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Epidemiology of *Burkholderia cepacia* complex colonisation in cystic fibrosis patients

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Epidemiology of Burkholderia cepacia *complex colonisation in cystic fibrosis patients. K. De Boeck, A. Malfroot, L. Van Schil, P. Lebecque, C. Knoop, J.R.W. Govan, C. Doherty, S. Laevens, P. Vandamme, on behalf of the Belgian* Burkholderia cepacia *study group. ©ERS Journals Ltd 2004.*

ABSTRACT: In Belgian cystic fibrosis (CF) clinics, sputum samples are evaluated on selective MAST medium routinely every 3 months. In this study, in 1993 and 1999, isolates were further examined by *recA* restriction fragment length polymorphism analysis and pulsed-field gel electrophoresis of genomic DNA restricted with *SpeI*.

In 1993, 12 patients were colonised with *Burkholderia cepacia* complex (Bcc): *B. cenocepacia* (n=6), *B. multivorans* (n=3), *B. stabilis* (n=3). Four patients were colonised with the same *B. cenocepacia* strain; two with the same *B. stabilis* strain. After 5 yrs, three *B. cenocepacia*- and one *B. multivorans*-colonised patients had died.

In 1999, Bcc was isolated in 12 patients: *B. multivorans* (n=9), *B. stabilis* (n=1) and *B. cenocepacia* (n=2). Three patients were colonised by the same *B. multivorans* strain. Compared to matched controls, the 5 yr outcome was poor; four *B. cepacia* patients died and none of the control patients died. Lung-function evolution was poor.

In conclusion, the rate of colonisation in Belgian cystic fibrosis patients is stable and low. *Burkholderia cenocepacia* was most prevalent in 1993; *Burkholderia multivorans* in 1999. The cross-infection rate is low. Three patients had transient colonisation. The impact of *Burkholderia cepacia* complex on morbidity in the Belgian cystic fibrosis population is high and not limited to *Burkholderia cenocepacia*.

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Chronic respiratory tract infections with intercurrent acute exacerbations are the hallmark of cystic fibrosis (CF) lung disease. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are recovered most frequently from respiratory secretions of CF patients [1]. *Burkholderia cepacia* infection is much less common, but it is notorious for being associated with cross infection and the possibility of rapid deterioration, known as the cepacia syndrome [2–4]. *B. cepacia* colonisation frequency differs greatly between CF centres; overall, the prevalence is low, but the impact on individual patient survival may be very high [5]. Furthermore, the possibility of *B. cepacia* colonisation induces a high level of anxiety in the CF population.

Optimal isolation of *B. cepacia* from CF sputum requires the use of selective culture media [6]. Remarkable heterogeneity among presumed *B. cepacia* strains and the lack of reliable identification schemes makes correct identification of *B. cepacia* colonisation a complex problem [5, 6]. Several distinct genomovars are collectively referred to as *B. cepacia* complex (Bcc) [7]. The most commonly isolated Bcc species in CF patients are *B. multivorans, B. cenocepacia* and *B. stabilis*. A rapid deterioration and patient-to-patient transmission is probably more likely with *B. cenocepacia* infection and colonisation [8, 9]. A Belgian surveillance study in 1993 revealed that 12 out of 465 Belgian CF patients were Bcc colonised. These strains belonged to three different genomovars *i.e. B. multivorans*, *B. cenocepacia* and *B. stabilis*) [10].

The aims of the present study were to critically re-evaluate the prevalence of Bcc colonisation in Belgian CF patients during a new national 1-yr surveillance study to determine the genomovar status of the isolates and to evaluate the clinical status of patients colonised with Bcc, as compared to patients not colonised with Bcc. In addition, the clinical course of CF patients, colonised during the 1993 surveillance study, was evaluated.

