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Burkholderia cepacia complex infection in an Adult Cystic Fibrosis unit in Madrid

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ABSTRACT

Introduction: Burkholderia cepacia complex have emerged as significant pathogens in cystic fibrosis (CF) patients due to the risk of cepacia syndrome and the innate multi-resistance of the microorganisms to antibiotics. The aim of this study was to describe the antimicrobial susceptibility profiles, the genotypes and subtypes of BCC, and the clinical evolution of CF patients with BCC.
Methods: The lung function and Brasfield and Shwachman score were assessed in 12 patients. BCC were identified and susceptibility was studied by MicroScan (Siemens). Species and genospecies of BCC were confirmed by molecular methods in a Reference Centre (Majadahonda).
Results: BCC were identified in 12 of 70 patients (17.1%) over a ten year period. The mean age to colonization by BCC was 24.4 years (SD: 7.71). *B. cenocepacia* was isolated in 4 patients (33.3%), *B. contaminans* was isolated in 3 patients (25%), both *B. vietnamiensis* and *B. stabilis* were isolated in 2 patients (16.7%), and *B. cepacia*, *B. multivorans* and *B. late* were isolated in one patient (8.3%). Among the *B. cenocepacia*, subtype IIIa was identified in two strains, and subtype IIIb was identified in the other two strains. There was susceptibility to meropenem in 90% of BCC, 80% to cotrimoxazole, 60% to minocycline, 50% to ceftazidime, and 40% to levofloxacin.
Conclusions: B. cenocepacia was the most prevalent species among the BCC isolated in CF adult patients, and subtypes IIIa and IIIb were identified in the 50% of the strains. Meropenem and cotrimoxazole showed the best activity.

Infección por *Burkholderia cepacia* complex en una Unidad de Fibrosis Quística de adultos en Madrid

RESUMEN

Introducción: Burkholderia cepacia complex (BCC) es un patógeno emergente significativo en pacientes con fibrosis quística (FQ), debido al riesgo de síndrome cepacia y debido a su multirresistencia a los antimicrobianos. El objetivo de este estudio fue describir la sensibilidad antimicrobiana, los genotipos y subtipos de BCC, y estudiar la evolución clínica de los pacientes con FQ.
Métodos: Se estudió la función pulmonar de 12 pacientes, y la puntuación Brasfield y Shwachman. BCC fue identificado y se estudió la sensibilidad mediante MicroScan (Siemens). Las especies y genoespecies de BCC se confirmaron mediante métodos moleculares en un centro de referencia (Majadahonda).
Resultados: BCC se identificó en 12 de 70 pacientes (17,1%) durante 10 años. La edad promedio de colonización por BCC fue de 24,4 años (DE: 7,71). *B. cenocepacia* se aisló en 4 pacientes (33,3%), *B. contaminans* se aisló en 3 pacientes (25%), *B. vietnamiensis* y *B. stabilis* se aisló cada una en 2 pacientes (16,7%), y *B. cepacia, B. multivorans* y *B. late* se aisló en un paciente (8,3%) cada una. Entre las cepas de *B. cenocepacia*, el subtipo IIIa se identificó en 2 cepas y el subtipo IIIb se identificó en otras 2. El 90% de las cepas de BCC fueron sensibles al meropenem, el 80% al cotrimoxazol, el 60% a la minociclina, el 50% a la ceftazidima y el 40% al levofloxacino.
Conclusiones: B. cenocepacia fue la especie más prevalente entre los aislados de BCC en pacientes adultos, y los subtipos IIIa y IIIb se identificaron en el 50%. El meropenem y el cotrimoxazol fueron los antibióticos más activos.

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Introduction

Burkholderia cepacia complex (BCC) has emerged as significant pathogens in cystic fibrosis (CF) patients due to the risk of cepacia syndrome (a fatal necrotizing pneumonia with bacteremia), the organism's innate multi-resistance to antibiotics, and the transmissibility of bacterial strains between patients by social contact.[1] Close and prolonged contact among CF patients and sharing nebulizers facilitates BCC acquisition and transmission.[2,3] It is proven that BCC bacteria acquisition is associated to hospitalization and cross-infection by social contact CF.

