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Burkholderia respiratory tract infections in Italian patients with cystic fibrosis: Molecular characterization

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Summary *Objectives:* To characterize by molecular techniques *Burkholderia* strains responsible for respiratory tract infections in cystic fibrosis (CF) patients (children and adults), to assign the *Burkholderia cepacia* complex (Bcc) isolates to a genomovar and to assess the presence of *cblA* and *esmR* genes in bacteria. Unique or sequential *Burkholderia* isolates ($n = 48$) that had been collected from eight CF children and 17 adults over several (4–6) years were investigated; moreover 11 reference strains were analyzed.
Methods: The microorganisms were identified by using biochemical methods, genotyped by pulse field gel electrophoresis (PFGE) and random-amplified polymorphic DNA fingerprinting–PCR (RAPD–PCR), and assessed by PCR assays for the genomovar and *cblA* and *esmR* genes of Bcc.
Results: Among isolates 70.8% were identified as Bcc genomovar III-A; one child was infected by *Burkholderia ambifaria* and four adults were colonized with *Burkholderia gladioli*. The *cblA* gene was not detected in any of the isolates, while the *esmR* gene was detected in the 52.1% of the strains, all belonging to genomovar III-A.
Conclusion: Molecular analysis of strains revealed in CF patients a colonization with a persistent *Burkholderia* flora with strains of one genotype. The prevalence of Bcc of genomovar III-A in the two categories of patients and of *B. gladioli* in four adults demonstrated that transmission may have occurred between subjects. Moreover the *B. ambifaria* infection demonstrated in a child may be environmentally derived.

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Introduction

Burkholderia species are widely distributed in the natural environment and several species also cause disease.[1] Correct identification of this genus is difficult, but recently[2] a *recA* gene-based approach was developed to identify the entire *Burkholderia* genus. In this genus, closely related species, referred collectively to the *Burkholderia cepacia* complex (Bcc), are recognized as opportunistic human pathogens that cause numerous outbreaks, particularly among cystic fibrosis (CF) patients. They cause a rapid decline in pulmonary function, with ensuing increased morbidity and mortality.[3] Because only a limited number of antibiotics are effective against Bcc, complete eradication of infection is rare.[4]

Taxonomic studies based on traditional phenotypic and genotypic tests[5–8] have demonstrated that Bcc includes nine genomic species or genomovars: *B. cepacia* (formerly genomovar I), *Burkholderia multivorans* (genomovar II), *Burkholderia cenocepacia* (genomovar III, divided into four *recA* clusters, III-A, III-B, III-C and III-D), *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).

Various markers have been associated with transmissible strains of Bcc, such as the *cblA* and *esmR* genes, which encode the protein for cable pilus production and the *B. cepacia* epidemic strain marker BCESM.[9,10]

With these recent developments in genotyping techniques, we examined 48 *Burkholderia* strains isolated from eight CF children and 17 adults (over the last 4 and 6 years, respectively). These subjects were admitted to hospital for periodic controls in two different centres, one in the city of Torino (northwest Italy), the other located in Torino city outskirts. Moreover 11 reference strains were assessed.

The strains identified as belonging to the *Burkholderia* genus by using biochemical methods were analyzed by the followings: (I) genotyping was effected by pulsed-field gel electrophoresis (PFGE) and random-amplified polymorphic DNA fingerprinting—PCR (RAPD—PCR); (II) the genomovar of Bcc was assessed by PCR assays; and (III) the *cblA* and *esmR* genes of Bcc were detected by PCRs.

