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Six-Year Molecular Analysis of *Burkholderia cepacia* Complex Isolates among Cystic Fibrosis Patients at a Referral Center for Lung Transplantation

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Over a 6-year period, *Burkholderia cepacia* complex species were isolated from cystic fibrosis (CF) patients receiving care at The University of North Carolina Hospitals (clinic CF patients) and from those referred from other treatment centers. Fifty-six isolates collected from 30 referred patients and 26 clinic CF patients were characterized by pulsed-field gel electrophoresis (PFGE) and were assayed by PCR to detect the cable pilin gene, *cblA*. PFGE results indicated that six separate clusters (clusters A to F) were present among the 56 isolates and that three clusters (clusters A, B, and E) consisted only of isolates from referred patients infected with *B. cepacia* complex isolates prior to referral. However, one cluster (cluster C) consisted of isolates from four CF patients, and hospital records indicate that this cluster began with an isolate that came from a referred patient and that spread to three clinic CF patients. Cluster D consisted of two isolates from clinic CF patients, and hospitalization records are consistent with nosocomial, patient-to-patient spread. *cblA* was present in only 4 of the 56 isolates and included isolates in cluster E from the referred patients. Our results indicate a lack of spread of a previously characterized, transmissible clone from referred patients to our clinic CF population. Only two instances of nosocomial, patient-to-patient spread could be documented over the 6-year period. An additional spread of an isolate (cluster F) from a referred patient to a clinic patient could not be documented as nosocomial and may have been the result of spread in a nonhospitalized setting. The majority (36 of 56) of our *B. cepacia* complex-infected CF patients harbor isolates with unique genotypes, indicating that a diversity of sources account for infection. These data suggest that CF patients infected with *B. cepacia* complex and referred for lung transplantation evaluation were not a major source of *B. cepacia* complex strains that infected our resident CF clinic population.

The *Burkholderia cepacia* complex is composed of at least seven closely related species or genomovars consisting of *B. cepacia* (genomovar I), *B. multivorans* (genomovar II), *B. stabilis* (genomovar IV), *B. vietnamiensis* (genomovar V), and *B. ambifaria* (genomovar VII), and genomovars III and VI, with species designations for genomovars III and VI still pending (4, 8).

Nearly 4% of cystic fibrosis (CF) patients in the United States are infected with a member of the *B. cepacia* complex (13). Of these patients, some will develop the cepacia syndrome, a rapidly fatal necrotizing pneumonia with bacteremia (9, 10, 26). Previous studies have shown that transmissible clones of *B. cepacia* exist, and subsequent infection with these clones of both healthy uninfected CF patients and CF patients seeking lung transplantation can be devastating (9, 11, 12, 22, 25). Therefore, screening of CF patients for the detection of *B. cepacia* complex and management of patients once infection is documented are extremely important. Furthermore, CF patients infected with *B. cepacia* complex isolates are stigmatized, being segregated from the general CF patient population in terms of clinical care and social interaction. In many North American transplant centers, infection with *B. cepacia* complex is a strict contraindication for lung transplantation in CF patients (14).

There are more than 120 lung transplantation centers in North America. The University of North Carolina (UNC) Hospitals is one of the few CF centers in North America that will perform lung transplantation for CF patients infected with *B. cepacia* complex. For this reason, about 14% of adult CF patients (i.e., roughly three times the national percentage) referred to the UNC Hospitals for double lung transplantation are infected with *B. cepacia* complex.

Some transmissible clones of *B. cepacia* complex exist, such as the cable pilin-positive (*cblA*$^+$) electropherotype (ET) ET 12 clone responsible for epidemic transmission in both Canada and the United Kingdom (7, 9, 11, 21, 22, 25). Therefore, we believed that it was important to study the CF patient population at the UNC Hospitals by asking three questions. First, have referred patients brought a previously characterized, transmissible clone into our (the UNC Hospitals) center, and has it been transmitted to our clinic CF patient population? Second, are any new, previously uncharacterized, transmissible

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TABLE 1. Summary of genotypes and clusters of *B. cepacia* complex isolates recovered from January 1995 to March 2001