The taxonomy of the genus *Burkholderia* has undergone several major revisions over the last decades. In the mid-1990s, "*B. cepacia*" strains were shown to belong to at least five different species, which were collectively referred to as the *B. cepacia* complex.[4] Further taxonomic analyses revealed that even more species were present within the BCC and currently 17 *B. cepacia* complex species have been described[4–7]: *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietanmiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina*, *B. pyrrocinia*, *B. ubonensis*, *B. latens*, *B. diffusa*, *B. arboris*, *B. seminalis*, *B. metallica*, *B. lata* and *B. contaminans*.[8]

Except for *B. ubonensis*, all of these species have been isolated from sputum of CF patients,[6,7] with *B. cenocepacia* and *B. multivorans* as predominant.[9]

B. cenocepacia is subdivided in 4 subtypes (IIIa, IIIb, IIIc and IIId), that are encoded by four alleles of the gen *recA* and there are differences in the frequency and virulence of the strains. Furthermore, *B. cenocepacia* is considered to be one of the most serious pathogens because it is frequently associated with reduced survival and highest risk of developing fatal cepacia syndrome.[10] BCC species are intrinsically resistant to many antibiotics such as aminoglycosides and polymyxin B and often require combination therapy to suppress infection in CF.[11]

Infections with BCC bacteria in CF patients are often correlated with increased morbidity and mortality, and the innate resistance of these organisms to a broad range of antibiotics complicates the treatment of infected patients.[12,13] This resistance is caused by various mechanisms, including limited permeability, changes in lipopolysaccharide structure and the presence of several multidrug efflux pumps, inducible chromosomal beta-lactamases and altered penicillin-binding proteins. In addition, *in vitro* biofilm formation has been described for multiple *B. cepacia* complex strains and this may contribute to their ability to survive in the CF lung environment by providing additional protection against antibiotics.[14–16]

Treatment of BCC infected patients should be based preferably on the results of susceptibility tests and often include combination therapy with two or three antibiotics showing synergistic activity.[12,17,18] *In vitro* susceptibility studies on BCC strains show that breakpoint concentrations of ceftazidime, ciprofloxacin, meropenem, tetracyclines or high doses of tobramycin have a bacteriostatic activity against a considerable fraction of these strains.[19–21] Consequently, these antibiotics are often used to treat BCC infected CF patients. In addition cotrimoxazol is still frequently used in the treatment of chronic BCC infections, although susceptibility testing of these complementary antibiotics revealed a poor activity against many BCC strains.[18,22]

The goal of this study was to assess the isolated and the susceptibility of BCC and to analyze the clinic repercussions.

Methods

The sputum of patients with CF were analyzed to final BCC isolates, at the adult CF Unit in the Hospital La Princesa that has been operating since March 1997. These samples were processed in microbiology department in the standard procedure[23]; we used specific selective medium and quantitive streak, using the conventional procedure of serial dilution for the sample. The sputum underwent on a process of homogenization with N-acetylcystein before culturing. In our laboratory, the culture mediums used were: blood agar, bacitracin chocolate agar, manitol-salt agar, MacConkey agar, Sabouraud cloranfenicol agar and selective medium for *B. cepacia* called BCSA (Biomèrieux). The incubation time for plates was from 3 to 5 days at 35 °C. Bacitracin chocolate agar was incubated in CO_2 atmosphere.

The preliminary identification of the BCC strains was performed by MicroScan (Siemens) and Api 20 NE (Biomerieux). These procedures were performed according to the manufacturer's recommendations.[24]

Subsequently, the strains were remitted at Centro Nacional de Microbiología (Majadahonda, Madrid) for the confirmation and determination of the specie and genospecie. For this study, the following methods were carried out: Api 20 NE (biomerieux, Marcy l'Etoile) and GN2 Microplate (BIOLOG, Hayward, CA) and molecular methods. All procedures were performed according to the manufacturer's recommendations.

Chromosomal DNA extraction

DNA extraction was performed with the commercial kit QIAamp DNA Mini Kit (QIAGEN, GmbH, Hilden, Germany) following the manufacturer's instructions.