Materials and methods

Patients and bacterial strains

The 48 clinical (unique or sequential) isolates were obtained from the expectorates of eight children affected by CF attending the Cystic Fibrosis Centre of the Regina Margherita Hospital (Torino, Italy) and 17 adults with CF attending the Cystic Fibrosis Centre at S. Luigi Gonzaga Hospital in Orbassano (Torino, Italy), centres that are ~15 km apart. The reference Bcc strains examined were ATCC 17759 (Bcc genomovar I), kindly given by C. Van Pelt (Rotterdam), LMG 17588 (Bcc genomovar II), 13011 (Bcc genomovar III-B), 18888 (Bcc genomovar IV), 16232 (Bcc genomovar V), 18941 (Bcc genomovar VI), 19467 (Bcc genomovar VII), 16670 (Bcc genomovar VIII) and 14191 (Bcc genomovar IX), obtained from BCCM/LMG bacteria collection (Gent, Belgium), the strain CF5610 (Bcc genomovar III-A, *cblA*+ ET12 lineage strain, from John Govan's collection, Edinburgh, kindly sent by F. Clode, PHLS, London). Moreover the LMG 2216 strain (*B. gladioli*) was assessed.

Bacteria were plated on OFPBL agar (Becton—Dickinson) and on Müeller Hinton agar (Oxoid) and liquid cultures effected in Trypticase Soy Broth (Oxoid) at 28 °C for 48 h; the isolates were tentatively identified as Bcc using commercial tests, including API 20NE kit (BioMèrieux) and Vitek Auto Microbic System (BioMèrieux).

Pulsed-field gel electrophoresis (PFGE)

DNA macrorestriction and PFGE of *Burkholderia* strains were carried out as previously described[11] with some modifications. Bacteria were incubated in Trypticase Soy Broth, then centrifuged at 3000 rpm for 10 min; the pellet was washed in 1 ml of SE buffer (25 mM EDTA, 75 mM NaCl, pH 7.4), resuspended in 150 μl of the same buffer and diluted. The bacterial suspension was included in 150 μl 2% low melting point agarose (Bio-Rad) in SE buffer; agarose plugs were prepared and maintained at 4 °C. Bacteria were lysed by incubating the plugs in lysis solution containing 0.5 mg ml^{-1} proteinase K (Sigma), 0.5 M EDTA, 1% *N*-laurylsarcosine (Sigma) for 16 h at 37 °C. Plugs were washed six times by gentle agitation for 1 h in TE buffer and stored in TE buffer at 4 °C, then incubated three times in 1 ml of pre-*Xba*I buffer (6 mM TrisHCl, 150 mM NaCl, 6 mM $MgCl_2$, 1 mM DTT, pH 7.9) for 30 min at 37 °C and treated overnight at 37 °C with 10 U *Xba*I (Sigma—Aldrich). The gel was

prepared using 1% pulsed-field-certified agarose (Bio-Rad) in 0.5× TBE buffer (45 mM Tris—borate and 1 mM EDTA) and sealed with 0.75% low melting point agarose. A 50—1000 Kb molecular weight marker (Sigma) was used. PFGE was performed using the CHEF Mapper XA System (Bio-Rad). The gel run was performed at 6.0 V cm^{-1} for 23 h at 14 °C with initial time pulse of 1 s and final time pulse of 30 s. Gel images of the genomic DNAs were digitized by using a Kodak Image Station 2000R following ethidium bromide staining. The clonality was examined according to the recommendations[12]; i.e., isolates differing in more than six bands were considered sufficiently divergent to warrant separate pattern designations, whereas those that differ by one to three (a—c subtypes) or four to six bands should be considered, respectively, closely or possibly related. Reproducibility was checked by repeating PFGE runs.

Random-amplified polymorphic DNA (RAPD)

Template DNA was prepared from bacteria grown for 48 h at 28 °C on Müeller Hinton agar plates. Crude DNA extracts were obtained by suspending five colonies in 100 μl sterile distilled water, vortexing, centrifuging at 3000 rpm for 5 min and using the supernatant. We used primers 208, 270 and 272 (5′-ACGGCCGACC-3′; 5′-TGCGCGCGGG-3′; and 5′-AGC GGGCCAA-3′, respectively) and the conditions described.[13] The amplified DNA fragments were separated in a 1.5% agarose gel (Invitrogen) in 1× TBE with 0.5% ethidium bromide (10 mg ml^{-1}, Sigma) for 1 h and 40 min at 6 V cm^{-1}. RAPD fingerprints were analyzed by Kodak Image Station 2000R and the clonality was evaluated based on guidelines reported.[14] Isolates that differed by two or more prominent bands were assigned to different major types, whereas isolates that differed by one to two faint bands were considered minor variant types (a and b) of a given major type. Reproducibility of the random PCR patterns was assessed by repeating PCR runs.