Genotype	Patient no.	Genomovar	Cluster	Area	*cblA*[a]
1	1	III	A	Michigan	Neg
1	2	III	A	Michigan	Neg
1	3	III	A	Michigan	Neg
1a	4	III	A	Michigan	Neg
1	5	III	A	Nebraska	Neg
1b	6	III	A	Michigan	Neg
2	7	III	B	New York	Neg
2	8	III	B	New York	Neg
2	9	III	B	New York	Neg
2	10	III	B	New Jersey	Neg
3	11	*B. multivorans* (II)	C	Florida	Neg
3	12	*B. multivorans* (II)	C	UNC Hospitals	Neg
3	13	*B. multivorans* (II)	C	UNC Hospitals	Neg
3	14	*B. multivorans* (II)	C	UNC Hospitals	Neg
4	15	III	D	UNC Hospitals	Neg
4	16	III	D	UNC Hospitals	Neg
5	17	III	E	Nova Scotia	Pos
5	18	III	E	Nova Scotia	Pos
6	19	III	F	UNC Hospitals	Neg
6	20	III	F	New Jersey	Neg
7	21	III	Distinct genotype		Pos
8	22	*B. cepacia* (I)	Distinct genotype		Pos
9–30	23–44	*B. multivorans* (II)	Distinct genotypes		Neg
31–38	45–52	III	Distinct genotypes		Neg
39–41	53–55	*B. vietnamiensis* (V)	Distinct genotypes		Neg
42	56	*B. cepacia* (I)	Distinct genotype		Neg

[a] Neg, negative; Pos, positive.

clones evident among our referred patients, and has transmission from referred patients to our local clinic CF population occurred? Third, how much intracenter spread of *B. cepacia* complex has occurred?

MATERIALS AND METHODS

Characterization of UNC Hospitals CF center and infection control. All *B. cepacia* complex isolates were recovered from CF patients between 1 January 1995 and 1 March 2001. We studied two distinct patient populations from which *B. cepacia* complex organisms were isolated. One consisted of 30 CF patients who were referred to the UNC Hospitals, often for lung transplantation evaluation, already infected with *B. cepacia* complex. The second population was of 26 CF patients who received their routine care at the UNC Hospitals and who became newly infected during a study period from 1 January 1997 to March 2001. For these patients to be considered "newly infected," prior cultures of samples from these patients at our institution had to be negative for *B. cepacia* and the organism had to be isolated during the study period.For the year 2000, 451 CF patients received care at the UNC Hospitals, with 251 of these patients being pediatric patients, while 200 were adult patients. Nineteen (9.5%) of the 200 adult patients and 8 (3.2%) of the 251 pediatric patients were infected with *B. cepacia* complex. A total of 107 CF patients are awaiting lung transplantation at the UNC Hospitals, with 15 (14%) of these patients infected with *B. cepacia* complex. As part of the evaluation of possible nosocomial transmission among CF patients whose isolates were in clusters C, D, and F, all patient charts were reviewed by a nurse trained in infection control. The review included production of a time line of all hospital admissions including hospital location, clinic visits, physical and occupational therapy visits, pulmonary rehabilitation visits, and evaluations in specialty clinics (e.g., pulmonary function laboratory, radiology, and transplantation clinics). Patients with known *B. cepacia* complex colonization requiring inpatient hospitalization were admitted to private rooms and placed on contact precautions. When pediatric patients with *B. cepacia* complex were seen in the outpatient clinic, they either were scheduled to be seen on a different day than other CF patients or were scheduled to be seen at the end of the day, after all patients not infected or colonized with *B. cepacia* had left the facility. Until 1998, adult CF patients were seen in the general pulmonary clinics in order to minimize contact with each other. In 1998, the formation of a dedicated adult CF clinic prompted the institution of several infection control measures, including patient education, masking of all patients upon arrival at the clinic and in all common areas, strict adherence to hand-washing procedures, and disinfection of rooms between patients. The UNC Hospitals Lung Transplant Clinic adopted similar infection control guidelines in 2000.

***B. cepacia* isolation, PFGE, and *cblA* analysis.** All strains were isolated on either *Pseudomonas cepacia* agar or *B. cepacia* selective agar prepared in-house and were further characterized biochemically and by PCR genomovar analysis as described previously (6, 16, 19). Once a member of the *B. cepacia* complex was isolated from a CF patient, a subculture was prepared from a single colony and stocks were prepared in skim milk and frozen at −70°C.

