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Original Article

Genetic relatedness of *Burkholderia contaminans* clinical isolates from cystic fibrosis and non-cystic fibrosis patients in Argentina

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Abstract

Introduction: The *Burkholderia cepacia* complex (BCC) bacteria are opportunistic pathogens that cause nosocomial infections and are especially dangerous for cystic fibrosis (CF) patients. *Burkholderia contaminans* is an emerging BCC species isolated from CF patients that also occurs as a contaminant in pharmaceutical and personal care products, sometimes linking it with outbreaks.

Methodology: A total of 55 *B. contaminans* isolates from CF and non-CF patients in Argentina were identified by *recA* sequencing and MALDI TOF MS. A standardized Pulsed Field Gel Electrophoresis (PFGE) protocol was set up in order to assess genetic diversity, outbreak investigations, and possible clone persistence.

Results: All isolates were identified as *B. contaminans* by both MALDI-TOF MS and recA sequence analysis. PFGE has enabled us to compare and determine the genetic relationship between *B. contaminans* isolates. Isolates were distributed in different PFGE clusters with evidence of the presence and persistence of a clone, over a period of 3 years, in the same hospital. This large hospital outbreak involved CF and non-CF patients. Moreover, PFGE results showed a good correlation between sporadic or outbreak-related isolates and the available epidemiological information.

Conclusions: These findings highlight the importance of *B. contaminans* in Argentina and provide evidence for encouraging the surveillance of highly transmissible clones. The study also contributes to global knowledge about *B. contaminans* infections.

Key words: *Burkholderia contaminans*; MALDI-TOF MS; PFGE; *Burkholderia* typing; recA; cystic fibrosis.

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Introduction

Burkholderia cepacia complex (BCC) is a group that comprises of Gram-negative, rod-shaped, motile, and non-spore forming bacteria that have been identified in many diverse ecological niches [1]. BCC comprises of at least 24 species widely distributed in nature showing an extensive nutritional versatility which contributes to their long-term survival under unfavorable conditions including aqueous environments. Furthermore, BCC bacteria are opportunistic pathogens that cause nosocomial infections that can arise from contaminated disinfectants, anesthetic solutions, distilled water, and aqueous chlorhexidine solutions [2,4]. Contamination of sterile and nonsterile pharmaceuticals with BCC has caused numerous nosocomial outbreaks in health care facilities, presenting a health threat, particularly for immunocompromised individuals around the world [2]. These species have a complex genome with three chromosomes and a high capacity for rapid mutation and adaptation [2]. BCC members pose a significant threat to individuals with cystic fibrosis (CF) leading to chronic infections in the lungs of these patients increasing their morbidity and mortality [3,4]. Due to resistance to a wide range of antibiotics in all BCC species, the prevention of infections in health care settings is a critical issue.

Burkholderia contaminans is an emerging BCC specie isolated from CF and non-CF patients and sometimes linked with outbreaks as a contaminant of pharmaceutical and personal care products [2,5,6]. Analysis of BCC species diversity among CF patients from Argentina established that *B. contaminans* is the most prevalent species recovered [7,8]. *B. contaminans* infections in CF are often of transient nature, nevertheless some patients develop chronic infection

with lung function declines and general condition worsening [9,10]. *B. contaminans* is also the most frequent species within BCC recovered from industrial products and settings in Argentina [11].

Identification and subtyping of BCC species is essential for clinical therapy and prognosis, particularly in CF patients and epidemiological research, as well as for providing information to detect persistent clones or outbreaks. Correct species identification of BCC requires molecular tools, such as *recA* gene sequencing. However, despite their good accuracy, molecular techniques are expensive, time-consuming, and technically demanding for clinical laboratories. The speed and low cost of bacterial identification by MALDI-TOF MS make it an attractive technology in the clinical microbiology laboratory which allows the identification of a broad range of microorganisms. MALDI-TOF MS shows resolution limitations among some closely related species, sometimes associated with a small number of reference strains included in the database [12]. MALDI-TOF MS correctly identified most BCC isolates at the species level but failed in the identification of *B. contaminans* due to the absence of a mass spectrum profile of this species in the Biotyper 3.0 [13]. Recently, we created a reliable reference database for *B. contaminans* showing good accuracy for the identification of this species [14].

