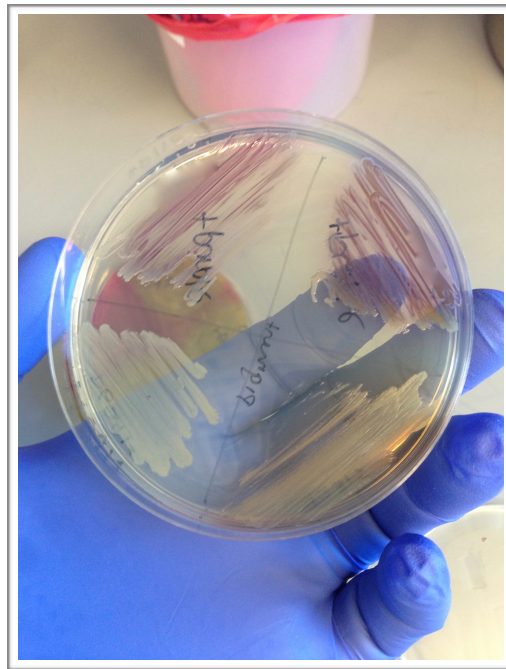


Canadian *Burkholderia cepacia* complex research and referral repository



2016 Annual Report: August 1st 2015 to July 31st 2016



a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA



Summary

We are delighted to present the 2015/16 Canadian *Burkholderia cepacia* complex research and referral repository (CBCRRR) annual report. Thanks to an enhanced grant received from Cystic Fibrosis Canada, as of 1st April 2015 we have been providing *Burkholderia* identifications **free of charge** to all Canadian CF clinics.

This year we have received 70 isolates from referring hospitals. Of these 63 isolates were from 51 CF patients. Several trends continue from previous years:

- ***B. multivorans* continues to be the most commonly identified species.**
- **Identifications of new cases of *B. cenocepacia* continue, however these are usually not caused by the epidemic strain types previously present in Canada.**
- **Based on prevalence reported in the Canadian CF Data Registry reports, there are still infections for which we do not routinely receive isolates. It is also likely that many clinics send us the initial isolate only. However because of the risk of species replacement we strongly encourage all clinics to submit isolates on an annual basis for all patients culturing *B. cepacia* complex.**

Did you know? The 2014 revised infection control guidelines recommend submission of *Burkholderia* isolates or other Gram-negatives for which the identification is equivocal on an **at least annual** basis for each patient. The CBCRRR is the Canadian resource for this.

Submitting isolates to us helps you because:

- i) we can alert you to species replacement
- ii) we store the isolates indefinitely so we can answer any future questions you might have
- iii) we can perform comparative strain typing to determine if any patients share strains (this service is available now and if you have a need for it please contact us).

Submitting isolates also helps people with CF by providing clinically relevant samples for understanding both epidemiology and conducting research.

To submit isolates visit: <http://cupic.cfri.ca/research/cbccrrr.html> or contact Dr. James Zlosnik: jzlosnik@cfri.ca