Pulsed-field gel electrophoresis (PFGE) was conducted as described previously, with the following revisions (7). All cultures were grown overnight in tryptic soy broth and adjusted to an optical density at 600 nm of 1. One milliliter was removed and centrifuged for 5 min to pellet the cells. The cells were then resuspended in formalin (3.8% [vol/vol]) (5) and allowed to sit on ice for 1 h. The cells were pelleted and rinsed three times in TE (10 mM Tris-HCl, 10 mM EDTA [pH 8.0]) before being placed in agarose blocks. Genomic DNA was restricted with *Spe*I (New England Biolabs); and electrophoresis was conducted for 24 h in 0.5× TBE (Tris-borate, EDTA), with initial and final pulse times of 1.2 and 54 s, respectively, by using a CHEF Mapper system (Bio-Rad). An ET 12 strain of *B. cepacia* complex was kindly provided by R. Goldstein, Boston University School of Medicine (25). Gels were analyzed visually according to the criteria of Tenover et al. (27).

All isolates of *B. cepacia* complex were tested by PCR for the presence of the cable pilin gene, *cblA*, as described previously (22).

RESULTS

Fifty-six *B. cepacia* complex isolates cultured from 56 CF patients were characterized for their PFGE patterns (genotypes) after digestion with *Spe*I (Table 1). Genotypes were considered related if they differed by three or fewer bands (27). In total, 42 distinct genotypes were present, which likely indicates a wide diversity in the sources of infection. There were 26 genomovar II (*B. multivorans*) isolates, 25 genomovar III isolates, two genomovar I (*B. cepacia*) isolates, and three genomovar V (*B. vietnamiensis*) isolates. Our PFGE analysis revealed that six clusters of PFGE patterns were present within

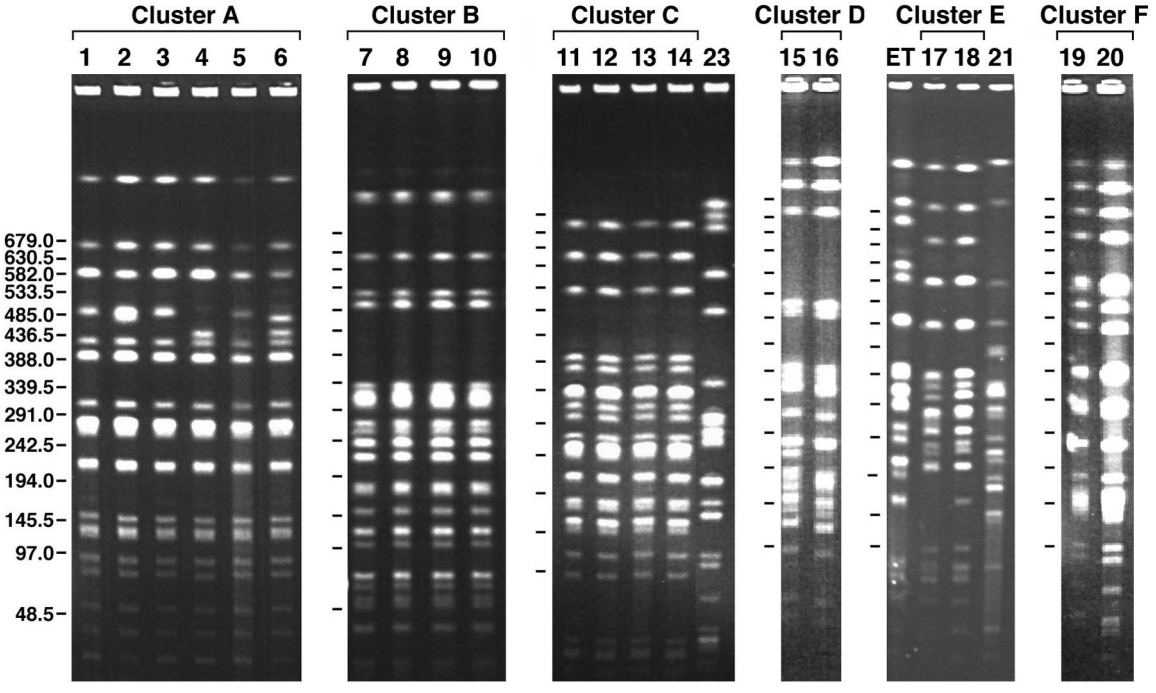

FIG. 1. PFGE banding patterns of six clusters (clusters A to F) of *B. cepacia* complex isolates recovered from CF patients at the UNC Hospitals CF center. The cluster C gel contains a *B. multivorans* strain designated 23 for purposes of comparison. The cluster E gel contains the Toronto strain, designated ET, and a *cblA*$^+$ genomovar III strain, designated 21, for the purposes of comparison. Numbers on the left are molecular weights.