The ubiquitous nature of *B. contaminans* requires standardized methodologies to assess the genetic relatedness among isolates in order to identify specific clones, persistence events, and confirm potential sources of infection. PCR-based and pulsed field gel electrophoresis (PFGE) was used for outbreak studies showing a good correlation with epidemiological investigations [5,6,15]. Both techniques allowed the confirmation of clonal identity among *B. contaminans* isolates from clinical specimens and contaminated products from hospital outbreaks in USA and France [5,6]. Speert *et al*. revealed remarkable differences among BCC strains clustered by RAPD and PFGE among CF-patient isolates from different provinces of Canada [15].

In the past few years, techniques based on whole-genome sequencing (WGS) have been used to evaluate phylogenetic relationships. However, this technique is not fully available for routine use in clinical microbiology laboratories in developing countries, and the sequence analysis is still complicated [9,16]. PFGE has proved to be a very useful tool for epidemiological studies. Therefore, in laboratories where PFGE is available, setting a reliable and efficient PFGE protocol would allow rapid detection and confirmation of clones preventing further spread of *B. contaminans* infections. The aim of this study was to evaluate the genetic relatedness among a collection of *B. contaminans* isolates recovered from CF and non-CF patients from Argentina by setting up a PFGE protocol designed for this species.

Methodology

Bacterial isolates

All *B. contaminans* clinical isolates (n = 55) submitted for bacterial identification to Bacteriologia Especial Laboratory between 2011 and 2014 were studied. Isolates were from 47 patients admitted at 14 hospitals distributed in 9 provinces of Argentina. Isolates were recovered from CF (n = 22) or non-CF patients (n = 33).

According to the available epidemiological information the isolates were also classified as sporadic cases (n = 37) or outbreak-related isolates (n = 18) when recovered from the same hospital up to 7 days apart from each other. Up to 5 isolates were recovered from a single CF patient, named CF (a) to CF (e), from different exacerbation episodes.

Species identification

Phenotypic characterization was carried out according to the method of Henry *et al.* [17], including production of yellow-green pigment and the presence of β-hemolysis. Identification was performed by *recA* gene sequencing as described by Mahenthiralingam *et al.* [18]. Briefly, each amplicon was purified using the AccuPrep PCR Purification Kit (Bioneer Corporation, California, USA) according to the manufacturer's protocol and sequenced with the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Massachusetts, USA) and the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Massachusetts, USA). *recA* DNA sequences were compared using BLASTN program against BCC sequences deposited in GenBank database. Identification at species level was accepted with those sequences showing > 99% of identity [19,20]. A representative isolate from those associated with each outbreak was sequenced. GenBank accession numbers for the 44 *recA* gene sequenced are listed in Table 1.

A phylogenetic tree was constructed using the distance-based neighbor-joining method and performing 1000 bootstraps replicates to statistical robustness by MEGA6 software. *B. contaminans* LMG 16227 (AM922301), *B. cepacia* ATCC 25416 (AF143786), and *B. vietnamensis* LMG 10929 (CP009631) were included as reference sequences.

Table 1. Burkholderia contaminans isolates examined.

