MAJOR ARTICLE

Infection with *Burkholderia cepacia* Complex Genomovars in Patients with Cystic Fibrosis: Virulent Transmissible Strains of Genomovar III Can Replace *Burkholderia multivorans*

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Infection with *Burkholderia cepacia* complex in patients with cystic fibrosis (CF) results in highly variable clinical outcomes. The purpose of this study was to determine if there are genomovar-specific disparities in transmission and disease severity. *B. cepacia* complex was recovered from 62 patients with CF on ⩾1 occasions (genomovar III, 46 patients; genomovar II [*B. multivorans*], 19 patients; genomovar IV [*B. stabilis*], 1 patient; genomovar V [*B. vietnamiensis*], 1 patient; and an unclassified *B. cepacia* complex strain, 1 patient). Patient-to-patient spread was observed with *B. cepacia* genomovar III, but not with *B. multivorans*. Genomovar III strains replaced *B. multivorans* in 6 patients. Genomovar III strains were also associated with a poor clinical course and high mortality. Infection control practices should be designed with knowledge about *B. cepacia* complex genomovar status; patients infected with transmissible genomovar III strains should not be cohorted with patients infected with *B. multivorans* and other *B. cepacia* genomovars.

The gram-negative bacterium *Burkholderia* (formerly *Pseudomonas*) *cepacia* [1] is a problematic pulmonary pathogen in patients with cystic fibrosis (CF). The organism is highly virulent in certain patients with CF [2], and there is substantial evidence that *B. cepacia* may spread from one patient with CF to another, both within and outside the hospital [3, 4]. Spread may be dependent on a number of risk factors, including bacterial strain type [5–9], patient behavior [9], use of contaminated therapeutic devices [10], and treatment center infection control practices [11]. To reduce spread of the organism, isolation or segregation of patients who are infected with *B. cepacia* has been recommended [4, 12]. Recent changes in the taxonomy of the species *B. cepacia* [13] have further complicated our understanding of the epidemiology of *B. cepacia* respiratory infection in patients with CF. Vandamme et al. [13] demonstrated that 5 distinct genomic species (referred to as "genomovars" [14]) are present among strains classified as *B. cepacia*. Three of the genomovars have been assigned species designation because of unique phenotypic features (genomovar II, *Burkholderia multi-*

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vorans [13]; genomovar IV, *Burkholderia stabilis* [15], and genomovar V, *Burkholderia vietnamiensis* [13]). Until differential tests are found, the remaining 2 groups are referred to as genomovars I and III, and all 5 genomovars are grouped in the *B. cepacia* complex [13].

Infection of patients with CF with *B. cepacia* complex is associated with a poor prognosis [16], but the risk attributable to infection with each genomovar and species has not been determined. Understanding these risks is vital for CF treatment centers to develop rational infection control policies and therapeutic approaches for *B. cepacia* complex–infected individuals. The epidemiology of infection with *B. cepacia* complex is poorly understood, in part because of difficulties with identification of *B. cepacia* [17] and the complexities of genomovar analysis [13]. Retrospective examination of strain collections suggests that the propensity of *B. cepacia* strains to spread among patients with CF is mainly associated with strains of genomovar III, although outbreaks linked to infection with *B. multivorans* strains in the United Kingdom and France have been reported [13, 18, 19]. The clinical outcomes of *B. cepacia* infections in patients with CF may also differ widely [20].

We speculated that strain and genomovar heterogeneity accounts for the extraordinary clinical variation among infected patients with CF. The purpose of this study was to characterize the natural history of infection with strains of the different genomovars and to determine if there is genomovar-specific disparity in transmission and disease severity. We have collected all isolates of *B. cepacia* from patients with CF who have attended treatment centers in Vancouver since 1981. All isolates were evaluated for genomovar status by *recA* gene polymorphism [21] to complement conventional genomovar testing [13]. In this study we report on a systematic study of the natural history of *B. cepacia* complex infection in a defined CF patient population.

METHODS

Study population. *B. cepacia* isolates were recovered from patients with CF attending either the pediatric clinic (British Columbia's Children's Hospital) or adult clinic (St. Paul's Hospital, or Shaughnessy Hospital until September 1993) in Vancouver. Data collected from June 1981 through June 1998 are presented in this study. Approximately 450 patients with CF were treated during this period. A total of 281 *B. cepacia* complex isolates were recovered from 62 patients. Sputum cultures were performed at every clinic visit (3- to 6-month intervals) for each patient.