the CF population at the UNC Hospitals during this study period (Fig. 1). Three clusters consisted of strains from multiple patients (clusters A to C), while clusters D to F consisted of isolates from only two patients each. The cluster A genotype occurred in six patients, five of whom had previously been seen at CF centers in Michigan and who were infected prior to care at the UNC Hospitals. The fifth patient whose isolate was in this cluster was also infected with *B. cepacia* prior to referral, but the patient was originally from Nebraska and it is not known whether there is a link between this patient and the other patients whose isolates were in this cluster. Isolates from patients 1 through 3 and 5 shared identical genotypes, while isolates from patient 4 differed from those from patients 1 through 3 and 5 by two bands and isolates from patient 6 differed from those from patients 1 through 3 and 5 by three bands. All were genomovar III isolates.

Likewise, cluster B isolates (Fig. 1) occurred in those referred patients seen previously at CF centers within the metropolitan New York City-New Jersey area and infected prior to care at the UNC Hospitals. Again, all were genomovar III isolates, and there was no evidence of the spread of this cluster to any of our clinic CF patients.

Interestingly, cluster C (Fig. 1) began with patient 11 from Florida, who sought care at the UNC Hospitals for a possible double lung transplantation and who was already infected with *B. multivorans*. This patient had a lengthy hospitalization from 7 June 1998 until 13 August 1998 and again from 21 August 1998 until 13 March 1999 (Fig. 2). Patient 12 was also hospitalized from 5 through 11 August 1998, during the hospitalization of patient 11, and subsequently became culture positive for *B. multivorans* on 5 November 1998. On the same date, patient 12 was also seen in the adult CF clinic. Patient 13 was a pediatric patient hospitalized from 29 October 1998 until 12 November 1998 and was later (11 February 1999) culture positive for *B. multivorans*. Patient 14 was hospitalized from 13 through 21 December 1999 and had an overlapping hospitalization with patient 12. Patient 14 was then culture positive for *B. multivorans* on 25 May 2000. Although these patients did not share the same hospital floor, they did share common hospital services (physical therapy, pulmonary function laboratory, and radiology). Since the *B. multivorans* isolates from all of these patients share the same genotype and overlapping hospitalizations were evident, we conclude that nosocomial, patient-to-patient spread occurred among these patients either directly or indirectly.

Three small clusters (clusters D to F) (Fig. 1) consisting of isolates from two patients each were also detected by PFGE, and isolates in one of these clusters (cluster E) were from siblings who were infected with *B. cepacia* and who had been referred to the UNC Hospitals (Fig. 1). As with cluster C isolates, the isolates in cluster D were from two patients who also had overlapping hospitalizations on the same hospital unit that were clearly documented (data not shown). These two patients represent part of the UNC Hospitals CF clinic population. The isolates in cluster F consisted of isolates from patient 19, who became infected while receiving care at the UNC Hospitals, and patient 20, who was from New Jersey and who came to the UNC Hospitals already infected with *B. cepacia* genomovar III. We could find no evidence of overlapping hospitalizations or shared hospital services between these two patients. Therefore, we suspect that patient 19 may have

