Clonality and antimicrobial susceptibility of *Burkholderia cepacia* complex isolates collected from cystic fibrosis patients during 1998-2013 in Bern, Switzerland

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SUMMARY

For the first time, we analyzed the clonality and susceptibility of *Burkholderia cepacia* complex isolates (n=55) collected during 1998-2013 from 44 Swiss cystic fibrosis (CF)-patients. *B. cenocepacia* (n=28) and *B. multivorans* (n=14) were mainly of sequence type (ST) 833 and ST874, respectively; *B. contaminans* isolates were of ST102. Overall, the following $MIC_{50/90}$s (mg/l) were obtained: piperacillin/tazobactam (≤4/≥128), ticarcillin/clavulanate (≥256/≥256), ceftazidime (2/≥32), aztreonam (16/≥32), meropenem (2/8), tobramycin (8/≥16), minocycline (≤1/16), levofloxacin (≤0.5/≥16), and trimethoprim/sulfamethoxazole (≤0.5/4). This is the first survey providing information on the clonality of *Bcc* detected in Switzerland. Species identification and antimicrobial susceptibility tests should always be routinely performed to adapt more targeted therapies.

KEY WORDS: Bcc, MIC, MLST, *Burkholderia*, Cystic fibrosis, Clonality.

Received November 5, 2014 *Accepted* January 31, 2015

The pathological airways condition of cystic fibrosis (CF)-patients favors chronic colonization/infection by bacterial species such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae, Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex (*Bcc*) (Doring *et al.,* 2012).

B. multivorans and *B. cenocepacia* are the most frequently detected *Bcc* species in CF-patients (Doring *et al.,* 2012). Their eradication is often unsuccessful and exacerbation results in high morbidity and mortality, mostly because of the *Bcc* natural multidrug resistance (MDR) pattern, the ability to form biofilm, and to invade epithelial cells or macrophages. Furthermore, these pathogens are highly transmissible in both clinical and community settings (Lipuma, 2010). For instance, *B. cenocepacia* sequence type (ST) 28, belonging to clonal complex (CC) 31, and *B. multivorans* ST16 are important clones spreading in different countries (Baldwin *et al.,* 2005).

Despite the natural MDR phenotype and the fast development of further antibiotic resistance, the paucity of data describing the antimicrobial susceptibility of *Bcc* is surprising. In particular, studies determining the minimum inhibitory concentration (MIC) of several classes of antibiotics with a reproducible and standardized methodology are scarce and most analyses were published prior to the differentiation of the species within *Bcc* (King *et al.,* 2010, Nzula *et al.,* 2002, Leitao *et al.,* 2008, Peeters *et al.,* 2009). As a consequence, the MIC distributions of clinically relevant antibiotics for the main *Bcc* species found in CF-patients are not available (http://mic.eucast.org/Eucast2/). This lack of data hinders a prediction of treatment outcome based on the MIC values.

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To fill in this gap of knowledge, we retrospectively analyzed the *in vitro* activity of 19 antimicrobials against a collection of *Bcc* isolates detected in CF-patients during January 1998 to March 2013. Isolates were collected at the Laboratory of Clinical Microbiology of the Institute for Infectious Diseases, University of Bern (Switzerland) that processes samples from a network of hospitals located in the city of Bern. From the same patient the first isolate per year was included in the study.

Briefly, all *Bcc* strains were cultivated on blood agar plates (Oxoid) at 35°C overnight. Identification of grown colonies was confirmed by matrix-assisted laser desorption ionization time of flight mass spectrometry (microflex LT, Bruker Daltonics) and sequencing of the *recA* gene (Lupo *et al.,* 2015, Baldwin *et al.,* 2005). MICs were obtained with microdilution GNX2F panels (Trek Diagnostics) using cation-adjusted Mueller-Hinton broth (Difco). *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains. The panels include the antibiotics recommended for treatment of infections due to *Bcc* in CF-patients by the European Consensus Study Group, ECSG (piperacillin/tazobactam, ticarcillin/clavulanate, ceftazidime, meropenem, aztreonam, doxycycline, trimethoprim/sulfamethoxazole, and tobramycin) (Doring *et al.,* 2012).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) does not provide interpretative criteria for the susceptibility results of *Bcc* species (EUCAST, 2014). Therefore, MICs of ticarcillin/clavulanate, ceftazidime, meropenem, minocycline, levofloxacin and trimethoprim/sulfamethoxazole were interpreted according to the 2014 Clinical and Laboratory Standards Institute (CLSI) criteria established for *B. cepacia*, whereas the other antibiotics (including the remaining suggested by the ECSG) were tentatively interpreted with the CLSI criteria set for *P. aeruginosa* when available (CLSI, 2014). Results were stratified taking into account three groups of organisms (*B. cenocepacia*, *B. multivorans* and "other species"). The clonality of randomly selected isolates (20 *B. cenocepacia*, 14 *B. multivorans* and 6 *B. contaminans*) was determined by multilocus sequence typing (MLST) (http://pubmlst.org/bcc/).