Methods

In Belgium, since 1993 CF centres have used selective MAST medium (Mast Diagnostics, Meyerside, UK) to inoculate all sputum samples and upper airway samples from CF patients. Sputum samples are examined routinely every 3 months. A throat swab is taken in patients unable to produce sputum. All 11 Belgian health facilities caring for CF patients agreed to participate in the current study and to send all *B. cepacia*-like isolates to the reference laboratory between February 1999 and February 2000. The isolates were first examined by means of whole-cell protein electrophoresis, and compared to a database comprising a large number of Gram-negative, nonfermentative bacteria, isolated from CF and environmental specimens [11–15]. Extensive taxonomic studies have revealed that this method allows species-level identification of a wide range of Gram-negative nonfermenting bacteria, commonly misidentified as Bcc, and confirms the identification of putative Bcc isolates at the complex level [14, 15]. The method has proved to be insufficiently discriminatory to identify all species within Bcc [14, 15]. Therefore, Bcc bacteria were further examined by means of *recA* restriction

fragment length polymorphism (RFLP) analysis [16] and pulsed-field gel electrophoresis (PFGE) of genomic DNA restricted with *SpeI* [17]. Non-Bcc bacteria were further examined by using whole-cell fatty acid analysis [7]. Presence of the cable pilus subunit gene [18] and the epidemic strain marker BCESM [19] were determined by PCR using established primers.

For every patient colonised by Bcc, a control patient was identified from the same CF centre as the index patient and matched for sex, pancreatic status and genotype (a homozygous F508del patient was chosen for a F508del homozygous index case, a heterozygous F508del/other severe mutation was chosen for a heterozygous F508del/other severe mutation index case; mutations were considered severe when associated with pancreatic insufficiency and reported as such in the Toronto CF consortium database [20]. Patients were also matched for respiratory colonisation status, with chronic *P. aeruginosa* colonisation being defined as at least three sputum cultures positive for *P. aeruginosa* over the course of 6 months [21]. If several patients qualified, the patient with the birth date closest to the index patient was chosen. There was no difference in mean age between index cases and controls (table 1).

For both index and control patients, body mass index (BMI) was calculated and expressed as SD score [22]. Maximal expiratory flow manoeuvres were performed according to 1994 American Thoracic Society criteria [23]. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were expressed as per cent of the normal value, according to Zapletal for children and the European Community for Coal and Steel for adults [24]. For the time period outside the surveillance year, lung function data were extracted from the patient's chart. For every Bcc-colonised patient, the time of first colonisation was determined from the patient's chart. Data on all reported patients are included in the Belgian CF database. These patients all sign an informed consent and agree that their clinical data may be analysed and reported in an anonymous way in clinical studies.

Median age of colonised patients was compared to median age of CF patients included in the Belgian CF registry. The age distribution for the Belgian CF population in 1999 was not normal and, therefore, for this purpose, median age is reported and Mann-Whitney U-test was used for analysis.

Since 1995, specific infection control measures have been taken in all Belgian centres to prevent patient-to-patient spread of Bcc. These include: stressing hand wash policy for all patients and personnel; individual rooms for all hospitalised CF patients; and isolation during hospital admission, as well as during outpatient clinic visits for patients colonised with Bcc.

Results

MAST isolates during study period 1999

During the study period, the sputum or throat samples of 650 CF patients were evaluated on selective media. In eight of the 11 participating centres, Bcc-like strains were isolated and sent to the reference laboratory. A total of 67 isolates from 26 different patients were examined. Twelve patients from six different centres were genuinely colonised with Bcc: *B. multivorans* (n=9), *B. stabilis* (n=1) and *B. cenocepacia* (n=2). The *recA* RFLP type of each isolate is listed in table 2. None of the *B. cepacia* isolates carried the BCESM or cable pilus genes. In one patient colonised with *B. cenocepacia*, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* were also isolated. Other bacteria isolated on MAST

Table 1. – Data on *Burkholderia cepacia* complex-colonised patients (Bcc) and their controls (Con)