PCR analysis

Amplification of genes was performed in a final volume of 25 μl, using the kit PuReTaq Ready-To-Go PCR Beads (Amersham Biosciences, Buckinghamshire, UK), and containing 5 μl of extracted DNA and 10 pmol of each primer: fD1 and rP2[25] for *16S rDNA*, and BCR1 and BCR4[26] for *recA*. Thermal cycling was carried out in a TaKaRa PCR Thermal Cycler v. III mod TP600 (TAKARA BIO Inc., Otsu, Shiga) under the following conditions for *16S rDNA*: 94 °C for 5 min for the first cycle, 35 cycles of 15 s at 94 °C, annealing for 15 s at 55 °C, and extension at 72 °C for 1 min and 50 s. Conditions for amplification of *rec*A were as follows: 94 °C for 5 min, 30 cycles of 30 s at 94 °C, annealing for 45 s at 55 °C, and extension at 72 °C for 10 min.

We visualized 2 μl of each PCR product by agarose gel electrophoresis with agarose concentration adjusted at 1.5% and using 1× TAE buffer. Molecular size markers were included on all gels: Marker X 0.07–12.2 kbp (Roche Applied Sciences, Mannheim, Germany) and GeneRuler 100 bp DNA Ladder (Fermentas GmbH, St. Leon-Rot, Germany) for *16s rDNA* and *recA* products, respectively.

Strains identified as *B. cenocepacia* were subjected to PCR method with specific primers for RecA-IIIA group (BCRG3A1 and BCRG3A2) and RecA-IIIB (BCRG3B1 and BCRG3B2) under conditions previously described.[24]

Ten μl of PCR products were visualized by agarose gel electrophoresis at the same conditions described above.

Nucleotide sequence analysis

16s rDNA and *recA* PCR products were sequenced using fD1 and rP2 and BCR1 as primers, respectively. Sequencing reactions were prepared by means of Big Dye Terminator v 3.1 (Applied Biosystem, USA) in a final volume of 10 μl in according with the manufacturer's instructions, and analyzed with ABI PRISM 3100 genetic analyzer capillary electrophoresis system (Applied Biosystem, USA). Sequences were assembled using SeqMan 3.61 software (DNA Star, Inc, Madison, WI, USA). Analysis also involved the use of

Table 1
Isolations of the various *Burkholderia cepacia* complex species and co-colonization with other CF-pathogens in patients chronically.

Patient	Isolation 1	Isolation 2	Co-colonization	Observations
1	*B. contaminans*		*H. influenzae*	
2	*B. cepacia*		*P. aeruginosa*	
3	*B. cenocepacia* (sub. IIIa)		–	
4	*B. multivorans*		*S. aureus* *H. influenzae*	
5	*B. vietnamiensis*		*S. aureus*	
6	*B. stabilis*		*S. aureus*	
7	*B. cenocepacia* (sub. IIIb)		*S. aureus*	
8	*B. cenocepacia* (sub. IIIb)	*B. lata*	*P. aeruginosa*	Eradicated
9	*B. stabilis*	*B. contaminans*	*S. aureus*	
10	*B. cenocepacia* (sub IIIa)		–	Transplanted and died
11	*B. vietnamiensis*		*S. aureus*	Eradicated and transplanted
12	*B. contaminans*		–	

Basic Local Alignment Search Tool (BLAST: www.ncbi.nlm.nih.gov) to establish the correct gene identity.

PFGE analysis

Strains relationship was analyzed by pulsed-field gel electrophoresis (PFGE). Plugs preparation, lysis, cell washing and restriction digestion were performed as described previously[25,26] with slight differences. The restriction enzyme *XbaI* (40 U, Fermentas GmbH, St. Leon-Rot, Germany) was used. PFGE was performed following the protocol described for *Stenotrophomonas maltophilia* by Valdezate et al.,[27] and using DRIII Chef System (Bio-Rad Laboratories, Hercules, USA) and lambda phage concatemers (Biolabs, New England, UK) as molecular weight marker. Images were obtained with Quantity One v. 4.6.1 software (BioRad). Images analysis were performed visually and isolates were regarded as genotypically indistinguishable if they had an identical banding pattern.