Strain ID	Sample	CF condition*	Sporadic / Outbreak classification	Hospital	MALDI-TOF MS best match ID	MALDI-TOF MS score value	PFGE cluster	Gen Bank accession number	recA group
265/11	Sputum	CF	Sporadic	Htal. Cordoba 2	*B. contaminans*	2.42	BX3	KT601572	recA type 1
311/11	Sputum	CF(e)	Sporadic	Htal. La Pampa-1	*B. contaminans*	2.45	NT	KT601581	recA type 2
26/12	Sputum	CF	Sporadic	Htal. Buenos Aires 1	*B. contaminans*	2.25	BX4	KT583154	recA type 2
154/12	Blood	non-CF	Sporadic	Htal. Rio Negro 1	*B. contaminans*	2.49	BX7	KT583153	recA type 1
231/12	Sputum	CF(a)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.51	BX3	KT583150	recA type 1
310/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.52	BX3	KT583149	recA type 1
311/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.27	BX3	NA	NA
312/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.27	BX3	NA	NA
313/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.57	BX3	NA	NA
314/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.24	BX3	NA	NA
315/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.41	BX3	NA	NA
316/12	Blood	non-CF	Outbreak	Htal. Cordoba 1	*B. contaminans*	2.31	BX3	NA	NA
341/12	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.69	BX3	KT598205	recA type 1
351/12	Sputum	CF	Sporadic	Htal. Buenos Aires 1	*B. contaminans*	2.43	NT	KT601573	recA type 2
353/12	Urine	non-CF	Sporadic	Htal. Buenos Aires 1	*B. contaminans*	2.39	BX3	KT601574	recA type 1
421/12	Unknown	non-CF	Sporadic	Htal. Misiones 2	*B. contaminans*	2.4	BX3	KT601575	recA type 1
425/12	Sputum	CF	Sporadic	Htal. Buenos Aires 1	*B. contaminans*	2.26	NT	KT583145	recA type 2
450/12	Sputum	CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.18	BX3	KT583143	recA type 1
460/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.4	BX1	KT583156	recA type 1
461/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.54	BX1	KT601576	recA type 1
462/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.33	BX1	NA	NA
463/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.31	BX1	NA	NA
464/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.22	BX1	NA	NA
465/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.41	BX1	NA	NA
466/12	Blood	non-CF	Outbreak	Htal. Misiones 1	*B. contaminans*	2.34	BX1	NA	NA
173/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.39	BX3	KT583155	recA type 1
211/13	Blood	non-CF	Sporadic	Htal. Chaco-1	*B. contaminans*	2.39	BX1	KT583152	recA type 1
212/13	Blood	non-CF	Sporadic	Htal. Chaco-1	*B. contaminans*	2.21	BX2	KT583151	recA type 1
384/13	Sputum	CF(a)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.5	BX3	KT583146	recA type 1
385/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.39	BX3	KT583144	recA type 1
386/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.58	BX3	KT598206	recA type 1
426/13	Sputum	CF(a)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.36	BX3	KT598208	recA type 1
447/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.38	BX3	KT598209	recA type 1
465/13	Sputum	CF(e)	Sporadic	Htal. La Pampa 1	*B. contaminans*	2.13	NT	KT583141	recA type 2
472/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.4	BX3	KT583140	recA type 1
497/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.18	BX3	KT583157	recA type 1
506/13	Sputum	CF(d)	Sporadic	Htal. BAC 1	*B. contaminans*	2.5	BX4	KT583158	recA type 2
512/13	Sputum	CF(a)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.52	BX3	KT583159	recA type 1
545/13	Blood	CF(b)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.38	BX3	KT583136	recA type 1
546/13	Blood	non-CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.39	BX3	KT583135	recA type 1
575/13	Sputum	CF(d)	Sporadic	Htal. BAC 1	*B. contaminans*	2.48	BX4	KT601577	recA type 2
632/13	Blood	non-CF	Outbreak	Htal. BAC 2	*B. contaminans*	2.28	NT	KT598210	recA type 2
634/13	Blood	non-CF	Outbreak	Htal. BAC 2	*B. contaminans*	2.29	NT	KT601578	recA type 2
635/13	Blood	non-CF	Outbreak	Htal. BAC 2	*B. contaminans*	2.44	NT	KT601579	recA type 2
636/13	Blood	non-CF	Outbreak	Htal. BAC 2	*B. contaminans*	2.48	NT	KT598211	recA type 2
191/14	Blood	non-CF	Sporadic	Htal. Buenos Aires 2	*B. contaminans*	2.48	BX6	KT598204	recA type 2
193/14	Blood	non-CF	Sporadic	Htal. Buenos Aires 2	*B. contaminans*	2.21	BX6	KT601582	recA type 2
341/14	Sputum	CF	Sporadic	Htal. BAC 1	*B. contaminans*	2.31	BX7	KT601581	recA type 1
369/14	Sputum	CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.64	NT	KT583148	recA type 2
371/14	Sputum	CF(c)	Sporadic	Htal. Cordoba 2	*B. contaminans*	2.37	NT	KT583147	recA type 2
423/14	Sputum	CF	Sporadic	Htal. Buenos Aires 1	*B. contaminans*	2.42	NT	KT598207	recA type 2
452/14	Sputum	CF(c)	Sporadic	Htal. Cordoba 3	*B. contaminans*	2.22	NT	KT583142	recA type 2
476/14	Sputum	CF(a)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.27	BX3	KT583139	recA type 1
477/14	Sputum	CF(b)	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.46	BX3	KT583138	recA type 1
480/14	Sputum	CF	Sporadic	Htal. Cordoba 1	*B. contaminans*	2.4	BX3	KT583137	recA type 1