Patient no.	Centre	M/F	*P. aeruginosa*	Bcc gen.	Genotype		Age yrs		FVC % pred		FEV1 % pred		BMI SD score	
					Bcc	Con	Bcc	Con	Bcc	Con	Bcc	Con	Bcc	Con
1	E	M	Yes	M	F508del/F508del	F508del/F508del	32	27	66	83	57	51	1.95	2.04
2	E	M	No	M	F508del/F508del	F508del/F508del	15	15	33	74	30	54	-2.13	-1.36
3	E	F	Yes	M	F508del/W401X	F508del/2183-2A>G	18	16	38	109	20	84	-3.72	-0.15
4	F	F	Yes	M	F508del/F508del	F508del/F508del	13	13	60	86	45	85	-0.82	-1.23
5	F	M	No	S	F508del/F508del	F508del/F508del	21	20	85	57	60	39	0.88	-1.07
6	H	F	Yes	M	F508del/2335delA	F508del/E60X	22	22	73	77	55	32	-1.32	-1.94
7	H	F	Yes	M	F508del/F508del	F508del/ F508del	32	32	38	102	15	84	-1.97	-1.26
8	H	M	Yes	M	F508del/F508del	F508del/F508del	20	20	68	87	50	91	-1.72	-1.38
9	H	F	Yes	M	F508del/W401X	F508del/G542X	19	19	45	105	21	96	-2.00	-1.50
10	D	F	Yes	M	F508del/F508del	F508del/F508del	14	14	51	45	35	37	-0.86	-0.85
11	I	M	No	C	F508del/F508del	F508del/F508del	7	6	86	113	93	91	-2.37	0.48
12	C	M	Yes	C	F508del/F508del	F508del/F508del	33	27	35	19	14	21	-0.52	-2.01
Mean±SEM							20±2	19±2	57±6**	80±8	41±7*	64±8	-1.22±0.43	-0.85±0.33

Con are matched for centre (E: St. Vincentiusziekenhuis, Antwerpen; F: University Hospital Gasthuisberg, Leuven; H: Academisch Ziekenhuis Jette, Brussels; D: Hôpital Erasme, Brussels; I: Zeepreventorium, De Haan; C: Clinique Universitaire Saint Luc, Brussels), sex (M: male; F: female) and chronic *Pseudomonas aeruginosa* colonisation status. Bcc genomovar (gen.) is indicated for each patient (M: *multivorans*; S: *stabilis*; C: *cenocepacia*). FVC: forced vital capacity; FEV1: forced expiratory volume in one second; BMI: body mass index. *: $p<0.05$; **: $p<0.01$.

Table 2. – *RecA* restriction fragment length polymorphism (RFLP) type by patient number

	Patient no.											
	1	2	3	4	5	6	7	8	9	10	11	12
RecA RFLP type	F	Se21	Se21	F	J	F	Se21	F	F	F	I	H

Results of *recA* RFLP by digestion with *Hae* III revealed 10 distinct patterns and each pattern was assigned an alphabetical code (A–J) [16]. Se21 is a new type, not previously reported.

medium not belonging to Bcc complex were identified as *Sphingobacterium* sp. [1], *Chryseobacterium meningosepticum* [2], *P. aeruginosa* [4] and *Pandoraea pnomenusa* [2]. Five strains could not be identified.

Typing results using pulsed field gel electrophoresis

An overview of typing results for Bcc isolates is given in figure 1. Typing results confirmed that the patients remained colonised by the same strain during the course of the study (data not shown). Three patients were colonised by an identical strain; two patients were followed at centre E (PFGE type III) and one was followed at centre H (PFGE type III′). The PFGE DNA profile of the latter isolate differed in four DNA fragments from the profiles of the former two isolates. Such a limited degree of genomic variability is well known to occur in genomically versatile organisms like Bcc bacteria [25], and unrelated isolates differ in larger numbers of DNA fragments, as can be seen in figure 1. The centres E and H are 50 km apart and the three patients claim not to have any contact with each other. Three colonised patients had already been detected as positive during the 1993 cohort study. Typing results indicate that these patients continue to be colonised by the same *B. cepacia* strain. In one patient, during 2002, a temporary switch from colonisation by *B. stabilis* to colonisation by *B. multivorans* occurred. At present, the patient is again colonised with *B. stabilis*, as he has been for 10 yrs.