Antibiotic susceptibility was performed by microdilution with MicroScan and disk diffusion simultaneously. Both methods were considered as CLSI breakpoints.[28] For ciprofloxacin and imipenem, the breakpoints of levofloxacin and meropenem were used, respectively. The following antibiotics were studied: ceftazidime, ciprofloxacin, levofloxacin, cotrimoxazol, minocycline, imipenem and meropenem.

Patients with BCC were studied with followings variables: age (at the beginning and currently), sex, weight (at the beginning and currently), gen mutation *Cystic Fibrosis Transmembrana Regulator*, evolution of respiratory function that was determined by the percentage according to theoretical value of the volume breathing out in the first second (FEV1) from their first isolation as well as the radiological punctuations of Brasfield and clinics of Shawchman at the beginning of the BCC isolation and current.

Co-colonization with other microorganisms was assessed.

The Brasfield score was evaluated with 0–5 (from low to high) according to radiological signs: air entrapment, linear shadows, nodular cystic lesions, segmental or lobar consolidation and the overall impression of the severity. The global punctuation obtained was subtracted to 25. The lowest value achieved corresponded to more severe radiology. The chest radiology and its Brasfield score correspondent were conducted each year.

Clinical score Shwachman evaluated 4 items with maximum punctuation of 25 each: general activity, physical examination, growth and nutrition and chest-X-ray. The ideal punctuation was 100 and the status of the patients was classified according to the punctuation: excellent (86–100 points), good (71–85 points), slight (56–70 points), moderate (40–55 points) or serious (less than or equal to 40).

Results

BCC was isolated in 12 of 70 adult CF patients (17.1%) during 10 years. Two of the patients had a lung transplant, one of them died after the transplant and the other, BCC was eradicated in 2005 before the transplant that was in 2011. BCC was eradicated in other patient in 2009. These patients were studied only for a few months, and so clinic evolution was not registered. *B. cenocepacia* was isolated in 4 patients (33.3%), *B. contaminans* in 3 patients (25%), *B. stabilis* in 2 patients (16.7%), *B. vietnamiensis* in 2 patients (16.7%), *B. cepacia* in one patient (8.3%), *B. multivorans* in one patient (8.3%) and *B. lata* in one patient (8.3%). Among *B. cenocepacia*, the subtype IIIa was identified in two patients out of 4 (50%), and subtype IIIb in the other two patients (50%). One patient had at first *B. cenocepacia* and then had a *B. lata*. Similarly, other patient had *B. stabilis* and then had *B. contaminans*. In our study, 50% of patients with BCC had *Staphylococcus aureus* strains (Table 1).

PFGE analysis shows that the same strain was isolated in each CF patient but it was different between patients, so this certifies that there was no cross transmission.

90% of BCC were sensible to meropenem, 80% to cotrimoxazol, 60% to minocycline, 50% to ceftazidime and 40% to levofloxacin, 20% to ciprofloxacin, and 10% to imipenem.

The 50% of the CF patients were male and the average age that these patients had their first isolation of BCC was 24.4 (SD: 7.71). The 41.7% of the patients had F508del/other mutation, 33.3% had F508del/F508 mutation and 25% had other/other mutation.

At the beginning Brasfield and Shwachman score was calculated for all patients included in this study, and the average punctuation was 18.6 and 82.3. However, the current Brasfield and Shwachman score is conducted with patients colonized. The average punctuation was 21.1 and 81. Only 1 patient had diabetes and 6 patients had pancreatic insufficiency. Table 2 shows the clinical characteristics of the CF patients who had BCC.

Fig. 1 shows the evolution of lung function (%FEV1) of the patients who had more than an isolation of BCC.

Discussion

There are few studies related to clinical evolution of patients with an isolated species of BCC, and there is a general idea that the *B. cenocepacia* species is associated to greater morbidity and mortality in CF patients.[19] Therefore, there are not many recent data about patients with BCC in Spain. A study in Hospital Universitario de Cruces shows an increase of colonization incidence with BCC.[29]

It is important that identification of BCC species is carried out in specialized center, because facultatives help to supervise a close

Table 2
Clinical characteristics of cystic fibrosis patients.

