Epidemiology and Clinical Course of *Burkholderia cepacia* Complex Infections, Particularly Those Caused by Different *Burkholderia cenocepacia* Strains, among Patients Attending an Italian Cystic Fibrosis Center

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In this study, the epidemiology of *Burkholderia cepacia* complex (Bcc) recovered from the sputum of 75 patients attending the Genoa Cystic Fibrosis (CF) Center at the Gaslini Children's Hospital (Genoa, Italy) was investigated, and the clinical course of the CF patients infected with the different species and genomovars of Bcc was evaluated. All isolates were analyzed for genomovar status by *recA* gene polymorphism and subsequently random amplified polymorphic DNA fingerprinting. *Burkholderia cenocepacia* is the predominant species recovered from the CF patients infected with Bcc at the Genoa CF Center. Of the other eight species comprising the Bcc, only a few isolates belonging to *B. cepacia* genomovar I, *Burkholderia stabilis*, and *Burkholderia pyrrocinia* were found. Of the four *recA* lineages of *B. cenocepacia*, most patients were infected by epidemic strains belonging to lineages IIIA and IIID, whereas only a few patients harbored IIIB strains. Patient-to-patient spread of Bcc among CF patients was mostly associated with *B. cenocepacia*, in particular with strains belonging to *recA* lineages IIIA and IIID. The mortality of CF patients infected with Bcc at the Genoa CF Center was significantly higher than mortality among CF patients not infected with Bcc. All of the deaths were associated with the presence of *B. cenocepacia*, except the case of a patient infected with *B. cepacia* genomovar I. Within *B. cenocepacia*, infection with epidemic strains belonging to lineages IIIA and IIID was associated with higher rates of mortality than was infection with lineage IIIB strains. No significant differences in lung function, body weight, and mortality rate were observed between patients infected with epidemic strains belonging to either *B. cenocepacia* IIIA or *B. cenocepacia* IIID.

Bacteria belonging to the *Burkholderia cepacia* complex (Bcc) are important opportunistic human pathogens in persons with cystic fibrosis (CF) or chronic granulomatous disease. Generally, CF patients colonized with Bcc show a significantly reduced long-term survival compared to CF patients not colonized with Bcc (7). Lung infections with Bcc in certain patients with CF result in progressive, invasive, fatal bacteremic disease, the so-called “cepacia syndrome” (15). Furthermore, these bacteria have a potential for patient-to-patient spread, both within and outside the hospital, raising questions about optimal measures for infection control.

Advances in the taxonomy of Bcc revealed that it comprises at least nine phenotypically similar species or genomovars, i.e., *B. cepacia* (genomovar I), *Burkholderia multivorans* (genomovar II), *Burkholderia cenocepacia* (genomovar III), *Burkholderia stabilis* (genomovar IV), *Burkholderia vietnamiensis* (genomovar V), *Burkholderia dolosa* (genomovar VI), *Burkholderia ambifaria* (genomovar VII), *Burkholderia anthina* (genomovar VIII), and *Burkholderia pyrrocinia* (genomovar IX), which can

be differentiated on the basis of molecular and biochemical tests (4, 8, 17, 18, 19). The taxonomic complexity of Bcc raises many questions about the clinical significance of each species belonging to the complex; so far, the risk attributable to infection with the different genomovars is not well understood. Understanding this risk is vital for CF treatment centers to improve infection control policies and therapeutic approaches for Bcc-infected individuals. Several studies (9, 12, 14) pointed out that *B. multivorans* and *B. cenocepacia* account for the majority of isolates from CF patients. In particular, it has been observed that *B. cenocepacia* comprises the most virulent and transmissible bacterial clones; in fact, certain strains belonging to this species are associated with a poor clinical course and high mortality among CF patients (12).

