The Journal of Microbiology, June 2007, p. 275-279
Vol. 45, No. 3

NOTE

Burkholderia cepacia Complex Infection in a Cohort of Italian Patients with Cystic Fibrosis

Antonietta Lambiase[1]*, Valeria Raia[2], Stefania Stefani[3], Angela Sepe[2], Pasqualina Ferri[2], Paolo Buonpensiero[2], Fabio Rossano[1], and Mariassunta Del Pezzo[1]

[1]*Department of Biology and Pathology Cellular and Molecular "Luigi Califano"*
[2]*Department of Pediatrics, Regional Cystic Fibrosis Center, University of Naples "Federico II", Pansini Street 5-80131, Italy*
[3]*Department of Microbiological and Gynecological Sciences, University of Catania, Italy*

(Received March 7, 2007 / Accepted June 1, 2007)

The aims of this study were to detect *Burkholderia cepacia* complex (Bcc) strains in a cohort of Cystic Fibrosis patients (n=276) and to characterize Bcc isolates by molecular techniques. The results showed that 11.23% of patients were infected by Bcc. *Burkholderia cenocepacia* lineage III-A was the most prevalent species (64.3%) and, of these, 10% was cblA positive and 50% esmR positive. Less than half of the strains were sensitive to ceftazidime, meropenem, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole. About half of the strains (41%) had homogeneous profiles, suggesting cross-transmission. The infection by *B. cenocepacia* was associated to a high rate of mortality (p=0.01).

Keywords: cystic fibrosis, *Burkholderia cepacia* complex, rec-A gene, PFGE

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative bacteria reported as opportunistic pathogens in Cystic Fibrosis (CF) patients and can be associated to a greater deterioration in lung function (LiPuma, 1998). It is accepted that different clinical outcomes can occur in CF patients with Bcc infection, ranging from a chronic asymptomatic infection to an acceleration of pulmonary decline up to death identified as "cepacia syndrome" (Tablan *et al.*, 1985; Gilligan, 1991; LiPuma, 2005). Bcc is described as a group of at least nine genetically distinct organisms (genomovar) (Coenye *et al.*, 2001; Vandamme *et al.*, 2002; Vermis *et al.*, 2004; Mahenthiralingam and Vandamme, 2005): *B. cepacia* (genomovar I), *B. multivorans* (genomovar II), *B. cenocepacia* (genomovar III), *B. stabilis* (genomovar IV), *B. vietnamiensis* (genomovar V), *B. dolosa* (genomovar VI), *B. ambifaria* (genomovar VII), *B. anthina* (genomovar VIII), and *B. pyrrocinia* (genomovar IX). For *B. cenocepacia*, at least four lineages have been recognized (IIIA, IIIB, IIIC, and IIID) (Mahenthiralingam *et al.*, 2000; Vandamme *et al.*, 2002). Two genetic markers have been associated to the high transmissibility and virulence of some Bcc strains among CF patients: the cable pilin gene (cblA gene) (Goldstein *et al.*, 1995; Sajjan *et al.*, 1995; Sun *et al.*, 1995) and the *B. cepacia* epidemic strain marker (esmR) (Mahenthiralingam *et al.*, 1997).

The aims of this study were to investigate the distribution and molecular properties of Bcc strains isolated in a cohort of CF patients and to identify some clinical outcomes closely related to Bcc infection. Therefore, a PCR-RFLP method to characterize the organisms in Bcc was used, the transmissibility markers (cblA, esmR) were detected and DNA-fingerprinting was carried out.

