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., 2000; 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 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 HaeIII (BsaRI) and MspI, 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>
<thead>
<tr>
<th>Strain</th>
<th>Isolation date (month/year)</th>
<th>Patient age</th>
<th>Clinical source</th>
<th>Inpatient/ outpatient</th>
<th>Lys</th>
<th>Orn</th>
<th>Suc</th>
<th>Ado</th>
<th>Gel</th>
<th>Esc</th>
<th>42 °C</th>
<th>β-h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC92</td>
<td>05/2002</td>
<td>1 year</td>
<td>Skin (osteomyelitis)</td>
<td>Out</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC201</td>
<td>04/2003</td>
<td>10 days</td>
<td>Ocular</td>
<td>In/out*</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC202</td>
<td>06/2003</td>
<td>76 years</td>
<td>Skin wound</td>
<td>In</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC203</td>
<td>06/2003</td>
<td>20 years</td>
<td>Skin wound</td>
<td>Out</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC204</td>
<td>07/2003</td>
<td>27 years</td>
<td>Skin wound</td>
<td>Out</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC205</td>
<td>09/2003</td>
<td>23 years</td>
<td>Ocular</td>
<td>Out</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BC111</td>
<td>07/2003</td>
<td>6 years</td>
<td>CF isolate</td>
<td>Out</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*New-born baby may have acquired the infection either as inpatient, from eye-drops following birth, or as outpatient.
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 BC204) by one nucleotide. The recA and opcL sequences have been deposited in GenBank (accession nos DQ166202 and DQ166203, respectively).

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

**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 pyrocinia) 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.
B. cepacia indicated in parentheses (I, stabilis et al alignment and tree construction were carried out as described previously (Plesa nucotide variations in http://jmm.sgmjournals.org 249 Pierre Cornelis3 and Craig Winstanley1 Maria Plesa,3 C. Anthony Hart,1 B. cenocepacia of BC92) clustered most closely with isolates (Fig. 3). However, the nucleotide variations in opcL would lead to one amino acid residue change between the new isolates and B. cenocepacia.

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).

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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