Recent works showed that *B. cenocepacia* is genetically highly heterogeneous, being composed of at least four phylogenetic lineages (IIIA, IIIB, IIIC, and IIID) based on the polymorphism of the *recA* gene (11, 18). So far, *recA* lineages IIIA and IIID have been detected exclusively in clinical specimens (11, 18), whereas *recA* lineage IIIC has been recovered only from soil (18). Only *recA* lineage IIIB has been found in both clinical specimens and natural habitats (11, 18; S. Tabacchioni, A. Bevivino, C. Dalmastrì, and L. Chiarini, unpublished

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data), where it can be recovered in high numbers (5). At present, of the three lineages found among CF patients, limited knowledge is available about the prevalence and epidemiology of lineages IIIA and IIIB (1, 12, 14) and no epidemiological and clinical data are available for *recA* lineage IIID.

The purpose of this study was to characterize the natural history of infection with strains of the different species and genomovars of *Bcc* among patients attending the Genoa CF Center of Gaslini Children's Hospital in Genoa, Italy, and to determine if there is genomovar-specific disparity in the transmission and clinical outcome for infected patients, with particular attention to the strains belonging to the different *recA* lineages of *B. cenocepacia*. We have collected all *Bcc* isolates from patients with CF who have attended our center since 1984. All isolates were evaluated for genomovar status by *recA* gene polymorphism and, subsequently, randomly amplified polymorphic DNA (RAPD) fingerprinting to (i) assess the prevalence of *Bcc* species and genomovars in CF patients and (ii) study the clinical, epidemiological, and genetic relatedness of *Bcc* isolates in light of the most recent taxonomic developments. Mortality, lung function, and body weight changes of patients infected with strains belonging to different *recA* lineages of *B. cenocepacia* were also investigated.

MATERIALS AND METHODS

Bacterial strains and CF study population. *Bcc* isolates were recovered from patients with CF attending the Genoa CF Center located inside the Gaslini Children's Hospital (Genoa, Italy). Data collected from 1984 through 2001 are presented in this study. Three hundred twenty-six patients were in regular follow-up during this period. A total of 195 *Bcc* isolates were recovered from 75 patients. Sputum cultures were performed at every clinic visit (around 3-month intervals) for each patient. *Bcc* isolates were cultured from sputum, identified, and stored as described elsewhere (2). A patient was considered free of *Bcc* if three consecutive cultures over a period of >3 weeks or two separate hospitalizations failed to yield *Bcc* bacteria. Infection with *Pseudomonas aeruginosa* was also recorded for each patient, and culture and identification of this organism were carried out as described elsewhere (13).

RAPD typing. Each *Bcc* isolate was genetically typed by RAPD analysis as described by Mahenthiralingam et al. (10). RAPD fingerprints were compared by eye and computer software (one-dimensional image analysis software; Kodak, Rochester, N.Y.). Reproducibility was verified by RAPD fingerprinting of each isolate at least four times in independent experiments. The following criteria were used to define a strain or a subtype: isolates were considered unrelated (distinct strains) if there were more than six fragment (band) differences between RAPD profiles; isolates were considered related (subtypes of a common strain) if there were only three to six band differences between RAPD profiles; isolates were considered closely related, and thus belonging to the same strain, if there were no more than two band differences between RAPD profiles. A numerical strain type 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.

Genomovar analysis. The genomovar status of each isolate was determined by restriction fragment length polymorphism analysis of the *recA* gene and confirmed by means of PCR of the *recA* gene performed with genomovar-specific primers when available, according to the procedures previously described (2, 11, 17, 18).

PCR amplification of *esmR* and *cblA* genes. The 1.4-kb *esmR* sequence coding for the *B. cepacia* epidemic strain marker (BCESM) was amplified with the specific primers BCESM 1 and BCESM 2 by following the procedure described by Mahenthiralingam et al. (10). The 664-bp *cblA* DNA coding for the cable pilus was amplified with the primers CBL1 and CBL2 according to the procedure described by Clode et al. (3).