PCR analysis

The identification of the *Burkholderia* genomovar status was effected by PCR using nine *recA* primer pairs specific for genomovars I, II, III-A, III-B, IV, V, VI, VII and VIII.[5—8] For isolates whose identification remained unclear, the evaluation of 23S rDNA of the species *B. gladioli* by using the SS-PCR with primer pair LP1—LP4[15] was done. Detection by PCRs of the *B. cepacia* epidemic strain marker (BCESM) and cable pili subunit gene (*cblA*) was carried out as previously described.[9,10]

Results

The analysis effected to determine the species or genomovar status of *Burkholderia* revealed (Table 1) that among the 48 isolates tested 34 (11 from seven children and 23 from 13 adults) belonged to *B. cenocepacia* group III-A, one strain (sequentially isolated from a child) was identified as *B. ambifaria* (genomovar VII), and 11 strains, obtained from four adults, were negative for all Bcc genomovars and positive to the PCR analysis for *B. gladioli*. Among all strains 25, four from children and 21 from adults, belonging exclusively to *B. cenocepacia* group III-A, were positive for *esmR* gene (Table 1); on the contrary, no strain was *cblA*-positive. By PFGE (Table 1, Fig. 1) we observed that the isolates show four distinct banding patterns: P1, P2 (including four subtypes), P3 and P4 (including P4a, that differed in one band). The pattern indicated as P2 was the most prevalent (75 and 58.8% of the bacteria collected on the first occasion of microbiological diagnosis from children and adults, respectively). The P1 and P3 profiles were noted only in strains from adults, while one, indicated as not classified (NC) because resulted not digested with *Xba*I, was isolated from one child alone; strains characterized as belonging to the P4 profile were observed in few isolates. The results of RAPD analysis reported were obtained using the primer 272, chosen after test screening with primers 208, 270 and 272. Four banding profiles were obtained by the analysis of isolates: R1, R2 (including two subtypes, differing in one to two minor bands), R3 and R4; the RAPD profile designated as R2 was prevalent and present both in children and adults (Table 1, Fig. 1). The *Burkholderia* strains with patterns P2—R2 corresponded to *B. cenocepacia* group III-A, those not classified (NC) by PFGE and defined as R3 by RAPD belonged to *B. ambifaria* (genomovar VII), and those named P1—R1 belonged to the *B. gladioli* species. The reference Bcc strain group III-A (CF5610) also revealed a P2—R2 profile, whereas the other reference strains tested, belonging to different Bcc genomovars, had PFGE and RAPD patterns different from the isolates tested (not reported) except for the LMG 19467 strain (Bcc genomovar VII) whose RAPD profile was similar to the isolate of the same genomovar collected from a child (Table 1).

Discussion

The poor prognosis of CF is especially associated with an early onset of respiratory tract infection with a variety of bacterial species. Among these are

Table 1 Evaluation of different genetic characters of *Burkholderia* strains isolated from CF patients