NT: Non-typable; NA: Not Applied; *Those isolates with the same letter (*a, b, c, d* o *e*) were recovered from the same CF-patient.

MALDI-TOF MS

Protein spectra of *B. contaminans* were not included in the commercial database Biotyper v. 3.1 (Bruker Daltonics, Massachusetts, USA), therefore an extended database created in our laboratory [14] was included in order to improve MALDITOF MS performance for identification of BCC. Briefly, each isolate was cultured on trypticase soy agar plates and incubated at 37 °C for 18–24 hours. Bacterial cells were smeared onto stainless steel MALDI target/plate, and 1 µL of a saturated solution of MALDI matrix (alpha cyano-4-hydroxycinnamic acid matrix solution; Bruker Daltonics, Massachusetts, USA) was applied to each sample and dried. External calibration was performed using the Bacterial Test Standard (Bruker Daltonics, Massachusetts, USA) according to the manufacturer's instructions. Mass spectra ranging from 2,000 to 20,000 m/z were processed using FlexControl software version 1.3 and compared with Bruker Biotyper 3.1 software and library (database [DB] 5627 with 5627 entries) plus extended local database RENAEM V2.0. Identification scores of ≥ 2.00 indicated species-level identification, scores of 1.70 to 1.99 indicated genus-level identification, and scores of < 1.70 indicated no reliable identification. Additionally, the "10% rule" was applied, which states that any species scoring > 10% below the top-scoring match may be excluded [21].

Pulsed field gel electrophoresis (PFGE)

Isolates were grown in trypticase soy agar plates at 37 °C for 18-20 hours. Colonies were suspended in 2 mL of TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) and adjusted to an optical density of 0.4-0.45 using a MicroScan® Turbidity Meter (Dade Behring Inc., Illinois, USA). A volume of 300 µL of the suspension was incubated for 10 minutes at 42 °C and mixed with 300 µL of Seakem Gold Agarose (Cambrex, New Jersey, USA) and then pipetted into plug molds (Bio-Rad, California, USA). Plugs were incubated overnight in 5 mL of CBC buffer lysis (50 mmol/L Tris, 1% Sarkosyl, 50 mmol/L EDTA, pH 8.0) containing Proteinase K (0.5 mg/mL) at 54 °C in shaking-water bath. Then plugs were washed twice with sterile distilled water, followed by three washes with sterile TE buffer once every 30 minutes. Plugs were stored at 4 °C until use.

To set up the optimal PFGE protocol several conditions were assayed. For each plug, two slices (2 mm) were cut with a sterile blade. One slice was incubated for 3 hours with 30U of XbaI endonuclease (Fermentas, Massachusetts, USA) at 37 °C, and the other was treated overnight with 50 U of SpeI restriction enzyme (Promega, Wisconsin, USA) at 30 ºC, in both cases using buffer and reaction conditions recommended by the manufacturers. Electrophoresis was performed in a 1% agarose Seakem Gold Agarose (Cambrex, New Jersey, USA) gel prepared in 0.5% TBE buffer and run on a CHEF-DRIII system (Bio-Rad, California, USA). Running conditions were defined with 5 seconds and 35 seconds for initial and final switch times, respectively, for 18.6 hours at 6 V/cm and an angle of 120 degrees. Gels were stained with Gel Red (Biotium, California, USA) and visualized under UV light using the Gel-Doc System (Bio-Rad, California, USA) for image digitalization. XbaI and SpeI digestion showed DNA profiles with ~25 and ~20 bands, allowing a precise discrimination among unrelated strains and grouping those related. Considering brands, availability and general cost, XbaI enzyme was selected to digest plugs for all isolates. Some *B. contaminans* isolates were refractory to PFGE typing, although additional conditions were tested, i) formaldehyde treatment, ii) adding thiourea during electrophoresis, or iii) the use of other restriction enzymes.