Microbiology and molecular epidemiology. *B. cepacia* isolates were cultured from sputum, identified, and stored exactly as described elsewhere [7, 17]. A patient was considered free of *B. cepacia* if 3 consecutive cultures over a period >3 weeks or 2 separate hospitalizations failed to yield *B. cepacia* complex bacteria (referred to as "*B. cepacia*–negative" below). Infection with *Pseudomonas aeruginosa* was also recorded for each patient, and culture and identification of this organism were carried out as described elsewhere [22]. Each *B. cepacia* complex isolate was genetically typed by random amplified polymorphic DNA (RAPD) analysis as described elsewhere [7, 22]. One isolate representative of each RAPD-defined strain type was also genetically fingerprinted by macrorestriction of whole genomic DNA with the restriction enzyme *Spe*I, followed by pulsed-field gel electrophoresis (PFGE) [23, 24]. PFGE fingerprints were compared by eye and computer software (Molecular Analyst Fingerprinting; BioRad), and Tenover's criteria [25] were used to define a strain type.

Clusters defined by each method correlated perfectly, and a numerical strain type (ST) was assigned to ⩾2 isolates that were grouped by fingerprint analysis. Isolates producing genetic fingerprints that did not match others within the strain collection were designated as unique. All *B. cepacia* complex isolates were also tested for the presence of the *B. cepacia* epidemic strain marker and cable pilus gene *(cblA)* as described elsewhere [8].

Genomovar analysis. The genomovar of each genetically defined strain was initially determined by use of conventional whole-cell protein electrophoresis as described elsewhere [13]. Final genomovar status was then confirmed by analysis of the *B. cepacia* complex *recA* gene [21].

Statistical analysis. Comparison of the mean age of patients at the time of *B. cepacia* complex acquisition and mean duration of colonization was performed by independent *t* tests. For all data, the mean ± SE is shown.

RESULTS

Prevalence of* B. cepacia *complex infection in patients with CF from Vancouver. *B. cepacia* complex isolates were recovered from 62 patients with CF during the study period, for a prevalence of 13% (62 of 450 patients). In total, 68 different *B. cepacia* complex isolates were recovered, and >1 genomovar was recovered from each of 6 of the 62 patients. Genomovar III strains were recovered from 46 patients (74% of *B. cepacia*–infected patients). *B. multivorans* (genomovar II) was recovered from 19 patients (30% of *B. cepacia*–infected patients). Of the remaining patients, 1 was chronically infected with a *B. stabilis* (genomovar IV) strain (7 isolates over 4 years); a single culture was positive for *B. vietnamiensis* (genomovar V) for the second, and a *B. cepacia* complex strain that could not be subclassified by conventional or molecular analysis [13, 21] was recovered on 2 occasions from the third. Data for patients with CF who were newly infected with *B. cepacia* complex bacteria were analyzed in ⩽5-year blocks and are presented in figure 1.

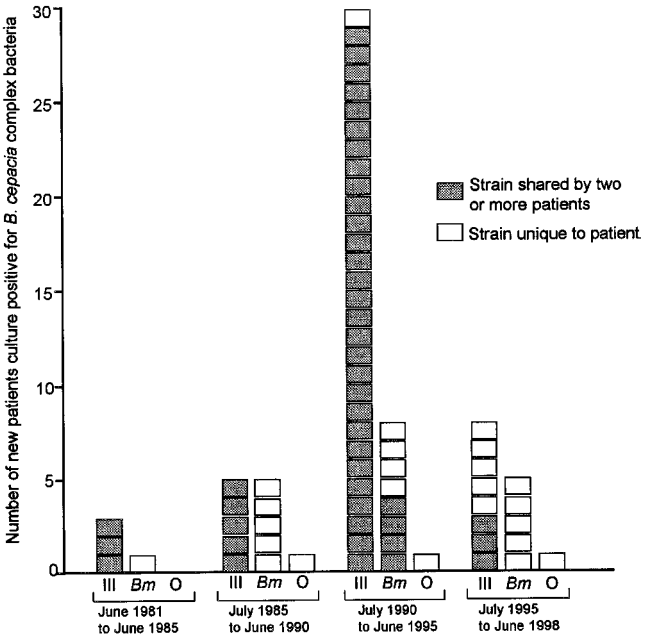

Figure 1. Prevalence of infection with each *Burkholderia cepacia* complex genomovar among patients with cystic fibrosis over the study period. Patients newly infected with genomovar III *(column III), B. multivorans (column Bm)* , and other *B. cepacia* complex genomovars *(B. stabilis, B. vietnamiensis,* or an unclassified genomovar; *column O),* are plotted by ≤5-year blocks. Blocks denoting patients infected with a strain shared by ≥2 patients are shaded to indicate the occurrences of patient-to-patient transmission.