Category of patients (number of strains)	Patients or isolates	Isolation	BCESM	Species or genomovar status	PFGE profile	RAPD profile
Children (14)	1c	I (02/96)	−	III-A	P2b	R2
		II (10/96)	−	III-A	P2d	R2b
		III (02/98)	+	III-A	P2c	R2b
		IV (10/98)	−	III-A	P2d	R2
		V (06/99)	−	III-A	P2d	R2
	2c	I (10/96)	+	III-A	P2c	R2a
	3c	I (01/00)	−	VII	NC[a]	R3
		II (03/00)	−	VII	NC[a]	R3
		III (05/00)	−	VII	NC[a]	R3
	4c	I (02/00)	+	III-A	P2a	R2b
	5c	I (10/98)	−	III-A	P2d	R2b
	6c	I (02/96)	−	III-A	P4	R3
	7c	I (10/96)	+	III-A	P2a	R2a
	8c	I (11/96)	−	III-A	P2	R2
Adults (34)	1a	I (04/99)	−	*B. gladioli*	P1	R1
		II (07/00)		*B. gladioli*	P1	R1
		III (07/01)	−	*B. gladioli*	P1	R1
		IV (01/03)	−	*B. gladioli*	P1	R1
		V (09/04)	−	*B. gladioli*	P1	R1
		VI (02/05)	−	*B. gladioli*	P1	R1
	2a	I (10/00)	+	III-A	P2d	R2
		II (04/03)	+	III-A	P2c	R2
	3a	I (04/99)	−	*B. gladioli*	P1	R1
		II (02/05)	−	*B. gladioli*	P1	R1
	4a	I (/03/99)	−	III-A	P4a	R3
	5a	I (06/99)	+	III-A	P2a	R2a
		II (05/01)	+	III-A	P2a	R2a
		III (04/03)	+	III-A	P2a	R2a
	6a	I (07/99)	−	*B. gladioli*	P1	R1
		II (01/05)	−	*B. gladioli*	P1	R1
	7a	I (07/00)	+	III-A	P3	R4
	8a	I (08/00)	+	III-A	P2a	R2a
	9a	I (11/00)	+	III-A	P2d	R2b
		II (06/01)	+	III-A	P2d	R2b
		III (02/02)	+	III-A	P2a	R2b
		IV (02/03)	+	III-A	P2a	R2a
		V (02/05)	+	III-A	P2d	R2b
	10a	I (09/99)	−	*B. gladioli*	P1	R1
	11a	I (09/99)	−	III-A	P4a	R3
	12a	I (01/02)	+	III-A	P2a	R2
		II (11/02)	+	III-A	P2	R2
	13a	I (11/04)	+	III-A	P2b	R2
	14a	I (11/04)	+	III-A	P2a	R2
		II (02/05)	+	III-A	P2a	R2
	15a	I (11/04)	+	III-A	P2b	R2
		II (01/05)	+	III-A	P2b	R2
	16a	I (01/05)	+	III-A	P2d	R2
	17a	I (01/05)	+	III-A	P2a	R2

[a] NC, not classified because not restricted with *Xba*I enzyme.

included several species of the genus *Burkholderia* whose incidence appears to be increasing; of the emerging pathogens, Bcc and *B. gladioli*, as also demonstrated in the present work, are prominent.[16] Between these microorganisms there is a high degree of phenotypic similarity and accurate identification is difficult. The use of a procedure based on a species-specific PCR for the identification