TIFF images were analyzed by Bionumerics software (version 5.1, Applied Maths, Belgium) and normalized using *Salmonella* Braenderup H9812 as reference pattern. Band-based dendrogram were constructed using Dice's coefficient and an unweighted pair group method using arithmetic averages (UPGMA), band optimization was set to 1.5 % and tolerance to 1.5 %. A cut off of 85% of similarity was used for clustering.

Results

All 55 *B. contaminans* isolates grew on MacConkey agar, were positive for oxidase, catalase, lysine decarboxylase but no DNAse activity was detected. Isolates were capable of assimilating a wide range of carbohydrates and all of them showed yellow pigmentation and hemolytic activity.

All 55 isolates were identified as *B. contaminans* by MALDI-TOF MS using the extended database RENAEM v2.0. These results were 100% concordant with the 44 *recA* sequences analyzed. All isolates were identified with score values of ≥ 2.00 and were in compliance with the 10% rule. Both parameters are those recommended by the manufacturer for standardized identification of other pathogens, therefore the identification of *B. contaminans* isolates by MALDI-TOF MS showed reliable results.

All the *recA* gene sequences obtained showed > 99% of sequence similarity with *B. contaminans* LMG

16227 (AM922301) reference strain confirming the molecular identification at the species level. The *recA* sequence analysis showed that *B. contaminans* isolates from CF and non-CF shared a high degree of sequence similarity and were clustered into two groups (Figure 1). These two groups were named "recA type1" and "recA type2" and showed high bootstrap values (98%). Twenty-seven isolates were grouped as "recA type1" and were recovered from 5 geographically distant provinces and Buenos Aires City, showing a wide distribution of this cluster across the country. Seventeen isolates clustered as "recA type2" were submitted from four nearby districts, Córdoba, La Pampa and Buenos Aires provinces plus Buenos Aires City (Figure 1).

Figure 1. Phylogenetic tree of recA gene sequences of isolates and BCC type strains. The tree was constructed using the Neighbor-Joining method (performed using MEGA6 software). Bootstrap values are shown for 1000 replicates. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site.

Evaluation of different PFGE conditions allowed to define an optimal XbaI-PFGE protocol, described in the material and methods section. Nevertheless, 12 out of 55 *B. contaminans* isolates were refractory to PFGE typing yielding degraded DNA results, presumably due to the presence of high DNase activity [22]. Analysis among the 43 PFGE-typeable isolates allowed to identify 12 XbaI-profiles. A UPGMA dendrogram was constructed based on the band similarity in order to evaluate the relationship among the isolates (Figure 2). Two cut-off values, 85% and 90% of similarity were evaluated to correlate with the epidemiological data. Applying an 85% of similarity value as a cut-off, the profiles obtained were grouped in six clusters, while using a 90% cut-off threshold the isolates were grouped in nine clusters. While considering the epidemiological data, an 85% similarity cut-off showed a satisfactory agreement (Figure 2).

Sixty percent of the isolates (n = 26) were assigned to a major cluster, named BX3 (Figure 2). The *recA* sequences of 20/26 BX3 cluster isolates were indistinguishable and classified as "recA type1", showing a good correlation between both approaches (Figure 2). Remarkably, 92% of the isolates grouped in BX3 corresponded to isolates recovered during a 38 months period from Hospital Cordoba 1. The results of this study suggest that the BX3 clone was capable of persisting in the hospital setting for more than 38 months causing infection in CF and non-CF patients and was also responsible for a nosocomial outbreak in 2012. Moreover, this clone was detected at the same hospital until 2018 showing its persistence capability (data not shown). Additionally, BX3 clone was recovered from two chronically infected CF patients, A and B, during different respiratory exacerbation events (Table 1). Based on these results there was confirmed long-term culture positivity (> 1-year persistence) in these two CF patients.