Molecular epidemiology of* B. cepacia *genomovar III. Twelve strain types were found among the 46 patients with CF who were infected with *B. cepacia* genomovar III. Four genomovar III strain types (ST01, ST02, ST04, and ST06) were each recovered from ≥6 patients and were presumed to have spread from patient to patient. The genetic fingerprints obtained by PFGE of these predominant strain types are shown in figure 2*A*. Strain type ST02 was *cblA*-positive and belonged to the major CF lineage (ET12) that infects patients in the United Kingdom and Canada [6, 18]. All but 1 of the 9 patients were infected with the ET12 strain before moving to British Columbia. The remaining 3 transmissible genomovar III strain types were *cblA*-negative. All strains that had spread among patients encoded the *B. cepacia* epidemic strain marker and also belonged to the *recA* genomovar III-A lineage [21]. This *B. cepacia* complex phylogenetic group was dominant within the Vancouver CF patient population, accounting for 82% of genomovar III infections and 61% of all cases involving *B. cepacia* complex.

Socializing among patients may have contributed to the spread of the 4 genomovar III-A strain types. Evidence of this mode of spread was observed in the epidemiology of *B. cepacia* strain ST04. A total of 6 patients were considered to be a "social cohort" and interacted with one another extensively, inside and outside of the hospital. The strain was acquired by all 6 patients within a 3-year period. Evidence of spread due to social contact was also observed for genomovar III strain ST06. For 7 years, this strain was recovered from only 1 patient. During adolescence, he socialized in the hospital extensively with a number of other contacts with CF; 3 of these contacts subsequently became infected with strain ST06.

Segregation (separate rooms and treatment areas) and intensive education of *B. cepacia*–infected patients with CF and caregivers were introduced in November 1995 at the Vancouver treatment centers. New acquisitions of transmissible genomovar III strains ST01, ST02, ST04, and ST06 were significantly reduced in the ensuing 2 years (figure 1). The majority of genomovar III infections that occurred after November 1995 were caused by genetically unique strains (figure 1).

Genomovar III-B strains were recovered from 6 patients, most of whom were infected with genetically distinct strains that did not spread to other patients during the study period. Patients 21 and 34 were each transiently culture-positive for the same genomovar III-B strain type (ST73). However, the isolates were recovered only once from each patient, 3 years apart. Both patients attended the pediatric treatment center, but they had no known contact with one another. The unique genomovar III-B strain recovered from patient 27 was the only genomovar III strain that lacked the *B. cepacia* epidemic strain marker DNA. No deaths were associated with strains of *recA* subgroup genomovar III-B.

***Molecular epidemiology of* B. multivorans.** Seventeen different strains were recovered from 19 patients with CF who were infected with *B. multivorans.* The diversity in *B. multivorans* genetic fingerprints is shown in figure 2*B*. Only 2 strain types, ST49 and ST15, were recovered from >1 patient. *B. multivorans* strain ST49 (figure 2*B*) was transiently recovered from patients 51 and 52, who were siblings; both siblings lost this strain and were later classified as *B. cepacia* complex–negative. *B. multivorans* type ST15 was recovered from patients 15 and 41 only once each. Among the remaining 15 *B. multivorans* strains recovered from patients with CF, there was no evidence of spread during the 17-year study period. None of the *B. multivorans* strains examined harbored the cable pilus subunit gene or *B. cepacia* epidemic strain marker DNA.

Mortality associated with* B. cepacia *genomovar III and* B. multivorans *infection. A summary of the epidemiological characteristics of patients with CF infected with genomovar III or *B. multivorans* is presented in table 1. Patients with genomovar III infection were significantly older than those infected with *B. multivorans.* The mean duration of infection recorded within the study period was not statistically different for the 2 groups of patients. However, the number of patients who were transiently infected with *B. multivorans* (10 out of 19 patients) exceeded the number transiently infected with genomovar III strains (9 out of 46), which generally caused chronic infection.