Clinical patient data

All 12 index patients had pancreatic insufficiency. Nine patients were homozygous F508del, and three patients were heterozygous for F508del and another mutation known to be associated with a severe phenotype. Three patients were not colonised by *P. aeruginosa* or other nonfermentative Gram-negative bacteria at the time of Bcc acquisition (table 1). In one 7-yr-old patient, colonisation by *B. cenocepacia* was transient during the study period. However, this patient became positive again in 2001.

Table 3. – Five-yr evolution of forced expiratory volume in one second in *Burkholderia cepacia* complex-colonised patients (Bcc) and control patients

	Years of colonisation						
	-1	0	1	2	3	4	5
Controls	63±8	62±8	62±7	62±7	59±8	60±7	59±7
Bcc	53±9	50±7	45±8	46±6	54±10	51±8	46±5
Surviving Bcc patients n	12	12	12	11	10	8	8

Data are presented as mean±SEM in surviving patients and their controls unless otherwise stated.

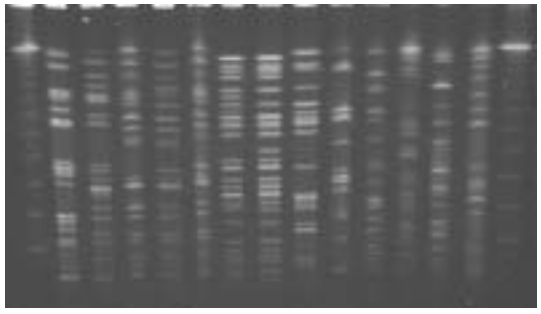

Fig. 1. – Pulsed-field gel electrophoresis (PFGE) pattern of the *Burkholderia cepacia* complex isolates in the 12 patients as indicated by their number in table 1 in 1999. Different PFGE banding patterns are numbered sequentially. Types III′ and IV′ differ in 4 and 3 fragments, respectively, from the types III and IV banding patterns. Patients 4, 8 and 5 were included in the 1993 study and were identified as having PFGE type IV′, VI and VIII respectively. For patient 4, the 1993 isolate with IV′ banding pattern is also shown which differs slightly from type IV. For patients 5 and 8, the PFGE pattern of the strain was identical in 1993 and 1999. Number of samples: patient 1, n=5; patient 2, n=6; patient 3, n=2; patient 4, n=2; patient 5, n=2; patient 6, n=3; patient 7, n=1; patient 8, n=8; patient 9, n=4; patient 10, n=2; patient 11, n=1; patient 12, n=8.

Median age (quartile ranges) of Bcc-colonised patients was 19.5 yrs (14.4–24.5) compared to 15.6 yrs (6.5–21.5) in the 1999 Belgian CF cohort ($p<0.05$, Mann-Whitney U-test; C. Sevens, Vrije Universiteit Brussels, Brussels, personal communication).

Clinical status in 1999 differed between Bcc-colonised patients and controls for lung function, but not for weight and BMI (table 1). FVC and FEV1 were significantly lower in colonised patients compared to control patients ((mean±SEM) FVC 57±6% *versus* 80±8%, unpaired t-test, $p<0.01$; FEV1 41±7% *versus* 64±8%, unpaired t-test, $p<0.05$).

At the end of the special surveillance period (*i.e.* February 2000), the mean duration of Bcc colonisation in the index cases was 3.8 yrs (range 0.5–7.25 yrs). Therefore, the 5-yr evolution was evaluated since the onset of colonisation with Bcc in the colonised patients, and in the corresponding time period in the control patients. The evolution is unfavourable in Bcc patients ($p<0.01$, Chi-squared test). None of the control patients died. Four *B. cepacia* colonised patients died: three were colonised with *B. multivorans*; one with *B. cenocepacia*. The latter died of *B. cepacia* sepsis 2 months post lung transplant. At the time of first isolation of *B. cepacia*, lung function values were lower in the index patients as compared to controls, but this difference was not statistically significant: FVC 65±7% *versus* 76±6%, unpaired t-test, $p=0.27$; FEV1 50±7% *versus* 62±8%, unpaired t-test, $p=0.15$). However, lung-function evolution from 1 yr before colonisation to 5 yrs after colonisation was significantly worse in the Bcc-colonised patients as compared to the matched controls ($p<0.01$, repeated measurement ANOVA) (table 3).

