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Short communication

Genomovar status, virulence markers and genotyping of *Burkholderia cepacia* complex strains isolated from Brazilian cystic fibrosis patients

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Abstract

Burkholderia cepacia complex isolates obtained by microbiological culture of respiratory samples from Brazilian CF patients were studied by *recA* based PCR, screened by specific PCR for virulence markers and genotyped by RAPD. Forty-one isolates of *B. cepacia* complex were identified by culture and confirmation of identity and genomovar determination obtained in 32 isolates, with predominance of *B. cenocepacia* (53.1%). Virulence markers were not consistently found among isolates. Genotyping did not identify identical patterns among different patients. *B. cenocepacia* was the most prevalent *B. cepacia* complex member among our patients, and cross-infection does not seem to occur among them.

Keywords: *Burkholderia cepacia* complex; Cystic fibrosis; Children; Virulence factors; Molecular epidemiology

1. Introduction

The *Burkholderia cepacia* complex has been recognized as a significant pathogen for CF patients since the early 1980s and consists of ten closely related species, also called genomovars [1]. Most specialized centres report a prevalence of *B. cepacia* complex of up to 10% [2–4]. *B. cenocepacia* is a member of the *B. cepacia* complex showing the highest prevalence among CF patients, followed by *B. multivorans*, although regional differences in genomovar distribution have been reported [5].

Highly transmissible strains of the *B. cepacia* complex have been identified in some CF centres, mainly in the United Kingdom and Canada, and genetic markers including cable pilus gene (*cblA*) and an insertion sequence named *B. cepacia* epidemic strain marker (BCESM) are commonly detected in these strains [6,7].

There is little published data regarding prevalence and genomovar distribution of *B. cepacia* complex strains isolated from Brazilian cystic fibrosis patients [8–10]. The Pediatric Pulmonology Unit of Instituto da Criança is one of the referral centres for CF care in Brazil and, although segregation of patients based on respiratory colonization has not been implemented to date, cross-colonization with *P. aeruginosa* strains has not been identified among patients [11]. The objective of the present study was to analyse *B. cepacia* complex strains isolated from CF patients attending our CF centre, by determining genomovar distribution, virulence-related genetic markers and the genotype of these isolates for epidemiological purposes.

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2. Materials and methods

2.1. Patients and samples

Samples identified as belonging to the *B. cepacia* complex in the microbiology laboratory during two periods (regular use of *B. cepacia* complex-selective medium) were studied: period A (September 2000 to April 2001) and period B (June 2003 to June 2004). A total of 140 CF patients attended our unit during these two periods, and the diagnosis of CF was based on international consensus guidelines [12]. Respiratory samples were collected from the patients during routine and unscheduled visits to the outpatient clinic and cultured on selective media, including *B. cepacia* complex-selective medium (Oxoid, Basingstoke, United Kingdom). Plates were incubated at 36 °C for 18 to 96 h.

During the first period of the study (period A), bacteria were identified using the Vitek automated system (bioMérieux Vitek Inc., St. Louis, MO, USA), with additional biochemical tests when indicated. During the second period of the study (period B), bacterial identification was performed by classical phenotypical testing.

The study was approved by the Ethics Committees of all Institutions involved.

2.2. DNA purification and PCR

Isolates identified as *B. cepacia* complex were submitted to DNA extraction as described elsewhere [8], and DNA was quantified in an ultraviolet spectrophotometer (BioPhotometer 6131, Eppendorf AG, Hamburg, Germany).

The genomovar status of the *B. cepacia* complex isolates was identified by sequential PCR targeting specific regions of the *recA* gene as previously described [13–15]. Positive controls consisting of DNA from the *B. cepacia* complex strain belonging to the genomovar tested were included in all reactions.

Virulence markers were identified by PCR targeting the *cblA* (cable pilus) and *esmR* (BCESM) genes as previously described [6,7]. DNA from the *B. cepacia* complex strain AU0355 (epidemic strain expressing both virulence factors) was included in the reactions as positive control.

