Enferm Infecc Microbiol Clin. 2013;31(10):665–668

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Brief report

Clonal diversity among *Burkholderia cepacia complex* isolates from cystic fibrosis patients in a reference unit

Laura Barrado[a], M. Teresa Martinez[b], Jennifer Villa[a], M. Ángeles Orellana[a], Esther Viedma[a], Fernando Chaves[a,*]

[a] *Servicio de Microbiología Clínica, Hospital Universitario 12 de Octubre, Madrid, Spain*
[b] *Unidad de Fibrosis Quística, Hospital Universitario 12 de Octubre, Madrid, Spain*

ARTICLE INFO

Article history:
Received 21 February 2013
Accepted 25 April 2013
Available online 11 July 2013

Keywords:
Burkholderia cepacia complex
Cystic fibrosis
Molecular epidemiology

ABSTRACT

Introduction: The epidemiology of *Burkholderia cepacia complex* (*Bcc*) in cystic fibrosis (CF) is not widely known.
Methods: All CF patients with *Bcc* between 2002 and 2011 were reviewed, and a molecular analysis of isolates was performed.
Results: The prevalence of *Bcc* infection was 7.2% (18/250). Molecular analysis of 16 *Bcc* isolates showed 5 species (7 *B. contaminans*, 6 *B. cepacia*, 1 *B. cenocepacia*, 1 *B. multivorans*, and 1 *B. stabilis*) and 13 sequence types. There were no cases of cross-transmission.
Conclusion: A high diversity of *Bcc* species was found in infected CF patients.

Diversidad clonal en aislamientos de *Burkholderia cepacia complex* de pacientes con fibrosis quística en una unidad de referencia

Palabras clave:
Burkholderia cepacia complex
Fibrosis quística
Epidemiología molecular

RESUMEN

Introducción: La epidemiología de *Burkholderia cepacia complex* (*Bcc*) en fibrosis quística (FQ) no es bien conocida.
Métodos: Se revisaron todos los pacientes con *Bcc* entre 2002-2011. Se realizó análisis molecular de los aislamientos.
Resultados: La prevalencia fue de 7.2% (18/250). El análisis molecular de 16 aislamientos representativos mostró 5 especies (7 *B. contaminans*, 6 *B. cepacia*, 1 *B. cenocepacia*, 1 *B. multivorans*, and 1 *B. stabilis*), y 13 tipos de secuencia. No hubo casos de transmisión cruzada.
Conclusiones: Se encontró una alta diversidad de especies de *Bcc* causantes de infecciones en FQ.

Introduction

Burkholderia cepacia complex (*Bcc*) is a closely related group of 17 bacterial species with similar phenotype but different genotype.[1] Colonization with *Bcc* is associated with a constant and accelerated decline in lung function and increased risk of death in cystic fibrosis (CF) patients. Particular species of the *Bcc* such as *Burkholderia cenocepacia* and *Burkholderia multivorans* have been associated with an increased transmission risk and virulence, and with a worse outcome following lung transplantation.[2]

In Spain, the prevalence of chronic *Bcc* infection in CF patients in 2009 was 3.5% (data collected from only 30% of the total CF population in Spain).[3] The distribution of *Bcc* species for this population is not well established, except for a recent report stating that *B. cenocepacia* was the most prevalent genomovar found in patients with CF (19.1%).[4] An increase in the number of CF *Bcc* cases in our center was identified during 2009–2010, for which, a decision was taken to investigate the clinical characteristics of CF patients with *Bcc*, the distribution of *Bcc* species, and the possibility of cross-transmission among the patients treated in our CF unit.

Methods

A retrospective study was performed on all CF patients colonized/infected with *Bcc* isolates from January 2002 to December

* Corresponding author.
E-mail address: fernando.chaves@salud.madrid.org (F. Chaves).

0213-005X/$ – see front matter © 2013 Elsevier España, S.L. All rights reserved.
http://dx.doi.org/10.1016/j.eimc.2013.04.025

Table 1
Baseline characteristics of patients with *Burkholderia cepacia complex* infection at time of acquisition and one year after the first isolation.