Clinical evolution of patients in the 1993 cohort

In 1993, 12 out of 465 Belgian CF patients tested were detected as colonised with Bcc. These patients were followed at three out of six participating centres. Six patients were colonised with *B. cenocepacia*, three with *B. multivorans* and three with *B. stabilis*. All clinical follow-up data could be collected in 11 patients. Transient colonisation was documented in three of these 11 patients. In all of the patients,

more than 10 sputum samples were grown onto MAST medium in an attempt to isolate Bcc. Five patients have died; four patients within 5 yrs after acquiring Bcc.

Four of the six patients colonised with *B. cenocepacia* have died. Three of these patients harboured the same (therefore called "epidemic") strain, as documented by PFGE. Of these, two patients died after prolonged colonisation (7 and 11 yrs). One patient died 2 yrs after becoming Bcc culture negative. The patient not carrying the epidemic strain died 2 yrs after lung transplantation, having remained positive for *B. cenocepacia* after transplant. Two *B. cenocepacia*-colonised patients are still alive; one is no longer culture positive, after 5 years of colonisation with the epidemic strain.

The three patients colonised with *B. multivorans* remained colonised for a long time. One was transplanted in March 1998, temporarily cleared but became positive again with a unique strain in 2001. Two patients remained positive and are also included in the 1999 cohort; one died in December 1999 and one underwent a lung transplant in August 2001.

No data are available in one patient for *B. stabilis*. The two other patients have been colonised by the same strain and are followed at the same centre. One young adult male is also included in the 1999 cohort and continues to be colonised with the same *B. stabilis* isolate. This patient suffers from moderate lung disease. One adolescent male became negative for *B. stabilis* 3 yrs before a right upper lobectomy performed in 1997 and continues to be negative ever since.

In three patients, transient colonisation is thus documented, even when several sputum cultures on selective media have been examined year after year.

Discussion

In 1999, only 12 out of 650 Belgian CF patients were colonised by Bcc. The prevailing genomovar was *B. multivorans*; only two patients were colonised by *B. cenocepacia*. The 5-yr evolution following onset of Bcc colonisation was poor: four of the 12 index cases died, although none of the matched controls died. In addition, lung-function evolution was significantly worse in index cases than in controls. In 11 out of 12 Belgian CF patients colonised with Bcc in 1993, the outcome could be evaluated: three patients had transient colonisation, five patients died, of whom four within 5 yrs of acquiring Bcc.

The Bcc colonisation rate in Belgian CF patients is low and remains low. In 1993, 12 patients out of 465 screened were Bcc positive. In the second national surveillance study, 12 out of 650 patients were Bcc positive. For the second study, all centres were able to cooperate and that study comprised all of the CF patients known in Belgium (the Belgian CF registry for 1999 contains data on 605 patients (C. Sevens, personal communication)). This suggests that there was no increase in Bcc colonisation in Belgian CF patients from 1993 to 2000.

In the Belgian CF community, the prevailing *B. cepacia* genomovar varied over time. In the 1993 cohort, six of the 12 patients were colonised with *B. cenocepacia*; the species most commonly associated with epidemic spread of disease and rapid deterioration [8, 9]. In the 1999 cohort, the majority (9 out of 12) of the patients were colonised with *B. multivorans*; only two patients were colonised with *B. cenocepacia*. This differs from the USA and Canada, where the majority of the isolates belong to *B. cenocepacia* [9, 26, 27]. In the study by CHEN *et al.* [27], 59 out of 60 Bcc isolates in CF patients were *B. cenocepacia*, and 57 of these 59 isolates represented the same clone. In Belgium, in between the two evaluation periods, strict isolation measures for Bcc-colonised patients have been instituted. These seem to have been successful so far.

