLC6), we further analyzed several epidemiologically unrelated *B. dolosa* isolates to better assess the genetic diversity within this species. Strains obtained from *B. dolosa*-infected CF patients receiving care in other US centers or in other countries were clearly distinct from strain SLC6 (Fig. 1C). Of great interest is our finding that strain SLC6 is now identified as the strain (represented by PC520 in Fig. 1C) implicated in the first reports of inter-patient spread and inapparent (i.e. sputum culture-negative) infection by ‘*B. cepacia*’ in CF in the early 1990s [24,25].

Our results may actually underestimate the degree to which non-*B. cenocepacia* strains are shared by CF patients. Because we were most interested in identifying relatively large clusters of shared strains, we chose to investigate only centers with at least six patients infected with non-*B. cenocepacia* species. Had we analyzed isolates from all patients whom we have confirmed as infected with *B. cepacia* complex, we expect that we would have identified more small (i.e. two or three patient) clusters with the same strain.

Further, we deliberately set a relatively high similarity coefficient cutoff value with which to define strain types in this study. The appropriate cutoff value to be applied in an epidemiologic study is difficult to determine a priori, as it is a function of the genetic diversity within the species investigated, the discriminatory power of the typing method used and the epidemiologic question being addressed [12]. In a previous study, a BOX-PCR similarity cutoff of 70% was deemed most appropriate when assessing the global population structure of *B. cenocepacia* [12]. In the present study, in which only intra-center comparisons were made of strains recovered during relatively short periods of time, a higher cutoff was necessary to provide a more rigorous definition of clonality. Had we used a less conservative (i.e. lower) cutoff value to define a strain, we most certainly would have identified more clusters, particularly amongst the *B. multivorans*-infected patients. Such a lower cutoff value may be appropriate in studies assessing larger-scale inter-center comparisons, which were beyond the scope of the present study. Further work will be required to better establish the most appropriate cut-

off(s) to be used, again depending on the specific study objectives.

We found that isolates belonging to the major strain types defined in this study (for which strain designations were provided) all clustered with approximately 90% similarity. We are confident that these isolates do indeed represent a single strain. In previous work we found that *B. cepacia* complex isolates serially recovered from the same patient over periods of up to 4 years also typically cluster by BOX-PCR at approximately 90%. Furthermore, PFGE analysis using published interpretive criteria [13] confirmed the clonality of the isolates we placed in the same strain type by BOX-PCR analysis.

The epidemiologic basis of our findings and their implications for infection control and clinical outcome are not entirely clear. Previous epidemiologic studies, supported by bacterial genotyping analyses, have provided compelling evidence for person-to-person spread of some *B. cepacia* complex strains. In the present study, however, we did not have access to information regarding specific contact among patients, either within treatment centers or in social settings. Further, on the basis of our data we cannot rule out acquisition from a common source or infection by a strain that dominates in the local environment. Surveys of environmental cultures from multiple treatment centers searching for common strain types were beyond the scope of the current study. If inter-patient transmission is primarily responsible for our findings, the factors that account for this apparent enhanced capacity for spread remain to be elucidated. *B. cenocepacia* strain ET12 is characterized by distinctive ‘cable pili’ [26], but this phenotype appears to be restricted to this strain only. ET12 and several, but not all, other ‘epidemic’ *B. cenocepacia* strains also contain the ‘*B. cepacia* epidemic strain marker’ (BCESM) [18]. In our study, neither cable pili nor the BCESM were found in any of the major clones identified (data not shown).

Although strains shared by multiple CF patients are often assumed to be more virulent in this population, large systematic clinical outcomes studies to support this contention are lacking. Similarly, a detailed assessment of patient clinical parameters relative to infecting strain was not possible within the context of our investigation. Thus, we must be careful to point out that we are not able to draw conclusions regarding the relative virulence of the common strain types identified.

In summary, we found strains of *B. cepacia*, *B. multivorans* and *B. dolosa* that are shared by multiple CF patients. Although the basis for these observations remains to be elucidated, the data suggest that *B. cepacia* complex other than *B. cenocepacia* are able to spread among CF patients. The microbiologic factors that account for this are the subject of ongoing investigation. Our data also indicate that the possibility of inter-patient transmission of non-*B. cenocepacia* strains should be taken into account in designing infection control measures in CF.

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