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Short communication

Transient isolation of *Burkholderia multivorans* and *Burkholderia cenocepacia* from a Brazilian cystic fibrosis patient chronically colonized with *Burkholderia vietnamiensis*

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Abstract

Fifteen serial *Burkholderia cepacia* complex isolates recovered over a period of 4 years from a single cystic fibrosis patient were analysed for genomovar status by means of *recA* sequence determination, and genetic relatedness by RAPD-PCR. Twelve isolates were assigned as *Burkholderia vietnamiensis*, two as *Burkholderia cenocepacia* and one as *Burkholderia multivorans*. *B. vietnamiensis* persisted in the airways during 4 years, except in three occasions when *B. cenocepacia* or *B. multivorans* were isolated. The patient was chronically colonized by *B. vietnamiensis* with the RAPD-profile 12 and transiently by the RAPD-profile 15.
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Keywords: *Burkholderia vietnamiensis*; *Burkholderia multivorans*; *Burkholderia cenocepacia*; Cystic fibrosis

1. Introduction

Bacteria belonging to the *Burkholderia cepacia* complex (BCC) are recognized as important opportunistic pathogens among patients with cystic fibrosis (CF). Multi-drug resistance, cross-infection due to patient-to-patient transmission or nosocomial spread, and the rapid and unexpected fatal clinical decline (cepacia syndrome) in some patients are factors that have pointed to BCC as a major threat to CF patients [1]. The BCC comprises a number of genomically distinct species. Nine genomovars have been described so far, and each one has received formal binomial designations, including *B. cepacia* (genomovar I), *Burkholderia multivorans* (genomovar II), *Burkholderia cenocepacia* (genomovar III), *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) [2,3].

Although strains from all the genomovars are potentially capable of causing human infections, differences seem to exist in their significance in CF patients. *B. cenocepacia* and *B. multivorans* have been described to account for the great majority of infections in CF [4]. *B. cenocepacia* appears more likely to spread from patient to patient, and to be more

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frequently associated with outbreaks than other genomovars [5]. Many CF patients have chronic respiratory infection/colonization by BCC whereas others have transient or intermittent respiratory infection/colonization [6]. Replacement of one BCC species, such as *B. multivorans*, *B. vietnamiensis*, *B. cepacia* or *B. dolosa,* by *B. cenocepacia*, with the subsequent deterioration of the patient's condition, has been documented in different epidemiological studies [7,8].

In the present study we assessed the characteristics of BCC isolates (including four mucoid isolates) recovered from a CF patient. The microbiological follow-up of the patient lasted 9 years, but BCC organisms were isolated over a period of 4 years. The genomovar status and genetic relatedness of different isolates were determined.

2. Material and methods

2.1. Sputum analysis

BCC isolates were obtained from a CF patient attending the Instituto Fernandes Figueira-FIOCRUZ—a cystic fibrosis centre located in Rio de Janeiro, Brazil. Since the CF diagnosis, the patient (a 13-year-old boy, at that time) was submitted to a regular multidisciplinary evaluation for 9 years (from November 1989 to November 1999). Thirty-three sputum samples were obtained through spontaneous expectoration, during both regular follow-up and exacerbation periods. The clinical samples were plated onto sheep blood agar, Eugon agar, cystine-lactose-electrolyte-deficient agar and *Pseudomonas* isolation agar (Difco Labs, Detroit, MI, USA) supplemented with polymixin B (300 μg ml^{-1}) (Sigma Co., St Louis, MD, USA). Preliminary identification of different morphotypes, as belonging to the BCC, was performed by using a panel of conventional phenotypic tests [9]. The other microorganisms were characterized at the genus and species level by routine conventional physiological methods [10].

2.2. B. cepacia complex reference strains

Strains BC 1254, BC 788, BC 818, BC 842, BC 825 and BC 1109, representative of genomovars I, II, IIIA, IIIB, IV, and V, respectively, were used as reference strains. Reference strains were kindly provided by Dr. Tyrone Pitt (Laboratory of Health-Care Associated Infection, Health Protection Agency, London).

2.3. Genomovar status

In order to identify the distinct genomovars, a 1043 bp PCR product corresponding to the *recA* gene was amplified by PCR using genomic DNA extracted using proteinase K and phenol as described previously by Sambrook et al. [11] from the isolates and the reference strains described above. The *recA* gene was amplified as described by Mahenthiralingam et al. (2000) were used [12]. DNA sequence was performed in both directions with the PCR primers and the aid of two additional primers, BCR3 and BCR4 [12]. DNA sequences for each sample were analysed by the Staden Package [13], and an online CAP3 sequence assembly program (http://pbil.univ-lyon1.fr/cap3.php). The consensus sequences obtained were aligned with the ClustalX software and used to construct phylogenetic trees with the ClustalX software by the neighbour joining method to allow genomovar identification [14]. *recA* reference sequences for genomovars VI–IX were obtained in GenBank and were also used for alignment and tree construction.

2.4. Molecular typing

Bacteria were grown overnight at 37 °C in BHI. An aliquot of the culture was diluted 1:10 in water immediately before use to determine the optical density at 600 nm. Cultures were then diluted to a final optical value of 0.5 and a 300-μl aliquot was centrifuged ($13{,}000 \times g$/2 min) and suspended in 900 μl of sterile distilled water. The suspension was then boiled for 10 min, briefly centrifuged, and used as the template source in amplification reaction [15]. RAPD reaction was carried out with primer 1254 (5′CCGCAGCCAA3′). PCR was performed in 30 μl reaction volumes containing 20 mM Tris–HCL (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 0,25 mM (each) dNTP (Invitrogen), 30 pmol of primer, 1.5 U of Taq DNA polymerase (Invitrogen) and 3 μl of bacterial lysate. Cycling parameters for amplification were as follows: 4 cycles of 94 °C for 5 min, 37° for 5 min, and 72 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 37 °C for 1 min, and 72 °C for 2 min and a final extension step at 72 °C for 10 min. Reaction products were analyzed by electrophoresis on 1.2% agarose gels and stained with 0.5 mg of ethidium bromide per ml. Gels were photographed under UV light. A 100-bp DNA ladder (Invitrogen) was used as a molecular weight marker [16].

3. Results

3.1. Genomovar status of the 15 serial isolates

During the first 6 years of the bacteriological follow-up (November 1989–November 1995), *Staphylococcus aureus*, non-mucoid and mucoid *Pseudomonas aeruginosa* were the only pathogens detected in the sputa. From December 1995 to September 1997, BCC isolates were found continuously in association with *P. aeruginosa* for two consecutive years. In the last 2 years of evaluation BCC isolates emerged and persisted chronically as the single pathogen and, on four occasions, the bacteria exhibited a mucoid morphotype. DNA sequence analyses of the *recA* gene showed that among the 15 BCC isolates examined, 12

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