The study was undertaken between January 2001 and December 2003 on 276 CF patients (131 males, 145 females, mean age 16.21 years, range 0.5-50 years), receiving care at the Regional CF Centre of Naples University "Federico II". Patients over 6 years of age had at least one lung function evaluation as measured by forced expiratory volume in 1 sec (FEV1) during each year of observation at clinic examination and/or hospital discharge expressed as a mean value of a three year survey; oxygen saturation measurement was noted for each examination. Chronic Bcc infection was defined as persistent presence of three Bcc positive cultures for at last 6 consecutive months (Frederiksen *et al.*, 1999). Similarly, chronic *Pseudomonas aeruginosa* (PA) infection was defined. Co-infection was defined as a positive sputum culture for more than one organism. Bcc infected patients were characterized for age and duration of infection by Bcc, co-colonization with PA, lung function and death. Comparison between the effect of PA and Bcc on decline in lung function was performed during the observation period. For the definition of multidrug-resistance, we used the one provided by the CF Foundation for PA strains, extending it to Bcc strains (Saiman *et al.*, 1996).

To isolate Bcc strains, sputum samples were incubated on BCSA agar (bioMérieux) at 37°C for up to 96 h. Isolates were identified by the Phoenix system (Becton Dickinson, BD); the API 20 NE identification system (bioMérieux) was used to confirm the identification.

* To whom correspondence should be addressed.
(Tel) 39-81-746-2530; (Fax) 39-81-746-2530
(E-mail) alambias@unina.it

For the antimicrobial sensibility test, the disk-diffusion method (Kirby-Bauer) and microbroth dilution assay using an automated system (Phoenix, BD) were used. Interpretative criteria for susceptibility were in accordance with the National Committee for Clinical Laboratory Standards criteria (NCCLS, M7-A4 document 1997). For the disk-diffusion method, the following drugs were assayed: amikacin, aztreonam, cefepime, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, imipenem, meropenem, gentamicin, levofloxacin, piperacillin, piperacillin-tazobactam and trimethoprim-sulfamethoxazole. The microbroth dilution assay was performed for ampicillin-sulbactam and tetracycline as well as those used for the disk-diffusion method.

DNA from Bcc bacterial cultures was extracted with phenol/chloroform. To characterize the strains in the Bcc, the Bcc rec-A gene was amplified using the primers bcr1 and bcr2 and the amplicons were digested with *Mnl*I and *Hae*III (New England Biolabs) restriction endonucleases, as previously described (McDowell *et al.*, 2001).

The presence of the cblA and esmR markers was detected using the primers cblA1 and cblA2 and the primers esmR1 and esmR2, as previously described (Sajjan *et al.*, 1995; Mahenthiralingam *et al.*, 1997).

DNA fingerprinting was carried out as previously described (Grothues *et al.*, 1988). DNA was digested with *Spe*I (New England Biolabs) and macrorestriction fragments separated using CHEF III (Biorad) at 10°C for 19 h, start time of 5 s and end-pulse time of 35 s, field strength of 6 V/cm. Macrorestriction was also carried out for three *B. cenocepacia* strains: one strain isolated from CF patients in Sicily, called the epidemic Sicilian strain (strain CG in Fig. 1), and two strains isolated from CF patients in Vancouver (strains 5393 and 6433 in Fig. 1) (Agodi *et al.*, 2001). These strains were used to establish if there was a clonal likeness between our strains and the strains recovered in Sicily and Vancouver. Fragment patterns were compared according to the criteria of Tenover (Tenover *et al.*, 1995).

In this study we compared patients chronically infected with Bcc (case group) with patients chronically infected with PA (control group). Both groups were comparable for age, gender, mean duration of infection, type of CFTR mutation, mean FEV1, chronic co-infection with meticillin-resistant *Staphylococcus aureus* (MRSA) and with multi-drug resistant Gram-negative bacilli, such as *Stenotrophomonas maltophilia* (SM).

Statistical analysis was carried out with t-test, chi-square test and Mann-Whitney test, using the SPSS software package. A p value of <0.05 was considered significant.