Cluster BX1 includes nine isolates from nine patients. Seven of them were recovered from blood samples and submitted from Hospital Misiones 1. These isolates were recovered less than 1 month apart from each other and yielded indistinguishable PFGE patterns confirming they were outbreak-related (Figure 2).The other two isolates were highly related, one derived from the same province and one from Chaco, located 350 km away. The *recA* sequences corresponding to these isolates belong to the "recA type 1" cluster.

The remaining isolates were grouped in four clusters (n): BX2 (1), BX4 (3), BX6 (2) and BX7 (2).

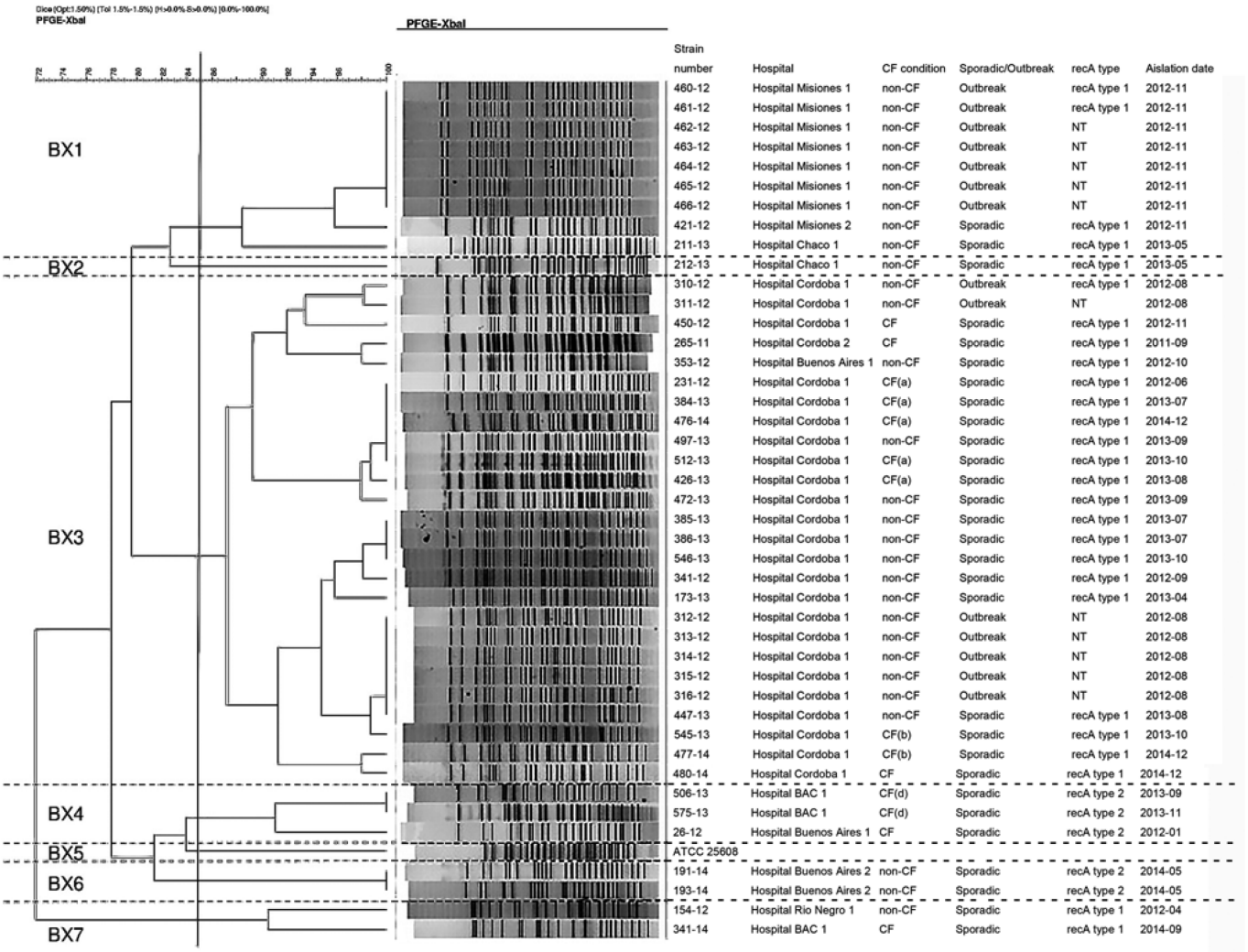
Two out of three isolates from BX4 cluster were recovered from the same patient (patient C), confirming a persistence event. Around 22% of the isolates were refractory to PFGE typing, however, it is not clear if it is a species' characteristic or of some circulating clones in our country. An overall correlation between *recA* sequence grouping and XbaI-PFGE classification was observed, although *recA*-typing showed a lower discriminatory power.