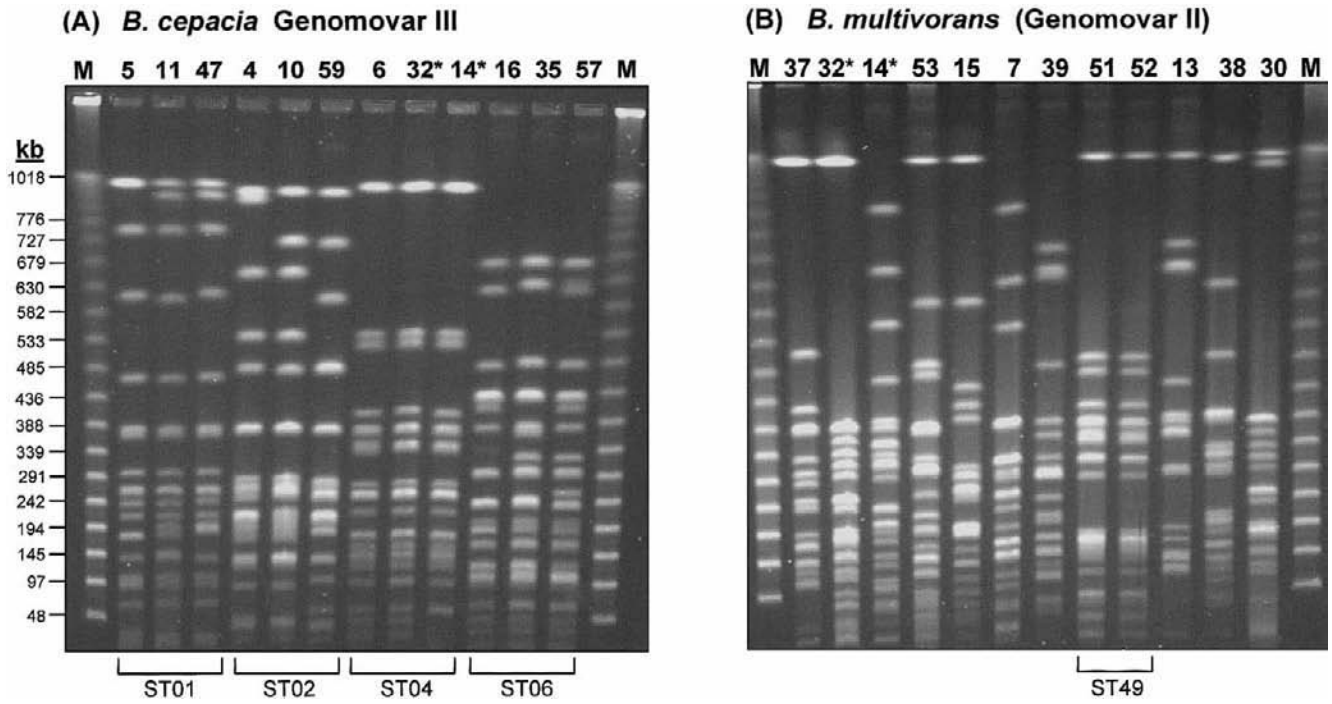

Figure 2. Pulsed-field gel electrophoretic (PFGE) fingerprints of *Burkholderia cepacia* complex bacteria. *A,* macorestriction fingerprints obtained from each of the predominant *B. cepacia* genomovar III strain types, ST01, ST02, ST04, and ST06. Strain fingerprints are shown for 3 patients infected with each major genomovar III strain, and the genetic type is indicated below each conserved group of 3 fingerprints. *B,* PFGE fingerprints for 11 strains of *B. multivorans* recovered from 12 patients. Strain type ST49 was transiently recovered from 2 patients (numbers 51 and 52), who were siblings. Patients 14 and 32 (indicated by *asterisks*) were initially infected with distinct *B. multivorans* strains *(B);* genomovar III strain ST04 was subsequently recovered from each of these patients *(A).*

The mean duration of *P. aeruginosa* infection prior to culture of *B. cepacia* complex was similar for the 2 groups. However, the number of patients infected with *P. aeruginosa* prior to infection with *B. cepacia* complex bacteria was marginally greater for cases involving genomovar III (35 out of 46 patients) than for those involving *B. multivorans* (9 out of 19).