In the study period, 31 of the 276 patients (11.23%; 11 males, 20 females; mean range 21.5 years) were identified as chronically Bcc infected. Molecular analysis of the CFTR mutation is shown in Table 1. Mean duration of Bcc infection was 5.55 years (range 6 months-17 years). The level of bacterial identification given by the two systems (Phoenix and API 20 NE) used in this study was "*Burkholderia cepacia*". On the basis of the identification of the rec-A gene, *B. cenocepacia* was the most prevalent pathogen, being isolated from 28 patients. The remaining three patients harbored strains of *B. cepacia*, *B. stabilis*, and *B. vietnamiensis*. Out of the 28 patients chronically infected

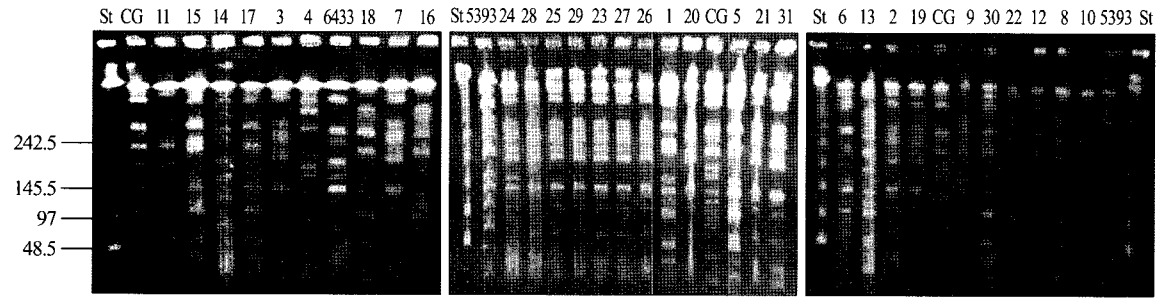

Fig. 1. PFGE fingerprinting of Bcc isolates. The numbers indicate each strain in the study (data shown in results and Table 2). Molecular size markers (a concatemer ladder of lambda phage DNA) were run in lane St. Sizes are indicated in kilobases.

Table 1. Type of CFTR mutation, co-infection by PA, clinical complications and number of patients who died from Bcc infection

Genomovar-type	No. of patients	CFTR mutation (type and no. of patients)	No. of patients co-infected by PA	No. of patients with IP*	Clinical complications	No. of died patients
B. cenocepacia/A	18	ΔF508 (4), ΔF508/Ot* (11), Ot/Ot (3)	8	18	3DLD[a], 4DD[b]	7
B. cenocepacia/B	10	ΔF508 (1), ΔF508/Ot (4), Ot/Ot (5)	6	10	1LD[c], 2DLD	2
B. cepacia	1	Ot/Ot	1	1	1DD	–
B. stabilis	1	ΔF508/Ot	1	1	–	–
B. vietnamiensis	1	Ot/Ot	1	1	–	–

*Ot: Other
*IP: pancreatic insufficiency
[a]Diabetes and liver diseases
[b]Only diabetes disease
[c]Only liver disease

Table 2. Genomovar-status, transmissibility markers, pulsed-type and antimicrobial susceptibility of 31 Bcc strains

