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CHRONIC INFECTION OF CYSTIC FIBROSIS PATIENT AIRWAYS BY A SINGLE CLONE OF *BURKHOLDERIA CEPACIA*: REPLACEMENT OF NON-MUCOID TO MUCOID MORPHOTYPE

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ABSTRACT

Mucoid *Burkholderia cepacia* morphotype emerged within a nine year follow-up of a cystic fibrosis patient. Clinical data suggested a linkage between the mucoid phenotype isolation and the deterioration of the patient's condition. Despite of the phenotypic variation, molecular typing showed that the patient was chronically infected with *B. cepacia* complex isolates belonging to a same genetic clone.

Key words: Cystic Fibrosis, *Burkholderia cepacia* complex, PFGE, RAPD-PCR.

INTRODUCTION

Burkholderia cepacia complex has emerged as a major pathogen in 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 *B. cepacia* complex as a major threat to CF patients (2,5). Mucoid (MUC) morphotype is thought to be relatively rare among *B. cepacia* complex CF isolates (1). There is no clear data about the possible contribution of the extracellular polysaccharide to the colonization and persistence of the microorganism in the infected host, as was described in patients infected with MUC *P. aeruginosa* (2). This study reports the emergence of a MUC *B. cepacia* complex morphotype in CF patient chronic infection attended at a reference CF center located in Rio de Janeiro, Brazil.

MATERIALS AND METHODS

B. cepacia complex isolates were obtained from a CF patient. Since the CF diagnosis, the patient was submitted to a regular multidisciplinary evaluation for nine years (from Nov/1989 to Nov/1998). During this period, 33 sputum samples were obtained and plated onto sheep blood agar, Eugon agar, cystine-lactose-electrolyte-deficient- (CLED) agar and Pseudomonas Isolation agar (Difco Labs, Detroit, MI, USA) added with polimixin (300 µg/ml) (Sigma Co., St Louis, MD, USA). Identification of *B. cepacia* complex isolates was performed by using biochemical tests (3). Genotyping of MUC and NM *B. cepacia* complex isolates were performed by PFGE and RAPD-PCR. PFGE was carried out as described previously (8) using *Xba*I endonuclease. Banding patterns were visualized by ethidium bromide staining and the profiles were interpreted according to Tenover rules (7). RAPD-PCR was performed as

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described by Pacheco *et al.* (6) using primer 1254 (nucleotide sequence 5'-CCGCAGCCAA-3').

RESULTS AND DISCUSSION

During first six years of the bacteriological follow-up (Nov/1989-Nov/1995), *S. aureus*, NM and MUC *P. aeruginosa* were the only pathogens detected in the sputa samples. After that, *B. cepacia* complex was found in association with *P. aeruginosa* for two consecutive years (Dec/1995-Sep/1997). In last year of evaluation (Oct/1997-Nov/1998), MUC *B. cepacia* complex was emerged and persisted chronically as a single pathogen. At the same period, respiratory infection exacerbation had become more frequent and the patient had to be hospitalized at shorter intervals. The computed tomography scanner of the thorax evidenced an increase in bronchiectasis and the pulmonary function test showed a degree II obstructive disturbance.

By both genotypic methods all *B. cepacia* complex strains shared similar DNA-banding profiles (Figs. 1 and 2) with little variations of one or two bands (Fig. 1 e.g. Lanes 4 and 9). Although, as described previously, strains isolated multiple times from the same patients should demonstrated such variability (7). Molecular typing procedures demonstrated that the patient was chronically infected by a single clone that had remained genetically stable over a period of three years in spite of exopolysaccharide (EPS) expression. Therefore, phenotypic changes in sequential isolates did not represent replacement by a new strain suggesting an adaptation of the primary colonizing isolates (4). The isolation of MUC *B. cepacia* complex is rare and to the best of our knowledge, the association between the chronic infection of MUC *B. cepacia* as a single bacterial pathogen with the decline of clinical outcome has not yet been reported. It is essential to evaluate the role of EPS biosynthesis and the possible factors that may lead to the changing of NM to MUC phenotype in the characteristic environment of the lungs of CF patients which could improve our knowledge about *B. cepacia* complex pathogenesis.

