## LUNG TRANSPLANTATION

# Prevalence and clonality of *Burkholderia cepacia* complex genomovars in UK patients with cystic fibrosis referred for lung transplantation

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**Background:** It has previously been reported that patients infected with *Burkholderia cenocepacia* (genomovar III) before lung transplantation have a poorer outcome than those with other *B cepacia* complex infections.

**Methods:** An extensive study was conducted to determine the prevalence and clonality of *B cepacia* complex genomovars isolated from patients referred for transplant assessment between 1989 to the present and, where appropriate, whether strain type was related to transplant outcome.

**Results:** Isolates from 29 patients were identified as *B cepacia* complex organisms by molecular analysis. Thirteen patients (45%) were infected with the highly transmissible ET-12 strain of *B cenocepacia recA* lineage III-A, while all remaining patients were infected with genetically unique *B cenocepacia*, *B multivorans*, and *B vietnamiensis* strains. All previously reported deaths following transplantation were associated with ET-12 infection.

**Conclusions:** The ET-12 strain is the predominant cause of *B cenocepacia* infections in patients with cystic fibrosis referred to our pulmonary transplant centre and is associated with poor transplant outcomes using standard treatment regimens.

*B*urkholderia cepacia is a life threatening pathogen in patients with cystic fibrosis (CF). The unique clinical syndrome of necrotising pneumonia and associated septicaemia known as "cepacia syndrome" is a feared complication of infection.[1] *B cepacia* infections have also proved highly transmissible leading to epidemics within many CF centres.[2]

Polyphasic taxonomic studies have shown that isolates previously classified as *B cepacia* comprise at least nine phenotypically similar genomovar species.[2] This group of organisms is collectively known as the *Burkholderia cepacia* complex. Analysis of the *recA* housekeeping gene has revealed that strains of *B cenocepacia* (formerly genomovar III) can be split into four distinct groups known as III-A, III-B, III-C, and III-D.[3] The clinical significance of these *B cenocepacia* subgroups is, however, unclear. Epidemiological studies have found a disproportionate distribution of *B cepacia* complex genomovars among patients, with *B cenocepacia* and, to a lesser extent, *B multivorans* (formerly genomovar II) the most prevalent organisms.[2] Most cases of "cepacia syndrome" are associated with *B cenocepacia*, although it has been unclear if this represents increased virulence or simply its high prevalence among patients. However, retrospective studies have shown that CF patients with *B cenocepacia* infections before transplantation have poorer post-transplant outcomes than patients infected with other genomovars, providing evidence that *B cenocepacia* is, on the whole, a more aggressive and virulent organism.[4][5]

Data on *B cenocepacia* specific virulence and transmissibility factors is at best limited. The *B cenocepacia* epidemic strain known as ET-12 is characterised by the expression of the *cblA* gene.[6] In addition, a conserved 1.4 kbp DNA fragment, the *B cepacia* epidemic strain marker (BCESM), has been described.[7] The ET-12 lineage is unique in bearing both the *cblA* gene and BCESM.[2] However, outbreaks have been observed involving strains that lack the *cblA* gene or both putative transmissibility markers.[8]

Our transplant unit serves CF units in Northern England, Scotland and both Northern and Southern Ireland. We conducted an extensive epidemiological study to determine the prevalence and clonality of isolates in these regions since 1989. *B cenocepacia* strain types were investigated for the presence of epidemic clones as well as putative transmissibility markers, and these findings were related to transplant outcome.

## METHODS

### Case finding
A retrospective review of the pulmonary transplant database at the Freeman Hospital from 1989 (programme start) to 2002 was performed. The management of transplant patients was as previously published.[9]

### Microbiology
Sputum was collected from patients during pre-transplant assessment and immediately before surgery. Seven days after transplantation bronchoalveolar lavage fluid was collected from recipients and presumed *B cepacia* complex bacteria were isolated by culture. Phenotypic analyses were performed using the API 20NE diagnostic test (Biomèrieux, Marcy l'Etoile, France).

### PCR based analyses
Genomic template DNA for molecular analysis was prepared from all isolates and their genomovar status determined using *recA* based methods.[10] Analysis of PCR and RFLP products by agarose electrophoresis was as previously described.[10] PCR based detection of the BCESM and the *cblA* gene was conducted as previously reported.[7][11]

## Pulsed field gel electrophoresis

*B cepacia* complex strains were genotyped by macrorestriction of whole genomic DNA with the restriction enzyme *Spe*I (New England Biolabs, UK) and the fragments were separated by pulse field gel electrophoresis (PFGE) (CHEF DRII System; Bio-Rad). Genotype patterns for *B cenocepacia* isolates were compared with those of the ET-12 index strain J2315. Investigators were blinded to the genomovar status of the *B cepacia* complex strains isolated.