Strains no.	Pulsed-types	Genomovar-type	cblA	esmR	Amk/ Gen	Atm	Sxt	Fep	Ctx	Caz	Mem/ Ipm	Cip/ Lvx	Pip	Pip	Chl
1	I 1	*B. cenocepacia*/A	-	+	R	R	R	R	R	R	R	R	R	R	R
2	I 2	*B. cenocepacia*/A	-	+	R	R	R	R	R	I	R	R	R	R	R
3	L 1	*B. cenocepacia*/A	+	+	R	R	S	R	R	I	R	R	R	R	S
4, 5	L 1	*B. cenocepacia*/A	-	+	R	R	R	R	R	R	R	R	R	R	R
6	L 1	*B. cenocepacia*/A	-	+	R	R	S	R	R	S	S	S	S	S	S
7	L 1	*B. cenocepacia*/A	+	+	R	R	R	R	R	R	R	R	R	R	R
8	M	*B. cenocepacia*/A	-	-	R	R	R	R	R	R	R	R	R	R	R
9	L 1	*B. cenocepacia*/A	-	+	R	R	R	S	S	S	S	S	S	S	R
10	L 2	*B. cenocepacia*/A	-	-	R	S	S	S	S	S	S	R	S	S	R
11	L 1	*B. cenocepacia*/A	-	+	R	S	S	I	S	S	S	S	S	S	I
12	L 2	*B. cenocepacia*/A	-	+	R	R	S	R	S	S	S	S	S	S	I
13	I 1	*B. cenocepacia*/A	-	-	R	R	R	R	R	S	R	R	R	R	R
14	H	*B. cenocepacia*/A	-	+	R	S	R	S	S	S	S	R	S	S	R
15	L 2	*B. cenocepacia*/A	-	+	R	R	R	R	R	R	R	R	R	R	R
16	L 2	*B. cenocepacia*/A	-	+	R	R	R	R	R	S	R	R	S	S	R
17	E	*B. cenocepacia*/A	+	+	R	R	R	R	R	R	R	R	R	R	R
18	L 2	*B. cenocepacia*/A	-	-	R	R	R	R	R	S	R	S	S	S	S
19	P	*B. vietnamiensis*	ND	ND	R	R	S	R	R	R	S	R	R	R	R
20	O	*B. cenocepacia*/B	-	+	R	R	S	R	R	R	R	R	R	R	I
21	G	*B. cenocepacia*/B	-	-	R	R	S	R	R	R	R	R	R	R	R
22	L 1	*B. cenocepacia*/B	-	+	S	R	S	S	S	S	R	S	S	S	S
23	N	*B. stabilis*	-	+	R	R	S	R	R	R	R	R	R	R	R
24	B	*B. cenocepacia*/B	-	-	R	R	S	R	R	R	R	R	R	R	R
25	C	*B. cenocepacia*/B	-	+	R	R	S	R	R	R	R	R	R	R	R
26	D	*B. cenocepacia*/B	+	-	R	R	R	R	R	R	R	R	R	R	R
27	E	*B. cenocepacia*/B	-	+	R	R	R	R	R	R	R	R	R	R	R
28	F	*B. cenocepacia*/B	-	+	R	R	R	R	R	R	R	R	R	R	R
29	G	*B. cenocepacia*/B	+	+	R	R	S	R	R	R	S	S	S	S	R
30	E	*B. cenocepacia*/B	-	-	R	R	R	S	S	S	I	R	S	S	S
31	H	*B. cepacia*	+	+	R	R	R	R	R	R	R	R	R	R	S

Amk=*Amikacin*; Atm=*Aztreonam*; Fep=*Cefepime*; Ctx=*Cefotaxime*; Caz=*Ceftazidime*; Cip=*Ciprofloxacin*; Chl=*Chloramphenicol*; Gen=*Gentamicin*; Ipm=*Imipenem*; Lvx=*Levofloxacin*; Mem=*Meropenem*; Pip=*Piperacillin*; Tzp=*Piperacillin-Tazobactam*; Sxt=*Trimethoprim-Sulfamethoxazole*
ND: no determined

by *B. cenocepacia*, 18 (64.3%) harbored isolates belonging to recA lineage IIIA, 10 (35.7%) harbored isolates belonging to recA lineage IIIB. No patient was infected with more than one Bcc strain. Seventeen chronically Bcc infected patients were also chronically infected by PA (Table 1).

Twenty of the thirty-one Bcc isolates were multiresistant. Beta-lactam antibiotics showed moderate activity and only ceftazidime seemed to be the most efficacious. Aminoglycosides and quinolones were not very active. Carbapenems, piperacillin, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole were efficacious (Table 2).

CblA gene was present in six of the 30 isolates examined; one of these was *B. cepacia*, the others were *B. cenocepacia*. The presence of the esmR gene was observed in 22 of the 30 isolates. Out of the 22 strains found positive for esmR, 20 were *B. cenocepacia*, one was *B. stabilis* and the one was *B. cepacia* (Table 2).