After amplification, PCR products were visualized by electrophoresis on 1.5% agarose gel stained with 0.5 mg/mL ethidium bromide under UV illumination using an automated system (AlphaImager®, Alpha Innotech Co., San Leandro, California, USA). A negative control consisting of sample without DNA was included in all reactions. All reactions were carried out under a laminar flow UV hood according to standard precautions to prevent PCR carry-over [16].

2.3. DNA sequencing

DNA sequencing of the *recA* gene was performed to confirm the results of the specific PCR. DNA sequencing was carried out using a panel of four primers (BCR 1–4) [13] and BUR1 and BUR2 primers [17], using the Big Dye Terminator kit (Applied Biosystems Incorporation, Foster City, CA, USA) in an automated DNA sequencer (ABI Prism 377, Applied Biosystems). The results obtained were compared to sequences deposited in the Genebank database using the BLAST program.

2.4. Molecular epidemiology (RAPD)

B. cepacia complex isolates were genotyped by the RAPD method using primer 272 (5′-AGCGGGCCAA-3′), as previously described [18]. After amplification, the RAPD products were visualized by electrophoresis on 7.5% acrylamide gel stained with 0.2% silver nitrate. The results were interpreted by direct visualization of the band patterns and isolates which differed by two or more major bands were considered sufficiently divergent to warrant separate strain designations.

3. Results

During the two periods of the study, a total of 672 respiratory samples were obtained from 140 CF patients attending our CF centre. *B. cepacia* complex isolates were identified by culture in 41 samples (35 sputum and 6 oropharyngeal swabs) from 21

Table 1
Genomovar determination by PCR, *recA* gene DNA sequencing and RAPD genotyping of isolates identified as members of the *B. cepacia* complex during the study ($n=32$)

Patient	Isolate	Period of isolation	Genomovar *recA* PCR	Epidemic strain marker (*esmR*)	RAPD typing	DNA sequencing of *recA* gene
B	2	B	IIIA	−	2	*B. cenocepacia*
C	3	B	IIIA	−	3	*B. cenocepacia*
D	4	A	IIIA	−	4	*B. cenocepacia*
D	5	B	IIIA	−	4	*B. cenocepacia*
E	6	A	I	−	5	*B. cepacia*
F	8	B	IIIA	−	7	*B. cenocepacia*
F	9	B	IIIA	−	7	*B. cenocepacia*
F	10	B	IIIA	−	7	*B. cenocepacia*
F	11	B	IIIA	−	7	*B. cenocepacia*
G	12	B	V	−	8	*B. vietnamiensis*
H	13	B	IIIA	−	9	*B. cenocepacia*
H	14	B	IIIA	−	9	*B. cenocepacia*
H	15	B	IIIA	−	9	*B. cenocepacia*
H	16	B	IIIA	−	10	*B. cenocepacia*
I	17	A	V	−	11	*B. vietnamiensis*
K	19	A	IIIB	+	13	*B. cenocepacia*
L	20	A	II	−	14	*B. multivorans*
L	21	A	II	−	NP	*B. multivorans*
L	22	B	II	−	14	*B. multivorans*
L	23	B	II	−	14	*B. multivorans*
L	24	B	II	−	14	*B. multivorans*
L	25	B	II	−	14	*B. multivorans*
M	26	B	II	−	15	*B. multivorans*
N	27	B	IIIA	+	16	*B. cenocepacia*
P	32	A	IIIA	−	18	*B. cenocepacia*
P	33	A	IIIA	−	18	*B. cenocepacia*
Q	34	B	II	−	19	*B. multivorans*
S	36	A	II	−	21	*B. multivorans*
S	37	B	II	−	21	*B. multivorans*
S	38	B	II	−	21	*B. multivorans*
S	39	B	II	−	21	*B. multivorans*
U	41	B	IIIA	−	23	*B. cenocepacia*

NP: not performed.