Comparison of clinical courses of patients infected with *recA* lineages IIIA and IIID. A subset of IIIA-infected patients was matched to a subset of IIID-infected patients according to age (± 1 year), infection with *P. aeruginosa* at *Bcc* acquisition, and gender. Changes in lung function (FEV₁) and body weight in the 2-year postacquisition period and mortality in the long-term period of the two subsets

TABLE 1. Incidence and prevalence rates of *Bcc* respiratory infections among CF patients over the study period^a

Yr	<i>Bcc</i> incidence	<i>Bcc</i> prevalence
1984–1988	4.78	10.87
1989–1993	4.77	18.98
1994–1997	2.17	11.05
1998–2001	0.48	7.50

^a Incidence and prevalence are reported as percentages of infected patients by 4- to 5-year periods.

were compared. Spirometry (FEV₁) and body weight measurements performed during stable outpatient clinic visits were collected for each patient of the two subsets.

Statistical analysis. Comparison of the mean age of patients at the time of *Bcc* acquisition and mean duration of colonization was performed by one-way analysis of variance. Comparison of mortality of the *Bcc*-positive and *Bcc*-negative CF population as well as within matched patient pairs infected with either IIIA or IIID strains was performed by using the chi-square test. Changes in the percentage of predicted FEV₁ and body weight in IIIA- and IIID-matched patients were compared by the Wilcoxon signed rank test. All analyses were carried out with GraphPad Prism software.

RESULTS

Prevalence of *Bcc* infection in patients with CF. *Bcc* isolates were recovered from 75 patients with CF during the study period, for a prevalence of 23% (75 of 326 patients). The incidence and prevalence rates over the study period are shown in Table 1. *B. cenocepacia* isolates were recovered from 68 patients (90% of *Bcc*-infected patients). Of the *B. cenocepacia*-infected patients, 29 were infected with isolates belonging to *recA* lineage IIIA (43% of *B. cenocepacia*-infected patients), 7 were infected with isolates belonging to *recA* lineage IIIB (10% of *B. cenocepacia*-infected patients), and 34 were infected with isolates belonging to *recA* lineage IIID (50% of *B. cenocepacia*-infected patients). Three patients were infected with a strain of *B. cenocepacia* (see below) which could not be assigned to any *recA* lineage. Five *B. cenocepacia*-infected patients harbored isolates belonging to more than one *recA* lineage; of these, 3 patients were infected with strains belonging to *recA* lineages IIIA and IIID, 1 patient was infected with isolates belonging to *recA* lineages IIIA and IIIB, and 1 patient was infected with isolates belonging to *recA* lineages IIIB and IIID. *B. stabilis* was recovered from 5 patients (6% of *Bcc*-infected patients). Of the remaining patients, 1 was infected with a *B. cepacia* genomovar I strain and 1 was infected with a *B. pyrocinia* strain. Data for patients with CF who were newly infected with *Bcc* bacteria were analyzed in 4- or 5-year blocks and are presented in Fig. 1.

Molecular epidemiology of *B. cenocepacia*. Four strain types (01, 02, 03, and 04) and eight unique genetic fingerprints were found among the 68 patients with CF who were infected with *B. cenocepacia* (Table 2). Two strain types (01 and 03) were each recovered from 26 patients or more and were presumed to have spread from patient to patient (Fig. 2).

Strain type 01, belonging to *recA* lineage IIIA, infected 26 patients. No subtypes were recognized among isolates recovered from these patients. The remaining three patients infected by *recA* lineage IIIA harbored strains which each had a unique genetic fingerprint. All isolates belonging to the epidemic IIIA strain harbored the BCESM marker. Of the three

FIG. 1. Prevalence of infection with each Bcc species among CF patients over the study period. Patients newly infected with strains belonging to *B. cenocepacia* IIIA (column A), *B. cenocepacia* IIIB (column B), *B. cenocepacia* IIID (column D), strain type 04 (column III), *B. stabilis* (column IV), *B. cepacia* genomovar I (column I), and *B. pyrrocinia* (column IX) are plotted by 4- to 5-year blocks. Bars denoting patients infected with a strain shared by two or more patients are white to indicate the occurrences of patient-to-patient transmission.