In total, we analyzed 55 isolates collected from 44 CF-patients: *B. cenocepacia* (n=28), *B. multivorans* (n=14), *B. contaminans* (n=8), *B. cepacia* (n=2), *B. ambifaria* (n=1), *B. seminalis* (n=1) and *B. stabilis* (n=1). The MLST analysis showed that 13 out of 20 *B. cenocepacia* isolates belonged to the novel ST833 (Table 1). Of these, one was isolated in 2007 and the remaining in 2008 from non-redundant patients suggesting the occurrence of a small outbreak that ended without any intervention. Five isolates found in three patients belonged to CC31 (ST250 and ST208). Several *B. multivorans* isolates collected from six patients in 1998 belonged to the novel ST874 indicating another small self-limiting epidemic event; the remaining *B. multivorans* isolates were associated to STs (ST22, ST180, ST188, and ST620) already reported in other countries. Six *B. contaminans* isolates found in two patients belonged to ST102, which is a worldwide ST found not only in CF-patients but also in environmental sources according to the *Bcc* PubMLST database.

As shown in Table 2, all *Bcc* isolates were resistant to ticarcillin/clavulanate, whereas most were susceptible to trimethoprim/sulfamethoxazole (89%), ceftazidime (86%), levofloxacin (84%), meropenem (82%), and minocycline (78%). However, significant differences were noted among the three species groups. *B. cenocepacia* isolates were at first susceptible to ceftazidime (93%) followed by trimethoprim/sulfamethoxazole (89%), levofloxacin (79%), meropenem and minocycline (both 75%). With the exception of ticarcillin/clavulanate, all *B. multivorans* were found fully susceptible to the antibiotics suggested by CLSI (CLSI, 2014). Isolates belonging to the "other species" group were mostly susceptible to meropenem, trimethoprim/sulfamethoxazole and levofloxacin (all 77%), followed by minocycline (62%) and ceftazidime (54%).

Therefore, in contrast with other investigations (Aaron *et al.,* 2000, Bonacorsi *et al.,* 1999, Nzula *et al.,* 2002), meropenem was not the most effective *in vitro* drug against the overall *Bcc* isolates detected in Bern. The other four antibiotics still represent a possible therapeutic alternative. In particular, ceftazidime resulted very active for most *Bcc* and its clinical implementation coupled with tobramycin could assure a positive

TABLE 1 - *Multilocus sequence typing of 40 randomly selected Bcc isolates collected from 33 cystic fibrosis patients during 1998-2013 in Bern, Switzerland*