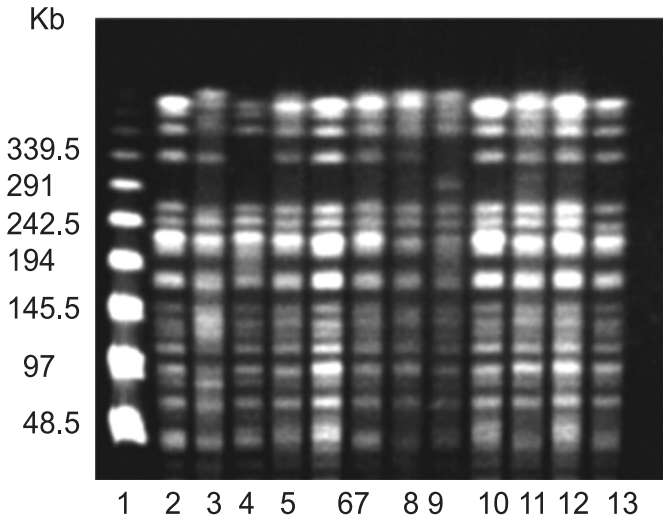

Figure 1. PFGE patterns of chromosomal DNA of sequential *Burkholderia cepacia* complex isolates after digestion with *XbaI*. Lanes: 1. Molecular size markers (in kilobases); Lanes 2 to 6, non-mucoid *B. cepacia* complex strains; Lanes 7-13, mucoid *B. cepacia* complex strains.

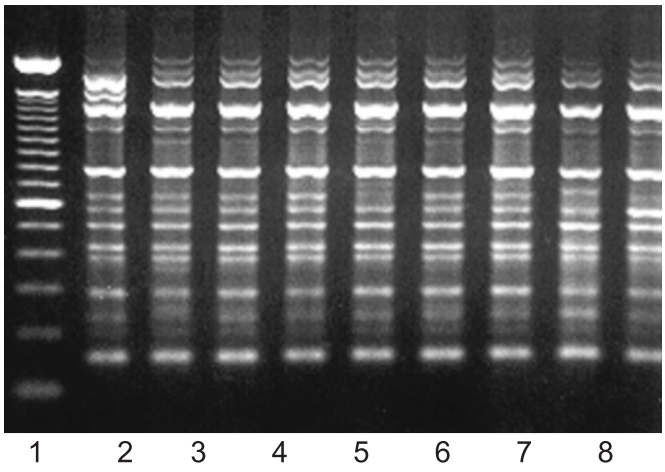

Figure 2. RAPD-PCR profiles of sequential *Burkholderia cepacia* complex isolates using 1254 primer. Lanes: 1. 1Kb Ladder (Gibco -BRL); Lanes 2 to 6, non-mucoid *B. cepacia* complex strains; Lanes 7-10, mucoid *B. cepacia* complex strains.

RESUMO

Infecção pulmonar crônica por um único clone de *Burkholderia cepacia*: substituição do morfotipo não mucóide por mucóide

O presente trabalho descreve a emergência de cepas mucoides do complexo *B. cepacia* em um paciente com Fibrose Cística dentro de um acompanhamento bacteriológico prospectivo de nove anos. Os dados clínicos sugerem a associação entre o isolamento do morfotipo mucoide e a deterioração clínica do paciente. Apesar da variação fenotípica, os testes moleculares mostraram que o paciente manteve-se cronicamente infectado por cepas de mesma origem clonal.

Palavras-chave: Fibrose Cística, complexo *Burkholderia cepacia*, PFGE, RAPD-PCR.

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