IIIA isolates with unique genetic fingerprints, only one harbored the BCESM marker. All IIIA isolates were *cblA* negative.

recA lineage IIIB isolates were recovered from 7 patients, 5 of which were infected with genetically distinct strains that did not spread to other patients during the study period. The remaining two patients were infected by the same strain type 02. RAPD profiles of strain type 02 and unique fingerprints are shown in Fig. 2. Four of the 7 isolates harbored the BCESM marker, and all isolates were *cblA* negative. Strain type 02 lacked BCESM.

All *recA* lineage IIID isolates, which were recovered from 34 patients, belonged to the same strain type 03. Within this strain type, two subtypes, named A and B, could be recognized (Fig. 2). Subtypes A and B infected 7 and 27 patients, respectively,

TABLE 2. RAPD strain types and unique genetic fingerprints of *B. cenocepacia* and *B. stabilis* and their characterization^d

Species or <i>recA</i> lineage (n)	RAPD strain type (n)	No. of unique genetic fingerprints	BCESM ^a	Cable pilus ^a
<i>B. cenocepacia</i> IIIA (29)	01 (26)	3	27	0
<i>B. cenocepacia</i> IIIB (7)	02 (2)	5	4	0
<i>B. cenocepacia</i> IIID (34)	03 (34) ^b	0	0	0
<i>B. cenocepacia</i> ^c (3)	04 (3)		0	0
<i>B. stabilis</i> (5)	05 (3)	2	0	0

^a Number of patients whose isolates had BCESM or cable pilus.

^b Of the 34 *B. cenocepacia* IIID-infected patients, 7 were infected with subtype A and 27 were infected with subtype B.

^c Isolates belonging to this strain type could not be assigned to any *recA* lineage of *B. cenocepacia*.

^d n, number of patients infected.

and were presumed to have spread from patient to patient. All isolates were *cblA* and *esmR* negative (Table 2).

Three patients were infected with the same strain type 04 (Fig. 2). Isolates belonging to this strain type could not be assigned to any *recA* lineage of *B. cenocepacia*. All isolates were *cblA* and *esmR* negative (Table 2).

To assess whether patients could be infected with different strains (genotypes) or subtypes of the same *recA* lineage, at least two isolates were typed (first and last available isolates) for all *B. cenocepacia*-infected patients and as many as 5 to 6 isolates recovered at 0.5- to 1.5-year intervals covering the entire infection period were typed for a subset of patients (five IIIA-, three IIIB-, and five IIID-infected patients). Results showed that all patients were infected by only one genotype or subtype per *recA* lineage (data not shown).

Using the genomovar status of each strain, a correlation between the risk of patient-to-patient cross-infection (number of cases associated with strains belonging to a particular *recA* lineage compared to remaining cases associated with strains belonging to the other *recA* lineages of *B. cenocepacia*) and genomovar status was made (1). The highest relative risk of cross-infection was associated with IIID isolates (1.13), whereas the risk associated with IIIA isolates was slightly lower (0.78); in contrast, the risk of cross-infection associated with IIIB isolates was extremely low (0.05).

Segregation (separate rooms and treatment areas) and intensive education of Bcc-infected patients with CF and caregivers were introduced in September 1993. After that date, new acquisitions of transmissible IIIA strain type 01 were limited to a single case (Fig. 1); other acquisitions concerned isolates representing unique genetic fingerprints. A reduction in the number of new acquisitions, although not so marked as

FIG. 2. RAPD fingerprints of *B. cenocepacia* IIIA strain type 01 (I), *B. cenocepacia* IIIB strain type 02 and unique fingerprints (II), *B. cenocepacia* IIID strain type 03 subtypes A and B (III), *B. cenocepacia* strain type 04 (IV), and *B. stabilis* strain type 05 and unique fingerprints (V). Lane M, 123-bp molecular size marker ladder; ST, strain type.

that of IIIA strain type 01, was also observed for the transmissible IIID strain type 03 (Fig. 1). In the case of *recA* lineage IIIB, acquisitions of bacteria with unique genetic fingerprints occurred all throughout the study period, whereas the unique case of cross-infection was observed after September 1993. Also, patients harboring strain type 04 were infected after September 1993. However, it has to be noted that after the introduction of segregation measures no evidence for overlapping hospitalizations, clinic visits, and hospital services of patients involved in cross-infection cases was found.