Sex frequency (%)	
Male	6 (50%)
Female	6 (50%)
Age average (SD)	
Primary infection ($n = 12$)	24.4 years (7.71)
Current ($n = 9$)	29 years (8.04)
Weight average (SD)	
Primary infection ($n = 12$)	57.9 kg (8.5)
Current ($n = 9$)	58.7 kg (8.6)
Mutation frequency (%)	
F508del/F508del ($n = 12$)	4 (33. 3%)
F508del/other ($n = 12$)	5 (41.7%)
Other/other ($n = 12$)	3 (25%)
Brasfield punctuation average	
Primary infection ($n = 12$)	18.6
Current ($n = 9$)	21.1
Shwachman punctuation average	
Primary infection ($n = 12$)	82.3
Current ($n = 9$)	81
Diabetes frequency (%)	
Current ($n = 9$)	0 (0%)
Pancreaty insuf. frequency (%)	
Current ($n = 9$)	6 (66.6%)

monitoring of these patients who are colonized by these species, as they can evolve adversely.

A study performed by Van Pelt et al.[30] shows that API 20 NE was more accurate than MicroScan. 90% of isolates were correctly identified using API 20NE *versus* 68% using MicroScan. They suggest the use of BCSA plates for the initial isolation of *B. cepacia* directly from clinical material. The sensitivity of these growth media appeared to be excellent (96%); the specificity was not 100%. It was quite striking to find that for the automated assays, as MicroScan, the accuracy was insufficient. The major outcome of the present analysis is the fact that molecular identification by PCR-RFLP analysis is superior to the biochemical and microbiological species identification procedures, although it should be emphasized that results obtained with API20 NE were satisfactory.[30]

In our study, the *B. cenocepacia*, *B. contaminans*, *B. vietnamiensis*, *B. stabilis*, *B. multivorans*, *B. cepacia* and *B. lata* were isolated from the respiratory secretions of 12 CF patients examined and *B. cenocepacia* was the most prevalent species like most of data described in European literature of CF patients.[19] However, this discovery does not agree with a Portugal study (Susana Correia et

Fig. 1. Evolution of lung function of the CF chronic patients from the first isolation of BCC in sputum.

cols), in which the most frequent species were *B. cepacia* 57% and *B. stabilis* 13%. It is maybe due to using non-sterile saline solutions intrinsically contaminated by *B. cepacia*.[19] These contaminated solutions were detected by Infarmed during a routine microbiological inspection.[19] In our study *B. cepacia* and *B. stabilis* were isolated in lower proportion like other studies in Europa and America.[19]

Among *B. cenocepacia* isolation, there was the same proportion between *B. cenocepacia* subtypes IIIa and IIIb.

One of the patients had *B. cenocepacia* as first isolation, *B. lata* as second isolation and then was eradicated. Another patient had *B. vietnamiensis* in 2004 that was eradicated, and lung transplantation was done in him in 2011, and another patient was isolated with *B. cenocepacia* subtype IIIa in 2004, but he died in the same year after a lung transplant. The survival results of lung transplant are worst in patients who are colonized with *B. cenocepacia*, so some units of transplant contraindicate the transplant in that case. It has been recognized that *B. cenocepacia* carries a worse prognosis compared with *B. multivorans* with shorter survival when matched with *Pseudomonas aeruginosa* controls.[31] Cepacia syndrome has been reported with both of these species.[26] The exact pathophysiology of this syndrome is poorly understood, and the precise mortality rate in not known, although it is thought to approach 100%.[32]

Our patients have low FEV_1 values. A great number of patients had already deteriorated prior to BCC isolation as a result of colonization by other pathogenic agents and disease progression. In clinically stable patients no significant alterations were seen in FEV_1 values and nutritional state. There is evidence that lung colonization might not be detected by standard cultures for some time (up to 2 years) after BCC acquisition.[33] In addition, it is not clear if, in cases of intermittent isolation, there is a re-infection by a new strain or if there is a recrudescence of the initial strain.[20] In the majority of cases in our different isolation, no replacement of the initial BCC strains was seen.