Mortality associated with *B. multivorans* infection was minimal in comparison with that associated with genomovar III (only 3 deaths occurred among patients with CF from whom *B. multivorans* was recovered). Two patients were infected with both *B. multivorans* and a genomovar III strain at the time of death. The third patient was culture-positive for *B. multivorans* once and died 4 years later, after being consistently culture-negative for *B. cepacia* complex. Of the 46 patients with CF from whom *B. cepacia* genomovar III strains were recovered, 20 died, and all of them harbored transmissible strains of the genomovar III-A lineage [21] (table 1). Eight pediatric patients died when infected with genomovar III-A strains before age 18 years; 12 adult patients died when infected after age 18 years. Six patients presented with symptoms of "*B. cepacia* syndrome" [2] prior to death (table 1). Of the remaining patients who died, all presented with terminal pulmonary decompensation and uncontrollable lung disease prior to death (table 1). The highest mortality was associated with genomovar III-A strain type ST04 (table 1). Fewer deaths in our patient population were associated with the highly transmissible ET12 *cblA*$^{+}$ strain type ST02 (table 1). No deaths were associated with infection by genomovar III-A strain type ST06 (table 1), even though this strain was associated with significant patient-to-patient spread.

Replacement of* B. multivorans *by genomovar III. Six patients were initially infected with *B. multivorans,* and each patient carried a unique strain (figure 2); in each case, the patients subsequently were infected with a genomovar III strain. Two of these patients were subsequently infected by a genomovar III-A strain of type ST04; one of them died shortly thereafter. Four other patients acquired strain ST04 by social interaction with the initial 2 infected patients. However, none of these patients acquired infection with the *B. multivorans* strains carried by these 2 patients, despite the fact that 1 of these patients remained coinfected with the *B. multivorans* strain and genomovar III strain ST04 for the next 2 years, until death. One patient acquired genomovar III strain ST01 4 years after testing positive for *B. multivorans* infection; this patient underwent double lung transplantation, survived *B. cepacia* septicemia, and is currently alive. Of the 3 remaining *B. multi-*

Table 1. Summary of epidemiological characteristics of patients with cystic fibrosis who had *Burkholderia cepacia* genomovar III or *Burkholderia multivorans* infection.

Parameter	Mean value ± SD or no. (%) of patients	
	Genomovar III (n = 46)	*B. multivorans* (n = 19)
Age (years) at acquisition	17.76 ± 1.11	13.2 ± 0.91 (P = .004)
Duration of infection (years)	2.29 ± 0.38	1.48 ± 0.61 (P = .25)
Transient infection	9 (20)	10 (53)
Cumulative mortality	20 (43)[a]	3 (16)[a]
Genomovar III, type 01	4 (44)	—
Genomovar III, type 02	5 (55)	—
Genomovar III, type 04	11 (70)	—
Terminal pulmonary decompensation	12 (26)	1 (5)[b]
B. cepacia syndrome	6 (13)	1 (5)[c]
Infected with *P. aeruginosa* prior to acquisition of *B. cepacia* complex	35 (76)	9 (47)
Duration of *P. aeruginosa* infection prior to acquisition of *B. cepacia* complex	5.4 ± 0.69	5.9 ± 1.15 (P = .70)

[a] Includes 2 patients who were colonized with both *B. multivorans* and genomovar III at death.
[b] Includes 1 patient who was colonized with both *B. multivorans* and genomovar III at death.
[c] Includes 1 patient who was colonized with both *B. multivorans* and genomovar III at death.

vorans–infected patients who became infected with genomovar III-A strains, 2 are alive but remain chronically infected with only the acquired genomovar III-A strain, and 1 is alive and currently *B. cepacia* complex–negative. Replacement of genomovar III-A infection by strains of other genomovars was not observed during the study period.

DISCUSSION

The clinical course and epidemiology of infection with *B. cepacia* complex among patients with CF in British Columbia appear to depend on the specific genomovar with which patients are infected. Strains of *B. cepacia* genomovar III were the most prevalent "novospecies" recovered and were also associated with epidemic spread, replacement of *B. multivorans* infection, cases of *B. cepacia* syndrome and high mortality. In contrast, evidence of patient-to-patient spread of *B. multivorans* was rarely observed over the 17-year study period. One sibling pair transiently shared the same *B. multivorans* strain; this is consistent with infection of CF sibling pairs with *P. aeruginosa* [26].