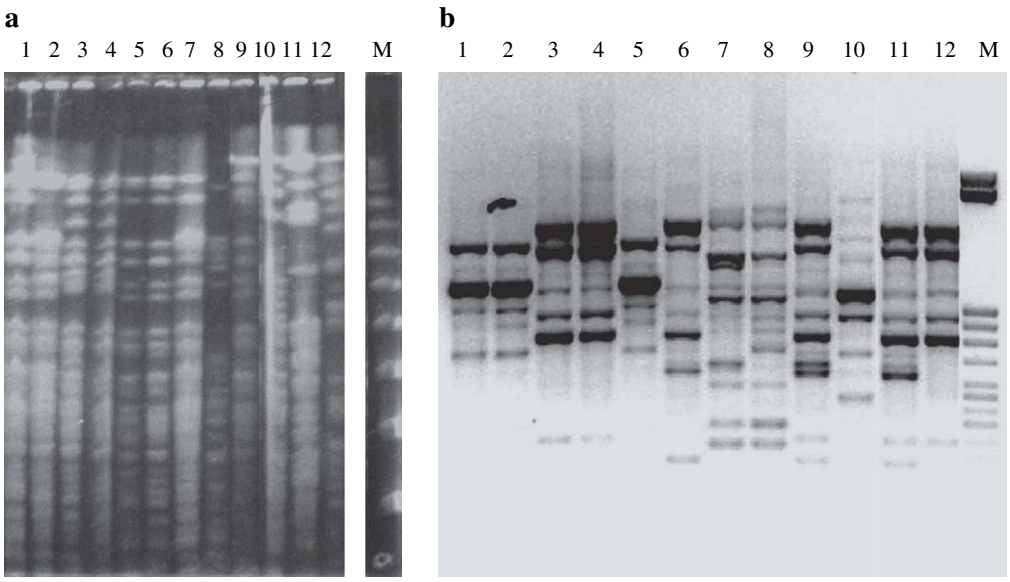

Figure 1 *B. cepacia* complex isolated from CF patients. (a) PFGE profiles of *XbaI*-digested genomic DNA. M: 50—1000 kb molecular weight marker (Sigma). From line 1 to 12: P2a, P2, P2d, P2d, P2a, P2a, P2c, P4, P2a, P2d, P3 and P1 profiles, respectively. (b) RAPD main profiles. M: molecular weight marker (Step Ladder 50 bp, Sigma). From line 1 to 12: R1, R1, R2, R2, R1, R2a, R4, R4, R2b, R3, R2a and R2 profiles, respectively.

of *B. gladioli*[15] is useful to clarify the role of this microorganism as a human pathogen in CF.

Our results revealed that *B. cenocepacia* (genomovar III-A) is the most prevalent (70.8 and 80% of all isolates or of the firstly examined strain for patient, respectively) in the samples obtained from CF patients. This is in line with other data derived from the analysis of CF patients,[13,17—24] but different from results reported in a Belgian study.[25] There is evidence that genomovar III-A strains are more invasive for respiratory epithelial cells and associated with greater morbidity and mortality.[26] Eleven strains (isolated from four different adults), that by biochemical analysis were presumptively identified as *B. cepacia*, resulted positive to the PCR assay for *B. gladioli* and revealed particular PFGE and RAPD patterns. These results confirm previous observations[27] indicating that *B. gladioli* is the species most frequently misidentified by commercial systems as Bcc and show that this species may determine adverse clinical outcome and person-to-person transmission. Moreover one child was colonized by *B. ambifaria* (genomovar VII); infection with this species, which is common in environmental samples, has not been previously reported, to our knowledge, in strains isolated from other Italian patients,[18—21] though found in small number in patients of other countries.[22,28—30] This suggests that this species may play a role as a human pathogen and its acquisition from the environment is possible.

Following up some patients over certain time period obtaining different isolates per patient, we noted that in all cases the infection was persistent. Only little genetic differences were identified in sequential isolates of some patients (1c and 9a); this fact might be due to antibiotic resistance variations. The PFGE and RAPD profiles P3—R4 and P4—R3 noted in singular isolates from four patients might be derived from isolates harbouring more than one *Burkholderia* strain or hybrids between Bcc and *B. gladioli*, as previously reported.[31]

Many Bcc genomovar III-A strains, isolated from patients submitted to controls in two different hospitals, were genotypically similar to the reference epidemic (ET 12 lineage) strain belonging to the same genomovar and associated with serious outbreaks among CF patients in UK. However, in the isolates tested the *cblA* gene was not demonstrated, perhaps owing to the instability of its sequence.[18] Isolates with a P2—R2 DNA profile and positive for *esmR* gene (part of a genomic cluster encoding virulence and metabolism-associated gene[32]) prevailed, suggesting the possibility of interpersonal transmission or nosocomial acquisition. CF patients may acquire other Bcc lineages also from the environment. In recent years, in fact, Bcc has been used in agriculture in biopesticides and bioremedial products,[1] most notably particular strains of *B. vietnamiensis* and *B. ambifaria* because of their low incidence of isolation from CF patients.[33]

Strategies introduced in different countries have limited the epidemic spread of Bcc[17,34,35]; so the implementation of stringent infection control measures is important to limit spread both within and outside hospital and epidemiological studies of *Burkholderia* species are critical to keep pace with the ecological and pathogenic interest in the genus.

