Identical *Burkholderia cepacia* complex strain types isolated from multiple patients attending a hospital in Brazil

The *Burkholderia cepacia* complex (BCC) is a group of bacteria comprising at least nine recognized species (or genomovars) (Mahenthiralingam *et al.*, 2005) and associated with various opportunistic human infections. BCC bacteria have been identified in infections of cystic fibrosis (CF) patients and assorted nosocomial infections. Epidemic spread of BCC strains amongst CF patients has been widely documented (Mahenthiralingam *et al.*, 2005). In addition, there have been several reports of outbreaks amongst non-CF patients (Agodi *et al.*, 2002; Souza *et al.*, 2004; Woods *et al.*, 2004; Magalhães *et al.*, 2003; Shehabi *et al.*, 2004). Relatively few studies have identified the BCC species types implicated in an outbreak, and these have generally used PCR-based diagnostic tests targeting the *recA* gene (Mahenthiralingam *et al.*, 2000). Agodi *et al.* (2002) identified episodes of cross-transmission involving *Burkholderia cenocepacia* and *Burkholderia stabilis* in non-CF patients. *B. cenocepacia* has also been implicated in interpatient spread in studies of patients with bacteraemia (Woods *et al.*, 2004), or bacteraemia and respiratory tract colonization (Shehabi *et al.*, 2004). In a previous study of bacteraemia in haemodialysis patients in Recife, Brazil,

B. cenocepacia and *Burkholderia vietnamiensis* were identified in a polyclonal outbreak (Magalhães *et al.*, 2003). A further study characterized strains as *B. cepacia* complex or unclassifiable using *recA* PCR-RFLP analysis (Souza *et al.*, 2004), and water was defined as the source of the outbreak.

Previously, we reported *B. cenocepacia* as the most prevalent species amongst a collection of non-CF BCC isolates from Brazil, and identified nine *B. cenocepacia* isolates, from different clinical sources and patients, but sharing a common genotype (Detsika *et al.*, 2003). We also identified isolates from six CF patients that shared the same atypical genotype not corresponding to recognized genomovars (Detsika *et al.*, 2003). Further isolates obtained from in- and outpatients attending the Hospital Portugues in Recife, Brazil, recovered on sheep blood agar or EMB plates, have been identified as belonging to the BCC by using a series of phenotypic tests (Henry *et al.*, 2001). Amongst these isolates are a collection of seven that share phenotypic characteristics (Table 1), with the exception of isolate BC203, which was positive for ornithine decarboxylase. The one CF isolate from this group, BC111 (Table 1), was

isolated from one of the six patients in our previous study from whom related isolates of unknown genomovar status were isolated (Detsika *et al.*, 2003). However, isolate BC111 is different from the previous isolate, which is exemplified by isolate BC14 (Detsika *et al.*, 2003).

The seven isolates could not be assigned to a known species using genomovar-specific *recA*-based PCR tests, but did yield *recA* PCR amplicons that were digested with *Hae*III (*Bsu*RI) and *Msp*I, as described previously (Mahenthiralingam *et al.*, 2000). The seven isolates shared common *recA* PCR-RFLP typing patterns with both enzymes (Fig. 1). In order to further confirm their relatedness, we amplified and sequenced a 417 bp region of the outer membrane lipoprotein *opcL* gene (Plesa *et al.*, 2004) of each isolate for comparison. The *opcL* sequences of isolates BC92, BC201, BC202, BC203, BC204 and BC205 were identical, and differed by 1 bp from the *opcL* sequence of isolate BC111. In addition, we sequenced an equivalent 788 bp region of the *recA* genes from strains BC111, BC202, BC203 and BC204. Over this region, the *recA* genes of strains BC202, BC203 and BC204 were identical, but they differed from the *recA* gene of strain

Table 1. Strains used in this study

Positive (+) or negative (–) results for lysine decarboxylase (Lys), ornithine decarboxylase (Orn), oxidation of sucrose (Suc), oxidation of adonitol (Ado), gelatin liquefaction (Gel), aesculin hydrolysis (Esc), no growth at 42 °C (42 °C) and β -haemolysis (β -h) are shown.