Characteristics ($n = 16$)	Time of acquisition	One year later	p
Mean ± SD age (year)	20 (±10.4)	–	
No. female (%)	9 (56.2)	–	
Body mass index (kg/m²)	19.8 (±2.4)	20.4 (±2.5)	0.52
No. (%) with CF transmembrane regulator mutation type			
F508del homozygote	7 (43.7)	–	
F508del heterozygote	6 (33.3)		
Other mutations (G542X/712-1G>T, 1811 + 1,6kBa, G1244E/1002-2A>G)	3 (16.6)		
No. (%) with:			
Pancreatic insufficiency	15 (93.7)	–	
Diabetes mellitus	5 (31.2)	–	
Liver disease	4 (25)	–	
Mean ± SD Pulmonary Function (%)[a]			
FEV1	70.7 (±18.7)	68.6 (±3.3)	0.10
FVC	81.8 (±16.9)	83.5 (±22.1)	0.62
No. (%) of hospitalized patients	9 (56.2%)	4 (25%)	0.15
No. (%) with antimicrobial exposure to:[b]			
≥3 groups	8/16 (50)	4/16 (25)	0.27
1 o 2 groups	7/16 (43.7)	11/16 (68.7)	0.28
Tobramycin (Inhaled)	9/16 (56.2)	6/16 (37.5)	0.48
Colistin (Inhaled)	10/16 (62.5)	6/16 (37.5)	0.29
No. (%) coinfected with other microorganism(s)			
Staphylococcus aureus	12 (75)	12 (75)	
Pseudomonas aeruginosa	7 (43.7)	9 (56.2)	
Other nonfermentative Gram-negative bacilli (*S. maltophilia*, *A. xyloxoxidans*)	2 (12.5)	1 (6.2)	
Aspergillus fumigatus	5 (31.2)	2 (12.5)	
Mycobacterium avium-intracellulare	1 (6.2)	1 (6.2)	

[a] FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.
[b] Antimicrobial exposure in the previous year of acquisition and one year after the first isolate. The groups of antimicrobial agents were: β-lactams, macrolides, fluoroquinolones, aminoglycosides, trimethoprim/sulfamethoxazole, and others.

2011 treated in the CF unit of the Hospital Universitario 12 de Octubre (Madrid). Clinical records were reviewed in order to collect demographic, clinical, and microbiology data. Stringent infection control measures were implemented to reduce cross-transmission, and included hand washing, use of gloves and masks, disposable mouthpieces and antimicrobial filters when a spirometry was performed.

Sputum samples were inoculated onto Columbia 5% blood, MacConkey, mannitol-salt, chocolate, and *B. cepacia* (BCSA) agar medium. The identification and the susceptibility to different antimicrobials were carried out using the microdilution method (Wider® System, Soria-Melguizo, Spain) and ε-test strips (AB, Biodisk, Solna, Sweden) for ceftazidime (CAZ), meropenem (MER), levofloxacin (LVX), minocycline (MC), chloramphenicol (CLO) and trimethoprim-sulfamethoxazole (SXT). The minimum inhibitory concentrations (MICs) were interpreted according to the Clinical Laboratory Standards Institute.[5]

Bcc strains were genotyped by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Xba*I.[6] PCR amplification of *rec*A[7] gene was used to identify at species level. Multilocus sequence typing[8] (MLST) was performed, besides to establish clonality, to confirm the identification. Sequencing reactions were run on an ABI Prism 3100 Genetic Analyzer, obtaining the taxonomic status by sequence analysis using GenBank and the *B. cepacia* MLST database (http://pubmlst.org/bcc/). Novel alleles and STs were submitted. Univariate analysis was performed by using the t test for continuous variables and the χ^2 or Fisher exact tests for categorical variables.

Results

Over the 10-year period of study, 70 *Bcc* isolates putatively identified by phenotypic methods from 18 CF patients were recovered from a total of 250 patients treated in our CF unit. The prevalence of *Bcc* infection was 7.2%. Clinical isolates from two patients were not available, and therefore removed from the study. Nine patients were considered as chronically colonized (at least three positive cultures obtained in one year) and seven as sporadically colonized (less than three positive cultures). Ten (62.5%) cases were detected in 2009–2010, and 12 patients (75%) were older than 16 years. Clinical parameters for the 16 *Bcc* infected patients did not demonstrate any significant trends with body mass index, lung function, hospitalization and antimicrobial usage unchanged after one year of infection (Table 1). We also compared these clinical parameters between chronic and sporadic colonizers, and we could not establish a relationship between *Bcc* infection and a worse outcome in the lung function, probably because we only analyzed the outcome one year after the first isolation, and this limited the power of this analysis. It should be noted that one patient with chronic ST208 *B. cenocepacia* infection, had lung transplantation. Following this, the patient had two episodes of *cepacia* syndrome, and finally, died after 8 months.

A total of 41 *Bcc* isolates phenotypically identified were available for molecular studies: 34 isolates from 9 chronically colonized patients and 7 from 7 sporadically colonized patients. *rec*A PCR-sequencing and MLST were performed in 16 *Bcc* isolates (one per patient) and showed 5 different species and 13 different sequence types (STs): 6 *B. cepacia* (ST9, ST717, ST718, ST719, ST720, ST721), 7 *B. contaminans* (ST102 [2], ST404 [2], ST482, ST716 [2]), 1 *B. cenocepacia* (ST208), 1 *B. multivorans* (ST712), and 1 *B. stabilis* (ST661) (Table 2). Six were novel STs (ST716, ST717, ST718, ST719, ST720, and ST721).