Discussion

An accurate and rapid species identification among BCC is essential to manage infections in CF patients in relation to applying appropriate therapy or infection control measures and for lung transplantation procedures. Sequencing of *recA* gene remains a proficient method to identify species belonging to the BCC [11]. In this study *recA* sequences of 44 *B. contaminans* showed > 99% of sequence identity, and were clustered into two highly related groups, "recA type 1" and "recA type 2". No association between CF/non-CF isolates and *recA* type clustering was observed. However, not all laboratories have molecular biology facilities to perform DNA sequencing. MALDI-TOF MS showed an accurate performance to identify *B. contaminans* at the species level [14]. Therefore, the use of optimized commercially MALDI-TOF MS databases including endemic pathogens, like *B. contaminans*, increases the usefulness and the impact in the clinical diagnosis.

In this work, we observed that *B. contaminans* BX3 cluster was a persistent clone at "Hospital Cordoba 1"

Figure 2. PFGE dendrogram showing the relationship between B. contaminans isolates. PFGE profiles of DNA digested with XbaI are shown and labeled with isolate number, hospital, recA type sequence, classification as CF or non-CF condition, epidemiological status (outbreak/sporadic), and date of isolation. B. cepacia ATCC 25608 was used as a reference strain. The similarities between isolates were evaluated using the Dice coefficient and the UPGMA clustering method. Cut off value for evaluating genetic similarity was ≥ 85%.



Strain number	Hospital	CF condition	Sporadic/Outbreak	recA type	Aislation date
460-12	Hospital Misiones 1	non-CF	Outbreak	recA type 1	2012-11
461-12	Hospital Misiones 1	non-CF	Outbreak	recA type 1	2012-11
462-12	Hospital Misiones 1	non-CF	Outbreak	NT	2012-11
463-12	Hospital Misiones 1	non-CF	Outbreak	NT	2012-11
464-12	Hospital Misiones 1	non-CF	Outbreak	NT	2012-11
465-12	Hospital Misiones 1	non-CF	Outbreak	NT	2012-11
466-12	Hospital Misiones 1	non-CF	Outbreak	NT	2012-11
421-12	Hospital Misiones 2	non-CF	Sporadic	recA type 1	2012-11
211-13	Hospital Chaco 1	non-CF	Sporadic	recA type 1	2013-05
212-13	Hospital Chaco 1	non-CF	Sporadic	recA type 1	2013-05
310-12	Hospital Cordoba 1	non-CF	Outbreak	recA type 1	2012-08
311-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
450-12	Hospital Cordoba 1	CF	Sporadic	recA type 1	2012-11
265-11	Hospital Cordoba 2	CF	Sporadic	recA type 1	2011-09
353-12	Hospital Buenos Aires 1	non-CF	Sporadic	recA type 1	2012-10
231-12	Hospital Cordoba 1	CF(a)	Sporadic	recA type 1	2012-06
384-13	Hospital Cordoba 1	CF(a)	Sporadic	recA type 1	2013-07
476-14	Hospital Cordoba 1	CF(a)	Sporadic	recA type 1	2014-12
497-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-09
512-13	Hospital Cordoba 1	CF(a)	Sporadic	recA type 1	2013-10
426-13	Hospital Cordoba 1	CF(a)	Sporadic	recA type 1	2013-08
472-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-09
385-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-07
386-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-07
546-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-10
341-12	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2012-09
173-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-04
312-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
313-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
314-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
315-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
316-12	Hospital Cordoba 1	non-CF	Outbreak	NT	2012-08
447-13	Hospital Cordoba 1	non-CF	Sporadic	recA type 1	2013-08
545-13	Hospital Cordoba 1	CF(b)	Sporadic	recA type 1	2013-10
477-14	Hospital Cordoba 1	CF(b)	Sporadic	recA type 1	2014-12
480-14	Hospital Cordoba 1	CF	Sporadic	recA type 1	2014-12
506-13	Hospital BAC 1	CF(d)	Sporadic	recA type 2	2013-09
575-13	Hospital BAC 1	CF(d)	Sporadic	recA type 2	2013-11
26-12	Hospital Buenos Aires 1	CF	Sporadic	recA type 2	2012-01
ATCC 25608					
191-14	Hospital Buenos Aires 2	non-CF	Sporadic	recA type 2	2014-05
193-14	Hospital Buenos Aires 2	non-CF	Sporadic	recA type 2	2014-05
154-12	Hospital Rio Negro 1	non-CF	Sporadic	recA type 1	2012-04
341-14	Hospital BAC 1	CF	Sporadic	recA type 1	2014-09