Strain	Isolation date (month/year)	Patient age	Clinical source	Inpatient/ outpatient	Lys	Orn	Suc	Ado	Gel	Esc	42 °C	β -h
BC92	05/2002	1 year	Skin (osteomyelitis)	Out	+	–	+	+	+	+	–	–
BC201	04/2003	10 days	Ocular	In/out*	+	–	+	+	+	+	–	–
BC202	06/2003	76 years	Skin wound	In	+	–	+	+	+	+	–	–
BC203	06/2003	20 years	Skin wound	Out	+	+	+	+	+	+	–	–
BC204	07/2003	27 years	Skin wound	Out	+	–	+	+	+	+	–	–
BC205	09/2003	23 years	Ocular	Out	+	–	+	+	+	+	–	–
BC111	07/2003	6 years	CF isolate	Out	+	–	+	+	+	+	–	–

*New-born baby may have acquired the infection either as inpatient, from eye-drops following birth, or as outpatient.

Fig. 1. *recA* PCR-RFLP patterns of new Brazilian isolates. The figure shows *recA* PCR products from the seven Brazilian isolates digested with (A) *Msp*I or (B) *Hae*III (*Bsu*RI). The isolates were BC92 (lane 1), BC111 (lane 2) and BC201–BC205 (lanes 3–7). M, 1 kb-Plus ladder (Invitrogen).

BC111 by one nucleotide. The *recA* and *opcL* sequences have been deposited in GenBank (accession nos DQ166202 and DQ166203, respectively).

Database searches using the *opcL* sequences indicated that the best matches with strains of known genomovar status were with *B. cenocepacia* (98 %). The best *recA* matches were found with isolates of unknown genomovar status (99 %). Sequence analysis indicated that these isolates would not yield amplicons with any of the previously published genomovar-specific primer sets (Mahenthiralingam *et al.*, 2000). The best matches with isolates of known genomovar status were with *Burkholderia ambifaria* (97 %). Based on the *recA* sequence, the new isolates (exemplified by BC204) cluster with several isolates of unknown genomovar status and are clearly distinct from the CF isolates of unknown genomovar status reported previously (exemplified by BC14) (Detsika *et al.*, 2003; Fig. 2). The strains HW, R-9929, LMG14095, CEP1061, LMG6860, ATCC17769 and ATCC17460 (*recA* sequence accession numbers AF456066, AF456020, AF456016, AF456011, AF456069, AF456008 and AF456019, respectively), which cluster with BC204 on the basis of *recA* sequence (Fig. 2), are all members of *B. cepacia* Group K (Vermis *et al.*, 2002; Payne *et al.*, 2005; E. Mahenthiralingam, personal communication). Based on the *opcL* sequence, the new isolates (exemplified by

Fig. 2. Phylogenetic relationships according to *recA* sequence comparisons. The dendrogram was constructed using sequences from representatives of known genomovars of the BCC (genomovar I, *B. cepacia*; II, *Burkholderia multivorans*; III, *B. cenocepacia*; IV, *B. stabilis*; V, *B. vietnamiensis*; VI, *Burkholderia dolosa*; VII, *B. ambifaria*; VIII, *Burkholderia anthina*; IX, *Burkholderia pyrrocinia*) and sequences giving best matches in BLASTN searches of the database, as described previously (Winstanley, 2004). BC204 is included as a representative of the new isolates.

Fig. 3. Phylogenetic relationships according to *opcL* sequence comparisons. Sequence alignment and tree construction were carried out as described previously (Plesa *et al.*, 2004). BC92 is included as a representative of the new isolates. Genomovar status is indicated in parentheses (I, *B. cepacia*; II, *B. multivorans*; III, *B. cenocepacia*; IV, *B. stabilis*; V, *B. vietnamiensis*; VI, *B. dolosa*; VII, *B. ambifaria*; VIII, *B. anthina*; IX, *B. pyrrocinia*). Bootstrap values are shown at nodes (based on 100 resamplings).

BC92) clustered most closely with isolates of *B. cenocepacia* (Fig. 3). However, the nucleotide variations in *opcL* would lead to one amino acid residue change between the new isolates and *B. cenocepacia*.

Thus, phenotypic and genotypic tests indicate that we have identified a group of related strains isolated from disparate sources and patients. The isolates do not conform to known genomovar status but belong to *B. cepacia* *recA* Group K, which may represent another species within the BCC (Payne *et al.*, 2005). The presence of common genotypes amongst such a diverse collection of isolates suggests that this strain type may be common in Brazil.

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