Molecular epidemiology of *B. stabilis*. One strain type (05) and two unique genetic fingerprints were found among the five patients with CF who were infected with *B. stabilis* (Table 2). The strain type 05 was recovered from three patients. Due to the transience of infection in these patients, only two patients infected with strain type 05 had overlapping infection periods; however, no overlapping hospitalizations and clinic visits for these two patients occurred. Moreover, the first cultures positive for *B. stabilis* were obtained after the introduction of segregation measures. The RAPD profiles of strain type 05 and the unique genetic fingerprints are shown in Fig. 2. All isolates were *cblA* and *esmR* negative (Table 2).

Clinical course of patients infected with *B. cenocepacia*. A summary of the epidemiological characteristics of CF patients infected with strains belonging to the different *recA* lineages of *B. cenocepacia* is presented in Table 3. Patients infected with IIIA isolates were generally younger at the time of acquisition than those infected with IIIB and IIID isolates. The mean duration of infection recorded within the study period was not statistically different for the three groups of patients. Most bacterial isolates caused chronic infection, although the percentage of patients transiently infected with IIID and IIIB

isolates was higher than that of patients transiently infected with IIIA isolates. Isolates belonging to strain type 04, which has not been assigned to any *recA* lineage, caused the chronic infection of one patient and the transient infection of two other patients.

Of the 75 patients who were colonized with Bcc, 33 (44%) had died from CF-related disease by December 2001 compared to 52 of 326 patients (15.95%) from the entire CF population, showing that Bcc-associated mortality was significantly higher than that associated with the entire CF population ($P < 0.01$). As far as *B. cenocepacia*-infected patients are concerned, 32 of 68 (47%) had died by December 2001; in particular, the mortality of IIIB-infected patients was minimal (14%) in comparison with IIIA- and IIID-infected patients (58 and 44%, respectively). As far as the IIID strain type 03 is concerned, it is worth noting that all deaths were associated with subtype B. No death was associated with strain type 04.

Since the mean age of patients infected with isolates belonging to the two major *recA* lineages IIIA and IIID was significantly different, 15 patients infected with IIIA strain type 01 were matched to 15 patients infected with IIID strain type 03 subtypes A and B according to gender, age, and infection with *P. aeruginosa* at Bcc acquisition (Table 4). Within pairs, the mean difference between birth dates was 3.07 ± 2.19 years. In this way, lung function and body weight changes as well as mortality were compared within each pair. Patients infected with IIID strain type 03 showed slightly lower postacquisition mortality than patients infected with IIIA strain type 01 (73.31 and 93.34%, respectively; $P = 0.22$) (Table 4). No significant differences concerning changes in pulmonary function and body weight in the 2-year postacquisition period between the

TABLE 3. Summary of epidemiological characteristics of CF patients infected with *B. cenocepacia* (IIIA, IIIB, and IIID strains) and *B. stabilis*

Parameter	Result (mean $\pm$ SD or no. [%] of patients) for ^a :			
	<i>B. cenocepacia</i> IIIA (n = 29)	<i>B. cenocepacia</i> IIIB (n = 7)	<i>B. cenocepacia</i> IIID (n = 34) ^b	<i>B. stabilis</i> (n = 5)
Age (yr) at acquisition	12.14 $\pm$ 6.45*	15.71 $\pm$ 4.93*†	17.09 $\pm$ 8.58† 18.67 $\pm$ 8.59 ^c 16.75 $\pm$ 8.70 ^d	18.40 $\pm$ 4.33†
Duration of infection (yr)	6.05 $\pm$ 4.72†	5.07 $\pm$ 4.46†	6.67 $\pm$ 4.02† 6.08 $\pm$ 3.29 ^c 6.80 $\pm$ 4.21 ^d	1.35 $\pm$ 1.75*
Transient infection	1 (3.22)	1 (14)	6 (17) 0 ^c 6 (22) ^d	5 (100)
Cumulative mortality	17 (58)	1 (14)	15 (44) 0 ^c 15 (55) ^d	0
Infection with <i>P. aeruginosa</i> prior to acquisition of Bcc	24 (77)	2 (28)	27 (79) 4 (57) ^c 23 (85) ^d	3 (60)