causing infections in both CF and non-CF patients. Environmental screening of *B. contaminans* isolates was not properly performed hence the possible acquisition of these infections from environmental sources could not be evaluated. In addition, we confirm an outbreak at "Hospital Misiones 1" of seven *B. contaminans* isolated from blood samples from seven non-CF adult patients. All patients were at the intensive care unit and four of them had cancer as an underlying disease. Therefore, the PFGE protocol described in this study was able to assess the genetic diversity of *B. contaminans* species and it constitutes a valuable tool for molecular surveillance in order to monitor the distribution and behavior of relevant clones.

B. contaminans infections in CF patients involved transient respiratory and chronic infections with long-term culture positivity. This study showed that CF patients could be colonized for long periods of time with a single clone. Moreover, *B. contaminans* septicemia causing the death of the infected patient was also described [9,24]. In our study, the relationship between the long-term carriage with epidemic BX3 clone in CF patients and morbidity was not evaluated. Surveillance investigation focused on monitoring the prevalence of dominant strains of *B. contaminans* within CF population should be performed.

The survival capability of *B. contaminans* and all BCC in hostile environments and contaminate pharmaceutical products is well known. Recently Lopez de Volder *et al.* [11] compared the *recA* sequences from *B. contaminans* recovered from pharmaceutical, cosmetic and beverages industries against clinical isolates and found 99.74-100% sequence identity among them, suggesting that contaminated massive consumption products could act as reservoir of this species [11]. One limitation of the present manuscript is the lack of *B. contaminans* isolates recovered from environmental sources in order to evaluate the possible role as reservoir of these clones.

B. contaminans has a low prevalence in CF patients worldwide, except in Argentina, Spain, and Portugal where 17-49% prevalence was reported [8,24,25]. Additionally, a polyclonal outbreak of BCC species amongst non-CF patients in Argentina has also been reported [26]. To the best of our knowledge, this is the first study analyzing the genetic relatedness by PFGE among a collection of *B. contaminans* isolates recovered from CF and non-CF patients from Argentina.

Conclusions

In conclusion, this study demonstrates the persistence of an epidemic clone of *B. contaminans* for over 38 months in a hospital setting not only causing an outbreak but also sporadic infections within CF and non-CF patients. We speculate that the incidence of these infections may be caused by environmental acquisition as well as patient to patient transmission. The lack of fully epidemiological information and an inadequate environmental screening of *B. contaminans* isolates restricts the complete understanding of the pathogenic behavior of this species. Nevertheless, the molecular typing of *B. contaminans* is crucial to define infection control strategies, especially in immunocompromised patients. Timely acquisition and communication of this information would allow the implementation of control measures for reducing the risk of cross-transmission including strict segregation of CF patients, and detection of potentially contaminated pharmaceutical products at hospitals, in order to avoid the dissemination of persistent clones such as BX3.

Unlike other BCC species, only little attention has been paid to the mechanisms of pathogenicity of *B. contaminans*. It is essential to understand the virulence factors present in this species, in addition to its distribution, transmissibility, and clinical impact.

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