Prevalence of Indeterminate Genetic Species of *Burkholderia cepacia* Complex in a Cystic Fibrosis Center in Argentina

The *Burkholderia cepacia* complex (BCC) represents a group of gram-negative bacilli usually found ubiquitously in the environment whose members are of significant pathogenic potential, particularly for patients with cystic fibrosis (CF) (3). In recent years, taxonomic advances have demonstrated that this group of bacteria consists of at least nine related genetic species (formerly designated “genomovars”) (9). Representative group of bacteria consists of at least nine related genetic species among the CF population in Argentina. Molecular techniques are required for an accurate identification of BCC species (7). Among these techniques, PCR-based diagnostic techniques are particularly valuable for patients with cystic fibrosis (CF) (3). In the clinical laboratory (7). In our experience examining sputum samples, the BCC species were often difficult to identify using only PCR and restriction fragment length polymorphism (RFLP) analyses (7). Therefore, the aim of this study was to determine the methods needed for identification of BCC species in sputum samples from our CF patients.

Thirty putative BCC isolates were recovered on BCSA medium (4) from sputum samples of 30 CF patients attending a CF center in Buenos Aires between 2002 and 2006. Chromosomal DNA was extracted either by phenol chloroform or boiling methods (8). Phenotypic characteristics were assigned according to the method of Henry et al. (5). The genetic species status of each isolate was determined by PCR of the recA gene and RFLP with HaeIII in combination with species-specific primers as previously described (7). To further characterize the isolates, the DNA sequence of the recA gene was determined (1) and the sequences analyzed using BLAST. The recA genes of *B. stabilis* LMG18870 and *B. cenocepacia* LMG16654 were also sequenced as control strains, obtaining 100% of identity with the sequences listed under GenBank accession numbers AF143789 and AF456025, respectively.

BCC strains were confirmed as present in the 30 CF patient isolates. Sequencing 348 bp of the recA gene allowed us to identify 14 isolates, which corresponded to *B. cenocepacia* (3), *B. cepacia* A and 4 *B. cenocepacia* B isolates, *B. cepacia* (4 isolates), *B. stabilis* (2 isolates), and *B. multivorans* (1 isolate). However, the recA nucleotide sequences in the remaining 16 BCC isolates exhibited high-level identity with two isolates of unknown genomic species status, also called indeterminate BCC species (Table 1). Although three of these indeterminate BCC isolates had the recA-RFLP H pattern that corresponds to *B. cenocepacia* and also yielded amplicons with specific primers for *B. cenocepacia* B (Table 1), the DNA sequence of their recA gene showed 100% identity with the sequence corresponding to GenBank accession number AY228543 (available in the GenBank database) and exemplified by isolate BC14, which was previously described in a study of isolates from Brazilian CF patients (2). The remaining 13 BCC isolates harbored the recA sequence, with 99 to 100% identity with the sequence of the species corresponding to GenBank accession number AF456112. The recA-RFLP pattern of these BCC isolates was H or J (corre-

### TABLE 1. Genotypic and phenotypic characteristics of indeterminate species of *Burkholderia cepacia* complex isolates

<table>
<thead>
<tr>
<th>B. cepacia complex strain</th>
<th>recA-RFLP pattern</th>
<th>Misidentification with specific primers</th>
<th>Best match GenBank accession no.</th>
<th>Presence or % of BCESM</th>
<th>Presence or % of ONPG</th>
<th>Presence or % of growth at 42°C</th>
<th>Presence or % of ornithine decarboxylase</th>
<th>Presence or % of esculin hydrolysis</th>
<th>Presence or % of pigment</th>
<th>Presence or % of β-hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>H</td>
<td>B. cenocepacia B</td>
<td>AY228543</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>92</td>
<td>H</td>
<td>B. cenocepacia B</td>
<td>AY228543</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FAV3</td>
<td>H</td>
<td>B. cenocepacia B</td>
<td>AY228543</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>101</td>
<td>Indeterminate</td>
<td>B. cenocepacia</td>
<td>AY228543</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>84</td>
<td>J</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>89</td>
<td>J</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>91</td>
<td>J</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>97</td>
<td>J</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>103</td>
<td>Indeterminate</td>
<td>B. cenocepacia</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>BCC28</td>
<td>H</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>BCC32</td>
<td>H</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>BCC34</td>
<td>H</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>BCC52</td>
<td>Indeterminate</td>
<td>B. cenocepacia</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>BCC57</td>
<td>H</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>Pt. 1111</td>
<td>Indeterminate</td>
<td>B. cenocepacia</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>180</td>
<td>J</td>
<td>B. stabilis</td>
<td>AF456112</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>B. stabiliis</td>
<td>AF456025</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* a, present; -, absent. Values in columns 5 to 12 represent percentages of strains giving positive results (adapted from Henry et al. [5]).

b BCESM, *Burkholderia cepacia* epidemic strain marker.

c ONPG, *o*-nitrophenyl-β-o-galactopyranoside.

d Indeterminate, the recA RFLP pattern could not be defined or no amplification with specific primers was obtained.
sponding to B. stabilis (Table 1), and for nine of these isolates a positive amplification product with the specific B. stabilis primers was obtained (Table 1). Moreover, the phenotypic characteristics of these isolates differed from those described for B. stabilis but resembled those of B. cenocepacia (Table 1). Indeed, most of them exhibited β hemolysis, formed a yellow-green pigment, and gave a positive result for the Burkholderia cepacia epidemic strain marker.

To identify a possible source of infection with BCC species in our hospital, air and surface samples were collected after an infected/colonized patient left the room. One hundred samples from 25 rooms were analyzed, and no evidence of BCC species was detected. Further studies are thus required to assess whether the environment around these patients represented a reservoir, especially for the indeterminate species of BCC, or whether patient-to-patient transmission had occurred.

In summary, phenotypic and genotypic tests indicated that 54% of the isolates clustered into two groups of indeterminate genetic species of BCC and that these species are prevalent among our CF patients. Molecular DNA-DNA hybridization studies should be carried out to assign the correct genetic species of BCC and that these species are prevalent among our CF patients.

D.C. M.A.V. holds a Canada Research Chair in Infectious Diseases and Microbial Pathogenesis.

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