^a Within each row, values followed by the same symbol (*, †) are not significantly different according to Fisher's protected least significant difference ($P > 0.05$).

^b Of the 34 *B. cenocepacia* IIID-infected patients, 7 were infected with subtype A and 27 were infected with subtype B of strain type 03.

^c Values refer to subtype A.

^d Values refer to subtype B.

two groups of patients were observed ($P = 0.19$ and 0.48 , respectively) (Table 4).

In our series of patients, cepacia syndrome was never observed and was only suspected in one patient. One year after Bcc acquisition, he showed acute pulmonary deterioration with signs of sepsis and death within 2 weeks, but we were not able to detect Bcc in the bloodstream, and no autopsy data are available. This patient was infected with *B. cenocepacia* IIID strain type 03 subtype B.

Clinical course of patients infected with *B. stabilis*, *B. pyrrocinia*, and *B. cepacia* (genomovar I). All patients from which *B. stabilis* was recovered were only transiently infected with this species; no deaths have occurred among these patients. One patient was chronically infected with *B. pyrrocinia*; another one, infected with *B. cepacia* genomovar I, died 3 years after being infected.

DISCUSSION

B. cenocepacia is the predominant species recovered from patients infected with Bcc at the Genoa CF Center. Of the remaining eight species, only a few isolates belonging to *B. cepacia* genomovar I, *B. stabilis*, and *B. pyrrocinia* were found.

The high prevalence of *B. cenocepacia* among CF patients is a common feature already observed in other studies (1, 9); in contrast, it is worth noting that no strains belonging to *B. multivorans* have been isolated from the sputum of the patients attending the Genoa CF Center. It is well known that in most North American and European CF populations, *B. multivorans* is one of the dominant Bcc species (9, 14); moreover, in a recent Italian study, *B. multivorans* has been recovered, although at low numbers (1). In addition, *B. ambifaria*, one of the prevalent Bcc species in soil and the rhizosphere of crop plants (5, 6), has not been found among the patients attending the Genoa CF Center.

Of the four *recA* lineages comprising *B. cenocepacia*, most patients harbored one of two epidemic strains belonging to lineages IIIA or IIID, whereas few patients were infected with *recA* lineage IIIB. The presence of these epidemic strains explains why *recA* lineages IIIA and IIID are dominant at the Genoa CF Center. It is worth noting that the epidemic IIIA strain as well as the other IIIA strains with unique genetic fingerprints are not genetically related to the highly transmissible ET12 lineage (data not shown). The high prevalence of *recA* lineage IIIA and low prevalence of *recA* lineage IIIB among CF patients seem to be characteristic of the Italian CF

TABLE 4. Matched variables and changes in lung function, body weight, and mortality of two subsets of IIIA- and IIID-infected patients^a

Parameter	Result for patients infected with lineage:		P value
	IIIA	IIID	
Mean age (yr) $\pm$ SD (range)	13.93 $\pm$ 6.32 (4–24)	14.40 $\pm$ 6.76 (5–25)	
No. of patients infected with <i>P. aeruginosa</i>	13	13	
% Change $\pm$ SD in:			
Predicted FEV ₁	−0.54 $\pm$ 14.18	−5.12 $\pm$ 17.27	0.19
Weight ^b	14.36 $\pm$ 18.62	13.64 $\pm$ 14.23	0.48
% Mortality	93.34	73.31	0.22

^a In the total sample (IIIA- and IIID-infected patients), there were 12 males and 18 females.