Cases were registered in which the same strain (with the same genotype), which persistently infected a patient over several years, was eradicated by antibiotic therapy or had no apparent impact on the clinical picture of other patients. It is not clear why strains of the different BCC species differ in their persistence, epidemiology and pathogen potential in CF and why the same strains can be associated to very different clinical evolutions.[19] It depends on factors inherent in each individual patient, on co-colonization by other pathogens and other factors still to be identified, stressing the importance of undertaking studies of this type.[19]

Antibiotic resistance is considered an important virulence factor of BCC organisms.[14] Although therapy is usually guided by antimicrobial susceptibility testing, eradication of BCC organisms is rarely achieved.[21] Multiple hypotheses have been formulated to explain this failure, including inadequate antibiotic concentrations or inactivation of the antibiotic in sputum, impaired host defenses in CF patients, biofilm formation, "inoculum" effect and *in vivo* growth rate of these organisms.[34]

Elke et al. reported in a Belgium study that meropenem, minocycline and ceftazidime were the most active antibiotics against BCC isolation and ciprofloxazin and trimethoprim/sulfamethoxazole had the lowest activity.[35] However, our study found that meropenem was the most active with 90% of susceptibility, followed by trimethoprim/sulfamethoxazole, minocycline and ceftazidime with 80%, 60% and 50%, respectively. Although BCC organisms are typically resistant against aminoglycosides, high doses of tobramycin inhibited the majority of tested strains. Nebulized tobramycin yielding high peak concentrations in sputum is increasingly used for treating CF patients.[36–38]

Consequently, these higher concentrations should be taken into account when evaluating the usefulness of this antibiotic.[35] Several reports confirm that nebulized tobramycin shows great promise
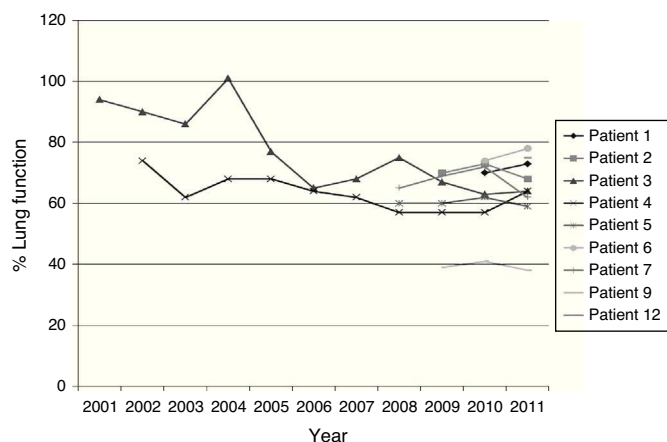
in the treatment of BCC-infected CF patients: for example, Weidmann et al.[39] recently described the complete eradication of BCC organisms from the lungs of CF patients by using a combination of nebulized tobramycin and amiloride. In addition, a combination therapy with nebulized and intravenous meropenem and tobramycin also resulted in the successful treatment of a female CF patient suffering from cepacia syndrome, although the sputum samples of the latter patient remained positive for *B. cenocepacia*.[39]

BCC species are intrinsically resistant to many antibiotics such as aminoglycosides and polymyxin B and often require combination therapy to suppress infection in CF.[11] The antibiotics polymyxin, gentamicin and vancomycin are used at high concentrations in *B. cepacia* Selective Agar, a highly effective medium for their isolation from CF sputum.[11] Nzula et al.[13] compared the antibiotic susceptibility of six BCC species and concluded that it was highly variable except for innate polymyxin resistance and not linked to the taxonomic status of the isolates examined. Efflux, the secretion of chromosomal beta-lactamases and the impermeability of the outer envelope of BCC bacteria have been implicated in antibiotic resistance.[14]

In contrast, the molecular basis for biocide resistance in BCC bacteria has been poorly studied despite these organisms being linked with many instances of contamination in disinfectants and other anti-infective solutions.[40]

The mission and therefore the challenge posed by the identification of BCC species for routine clinical microbiology laboratories are different. Strains isolated on selective media and tentatively identified as belonging to the BCC using commercial systems should be confirmed with the classical biochemical tests described.[41]

The early detection of BCC is extremely important both for the CF patient as well as for the CF community. However, a recent study[5] indicated that less than half of W.S. centers surveyed employ "*B. cepacia*"-specific selective media or incubate cultures for extended periods, both of which improve the yield of this organism. The use of these up-to-date culture techniques is technically not demanding and should be the expected standard of care in every CF center worldwide.