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References

1. Coenye T, Vandamme P. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* 2003;**5**:719—29.
2. Payne GW, Vandamme P, Morgan SH, LiPuma JJ, Coenye T, Weightman AJ, et al. Development of a *recA* gene-based identification approach for the entire *Burkholderia* genus. *Appl Environ Microbiol* 2005;**71**:3917—27.
3. Mahenthiralingam E, Baldwin A, Vandamme P. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. *J Med Microbiol* 2002;**51**:533—8.
4. Ryley HC, Doull IJM. *Burkholderia cepacia* complex infection in patients with cystic fibrosis: laboratory investigations, epidemiology and clinical management. *Rev Med Microbiol* 2003;**14**:14—24.
5. Coenye T, Vandamme P, Govan JRW, LiPuma JJ. Taxonomy and identification of the *Burkholderia cepacia* complex. *J Clin Microbiol* 2001;**39**:3427—36.
6. Vermis K, Coenye T, Mahenthiralingam E, Nelis HJ, Vandamme P. Evaluation of species-specific *recA*-based PCR tests for genomovar level identification within the *Burkholderia cepacia* complex. *J Med Microbiol* 2002;**51**:937—40.
7. Vandamme P, Holmes B, Coenye T, Goris J, Mahenthiralingam E, LiPuma JJ, et al. *Burkholderia cenocepacia* sp. nov. — a new twist to an old story. *Res Microbiol* 2003;**154**:91—6.
8. Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon *Burkholderia cepacia* complex. *Nat Rev Microbiol* 2005;**3**:144—56.
9. Sun L, Jiang R, Steinbach S, Holmes A, Campanelli C, Forstner J, et al. The emergence of highly transmissible lineage of cbl+ *Pseudomonas (Burkholderia) cepacia* causing CF centre epidemics in North America and Britain. *Nat Med* 1995;**1**:661—6.
10. Mahenthiralingam E, Simpson DA, Speert DP. Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. *J Clin Microbiol* 1997;**35**:808—16.
11. Wigley P, Burton NF. Genotypic and phenotypic relationships in *Burkholderia cepacia* isolated from cystic fibrosis patients and the environment. *J Appl Microbiol* 1999;**86**: 460—8.
12. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persin DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**: 2233—9.
13. Mahenthiralingam E, Campbell ME, Henry DA, Speert DP. Epidemiology of *Burkholderia cepacia* infections in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. *J Clin Microbiol* 1996;**3**:2914—20.
14. Segonds C, Bingen E, Couetdic G, Mathy S, Brahimi N, Marty N, et al. Genotypic analysis of *Burkholderia cepacia* isolates from 13 French cystic fibrosis centers. *J Clin Microbiol* 1997;**35**:2055—60.
15. Whitby PW, Pope LC, Carter KB, LiPuma JJ, Stull TL. Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. *J Clin Microbiol* 2000;**38**:282—5.
16. Goss CH, Rosenfeld M. Update on cystic fibrosis epidemiology. *Curr Opin Pulm Med* 2004;**10**:510—4.
17. McDowell A, Mahenthiralingam E, Dunbar KEA, Moore JE, Crowe M, Stuart Elborn J. Epidemiology of *Burkholderia cepacia* complex species recovered from cystic fibrosis patients: issues related to patients segregation. *J Med Microbiol* 2004;**53**:663—8.
18. Agodi A, Mahenthiralingam E, Barchitta M, Giannino V, Sciacca A, Stefani S. *Burkholderia cepacia* complex infection in Italian patients with cystic fibrosis: prevalence, epidemiology and genomovar status. *J Clin Microbiol* 2001;**39**: 2891—6.
19. Bevivino A, Dalmastri C, Tabacchioni S, Chiarini L, Belli ML, Piana S, et al. *Burkholderia cepacia* complex bacteria from clinical and environmental sources in Italy: genomovar status and distribution of traits related to virulence and transmissibility. *J Clin Microbiol* 2002;**40**:846—51.
20. Petrucca A, Cipriani P, Valenti P, Santapaola D, Cimmino C, Scoarughi GL, et al. Molecular characterization of *Burkholderia cepacia* isolates from cystic fibrosis (CF) patients in an Italian CF center. *Res Microbiol* 2003;**154**:491—8.
21. Manno G, Dalmastri C, Tabacchioni S, Vandamme P, Lorini R, Minicucci L, et al. 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* 2004;**42**:1491—7.
22. Reik R, Spiker T, LiPuma JJ. Distribution of *Burkholderia cepacia* complex species among isolates recovered from persons with or without cystic fibrosis. *J Clin Microbiol* 2005;**43**:2926—8.
23. Cunha MV, Leitao JH, Mahenthiralingam E, Vandamme P, Lito L, Barreto C, et al. Molecular analysis of *Burkholderia cepacia* complex isolates from a Portuguese cystic fibrosis center: a 7-year study. *J Clin Microbiol* 2003;**41**:4113—20.
24. Drevinek P, Cinek O, Melter J, Langsadl L, Navesnakova Y, Vavrova V. Genomovar distribution of the *Burkholderia cepacia* complex differs significantly between Czek and Slovak patients with cystic fibrosis. *J Med Microbiol* 2003; **52**:603—4.
25. De Boeck K, Malfroot A, Van Schil L, Lebecque P, Knopp C, Govan JRW, et al. Epidemiology of *Burkholderia cepacia* complex colonisation in cystic fibrosis patients. *Eur Respir J* 2004;**23**:851—6.
26. Keig PM, Ingham E, Vandamme PAR, Kerr KG. Differential invasion of respiratory epithelial cells by members of the *Burkholderia cepacia* complex. *Clin Microbiol Infect* 2002; **8**:47—9.
27. Blecker Shelly D, Spilker T, Gracely EJ, Coenye T, Vandamme P, LiPuma JJ. Utility of commercial systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. *J Clin Microbiol* 2000;**38**:3112—5.
28. LiPuma JJ, Spilker T, Gill LH, Campbell PW, Liu L, Mahenthiralingam E. Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am J Respir Crit Care Med* 2001;**164**:92—6.