## RESULTS

### Prevalence of *B cepacia* complex genomovars in CF patients

Thirty two patients referred to the Freeman Hospital Transplant Unit were found to have presumptive *B cepacia* complex infection based on phenotypic analysis. However, further analysis revealed that isolates from one patient were *Brevundimonas vesicularis*, while isolates from two patients were identified as *Alcaligenes xylosoxidans*. Sixteen of the remaining 29 patients (55%) were found to have *B cenocepacia* infections (14 (48%) *B cenocepacia* III-A and two (7%) *B cenocepacia* III-B), 11 patients (38%) were infected with *B multivorans*, while the remaining two patients (7%) had *B vietnamiensis* (formerly genomovar V) infections.

### Molecular epidemiology of *B cenocepacia* infection

Four strain types were found among the 16 patients infected with *B cenocepacia* (fig 1). Thirteen patients were infected with the *B cenocepacia* ET-12 epidemic strain. All ET-12 isolates contained both the *cblA* gene and BCESM. The three remaining *B cenocepacia* III-A and III-B strains were genetically unique. The unique *B cenocepacia* III-A strain lacked the *cblA* gene but was positive for BCESM. PFGE analysis of ET-12 isolates before and after transplantation from two representative patients revealed the persistence of the epidemic strain after surgery (fig 2).
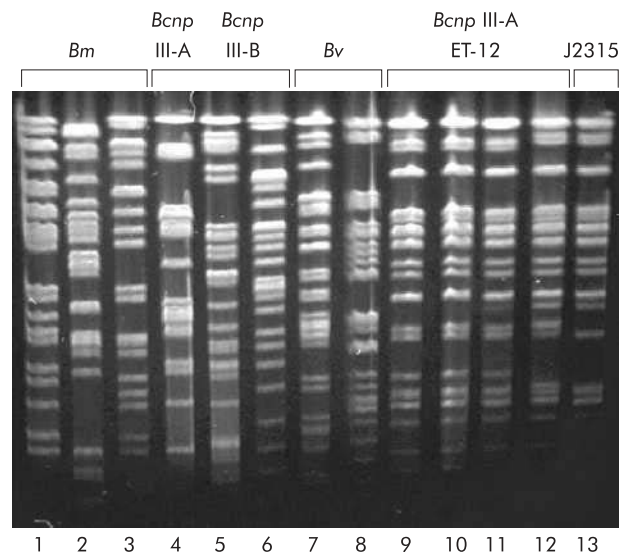


**Figure 1** Macrorestriction (*Spe*I) fingerprinting of *B cepacia* complex genomovar strains recovered from patients with CF attending The Freeman Hospital Transplant Unit. Lanes 1–3, representative sample of unique *B multivorans* (*Bm*) strains; lane 4, unique *B cenocepacia* III-A (*Bcnp* III-A) strain; lanes 5–6, unique *B cenocepacia* III-B (*Bcnp* III-B) strains; lanes 7–8, unique *B vietnamiensis* (*Bv*) strains; lanes 9–12, representative sample of *B cenocepacia* III-A (*Bcnp* III-A) ET-12 isolates; lane 13, J2315 Edinburgh index strain for ET-12 lineage. Restriction fragments were separated using pulse times of 2.9–35.4 seconds at 6 V/cm for 20 hours at 14°C.
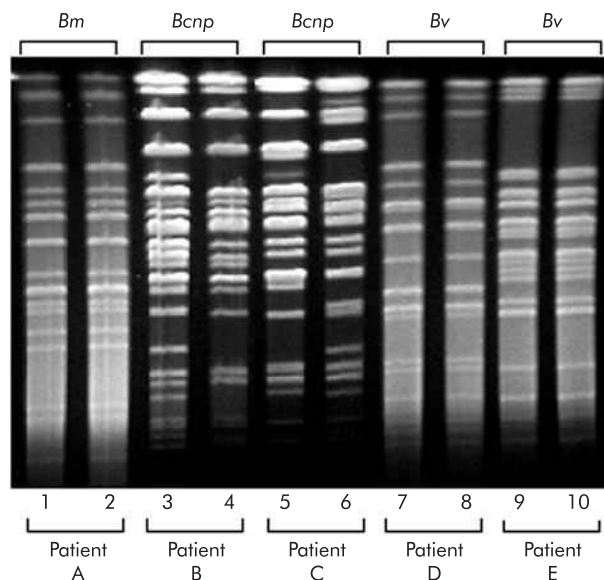


**Figure 2** Persistence of pre-transplant *B cepacia* complex strains in post-transplant cultures by macrorestriction (*Spe*I) fingerprinting. Pre- and post-transplant isolates from patient A (lanes 1–2: *B multivorans*, *Bm*), patient B (lanes 3–4: *B cenocepacia* III-A ET-12, *Bcnp*), patient C (lanes 5–6: *B cenocepacia* III-A ET-12; *Bcnp*), patient D (lanes 7–8: *B vietnamiensis, Bv*), and patient E (lanes 9–10: *B vietnamiensis, Bv*). Restriction fragments were separated using pulse times of 2.9–35.4 seconds at 6 V/cm for 20 hours at 14°C.