^b Changes in lung function and body weight are for a 2-year postacquisition interval.

centers (1). A similar distribution of the two *recA* lineages has been observed also in Canada (12). In contrast, in the United States, *recA* lineage IIIB is by far the most prevalent lineage among CF patients (9). Few data are available about the prevalence of *recA* lineage IIID in other CF centers. Data presented by Vandamme et al. (18) suggest a geographically limited diffusion of this *recA* lineage, in contrast with the broader diffusion of *recA* lineages IIIA and IIIB; in fact, so far IIID strains have been isolated only in Sweden, Argentina, and Italy. In Italy, the presence of IIID strains is not limited to the Genoa CF Center, as it has also been detected at other CF centers (1, 18).

The presence of strains common to multiple patients strongly suggests person-to-person transmission, as indicated by the decreased incidence after the introduction of increased infection control (see below). However, acquisition from a common source cannot be ruled out. This probable person-to-person transmission of Bcc among patients attending the Genoa CF Center was mostly associated with *B. cenocepacia* strains, particularly with the *recA* lineages IIIA and IIID. Before cohorts of Bcc-infected patients were introduced in 1993, the two highly transmissible IIIA and IIID strains, i.e., strain types 01 and 03, infected more than 80% of Bcc-infected patients. In contrast, all IIIB isolates showed unique genetic fingerprints, except one isolate which was shared by two patients. The transmissibility of *B. cenocepacia* has been often associated with the presence of the BCESM marker (10). In this study, we found that the epidemic IIIA strain possessed the BCESM marker, whereas the epidemic IIID strain lacked it. These findings confirm that the BCESM marker is not a very reliable marker for transmissibility within *B. cenocepacia*, as already suggested by LiPuma et al. (9). It is worth noting that the other marker commonly associated with virulence, particularly with *recA* lineage IIIA strains, i.e., the cable pilus, was absent in all *B. cenocepacia* strains.

The introduction of segregation measures in 1993 proved effective in limiting the spread of the transmissible strains among the patients attending the Genoa CF Center. Nowadays, the incidence of Bcc and, in particular, of *B. cenocepacia* is highly reduced, and new acquisitions mainly concern bacteria with unique genetic fingerprints. Nonetheless, several apparent cross-infection cases concerning *B. cenocepacia* and *B. stabilis* strains occurred after infection control measures had been introduced in 1993. Because of the stringency of the adopted infection control measures, it is conceivable that these cases occurred outside the CF center. As far as *B. stabilis* is concerned, Vandamme et al. (16) observed that this species is characterized by a remarkable genomic stability such that epidemiologically unrelated isolates may show very similar genetic fingerprints; therefore, it is difficult to establish whether the Genoa strain type 05 has really spread somehow from one patient to another or whether independent infection of three patients by genetically similar but epidemiologically unrelated strains has occurred.

The mortality of CF patients infected with Bcc was significantly higher than mortality among CF patients not infected with Bcc at the Genoa CF Center. In our study, most deaths of Bcc-infected patients were associated with the presence of epidemic *B. cenocepacia* strains. These observations suggest that certain strains with the potential to spread may represent

a greater hazard to humans than others. The clinical course of patients infected with epidemic IIIA or IIID strains (strain types 01 and 03) was further investigated to get a better understanding of the clinical significance of these two groups of bacteria, present in high numbers at the Genoa CF Center. Since the mean age in the two whole groups of patients was quite different, two subsets of IIIA- and IIID-infected patients, matched according to gender, age, and infection with *P. aeruginosa* at Bcc acquisition, were compared to each other as far as lung function, body weight, and mortality were concerned. According to our data, the two strains had a similar effect on the clinical course of infected patients.

In conclusion, our study confirms the prevalence of *B. cenocepacia* among Bcc-infected CF patients and the high percentage of mortality associated with this species. The major role of an epidemic strain belonging to the recently identified *recA* lineage IIID in spreading Bcc infection among CF patients has been recognized for the first time.

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