PFGE showed 13 major clones (clones F, B, N, G, O, P, I, L, M, H, E, C, and D). Clone L was the most frequently recovered, with two subtypes (L1 and L2): eight strains were L1 and five strains were L2. All thirteen strains belonging to this clone were *B. cenocepacia* (12 lineage IIIA and 1 lineage IIIB). Clone I and clone E were recovered in three patients each: clone I was present with two subtypes (I1 and I2) and both were *B. cenocepacia* lineage IIIA. Clone G and clone H were recovered from two patients each. The remaining pulsed-types were unique and each recovered in one patient (Table 2). No correlation between

Table 3. Characteristics of patients with chronic Bcc infection (case group) compared to patients with chronic PA infection (control group)

Parameters	PA chronic infected patients (n=31)	Bcc chronic infected patients (n=31)
Male/Female (n)	11/20	11/20
Mean age	20.65 years (13-35 years)	21.5 years (11-36 years)
Mean duration of infection	6 years (1-20)	5.55 years (6 months - 17 years)
DF508/DF508 (n)	10	5
DF508/other (n)	11	16
Other/other (n)	10	10
Mean Fev1 (%, range)	60 (23-111)	51.8 (13-105)
Mortality	1/31	9/31 *

3/9 had co-colonization with PA

strains of our Centre and the Sicilian and Vancouver strains was found (Fig. 1).

All 31 patients chronically Bcc infected showed pancreatic insufficiency at CF diagnosis. During the Bcc infection period, 11 (35.4%) patients showed CF complications, i.e. diabetes and liver disease. Seven of these patients were infected by *B. cenocepacia* lineage IIIA, three patients by *B. cenocepacia* lineage IIIB and one patient by *B. cepacia* (Table 1).

B. cenocepacia lineage IIIA was associated with a more severe prognosis as demonstrated by the development of the "cepacia syndrome" and the death of 7/18 patients colonized by this pathogen. Two patients colonized by *B. cenocepacia* lineage IIIB died due to severe respiratory insufficiency (Table 1). A higher mortality among patients with chronic Bcc infection, compared to a control group of patients chronically infected with PA, was found (p=0.01). The mean age of the case group and the control group were, respectively, 21.5±3.63 (range 11-36 years) and 20.65±3.78 (range 13-35 years) (p=0.37). In both groups, a male/female ratio of 11/20 was found. The mean duration of infection was 6±3.23 (range 1-20 years) in the control group and 5.55±2.87 (6 month-17 years) in the case group (p=0.564) (Table 3). The distribution of the CFTR mutation in both groups did not differ significantly (p=0.2). Patients with chronic Bcc infection showed FEV1 mean values significantly lower than those shown by patients with only chronic PA infection (p=0.03). Co-infection with PA in Bcc patients did not lead to a significant variation of FEV1 mean values (p=0.9). In the case group, one patient was chronically infected by MRSA and 5 patients by SM; in the control group, three patients were chronically infected by MRSA and 10 patients by SM (p= 0.77).

CF Foundation data show a prevalence of 3.1% of patients with Bcc infection with different findings in the prevalence and epidemiology among care centers (CF Foundation Patient Registry, 2003). The results of high prevalence of Bcc infection (11.23%) and high prevalence of *B. cenocepacia* lineage IIIA recovered in our cohort of patients have been similarly observed in other Italian Centers. In 2001, Agodi *et al.* (2001) indicated a prevalence of Bcc infection of 9.5% (65 out of 683 patients) in a study performed in four Italian CF Centers (Catania, Palermo, Gualdo Tadino and Milan) and the *B. cenocepacia* III-A was the most prevalent lineage (55%, 51 out of 92 isolates). In 2004, Manno *et al.* reported a prevalence of Bcc infection of 23% (75 out of 326 patients) in a study performed in another CF Center (Genoa), and of 68 patients infected by *B. cenocepacia*, 29 were infected by strains of the lineage III-A. In 2006, Allice *et al.* (2006), indicated a high percentage of *B. cenocepacia* III-A (70%, 34 out of 48 isolates) in a study performed in a Turin CF Center. Data from Italian reports are different from data of CF patients in the USA where *B. cenocepacia* lineage IIIB was the most prevalent (LiPuma *et al.*, 2001). CblA appears to be relatively specific for *B. cenocepacia* and not for transmissible Bcc strains in general. The high prevalence of strains positive for the esmR gene locus indicates the high capacity to spread among CF patients: it is well-known that esmR is the genetic marker identified among several epidemic strain types, and it should be considered as a putative marker for the presence of the Bcc strain with the potential for spread among patients with CF (Mahenthiralingam *et al.*, 1997). In our study, PFGE indicated the presence of 13 clones of Bcc strains: of these, the pulsed-type L was the most frequently observed suggesting a spread of strains. Therefore, the distribution of pulsed-type L was predominant in the *B. cenocepacia* III-A and all these strains were esmR positive. The source of these pathogens is unknown and it is noteworthy that these bacteria reside in the natural environment.