29. Kidd TJ, Bell SC, Coulter C. Genomovar diversity amongst *Burkholderia cepacia* complex isolates from an Australian adult cystic fibrosis unit. *Eur J Clin Microbiol Infect Dis* 2003;**22**:434—7.
30. Coenye T, Mahenthiralingam E, Henry D, LiPuma JJ, Laevens S, Gillis M, et al. *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. *Int J Syst Evol Microbiol* 2001;**51**:1481—90.
31. Baxter IA, Lambert PA, Simpson IN. Isolation from clinical sources of *Burkholderia cepacia* possessing characteristics of *Burkholderia gladioli*. *J Antimicrob Chemother* 1997; **39**:169—75.
32. Baldwin A, Sokol PA, Prkhill J, Mahenthiralingam E. The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*. *Infect Immun* 2004;**72**:1537—47.
33. Parke JL, Guriam-Sherman D. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu Rev Phytopathol* 2001;**39**: 225—8.
34. Muhdi K, Edenborough FP, Gumery L, O' Hickey S, Smith EG, Smith DL, et al. Outcome for patients colonised with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. *Thorax* 1996;**51**:374—7.
35. Festini F, Ballarin S, Loganes C, Codamo T, Doro R, Adamo A, et al. Prevention and control of respiratory tract infections in the network of Italian centers for cystic fibrosis. *Assist Inferm Ric* 2004;**23**:14—20.