Patient	*Isolate*	*Year of isolation*	*Specimen*	*ST (B. cenocepacia subgroup)*	*CC*	*Previous report*	
						Country	*Source*
B. cenocepacia							
#44	602058	1998	Sputum	250 (IIIA)	31	USA	CF-patients
#44	919456	2001	Sputum	250 (IIIA)	31	USA	CF-patients
#26	1257579	2005	Sputum	250 (IIIA)	31	USA	CF-patients
#20	1352420	2006	LRTS	208 (IIIA)	31	ESP, RUS, USA,	CF-patients
#26	1512265	2007	LRTS	250 (IIIA)	31	USA	CF-patients
#19	1555733	2007	LRTS	833[b] (IIIB)	469	None	None
#17	1610124	2008	LRTS	834[a] (IIIA)	None	None	None
#32	1564292	2008	Sputum	833[b] (IIIB)	469	None	None
#43	1559631	2008	LRTS	833[b] (IIIB)	469	None	None
#12	1565171	2008	LRTS	833[b] (IIIB)	469	None	None
#40	1574181	2008	LRTS	833[b] (IIIB)	469	None	None
#7	1563289	2008	LRTS	833[b] (IIIB)	469	None	None
#47	1562860	2008	LRTS	833[b] (IIIB)	469	None	None
#23	1587357	2008	Pharyngeal swab	833 [b] (IIIB)	469	None	None
#22	1584063	2008	Urine	833[b] (IIIB)	469	None	None
#15	1588860	2008	Urine	833[b] (IIIB)	469	None	None
#11	1588861	2008	Urine	833[b] (IIIB)	469	None	None
#29	1648163	2008	Urine	833[b] (IIIB)	469	None	None
#9	1604077	2008	Urine	833[b] (IIIB)	469	None	None
#28	1734146	2009	LRTS	834[a] (IIIA)	None	None	None
B. multivorans							
#6	642289	1998	Sputum	22[c]	22	CAN	Environment, CF-patients
#35	609975	1998	Pharyngeal swab	620	None	USA, AUS	CF-patients
#39	620768	1998	LRTS	874	None	None	None
#31	647228	1998	LRTS	874	None	None	None
#4	655039	1998	LRTS	874	None	None	None
#13	610183	1998	LRTS	874	None	None	None
#44	614620	1998	Sputum	874	None	None	None
#41	637589	1998	LRTS	874	None	None	None
#36	811244	2000	LRTS	180[d]	180	CZE, UK, FRA	Environment, CF-patients
#3	1689963	2009	Biopsy	180[d]	180		
#2	1064722	2003	Pharyngeal swab	873	None	None	None
#25	1996055	2011	Pharyngeal swab	188	None	CAN	CF-patients
#42	1971592	2011	Sputum	750-variant[e]	None	None	None
#33	800856	NA	Pharyngeal swab	875	None	None	None
B. contaminans							
#37	1229687	2005	LRTS	102	None	Sargasso Sea, CZE, ESP, BEL, AUT, BRA, ITA, USA, RUS	Environment, CF-patients
#37	1385245	2006	Pharyngeal swab	102			
#37	1609253	2008	Pharyngeal swab	102			
#5	1830253	2010	Sputum	102			
#37	1899744	2010	Pharyngeal swab	102			
#37	2191457	2013	Pharyngeal swab	102			

Note. NA: not available; LRTS, lower respiratory tract secretions (including tracheobronchial secretions and bronchoalveolar lavages); ST, sequence type; CC, clonal complex; CAN, Canada; AUS, Australia; CZE, Czech Republic; UK, United Kingdom; FRA, France; ESP, Spain; BEL, Belgium; AUT, Austria; BRA, Brazil; ITA, Italy; RUS, Russia; CF, cystic fibrosis. [a]Double locus variant (DLV) of ST633, ST844 and ST382 previously isolated from CF-patients in France and Brazil. [b]Single locus variant (SLV) of ST469. [c]SLV of ST652 previously isolates from CF-patients in Czech Republic. [d]SLV of ST419. [e]The alleles *gltB* and *phaC* could not have been amplified, all the remaining alleles were identical to ST750.

TABLE 2 - *Minimum inhibitory concentration (MIC) of 19 antibiotics against 55 Burkholderia cepacia complex isolates collected from specimens of 44 cystic fibrosis patients during 1998-2013 in Switzerland*