The prevailing Bcc genomovar has been reported in few countries. The Belgian data differ somewhat from that which is known in both Italy and the UK as well. A nationwide survey was not reported from these countries, but a large number of Bcc isolates from CF patients was studied. A high incidence of infection caused by a single *B. cenocepacia* epidemic clone, possessing the cable A gene and closely related to the USA–UK clone "ET12", was reported from two Sicilian CF centres [28]. A recent study on 117 patient isolates from 40 UK hospitals reported that *B. cenocepacia* is most prevalent, with most isolates belonging to the epidemic clone [29]. However, both in Italy and the UK, other Bcc species were isolated from CF patients. It appears that in the absence of epidemic spread of one particular strain, the incidence of Bcc infection is low (such as in Belgium) and the distribution of genomovars is more varied.

The possibility of patient-to-patient transfer of *B. cepacia* in the CF community has always been worrisome. From DNA fingerprinting by AP-PCR and PFGE results in the 1993 cohort, it was demonstrated that four Belgian patients with *B. cenocepacia* were colonised by the same strain. These patients all attended the same clinic [10]. Two patients with *B. stabilis* colonisation, attending distinct clinics, shared the same strain.

For the 1999 cohort, three patients with *B. multivorans* shared an identical strain. In the UK, most reports stress the importance of the epidemic *B. cenocepacia* strain; however, *B. multivorans* is second in prevalence and several clusters of cross-infection have been identified [29]. In addition, in Italy, patient-to-patient spread was observed with genomovars other than *B. cenocepacia* [28]. The fact that the number of patients sharing the same strain decreases, suggests that for Belgian patients cross-infection is at present less likely than acquisition from an environmental source. Bcc is being used in agriculture for plant disease control [30] and strains from all genomovars have been isolated from the environment [31].

Patients colonised with Bcc may remain colonised during prolonged periods of time. Three patients were colonised by the same strain from 1993–1999. However, transient colonisation does seem to occur. It was documented in three patients of the total group of 21 patients described in this paper. Since in these patients several sputum samples were grown on *B. cepacia* selective media, it is unlikely that the pathogen was missed. In some patients, however, Bcc was not isolated for several years and then their first strain reappeared up to 10 yrs later. In some of these patients, it cannot be excluded that reacquisition from the environment occurred. Reports from UK [32] and Canada [33] also incidentally mention transient Bcc infection, but a thorough evaluation of a cohort to assess the frequency of this event has not been reported previously.

Unlike the initial reports [2, 3] suggested, Bcc acquisition is not limited to patients with *P. aeruginosa* colonisation nor patients with severe lung disease. In the Belgian study, several patients were not colonised with *P. aeruginosa* prior to Bcc colonisation. At the onset of Bcc colonisation, there was no significant difference in lung function abnormality between Bcc positive patients and controls. However, in the 5 yrs following onset of colonisation, the mortality is higher and lung function deterioration is faster.

In this Belgian study, the mortality amongst Bcc-colonised patients is high; from the 1993 cohort, four of the six patients colonised with *B. cenocepacia* have died. One of the remaining six patients has died; this patient was included in the 1999 cohort. Four other patients out of the 1999 cohort have also died, whereas none of the control patients have died. Only the transplanted patient died with the typical febrile bacteraemic "cepacia syndrome". The big impact of Bcc colonisation on disease severity and mortality has been reported previously

[2, 3, 32] and is confirmed by the present study. This study in a low-Bcc-incidence country clearly corroborates that the Bcc problem is not limited to *B. cenocepacia*.

In conclusion, in the Belgian cystic fibrosis population, *Burkholderia cepacia* complex colonisation rate is low and stable. *Burkholderia multivorans* and *Burkholderia cenocepacia* colonisation is most common. Although most patients are colonised for a longer time, in some patients transient colonisation seems to occur. The impact of *Burkholderia cepacia* complex on morbidity and mortality in a cystic fibrosis population is high and not limited to *Burkholderia cenocepacia* isolates. In Belgian cystic fibrosis patients, most patients carry a different strain and the cross-infection rate is low.

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