Given the confirmed ability of *B. cenocepacia* to spread from one patient to another, a continuous epidemiological survey appears necessary in CF Centers. Current infection control guidelines recommend individual segregation of CF patients infected with Bcc bacteria (Saiman *et al.*, 2000; Saiman and Siegel, 2003). From 2002 to 2004, our Centre adopted a strict infection-control policy that segregated Bcc infected patient from non-infected patients. Based on these strategies, we registered a dramatic reduction of Bcc infection (7%, 21 out of 300 patients) (Lambiase *et al.*, 2006).

Data indicated in this note show that the study of epidemiological relatedness associated to evidence of molecular virulence markers should provide an excellent means for a better comprehension of these pathogens, as well as of probable patient-to-patient spread.

References

Agodi, A., E. Mahenthiralingam, M. Barchitta, V. Giannino, A. Sciacca, and S. Stefani. 2001. *Burkholderia cepacia* complex in-

fection in italian patients with cystic fibrosis: prevalence, epidemiology and genomovar status. *J. Clin. Microbiol.* 39, 2891-2896.

Allice, T., S. Scutera, M.G. Chirillo, and D. Savoia. 2006. Burkholderia respiratory tract infections in Italian patients with cystic fibrosis: molecular characterization. *J. Infect.* 53, 159-165.

Cystic Fibrosis Foundation. 2003. *Patient Registry: Annual Data Report*. Bethesda.

Coenye, T., P. Vandamme, J.R.W. Govan, and J.J. LiPuma. 2001. Taxonomy and identification of the *Burkholderia cepacia* complex. *J. Clin. Microbiol.* 39, 3427-3436.

Frederiksen, B., C. Koch, and N. Høiby. 1999. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish Cystic Fibrosis patients (1974-1995). *Pediatr Pulmonol.* 28, 159-166.

Gilligan, P.H. 1991. Microbiology of airway disease in patients with Cystic Fibrosis. *Clin. Microbiol. Rev.* 4, 35-51.

Goldstein, R., L. Sun, R.Z. Jiang, U. Sajjan, J. Forstner, and C. Campanelli. 1995. Structurally variant classes of pilus appendage fibers coexpressed from *Burkholderia* (*Pseudomonas*) *cepacia*. *J. Bacteriol.* 177, 1039-1052.

Grothues, D., U. Koopmann, H. Van der Hardt, and B. Tummler. 1988. Genome fingerprinting of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. *J. Clin. Microbiol.* 26, 115-123.

Lambiase, A., V. Raia, M. Del Pezzo, A. Sepe, V. Carnovale, and F. Rossano. 2006. Microbiology airway disease in a cohort of patients with cystic fibrosis. *BMC Infect. Dis.* 6, 4.

LiPuma, J.J. 1998. *Burkholderia cepacia*. Management issues and new insights. *Clin. Chest Med.* 19, 473-486.

LiPuma, J.J., T. Spilker, L.H. Gill, P.W. Campbell, L. Liu, and E. Mahenthiralingam. 2001. Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 164, 92-99.