In this study, we only eradicated *B. cenocepacia* in one patient; another patient died immediately post transplant, and neither patient had cepacia syndrome and suffered an important clinical deterioration, although the period of observation was short because they acquired BCC recently.

The improved diagnosis of infections caused by members of the BCC and other *B. cepacia*-like organisms will help with the interpretation of the results from clinical outcome studies, and by doing so will provide crucial information regarding the pathogenicity and/or transmissibility of specific strains involved.[5]

Conflict of interest

None of the authors have any conflict of interest to declare.

References

1. Norskov-Lauritsen N, Krogh Johansen H, Fenger MG, Nielsen XC, Pressler T, Olesen HV, et al. Unusual distribution of *Burkholderia cepacia* complex species in Danish cystic fibrosis clinics may stem from restricted transmission between patients. J Clin Microbiol. 2010;48:2981–3.
2. LiPuma JJ, Dase SE, Nielsol DW, Stern RC, Stull TL. Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. Lancet. 1990;336:1094–6.
3. Goval JRW, Brown PH, Maddison J, Doherty CJ, Nelson JW, Dodd M, et al. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. Lancet. 1993;342:15–9.
4. Vandamme P, Holmes B, Vancanneyt M, Coenye T, Hoste B, Coopman R., et al. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. Int J Syst Bacteriol. 1997;47:1188–200.
5. Coenye T, Vandamme P, Govan JRW, LiPuma JJ. Taxonomy and identification of the *Burkholderia cepacia* complex. J Clin Microbiol. 2001;39:3427–36.
6. Vanlaere E, LiPuma JJ, Baldwin A, Henry D, De Brandt E, Maahanthiralingam E, et al. *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. Int J Syst Evol Microbiol. 2008;58:1580–90.
7. Vanlaere E, Baldwin A, Gevers D, Hery D, De Brandt E, LiPumm JJ, et al. Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int J Syst Evol Microbiol. 2009;59:102–11.
8. Rose H, Baldwin A, Dowson CG, Mahenthiralingam E. Biocide susceptibility of the *Burkholderia cepacia* complex. J Antimicrob Chemother. 2009;63:502–10.
9. Mahenthiralingham E, Baldwin A, Vandamme P. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. J Med Microbiol. 2002;51:533–8.
10. Drevinek P, Mahenthiralingam E. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Eur J Clin Microbiol Infect Dis. 2010;16:821–30.
11. Chernish RN, Aaron SD. Approach to resistant gram-negative bacterial pulmonary infections in patients with cystic fibrosis. Curr Opin Pulm Med. 2003;9:509–15.
12. Speert DP. Advances in *Burkholderia cepacia* complex. Paediatr Respir Rev. 2002;3:230–5.
13. Nzula S, Vandamme P, Goman JR. Influence of taxonomic status on the in vitro antimicrobial susceptibility of the *Burkholderia cepacia* complex. J Antimicrob Chemother. 2002;50:265–9.
14. Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol. 2005;3:144–56.
15. Conway BAD, Venu V, Speert DP. Biofilm formation and acyl homoserine lactone production in the *Burkholderia cepacia* complex. J Bacteriol. 2002;184:5678–85.
16. Tomlin KL, Clark SRD, Ceri H. Green and red fluorescent protein vectors for use in biofilm studies of the intrinsically resistant *Burkholderia cepacia* complex. J Microbiol Methods. 2004;57:95–106.
17. Aaron SD, Ferris W, Henry DA, Speert DP, MacDonald N. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. Am J Respir Crit Care Med. 2000;161:1206–12.
18. Zhou J, Chen Y, Tabibi S, Alba L, Garber E, Saiman L. Antimicrobial susceptibility and synergy studies of *Burkholderia cepacia* complex isolated from patients with cystic fibrosis. Antimicrob Agents Chemother. 2007;51:1085–8.
19. Correia S, Nascimento C, Pereira L, Cunha MV, Sá-Correia I, Barrreto C. The clinical course of *Burkholderia cepacia* complex bacteria respiratoriy infection in cystic fibrosis patients. Rev Port Pneumol. 2008;14:5–26.
20. LiPuma JJ. *Burkholderia cepacia*. Management issues and new insights. Clin Chest Med. 1998;19:473–86.
21. Middleton PG, Kidd TJ, Williams B. Combination aerosol therapy to treat *Burkholderia cepacia* complex. Eur Respir J. 2005;26:305–8.
22. Bromley K. Antibiotic treatment for cystic fibrosis, report of the UK Cystic Fibrosis Trust Antibiotic Group. 2nd ed. UK: Cystic Fibrosis Trust; 2002.
23. Oliver A, Alarcón T, Caballero E, Canton R. Diagnóstico microbiológico de la colonización-infección broncopulmonar en el paciente con fibrosis quística. Enferm Infecc Microbiol Clin. 2009;27:89–104.
24. McGregor A, Schio F, Beaton S, Boulton V, Perman M, Gilber G. The MicroScan WalkAway diagnostic microbiology system-an evaluation. Pathology. 1995;27:172–6.
25. Weisburg WG, Barns S, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173:697–703.
26. Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, et al. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genovars I and III. J Clin Microbiol. 2000;38:3165–73.
27. Valdezate S, Vindel A, Maiz L, Baquero F, Escobar H, Cantón R. Persistence and variability of *Stenotrophomonas maltophilia* in Cystic Fibrosis in Madrid, 1991–1998. Emerg Infect Dis. 2001;7:113–22.
28. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement M100-S22. Clinical and Laboratory Standards Institute; 2012.
29. Gómez B, Gómez A, Martínez N, Santiago M, Sojo A, Barrón J, et al. Infección por el complejo *Burkholderia Cepacia* en la Fibrosis Química. Arch Bronconeumol. 2012;48:218.
30. Van Pelt C, Verduin CM, Goessens WHF, Vos MC, Tummler B, Segonds C, et al. Identification of *Burkholderia* spp. In the Clinical Microbiology Laboratory: comparison of conventional and molecular methods. J Clin Microbiol. 1999;37:2158–64.
31. Jones AM, Dodd ME, Govan JRW, Barcus V, Doherty CJ, Morris J, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. Thorax. 2004;59:948–51.
32. Tablan OC, Martone WJ, Doershuk CF, Stern RC, Thomassen MJ, Klinger JD, et al. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. Chest. 1997;91:133–4.
33. LiPuma JJ, Marks-Austin DA, Holsclaw DS, Speert DP. Inaparent transmission of *Pseudomonas (Burkholderia) cepacia* among patients with cystic fibrosis. Pediatr Infect Dis J. 1994;13:716.