Antibiotic	*Organism*[a]	*Number of isolates with corresponding MIC (mg/l)*[b]													*MIC*$_{50}$	*MIC*$_{90}$	*S%*[c,d,e]
		0.06	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*			
Ticarcillin/ clavulanate (TIM)	Overall *Bcc* isolates											1	2	52	≥256	≥256	0.0
	B. cenocepacia													28	≥256	≥256	0.0
	B. multivorans											1	2	11	≥256	≥256	0.0
	Other species													13	≥256	≥256	0.0
Piperacillin/ tazobactam (TZP)	Overall *Bcc* isolates							35			3	5	12		≤4	≥128	(63.6)
	B. cenocepacia							17			2	5	4		≤4	≥128	(60.7)
	B. multivorans							13			1				≤4	≤4	(92.9)
	Other species							5					8		≥128	≥128	(38.5)
Cefotaxime	Overall *Bcc* isolates						4	15	13	4	3	16			8	≥64	NA
	B. cenocepacia						1	5	9	3	2	8			8	≥64	NA
	B. multivorans						3	9	2						4	8	NA
	Other species							1	2	1	1	8			≥64	≥64	NA
Ceftazidime (CAZ)	Overall *Bcc* isolates				8		22	12	5	2	6				2	≥32	85.5
	B. cenocepacia				4		12	7	3	1	1				2	16	92.9
	B. multivorans				4		9	1							2	2	100
	Other species						1	4	2	1	5				8	≥32	53.9
Cefepime (FEP)	Overall *Bcc* isolates					10		14	9	5	17				4	≥32	(60.0)
	B. cenocepacia							10	6	3	9				8	≥32	(57.1)
	B. multivorans					10		1	1	2					≤1	16	(85.7)
	Other species							3	2		8				≥32	≥32	(38.5)
Meropenem (MER)	Overall *Bcc* isolates				11		17	17	7	3					2	8	81.8
	B. cenocepacia				6		9	6	4	3					2	8	75.0
	B. multivorans				3		4	7							2	4	100
	Other species				2		4	4	3						4	8	76.9
Imipenem (IMP)	Overall *Bcc* isolates				1		8	17	11	18					4	≥16	(16.4)
	B. cenocepacia						2	13	5	8					4	≥16	(7.1)
	B. multivorans								6	8					≥16	≥16	(0.0)
	Other species				1		6	4		2					2	≥16	(53.9)
Ertapenem	Overall *Bcc* isolates						12	16	27						4	≥8	NA
	B. cenocepacia						6	9	13						4	≥8	NA
	B. multivorans						5	5	4						4	≥8	NA
	Other species						1	2	10						≥8	≥8	NA
Doripenem (DOR)	Overall *Bcc* isolates					3	7	45							≥4	≥4	(18.2)
	B. cenocepacia					2	4	22							≥4	≥4	(21.4)
	B. multivorans					1	1	12							≥4	≥4	(14.3)
	Other species						2	11							≥4	≥4	(15.4)
Aztreonam (ATM)	Overall *Bcc* isolates							12	6	15	22				16	≥32	(32.7)
	B. cenocepacia							2	4	11	11				16	≥32	(21.4)
	B. multivorans							10	1	1	2				4	4	(78.6)
	Other species								1	3	9				≥32	≥32	(7.7)
Gentamicin (GEN)	Overall *Bcc* isolates				16			2	2	35					≥16	≥16	(32.7)
	B. cenocepacia				15					13					≤0.5	≥16	(53.6)
	B. multivorans									14					≥16	≥16	(0.0)
	Other species				1			2	2	8					≥16	≥16	(23.1)
Tobramycin (TOB)[f]	Overall *Bcc* isolates				16		1	1	5	32					8	≥16	(32.7)
	B. cenocepacia				15					13					≤0.5	≥16	(53.6)
	B. multivorans									14					≥16	≥16	(0.0)
	Other species				1		1	1	5	5					8	≥16	(23.1)
Amikacin (AK)	Overall *Bcc* isolates						12		4	3	4	32			≥64	≥64	(34.5)
	B. cenocepacia						11		4			13			8	≥64	(53.6)
	B. multivorans										2	12			≥64	≥64	(0.0)
	Other species						1			3	2	7			32	≥64	(30.8)