Lipuma, J.J. 2005. Update on the *Burkholderia cepacia* complex. *Curr. Opin. Pulm. Med.* 11, 528-533.

Mahenthiralingam, E., D. Simpson, and D.P. Speert. 1997. Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. *J. Clin. Microbiol.* 35, 808-816.

Mahenthiralingam, E., J. Bischof, S.K. Byrne, C. Radomski, J.E. Davies, Y. Av-Gay, and P. Vandamme. 2000. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J. Clin. Microbiol.* 38, 3165-3173.

Mahenthiralingam, E. and P. Vandamme. 2005. Taxonomy and pathogenesis of the *Burkholderia cepacia* complex. *Chron. Respir. Dis.* 2, 209-217.

Manno, G., C. Dalmastri, S. Tabacchioni, P. Vandamme, R. Lorini, L. Minicicci, L. Romano, A. Giannattasio, L. Chiarini, and A. Bevivino. 2004. 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. *J. Clin. Microbiol.* 42, 1491-1497.

McDowell, A., E. Mahenthiralingam, J.E. Moore, K.E.A. Dunbar, A.K. Webb, M.E. Dodd, S.L. Martin, B.C. Millar, C.J. Scott, M. Crowe, and J.S. Elborn. 2001. PCR-based detection and identification of *Burkholderia cepacia* complex pathogens in sputum from cystic fibrosis patients. *J. Clin. Microbiol.* 39, 4247-4255.

National Committee for Clinical Laboratory Standards. 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa, USA.

Saiman, L, N. MacDonald, J.L. Burns, N. Hoiby, D.P. Speert, and D. Weber. 2000. Infection control in cystic fibrosis: practical recommendations for the hospital, clinic, and social setting. *Am. J. Infect. Control.* 28, 381-385.

Saiman, L., N.E. MacDonald, and S. Whittier. 1996. The microbiological basis for antimicrobial therapy. *In* Tenth Annual North American Cystic Fibrosis Conference. Cystic Fibrosis Foundation. Orlando, USA.

Saiman, L. and J. Siegel. 2003. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens and infection control practices to prevent patient-to-patient transmission. *Am. J. Infect. Control.* 3 (Suppl).

Sajjan, U., L. Sun, R. Goldstein, and J. Forstner. 1995. Cable (Cbl) type II pili of cystic fibrosis-associated *Burkholderia* (*Pseudomonas*) *cepacia*: nucleotide sequence of the cblA major subunit pilin gene and novel morphology of the assembled appendage fibers. *J. Bacteriol.* 177, 1030-1038.

Sun, L., R. Jiang, S. Steinbach, A. Holmes, C. Campanelli, J. Forstner, Y. Tan, M. Riley, and R. Goldstein. 1995. The emergence of a highly transmissible lineage of cbl+ *Pseudomonas* (*Burkholderia*) *cepacia* causing Cystic Fibrosis centre epidemics in North America and Britan. *Nat. Med.* 1, 661-666.

Tablan, O.C., T.L. Chorba, D.V. Schidlow, J.W. White, K.A. Hardy, P.H. Gilligan, W.M. Morgan, L.A. Carson, W.J. Martone, J.M. Jason, and W.R. Jarvis. 1985. *Pseudomonas cepacia* colonization in patients with Cystic Fibrosis: risk factors and clinical outcome. *J. Pediatr.* 107, 382-387.

Tenover, F., R. Arbeit, R. Goering, P. Mickelsen, B. Murray, D. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* 33, 2233-2239.

Vandamme, P., D. Henry, T. Coenye, S. Nuzla, M. Vancanneyt, J.J. LiPuma, D.P. Speert, J.R.W. Govan, and E. Mahenthiralingam. 2002. *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. *FEMS Immunol. Med. Microbiol.* 33, 143-149.

Vermis, K., T. Coenye, J.J. LiPuma, E. Mahentheralingam, H.J. Nelis, and P. Vandamme. 2004. Proposal to accomodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. *Int. J. Syst. Evol. Microbiol.* 54, 689-691.