### Molecular epidemiology of *B multivorans* and *B vietnamiensis* infections

All *B multivorans* and *B vietnamiensis* strains were found to be unique by PFGE (fig 1). All strains were negative for the *cblA* gene and BCESM. PFGE analysis of pre- and post-transplant *B multivorans* and *B vietnamiensis* isolates again revealed the persistence of the infecting strain after surgery (fig 2).

### Strain types and post-transplant outcomes

PFGE analysis also revealed that all our previously described post-transplant deaths[4] were due to infections with the *B cenocepacia* ET-12 epidemic strain. The previously described patient group who were successfully transplanted all had pre-transplant infections due to genetically unique strains of *B multivorans* (n = 3) and *B vietnamiensis* (n = 2). These patients are all currently alive and well. Four of our post-transplant survivors had *B multivorans* or *B vietnamiensis* organisms in the BAL fluid for up to 1 year after surgery.

## DISCUSSION

Pulmonary transplantation has emerged as a highly successful treatment for end stage CF associated lung disease. There has been much debate regarding the role of transplantation in those infected with *B cepacia* complex, reflecting the variable outcomes seen in these patients. Many transplant units consider that infection with *B cepacia* complex before transplantation is an absolute contraindication for surgery. Currently, prevalence rates of *B cepacia* complex infection among CF patients transplanted at this unit are 15%.[4]

We have previously reported that poor outcomes following transplantation were associated with pre-transplant *B cenocepacia* infections, while infections with other genomovars were associated with excellent post-transplant prognoses,[4] providing an insight into the variable outcomes previously observed in transplant patients with ''*B cepacia*''. Data supporting our observations were also reported from North Carolina, USA.[5] Both studies provided evidence that the

moratorium placed against transplanting all CF patients infected with the *B cepacia* complex was not justified.

In this follow up study we have determined the prevalence and clonality of *B cepacia* complex isolates recovered from patients with CF referred for pre-transplant assessment from 1989 to the present. Selective culture and phenotypic analysis identified 32 patients with *B cepacia* complex infections, but PCR based molecular analyses revealed that only 29 were actually infected with *B cepacia* complex pathogens. False positive rates of identification highlight the importance of molecular analyses for accurate identification of *B cepacia* complex organisms.

Genotyping analysis in this study revealed the persistence of infection with the pre-transplant strain responsible for post-transplant infections, confirming the previous study by Steinbach *et al*.[12] This persistent infection may reflect, in part, the difficulty in clearing the fused pleural spaces often seen during surgery. An alternative explanation for the persistence of the pre-transplant strain may be ongoing paranasal sinus infection leading to infection of the graft. In this study we found that the majority of patients referred to our unit infected with the *B cepacia* complex were infected with *B cenocepacia*. Analysis of these isolates by PFGE revealed that 13 patients from geographically diverse CF centres were infected with the *B cenocepacia* III-A ET-12 clone. In contrast to the UK, the ET-12 strain is found infrequently in CF patients in the USA.

Our genotyping studies show that all our previously described post-transplant deaths related to infection with *B cepacia* complex were associated with the ET-12 strain of *B cenocepacia* III-A. Notably, the North Carolina study showed that poor transplant outcomes were associated with a variety of *cblA* negative, non-ET-12 *B cenocepacia* strains.[5] A separate study at that US centre found that most of the 56 patients assessed for transplantation who were infected with *B cepacia* complex harboured strains with unique genotypes.[13]

Since our discovery that the ET-12 strain of *B cenocepacia* III-A was associated with poor post-transplant survival and was the most prevalent strain among *B cenocepacia* infected patients awaiting transplantation, we have altered our previously described management of transplant patients infected with *B cenocepacia*.[9] We now omit T cell ablation at induction, reduce target trough cyclosporin levels, wash out the pleural cavities with the surface disinfectant taurolidine,[14] and commence a 48 hour multi-antibiotic regimen (dependent on recent sensitivity testing) similar to that used by the Toronto transplant group.[15] We have successfully transplanted two ET-12 infected patients with current survival times of up to 1 year. The antibiotic regimens used were aztreonam, clindamycin, gentamicin, and systemic taurolidine (250 ml 2% taurolidine intravenously qds) in one patient and clindamycin, chloramphenicol, and temocillin with nebulised taurolidine in the other.

In conclusion, the ET-12 epidemic clone was the most prevalent strain in patients infected with *B cenocepacia* referred to our unit for pre-transplant assessment. Although previously reported deaths following transplantation were associated with ET-12, successful transplantation of patients infected with this strain has been achieved using an altered management regimen. It remains to be determined whether these measures will provide safer management strategies for patients infected with other *B cenocepacia* strains.

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