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Antibiotic	*Organism*[a]	*Number of isolates with corresponding MIC (mg/l)*[b]													*MIC*$_{50}$	*MIC*$_{90}$	*S%*[c,d,e]
		0.06	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*			
Trimethoprim/ sulfa (SXT)	Overall *Bcc* isolates			39		7	3	3	3						≤0.5	5	89.1
	B. cenocepacia			21		2	2	2	1						≤0.5	2	89.3
	B. multivorans			12		2									≤0.5	1	100
	Other species			6		3	1	1	2						1	≥8	76.9
Doxycycline (DOX)[g]	Overall *Bcc* isolates					30	6	4	4	1	10				≤2	≥32	72.7
	B. cenocepacia					11	6	2	2		7				2	≥32	67.9
	B. multivorans					14									≤2	≤2	100
	Other species					5		2	2	1	3				4	≥32	53.8
Minocycline (MIN)	Overall *Bcc* isolates					34		9	6	6					≤1	16	78.2
	B. cenocepacia					14		7	5	2					≤1	8	75.0
	B. multivorans					13		1							≤1	≤1	100
	Other species					7		1	1	4					≤1	16	61.5
Tigecycline (TIG)	Overall *Bcc* isolates		15		18	12	3	6		1					0.5	4	NA
	B. cenocepacia		5		11	5	3	3		1					0.5	4	NA
	B. multivorans		4		5	5									0.5	1	NA
	Other species		6		2	2		3							0.5	4	NA
Ciprofloxacin (CIP)	Overall *Bcc* isolates		4		21	9	6	15							0.5	≥4	(61.8)
	B. cenocepacia		1		12	6	1	8							1	≥4	(67.9)
	B. multivorans				5	2	4	3							1	≥4	(50.0)
	Other species		3		4	1	1	4							0.5	≥4	(61.5)
Levofloxacin (LVX)	Overall *Bcc* isolates			24		16	6	3	6						≤0.5	≥16	83.6
	B. cenocepacia			8		10	4	2	4						2	≥16	78.6
	B. multivorans			11		2	1								≤0.5	2	100
	Other species			5		4	1	1	2						2	≥16	76.9

Note. NA: CLSI interpretative criteria for *B. cepacia* or *P. aeruginosa* not available. [a]Overall *Bcc* isolates (n=55); *B. cenocepacia* (n=28); *B. multivorans* (n=14); other species (n=13). Other species includes: *B. ambifaria*, (n=1); *B. cepacia*, (n=2); *B. contaminans* (n=8); *B. seminalis* (n=1); and *B. stabilis*, (n=1). [b]The grey areas delimit the concentration range tested with the microdilution GNX2F Trek panels. [c]MICs were interpreted as susceptible (S) according to the available 2014 CLSI criteria established for *B. cepacia* (CLSI, 2014) : TIM (S ≤ 16 mg/l), CAZ (S ≤ 8 mg/l), MER (S ≤ 4 mg/l), SXT (S ≤ 2 mg/l), LEV (S ≤ 2 mg/l), and MIN (S ≤ 4 mg/l). [d]MIC values for the other antibiotics were tentatively categorized using the CLSI criteria for *P. aeruginosa*: TZP and AK (S ≤ 16 mg/l), FEP and ATM (S ≤ 8 mg/ml), IMP and DOR (S ≤ 2 mg/l), GEN and TOB (S ≤ 4 mg/l), CIP (S ≤ 1 mg/l) (CLSI, 2014). These results are presented in parentheses. [e]Colistin and polymyxin B were tested as well, but they results were omitted from the table as all the isolates showed the same expected susceptibility value (i.e., MIC ≥ 8 mg/l). [f]All *B. cenocepacia* isolates of ST833 were susceptible to aminoglycosides. [g]MIC values for DOX were categorized with the same cutoff of MIN.

outcome (Latzin *et al.,* 2008). We also emphasize that the antimicrobials with an oral route of administration (trimethoprim/sulfamethoxazole, levofloxacin, and minocycline) may be used for the treatment of CF-patients (Doring *et al.,* 2012). However, we should note that other authors have observed higher resistance rates for such antibiotics. These overall differences could be due to the diverse *Bcc* species included in the studies (King *et al.,* 2010, Leitao *et al.,* 2008, Moore *et al.,* 2001, Zhou *et al.,* 2007, Bonacorsi *et al.,* 1999, Peeters *et al.,* 2009).

With regard to the antibiotics for which CLSI breakpoints for *B. cepacia* are not available (CLSI, 2014), we found that most β-lactams have scarce *in vitro* activity against *Bcc*. This is mainly due to the expression of chromosomal β-lactamases that are able to hydrolyze carbapenems (i.e., PenA, an inhibitor-resistant carbapenemase similar to KPC-2) (Papp-Wallace *et al.*, 2013). However, our *B. multivorans* isolates were less resistant to β-lactams than the other species (e.g., 93% susceptible to piperacillin/tazobactam: MIC_{90} ≤4 mg/l).

