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Letters to the Editor

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 strains of all BCC species have been isolated from pulmonary infections in CF patients, but several studies have indicated that *B. multivorans* and *B. cenocepacia* account for the majority of BCC isolates from these patients (1, 6). Furthermore, certain clones of *B. cenocepacia* and *B. dolosa* are particularly associated with poor clinical course and high mortality (1, 10).

Currently, there are no data about the distribution of BCC 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 tests targeting the *recA* gene are amenable for use 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. cenocepacia* 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[a]

B. cepacia complex strain	*recA*-RFLP pattern	Misidentification with specific primers	Best match GenBank accession no.	Presence or % of BCESM[b]	Presence or % of sucrose	Presence or % of ornithine decarboxylase	Presence or % of growth at 42°C	Presence or % of ONPG[c]	Presence or % of esculin hydrolysis	Presence or % of pigment	Presence or % of β hemolysis
83	H	*B. cenocepacia* B	AY228543	−	+	+	−	−	+	−	−
92	H	*B. cenocepacia* B	AY228543	−	+	+	−	−	+	−	−
FAV3	H	*B. cenocepacia* B	AY228543	−	−	+	−	+	−	−	−
101	Indeterminate[d]	Indeterminate	AY228543	−	−	−	−	−	−	−	−
84	J	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	−
89	J	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	+
91	J	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	+
97	J	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	+
103	Indeterminate	Indeterminate	AF456112	+	+	−	−	+	+	Yellow-green	+
BCC28	H	*B. stabilis*	AF456112	+	−	+	−	+	+	Yellow-green	+
BCC32	H	*B. stabilis*	AF456112	+	−	+	−	+	+	Yellow-green	+
BCC34	H	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	+
BCC52	Indeterminate	Indeterminate	AF456112	+	+	−	−	+	+	Yellow-green	−
BCC57	H	*B. stabilis*	AF456112	+	+	−	−	+	+	Yellow-green	+
Pt. 1111	Indeterminate	*B. cenocepacia* A	AF456112	−	+	+	−	+	−	−	−
180	J	*B. stabilis*	AF456112	+	−	−	−	+	+	Yellow-green	+
B. stabilis	J	*B. stabilis*	AF143789	0	0	100	0	0	0	0	0
B. cenocepacia B	H	*B. cenocepacia* B	AF456025	63	91	71	84	99	33	3	3

[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-D-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 status of the BCC species prevalent in our CF patients (9). This molecular surveillance study also revealed that Argentina and Brazil exhibit a particular local epidemiology that should be confirmed with a wider regional study.

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