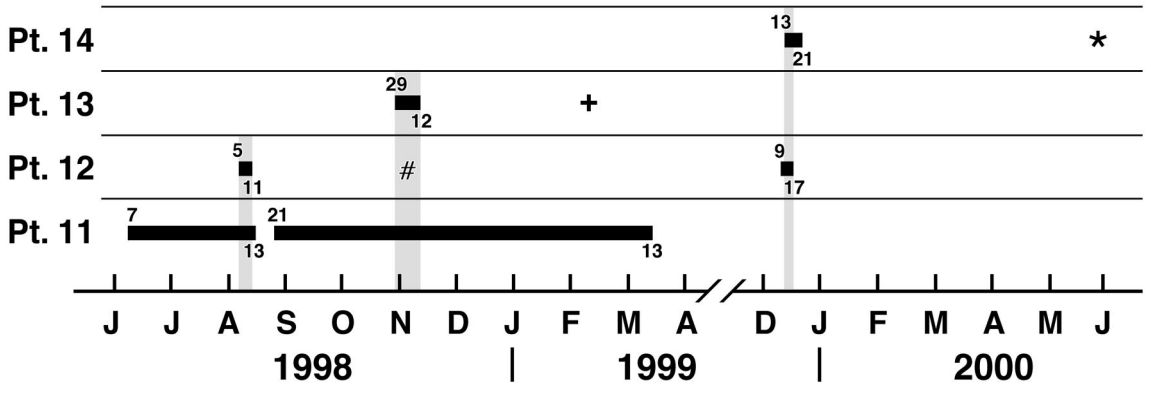

FIG. 2. Time line graph showing the hospitalization course of patients (Pt.) 11 to 14. Horizontal bars represent inpatient status, with the admission date given at the beginning of the bars and the discharge date given at the ends. Vertical bars represent dates of concurrent admission resulting in possible cross infection. #, +, and ∗, the dates that patients 12, 13, and 14, respectively, first became positive for *B. multivorans* infection (see text).

become infected with an isolate of the same genotype as that from patient 20 in a nonhospitalized setting (23).

Epidemic clone ET 12, isolated from CF centers in both the United Kingdom and North America, expresses cable pilin on its surface (25). For this reason, we performed PCR on our isolates to see if the *cblA* gene was present, especially in our cluster isolates. Our results show that the *cblA* gene was present in only four isolates, two of which were from cluster E (isolates from a sibling pair). A comparison of the *Spe*I restriction patterns between the *B. cepacia cblA*$^+$ isolates from the siblings (patients 17 and 18) and patient 21 with that of the highly transmissible Toronto strain is shown in Fig. 1. The results indicate that while the *Spe*I patterns for the isolates from the siblings are an exact match, the genotypes produced by isolates from the siblings, patient 21, and the ET 12 clone differ from each other by more than seven fragments and are therefore unrelated (25). A fourth patient infected with a *cblA*$^+$ strain also showed a unique *Spe*I genotype, as expected, since this isolate was a genomovar I strain (data not shown), while the other *cblA*$^+$ isolates were genomovar III strains (Table 1).

DISCUSSION

In an earlier study, we analyzed *B. cepacia* complex isolates from five transplant patients and 17 clinic patients and found no evidence of transmission of *B. cepacia* complex isolates among these patients (24). However, we did find a strain of *B. cepacia* complex in a patient referred for transplantation evaluation that resulted in the recognition of a large nosocomial outbreak at the institution from which he was referred (9). This caused us to be concerned that patients referred for transplantation might transmit *B. cepacia* complex strains to patients receiving their routine care for CF at our institution.

Of the six clones found in clusters in this study, isolates of four previously uncharacterized clones (clusters B, C, E, and F) were present in our referred patients, while cluster A has been reported previously (12) and cluster D isolates were not present in patients referred for transplantation. Cluster B isolates were present in four referred patients from the metropolitan New York City-New Jersey area. To our knowledge, there is no published study of an epidemic clone of the *B. cepacia* complex from CF patients from that geographic area. Therefore, we cannot comment on the transmissibility of this clone except to say that it was present in these four referred CF patients and did not spread to our clinic CF patients.

Cluster C comprised a clone of *B. multivorans.* Among the isolates in the *B. cepacia* complex, transmissibility is typically thought to be associated with *B. cepacia* genomovar III organisms (2, 7, 9, 11, 21, 22, 25). Person-to-person spread of *B. multivorans* is not as frequently described, and most patients infected with *B. multivorans* are infected with unique strains (1, 14). Overlapping hospitalizations could be traced for all four patients infected with cluster C isolates. Cluster E consisted of only two isolates from referred patients who were siblings, but the isolates were *cblA*$^+$. Again, there was no evidence of spread from these referred patients to our clinic CF patient population. Cluster F consisted of isolates from two patients and represented the spread of *B. cepacia* genomovar III from a referred patient to a clinic CF patient. However, we were unable to show any link between these two patients through overlapping hospitalizations, clinic visits, or hospital services and therefore consider this cluster not to have occurred nosocomially.