Two-thirds of the *Bcc* isolates were susceptible to doxycycline, whereas tigecycline showed lower MICs when compared to both doxycycline and minocycline (MIC_{90} of 4 mg/l *versus* ≥32 and 16 mg/l, respectively). However, one should be aware that *Bcc* possess efflux pumps that influence their susceptibility to tigecycline (Rajendran *et al.,* 2010). Such mechanism can also contribute to *Bcc* resistance to aminoglycosides. Therefore, we were not surprised to see that only one-third of our isolates (most of which were *B. cenocepacia* of ST833) had an MIC in the susceptible ranges established for *P. aeruginosa* (CLSI, 2014). In particular, as previ-

ously observed (Leitao *et al.,* 2008, Nzula *et al.,* 2002), tobramycin was scarcely active (MIC_{90} ≥16 mg/l). However, the association of this and other aminoglycosides with ceftazidime or other β-lactams seems beneficial for the treatment of infections due to *Bcc* (Avgeri *et al.,* 2009).

For three patients, we analyzed the *Bcc* isolates collected at each hospital admission. We observed that the first and last isolates from each patient belonged to the same sequence type (i.e., ST250 from two patients and ST102 from one patient) demonstrating the strong ability of these *Bcc* strains to permanently colonize/infect the lower respiratory tract of CF-patients. In one patient (#44), co-infection with *B. cenocepacia* ST250 and *B. multivorans* ST874 was observed. The isolates relative to each patient showed a fluctuation in the MIC values (Table

TABLE 3 - *Minimum inhibitory concentration (MIC) and multilocus sequence typing of Bcc isolates consecutively collected from three patients.*

Patient	*Sample*	*Month, Year*	*Isolate*	*Species*	*MIC (mg/l)*									*ST*
					TIM	*CAZ*	*MER*	*SXT*	*MIN*	*LVX*	*TZP*	*ATM*	*TOB*	
#26	Sputum	04/2005	1257579	*B. cenocepacia*	≥256	8	8	≤0.5	4	≥16	32	≥32	≥16	250
	Sputum	08/2005	1295854	*B. cenocepacia*	≥256	≥32	8	≤0.5	4	≥16	32	≥32	≥16	ND
	Sputum	09/2005	1299720	*B. cenocepacia*	≥256	4	8	≤0.5	4	≥16	≥128	≥32	≥16	ND
	Sputum	02/2006	1346522	*B. cenocepacia*	≥256	2	4	≤0.5	4	≥16	≥128	≥32	≥16	ND
	Sputum	05/2006	1372142	*B. cenocepacia*	≥256	4	8	≤0.5	8	≥16	64	16	≥16	ND
	Sputum	10/2006	1420025	*B. cenocepacia*	≥256	≥32	≥16	4	8	≥16	≥128	≥32	≥16	ND
	LRTS	06/2007	1494646	*B. cenocepacia*	≥256	8	8	≤0.5	≤2	≥16	≥128	≥32	≥16	ND
	LRTS	08/2007	1512265	*B. cenocepacia*	≥256	8	8	4	4	≥16	≥128	≥32	≥16	250
#37	LRTS	01/2005	1229687	*B. contaminans*	≥256	≥32	8	≤0.5	≤2	2	≥128	≥32	≤1	102
	Pharyngeal swab	09/2005	1299422	*B. contaminans*	≥256	16	4	2	8	2	≥128	≥32	≥16	ND
	Pharyngeal swab	02/2006	1349631	*B. contaminans*	≥256	4	2	≥8	16	4	≥128	≥32	8	ND
	Pharyngeal swab	06/2006	1385245	*B. contaminans*	≥256	8	4	1	≤2	≤1	≥128	≥32	≥16	102
	Pharyngeal swab	09/2006	1412712	*B. contaminans*	≥256	≥32	2	≤0.5	4	4	≥128	≥32	8	ND
	Pharyngeal swab	11/2006	1430625	*B. contaminans*	≥256	≥32	8	4	≤2	2	≥128	≥32	≥16	ND
	Pharyngeal swab	12/2006	1441653	*B. contaminans*	≥256	≥32	≥16	2	8	4	≥128	≥32	≥16	ND
	Pharyngeal swab	12/2007	1552885	*B. contaminans*	≥256	16	4	4	≤2	≤1	≥128	≥32	8	ND
	Pharyngeal swab	06/2008	1609253	*B. contaminans*	≥256	8	4	1	≤2	≤1	≥128	≥32	≥16	ND
	Pharyngeal swab	06/2009	1742005	*B. contaminans*	≥256	≥32	4	≤0.5	16	8	≥128	≥32	≥16	ND
	Pharyngeal swab	10/2010	1899744	*B. contaminans*	≥256	≥32	8	1	16	≥16	≥128	≥32	≥16	102
	Pharyngeal swab	06/2013	2191457	*B. contaminans*	≥256	≥32	8	≥8	16	≥16	≥128	≥32	≥16	102
#44	Sputum	04/1998	602058	*B. cenocepacia*	≥256	4	8	1	8	8	≥128	≥32	≥16	250
	Sputum	06/1998	614620	*B. multivorans*	≥256	4	2	≤0.5	≤2	≤1	32	4	≥16	874
	Sputum	07/1999	709191	*B. cenocepacia*	≥256	4	≥16	≥8	8	8	≥128	≥32	≥16	ND
	Sputum	11/2001	919456	*B. cenocepacia*	≥256	4	≥16	≤0.5	8	4	64	≥32	≥16	250