34. Desai M, Bühler T, Weller PH, Brown MR. Increasing resistance of plank-tonic and biofilm cultures of Burkholderia cepacia to ciprofloxacin and ceftazidime during exponential growth. J Antimicrob Chemother. 1998;42: 153–60.

35. Peeters E, Nelis HJ, Coenye T. In vitro activity of ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim/sulfamethoxazole against planktonic and sessile *Burkholderia cepacia* complex bacteria. J Antimi-crob Chemother. 2009;63:801–9.

36. Spoering AL, Lewis K. Biofilms and planktonic cells of *Pseudomonas aeru-ginosa* have similar resistance to killing by antimicrobials. J Bacteriol. 2001;183:6746–51.

37. Eisenberg J, Pepe M, Williams-Warren J, Vasiliev M, Montgomery AB, Smith AL, et al. A comparison of peak sputum tobramycin concentration in patients with cystic fibrosis using jet and ultrasonic nebulizer systems. Chest. 1997;111: 955–62.

38. Geller DE, Rosenfeld M, Waltz DA, Wilmott RW. Efficiency of pulmonary administration of tobramycin solution for inhalation in cystic fibrosis using an improved drug delivery system. Chest. 2003;123:28–36.

39. Weidmann A, Webb AK, Dodd ME, Jones AM. Successful treatment of cepacia syndrome with combination nebulized and intravenous antibiotic therapy. J Cyst Fibros. 2008;7:409–11.

40. Henry DA, Campbell ME, LiPuma JJ, Speert DP. Identification of *Burkholdria cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. J Clin Microbiol. 1997;35:614–9.

41. Jimenez L. Microbial diversity in pharmaceutical product recalls and environ-ments. PDA J Pharm Sci Technol. 2007;61:383–99.