PFGE analysis of the 41 *Bcc* isolates showed 16 different pulsotypes. Furthermore, those belonging to the same species (*B. cepacia* and *B. contaminans)* had different DNA patterns (Table 2). Thirty-four sequential isolates recovered from 9 patients (range 10 months–4 years) were analyzed by PFGE, and all patients harbored the same strain over the time.

Table 2
Distribution of *Burkholderia cepacia complex* species according to the PFGE patterns, sequence types and antimicrobial resistance patterns.

Species no. (%)	ID	Date of the first isolation	PFGE type[a]	Sequence type	Antimicrobial resistance pattern[b]
B. cepacia 6 (37.5%)	BCS 15	2003	O[a]	ST720	CLO
	BCS 14	2004	N[a]	ST719	LVX
	BCS 6	2009	F[a]	ST9	CLO
	BCS 16	2009	P[a]	ST721	CLO
	BCS 12	2010	L	ST717	–
	BCS 13	2010	M[a]	ST718	–
B. contaminans 7 (43.7%)	BCS 5	2008	E[a]	ST102	–
	BCS 4	2009	D	ST102	CAZ, MER, LVX, MIN, CLO
	BCS 11	2009	K	ST716	LVX, CLO
	BCS 1	2010	A[a]	ST482	LVX
	BCS 8	2010	H	ST404	MER, MIN, CLO
	BCS 9	2010	I	ST404	MER, CLO
	BCS 10	2010	J	ST716	CLO
B. cenocepacia 1 (6.25%)	BCS 7	2006	G[a]	ST208	MER, LVX, SXT
B. multivorans 1 (6.25%)	BCS 2	2002	B[a]	ST712	MER, LVX, CLO, SXT
B. stabilis 1 (6.25%)	BCS 3	2006	C	ST661	LEV, CLO

[a] This letter indicates isolates from chronically colonized patients.
[b] CLO, chloramphenicol; LVX, levofloxacin; MER, meropenem; MIN, minocycline; CAZ, ceftazidime; SXT, trimethoprim-sulfamethoxazole.

The percentages of resistance to antimicrobials were: CAZ (6.2%), MER (31.2%), LVX (37.5%), MC (12.5%), CLO (72.5%) and SXT (12.5%). The MICs were: CAZ (range 1–256 μg/ml, $MIC_{90\%}$: 8 μg/ml, $MIC_{50\%}$: 4 μg/ml), MER (range 0.38–32 μg/ml, $MIC_{90\%}$: >32 μg/ml, $MIC_{50\%}$: 1.5 μg/ml), LVX (range 0.38–32 μg/ml, $MIC_{90\%}$: 6 μg/ml, $MIC_{50\%}$: 1.5 μg/ml), MC (range 0.19–32 μg/ml, $MIC_{90\%}$: 6 μg/ml, $MIC_{50\%}$: 3 μg/ml), CLO (range 0.5–32 μg/ml, $MIC_{90\%}$: 32 μg/ml, $MIC_{50\%}$: 12 μg/ml) and SXT (range 0.047–32 μg/ml, $MIC_{90\%}$: 6 μg/ml, $MIC_{50\%}$: 0.38 μg/ml).

Discussion

This study detected a prevalence of 7.2% of *Bcc* infection in CF patients. Other European countries and United States have reported rates ranged between 0 and 11.5%.[3,9] The most prevalent species were *B. contaminans* (43.7%) and *B. cepacia* (37.5%). *B. cenocepacia* and *B. multivorans* represented 12.5%. However, other European countries, Australia, New Zealand, Canada, and United States, reported rates for *B. cepacia* between 0 and 11.2%, for *B. cenocepacia* between 45.1 and 91.8% and for *B. multivorans* between 0 and 51.6%.[10–12] A high clonal diversity was also found in the two more frequent species, *B. contaminans* and *B. cepacia*. *B. contaminans* belonging to ST102 has been involved in a widespread outbreak in the United States and Brazil in non-cystic fibrosis patients.[13] In Spain, ST102 has been reported as cause of an outbreak of subclinical mastitis in dairy sheep.[1,13,14] The *B. cenocepacia* isolate of this study did not belong to either the epidemic ET12 strain (ST28) or the Czech strain (ST32), which have been associated with serious outbreaks among patients in Canada and Europe.[10] Portugal seems to be the scenario where *B. cepacia* have been seen to be dominant in CF patients.[15] Since the environment will be source of *Bcc* infection in the absence of strain transmission, then we could speculate that environmental sources of *B. cepacia* may be more common in Portugal and Spain, and hence lead to it being dominant in CF in these two close countries. Differences in the prevalence of *Bcc* species, at the regional and local level, may result from a combination of several factors, as genetic diversity among *Bcc* species living in the natural environment, predominance of epidemic strains (belonging mostly to *B. cenocepacia*), antimicrobial pressure, and adherence to infection control measures.[10]