Note. LRTS, lower respiratory tract secretions (including tracheobronchial secretions and bronchoalveolar lavages); ST: sequence type; ND: not determined.

3). Unfortunately, the current data do not link the antibiotic susceptibility pattern to the therapeutic regimen(s) followed by the patients.

This is the first survey providing information on species and clonality of *Bcc* isolates detected in Swiss CF-patients. *B. cenocepacia* was the most frequently isolated species during the study period but *B. multivorans* and *B. contaminans* seem to have emerged in the last few years. Sporadic international pandemic clones and the occurrence of two small epidemic events (*B. cenocepacia* of ST833 and *B. multivorans* of ST874) were recorded. Our study also shows that the antibiotic susceptibility profile significantly varies among the *Bcc* species: *B. multivorans* exhibited susceptibility to almost all tested antibiotics, whereas *B. cenocepacia* and other species showed more drug resistance. Therefore, we highlight the importance of routine and constant performance of both species identification and antimicrobial susceptibility tests to adapt more targeted therapies and to impede any increased resistance in CF-patients.

This work constitutes a solid dataset for further epidemiological surveillance allowing a prompt response to epidemic events among CF-patients. Further surveys including larger collections of isolates from more centers caring for CF-patients should be planned in the near future to better comprehend the extent of the spread of *Bcc* at national level. Moreover, the lack of specific international and standardized methods to perform and interpret susceptibility results for *Bcc* species needs to be addressed. By evaluating optimal dosing and efficacy, randomized controlled clinical trials will provide important insights into the *in vivo* performance of antimicrobials recently suggested for the treatment of respiratory infections in CF-patients (Doring *et al.,* 2012).

ACKNOWLEDGMENTS

We thank Salome N. Seiffert for helping with the phenotypic tests and Dr Sara Droz for providing clinical isolates. We also express deep gratitude to Prof. Martin G. Täuber for the support with internal funds of the Institute of Infectious Diseases (IFIK). This work summarizes part of the Master Thesis of Enver Isis.

REFERENCES

Aaron S.D., Ferris W., Henry D.A., Speert D.P., Macdonald N.E. (2000). Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *Am. J. Respir. Crit. Care Med.* **161,** 1206-1212.

Avgeri S.G., Matthaiou D.K., Dimopoulos G., Grammatikos A.P., Falagas M.E. (2009). Therapeutic options for *Burkholderia cepacia* infections beyond co-trimoxazole: a systematic review of the clinical evidence. *Int. J. Antimicrob. Agents.* **33,** 394-404.