Whiteford et al. [27] reported an outbreak of *B. cepacia* among pediatric patients attending a clinic in Glasgow, which was associated with some deaths. The strain responsible for this epidemic has recently been classified as *B. multivorans* (genomovar II) [13], and hence the report by Whiteford et al. [27] contrasts with the lack of evidence of epidemic spread of *B. multivorans* in our study. The rapid spread of *B. multivorans* and short duration of the Glasgow epidemic [27] suggest that (1) transmission may have been due to factors other than patient contact or (2) the particular clone of *B. multivorans* may have greater potential for spread than strains we have encountered. Phylogenetic analysis of the *recA* gene of the index *B. multivorans* strain (C1576) in the Glasgow epidemic indicates that it is genetically distinct from *B. multivorans* strains present in the Vancouver CF population [21]. In agreement with the findings of Whiteford et al. [27], we observed that *B. multivorans* infection occurred predominantly in the pediatric CF patient population. Spread of *B. cepacia* strains among 4 adult patients with CF in Cardiff, Wales, was also reported [28]; the strain responsible for this outbreak was also subsequently found to be *B. multivorans* [18].

Finally, Segonds et al. [19] reported epidemic spread of 2 *B. multivorans* clones in multiple CF centers involving multiple patients and several cases of fatal septicemia. However, genetic typing of the latter strains was performed by PCR-ribotyping [19], which has limited discriminatory power for *B. cepacia* [7] and may have overrepresented the clonality of each outbreak. The behavior of *B. multivorans* strains therefore appears to vary greatly among different CF patient populations, but within the Vancouver CF patient population it did not present a significant infection control problem. Lack of nosocomial spread of *B. cepacia* strains was also observed in the Danish CF population [29]; it is interesting that these strains have been found to be *B. multivorans* (D. Henry and D. P. Speert, unpublished data), suggesting that other treatment centers have also encountered

B. multivorans strains with reduced risk of patient-to-patient spread. Transient *B. multivorans* infection was observed in half of the infected patients, suggesting that therapy may clear this organism or that it may be shed spontaneously in pediatric patients; transient infection was rarely observed with strains of genomovar III.

Genomovar III was the predominant species infecting patients in our study, corroborating other culture collection–based observations [13, 18]. Patient-to-patient spread of *B. cepacia* among Vancouver patients with CF was also specifically associated with genomovar III strains, of the genomovar III-A *recA* subgroup [21]. These strains presented a significant infection control problem, capable of replacing infection with *B. multivorans* and leading to poor prognosis. Ledson et al. [30] also recently reported cross-infection between patients with CF who were infected with *B. cepacia.* All 5 cases of cross-infection were due to a *B. cepacia* strain that encoded the cable pilus and belonged to the United Kingdom epidemic strain type [9, 18]. We report that replacement of infection is not solely linked to the *cblA*$^+$ strains; moreover, at our centers it was specifically associated with genomovar III-A strains [21], which encode the *B. cepacia* epidemic strain marker.

Prior to the occurrence of cross-infection, each CF patient examined by Ledson et al. [30] was infected with a unique *B. cepacia* strain; these data concur with our observations. Epidemic spread of genomovar III-B strains was not observed within the Vancouver CF patient population; however, strains of this genetic lineage have been associated with outbreaks of infection among patients with CF at several other centers [18]. These data also suggest that strains with the greatest potential for epidemic spread should now be sought by means of tests for the identification of genomovar [13, 21]; we observed that genomovar III-A was an excellent marker of epidemic spread. Other markers, such as *cblA* or *B. cepacia* epidemic strain marker, which are commonly associated with this "species" type but of which the natural prevalence and stability among the *B. cepacia* complex have not been fully determined, should be used as well [21].

In conclusion, we present the first systematic study of *B. cepacia* complex infection among a defined CF patient population. Infection with strains of genomovar III-A was associated with apparent patient-to-patient spread and high mortality. Nonetheless, one cannot draw firm conclusions about the attributable risk from infection with genomovar III versus *B. multivorans,* since the age of patients in the 2 groups was quite different. A case-control study to enable risk assessment is currently under way.

We have not encountered significant clinical or infection control problems in patients infected with *B. multivorans,* but other centers have reported more widespread transmission of strains of this new species [19, 27, 28]. Because our study is the first to systematically evaluate a CF patient population for the genomovar status of *B. cepacia* infection, it is perhaps too early to make definitive judgements on the risk that each genomovar poses to patients with CF. However, replacement of *B. multivorans* with strains of genomovar III-A is a significant clinical problem. Other groups have reported this phenomenon for the *cblA*$^+$ genomovar III-A strain [30]. In light of this apparent epidemiological difference between *B. multivorans* and genomovar III, we recommend that patients infected with *B. cepacia* genomovar III strains should not be cohorted with patients infected with other genomovars of the *B. cepacia* complex.

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