A closer analysis of the isolates in cluster A indicated that four of five referred patients from Michigan were infected with isolates with identical genotypes (cluster A) when they arrived at the UNC Hospitals and that the isolate from the fifth patient (Fig. 1, patient 4) possessed a genotype that differed from that of the isolates from the first four patients by only two bands. Digestion with *Xba*I and PFGE of the isolate from patient 4 allowed us to confirm by direct comparison with the PFGE results of Kumar et al. (12) that these isolates were part of the cluster of genetically related isolates from five CF centers in Michigan (data not shown). We were initially alarmed at finding this cluster among our referred patients. However, we found no evidence of the spread of isolates in this cluster to any of the other CF patients screened in this study. Cluster A also included an isolate from a single referred patient from Nebraska. We have not been able to verify whether this patient

attended some of the same CF camps or CF centers as the rest of the patients whose isolates were in cluster A.

One of the shortcomings of our study design was that we studied only a single isolate from each patient. We had two instances in which isolates from sibling pairs had different genotypes. Patient 14 is a sibling of patient 23, and both patients were infected with the same genomovar (*B. multivorans*) but the genotypes of the isolates were not related. In another instance, we noted that isolates from siblings (patient 10, whose isolate was in cluster B, and patient 20, whose isolate was in cluster F) differed in their genotypic patterns. Since we studied only one isolate from each patient, it is possible that the siblings may have been infected with isolates with common genotypes that were not detected. We previously showed that over time patients harbor *B. cepacia* complex isolates with the same genotype (24).

Overall, the results of our genotype analysis indicate that the spread of a previously characterized, transmissible clone of *B. cepacia* from referred patients to our clinic CF patient population has not occurred. Other studies have indicated that a high percentage of patients at CF centers often harbor endemic, transmissible clones (2, 12, 15, 17, 23, 25, 29). In contrast, our PFGE results did not indicate the presence of a common, transmissible clone among our clinic CF patient population. Our results more closely parallel those of a recent study (20) indicating that hospitals with a segregation policy tend to have patients infected with unique strains. Documented nosocomial spread involved 4 of 26 (15%) of our clinic *B. cepacia* complex-infected patients (patients 12 to 14, whose isolates were in cluster C, and patient 16, whose isolate was in cluster D).

A recent publication indicated that in the United Kingdom the *cblA* gene may be used as a marker to identify strains with an enhanced capacity for spread (3). We found the *cblA* gene in only 4 of the 56 *B. cepacia* isolates that we examined. Two of the four isolates were from referred siblings from Canada and were in cluster E. However, the genotype for cluster E isolates differed from that of the epidemic, *cblA*$^+$ ET 12 strain, also isolated from Canadian patients, by more than seven fragments, and they are therefore considered genetically unrelated. There was no evidence of spread of *cblA*$^+$ clones in our patient population. Our data are consistent with those of LiPuma and colleagues (18), who found that only 1 of 606 isolates carried the *cblA* gene. These data suggest that other factors are important in the transmissibility of the genomovar III organisms.

The most frequently recovered *B. cepacia* complex species from CF patients are genomovar III (18), and recent data indicate that CF transplant patients infected with genomovar III suffer higher rates of mortality than those infected with another genomovar (1). However, it is apparent from our study and those of others that *B. multivorans* can also be frequently recovered (8, 18, 28). In fact, our results indicate that *B. multivorans* was slightly more prevalent than genomovar III among our CF patient population (26 and 25 patients, respectively). Our results also revealed that 36 of the 56 CF patients seeking care at the UNC Hospitals harbor strains with unique genotypes, so their sources of infection are likely to be diverse.

In conclusion, our study of *B. cepacia* complex-infected CF patients indicated that transmission of an isolate from a referred patient to our clinic CF patient population occurred in only two instances (with isolates in clusters C and F), but nosocomial transmission could clearly be documented for only one of these isolates (a cluster C isolate). Intracenter transmission of isolates in one, two-patient cluster (cluster D) occurred within our clinic patient population. Our clinic patients were infected with a variety of different genotypes of the *B. cepacia* complex. These data suggest that CF patients who were infected with *B. cepacia* complex isolates and who were referred for lung transplantation evaluation were not a major source of the *B. cepacia* complex organisms that infected our resident CF clinic population.

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