Baldwin A., Mahenthiralingam E., Thickett K.M., Honeybourne D., Maiden M.C., Govan J. R., Speert D.P., Lipuma J.J., Vandamme P., Dowson C.G. (2005). Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. *J. Clin. Microbiol.* **43,** 4665-4673.

Bonacorsi S., Fitoussi F., Lhopital S., Bingen E. (1999). Comparative in vitro activities of meropenem, imipenem, temocillin, piperacillin, and ceftazidime in combination with tobramycin, rifampin, or ciprofloxacin against *Burkholderia cepacia* isolates from patients with cystic fibrosis. *Antimicrob. Agents Chemother.* **43,** 213-217.

CLSI. (2014). Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 24th Informational Supplement. M100-S24. Wayne, PA.

Doring G., Flume P., Heijerman H., Elborn J.s., Consensus Study G. (2012). Treatment of lung infection in patients with cystic fibrosis: current and future strategies. *J. Cyst. Fibros.* **11**, 461-479.

EUCAST. (2014). European Committee on Antimicrobial Susceptibility Testing - Clinical breakpoints, Version 4.0, 2014.

King P., Lomovskaya O., Griffith D.C., Burns J.L., Dudley M.N. (2010). In vitro pharmacodynamics of levofloxacin and other aerosolized antibiotics under multiple conditions relevant to chronic pulmonary infection in cystic fibrosis. *Antimicrob. Agents Chemother.* **54**, 143-18.

Latzin P., Fehling M., Bauernfeind A., Reinhardt D., Kappler M., Griese, M. (2008). Efficacy and safety of intravenous meropenem and tobramycin versus ceftazidime and tobramycin in cystic fibrosis. *J. Cyst. Fibros.* **7**, 142-146.

Leitao J.H., Sousa S.A., Cunha M.V., Salgado M.J., Melo-Cristino J., Barreto M.C., Sa-Correia I. (2008). Variation of the antimicrobial susceptibility profiles of *Burkholderia cepacia* complex clonal isolates obtained from chronically infected cystic fibrosis patients: a five-year survey in the major Portuguese treatment center. *Eur. J. Clin. Microbiol. Infect. Dis.* **27**, 1101-1111.

Lipuma J.J. (2010). The changing microbial epidemi-

ology in cystic fibrosis. *Clin. Microbiol. Rev.* **23**, 299-323.

Lupo A., Isis E., Perreten V., Endimiani A. (2015). Raw meat contaminated with epidemic clones of *Burkholderia multivorans* found in cystic fibrosis patients. *J. Cyst. Fibros.* **14**, 150-152.

Moore J.E., Crowe M., Shaw A., Mccaughan J., Redmond A.O., Elborn J.S. (2001). Antibiotic resistance in *Burkholderia cepacia* at two regional cystic fibrosis centres in Northern Ireland: is there a need for synergy testing? *J. Antimicrob. Chemother.* **48**, 319-21.

Nzula S., Vandamme P., Govan J.R. (2002). Influence of taxonomic status on the in vitro antimicrobial susceptibility of the *Burkholderia cepacia* complex. *J. Antimicrob. Chemother.* **50**, 265-269.

Papp-Wallace K.M., Taracila M.A., Gatta J.A., Ohuchi N., Bonomo R.A., Nukaga M. (2013). Insights into beta-lactamases from *Burkholderia* species, two phylogenetically related yet distinct resistance determinants. *J. Biol. Chem.* **288**, 19090-19102.

Peeters E., Nelis H.J., Coenye T. (2009). In vitro activity of ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim/sulfamethoxazole against planktonic and sessile *Burkholderia cepacia* complex bacteria. *J. Antimicrob. Chemother.* **64**, 801-809.

Rajendran R., Quinn R.F., Murray C., Mcculloch E., Williams C., Ramage G. (2010). Efflux pumps may play a role in tigecycline resistance in *Burkholderia* species. *Int. J. Antimicrob. Agents.* **36**, 151-154.

Zhou J., Chen Y., Tabibi S., Alba L., Garber E., Saiman L. (2007). Antimicrobial susceptibility and synergy studies of *Burkholderia cepacia* complex isolated from patients with cystic fibrosis. *Antimicrob. Agents Chemother.* **51**, 1085-1088.