Cases of cross-transmission among CF patients were not found although 62.5% of cases were detected in the last two years of study. Although the increase in 2009–2010 was remarkable, there were not changes in handling, processing or identification procedures in our laboratory that explained the increase in the number of *Bcc* cases. Probably, it could be related in part to having started to use inhaled colistin in our CF unit from 2005 (data not shown). We also investigated if the *Bcc* strains could persist in the same patient causing infection. In 9 patients, chronic infection involved a single strain. Bernhardt et al. found that replacement of the initial infecting strain occurred in 6.9% of patients infected with *Bcc* strains.[16]

Although our study refers only to a single CF unit, it illustrates the high diversity of *Bcc* species infecting CF patients. Given the great divergence in the different countries, it is important to know the local epidemiology due to the impact of this information on the clinical management of patients and on the implementation of appropriate infection control policies.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr. Joaquín R. Otero for reviewing the manuscript.
This study was supported by the Spanish Network for the Research in Infectious Diseases (RD06/0008) from the Instituto de Salud Carlos III and Fundación Mutua Madrileña (FMM 2011/0064).

References

1. Vanlaere E, Baldwin A, Gevers D, Henry D, De Brandt E, LiPuma 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 Pt 1:102–11.
2. Lynch JP. *Burkholderia cepacia* complex: impact on the cystic fibrosis lung lesion. Semin Respir Crit Care Med. 2009;30:596–610.
3. Viviani L, Zolin A, Olesen HV. Annual data report 2008–2009 of European Cystic Fibrosis Society Patient Registry. http://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports
4. Medina-Pascual MJ, Valdezate S, Villalón P, Garrido N, Rubio V, Saéz-Nieto JA. Identification, molecular characterisation and antimicrobial susceptibility of genomovars of the *Burkholderia cepacia* complex in Spain. Eur J Clin Microbiol Infect Dis. 2012;31:3385–96.

5. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing; Twenty-first Informational Supplement. M100-S21. Wayne, PA: NCCLS; 2011.
6. Wigley P, Burton NF. Genotypic and phenotypic relationships in *Burkholderia cepacia* isolated from cystic fibrosis patients and the environment. J Appl Microbiol. 2009;86:460–8.
7. Payne GW, Vandamme P, Morgan SH, Lipuma JJ, Coenye T, Weightman AJ, et al. Development of a *rec*A gene-based identification approach for the entire *Burkholderia* genus. Appl Environ Microbiol. 2005;71:3917–27.
8. Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ. Expanded multilocus sequence typing for *Burkholderia* species. J Clin Microbiol. 2009;47:2607–10.
9. LiPuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev. 2010;23:299–323.
10. Drevinek P, Mahenthiralingam E. Burkholderia cenocepacia in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Clin Microbiol Infect. 2010;16:821–30.
11. Kidd TJ, Douglas JM, Bergh HA, Coulter C, Bell SC. *Burkholderia cepacia* complex epidemiology in persons with cystic fibrosis from Australia and New Zealand. Res Microbiol. 2008;159:194–9.
12. Crowley D, Daly M, Lucey B, Shine P, Collins JJ, Cryan B, et al. Molecular epidemiology of cystic fibrosis-linked *Burkholderia cepacia* complex isolates from three national referral centres in Ireland. J Appl Microbiol. 2002;292:992–1004.
13. Mahenthiralingam E, Baldwin A, Drevinek P, Vanlaere E, Vandamme P, LiPuma JJ, et al. Multilocus sequence typing breathes life into a microbial metagenome. PLoS ONE. 2006;1:e17.
14. Berriatua E, Ziluaga I, Miguel-Virto C, Uribarren P, Juste R, Laevens S, et al. Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection. J Clin Microbiol. 2001;39:990–4.
15. Coutinho CP, Dos Santos SC, Madeira A, Mira NP, Moreira AS, Sá-Correia I. Long-term colonization of the cystic fibrosis lung by *Burkholderia cepacia* complex bacteria: epidemiology, clonal variation, and genome-wide expression alterations. Front Cell Infect Microbiol. 2011;1:12. http://dx.doi.org/10.3389/fcimb.2011.00012.
16. Bernhardt SA, Spilker T, Coffey T, LiPuma JJ. *Burkholderia cepacia* complex in cystic fibrosis: frequency of strain replacement during chronic infection. Clin Infect Dis. 2003;37:780–5.