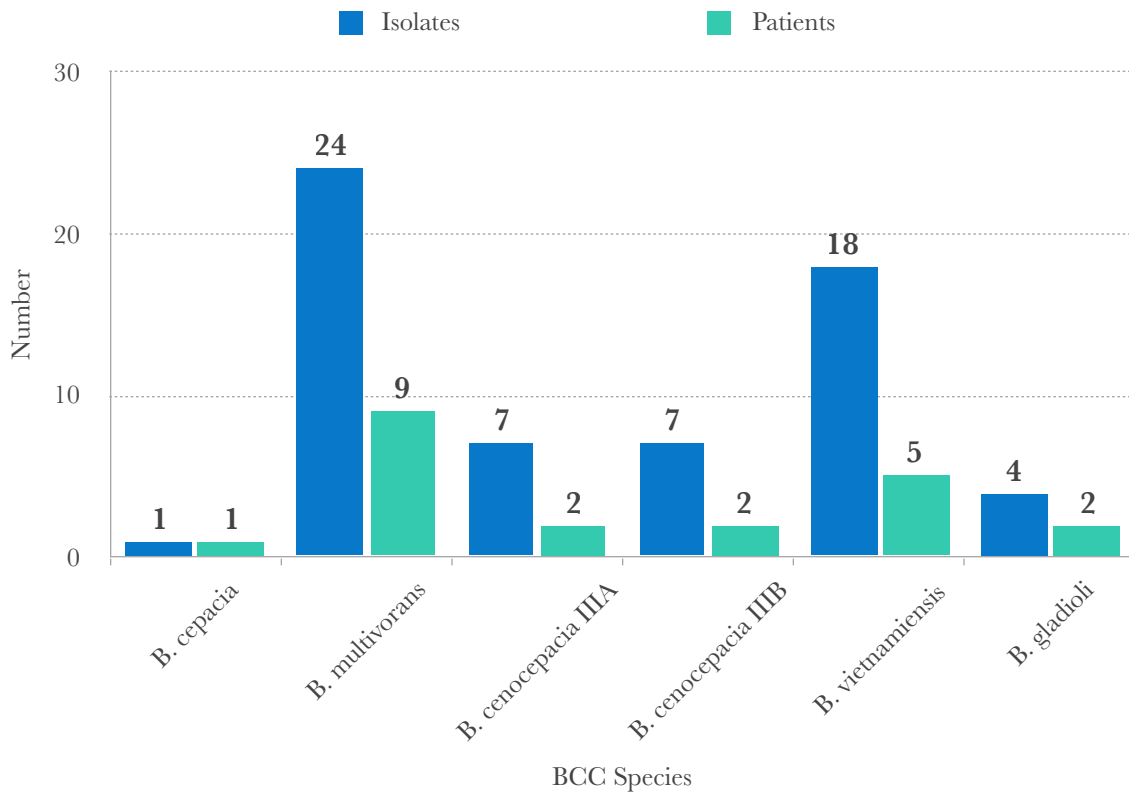
Overview of Identifications

Province	Clinic	# samples	# patients [# w.CF] (# new*)	Isolates ID'd as <i>B. cenocepacia</i> [# pt w. CF] (# new cases)	Isolates ID'd as <i>B. multivorans</i> [# pt. w. CF] (# new cases)	Other Isolates ID'ds [#pt w.CF] (# new cases)
AB	Calgary Adult	7	5 [5](3)	-	2 [2] (1)	3 x <i>B. gladioli</i> [2] (2) 2 x <i>Herbaspirillum</i> spp.[1] (0)
	Calgary Children's	1	1[1] (1)	-	1 [1] (1)	
	Edmonton	10	8 [7] (5)	2 [1] (1 non-CF)	3 [2] (1)	2 x <i>B. gladioli</i> [1] (0) 1 x <i>Pandoraea</i> spp. [1] (1) 2 x <i>Herbaspirillum</i> spp. [2] (2)
BC	BCCH	2	2 [1] (1)	1 [0] (1 non-CF)	1 [1] (0)	
	St Paul's	4	4 [4] (3)	-	-	1 x <i>B. cepacia</i> [1] (1) 3 x <i>Pandoraea</i> spp. [3] (2)
	Royal Jubilee	8	7 [7] (3)	2 [2] (1)	5 [4] (1)	1 x <i>Acidovorax</i> spp. [1] (1)
NL	Health Sciences Centre	2	2 [2] (2)	2 [2] (2)	-	-
NS	QE2	1	1 [1] (0)	-	-	1 x <i>B. contaminans</i> [1] (0)
ON	Hamilton Health Sciences	1	1 [1] (1)	-	1 [1] (1)	-
	Ottawa General	3	2 [2] (1)	2 [1] (1)	1 [1] (0)	-
QC	Chicoutimi	3	3 [3] (1)	-	2 [2] (1)	1 x <i>B. vietnamiensis</i> 1[1] (0)
	Centre mere-enfant soleil du chu de Qubec	1	1 [1] (1)	-	-	1 x <i>B. cepacia</i> 1
	Sainte-Justine	7	7 [2] (7)	1 [0] (1)	6 [2] (2 + 4 non-CF)	-
	Hotel Dieu	8	4 [4] (1)	-	6 [3] (0)	2 x <i>B. gladioli</i> [1] (1)
	IUCPQ	11	9 [9] (3)	4 [3] (1)	3 [3] (1)	3 x <i>B. gladioli</i> [2] (1) 1 x <i>B. diffusa</i> [1] (0)
	Montreal Chest	1	1 [1] (0)	-	1 [1] (0)	-
Total		70	58 [51] (33)	14 [9] (6 + 2 non-CF)	32 [23**] (9 + 4 non-CF)	24 [19] (12)

Notes: * new = patient for whom we have not previously received an isolate; in the case of some clinics where we have recently started receiving isolates these can represent established infections rather than new acquisitions. A new isolate may also represent a patient who has moved into a centre with an infection from another centre that had not sent us the initial isolates and therefore may also not be truly 'new'. ** we received samples from one person from both BCCH and Royal Jubilee, so the total number of unique people with *B. multivorans* is 22.

Isolates from the Vancouver clinics

As a matter of routine, we have been collecting all BCC isolates from the Vancouver clinics since 1981. We now save all isolates sent to us - which could be up to four times a year if a patient routinely attends clinic. During the period of this report we saved 61 isolates from 21 people with CF, 4 of which appear to be new acquisitions (all different species). Below is a summary of these isolates:



Report Summary

- This year continues the now well established trend of *Burkholderia multivorans* being the most commonly identified species in samples sent to us, accounting for just under 46% of isolates sent to us (both in terms of isolates and individual people with CF). *B. cenocepacia* was the second most identified species.
- We continue to see a limited number of samples for which the preliminary identification of *B. cepacia* complex turns out to be incorrect, as evidenced by some of the *Herbaspirillum spp.* and *Pandoraea spp.* identifications.
- New acquisitions of *B. cepacia* complex in people with CF are continuing, despite infection control. The source of these infections is unclear, but strain-typing indicates that in most cases this does not seem to be from other people with CF.
- This year, for the first time, we have conducted strain-typing by MLST on the isolates sent to us.
- There are still a number of clinics from whom we do not routinely receive isolates. We currently receive all the isolates for our local Vancouver clinics. Additionally, we have seen in recent years excellent submission from clinics in Alberta, Quebec and Nova Scotia.
- The CBCRRR is unlikely to be able to continue operating from its current location after Dr. Speert retires in 2017. Discussions are underway with CF Canada about the future of the CBCRRR and we are optimistic that it will transition to a new centre and leadership at some point in the next year. Clinics will be appraised of any changes when they are known, until then they can and should continue to use the service.

Did you know: the CBCRRR is available to help CF clinics and their microbiology labs at any time with regards to *Burkholderia spp.* bacteria.

To discuss your needs contact Dr. James Zlosnik: jzlosnik@cfri.ca

Summary of isolates submitted to CBCRRR

The CBCRRR was established by Dr. Speert in 1994 at the University of British Columbia and has been funded by Cystic Fibrosis Canada since 2001. We have been operating under the revised funding formula from CF Canada since April 1st 2015. In review of the proposal for this funding, some of the reviewers felt it was unlikely we would see all isolates from all CF people in Canada culturing BCC. We acknowledge that there are several clinics that do not send us isolates and we are trying to address that so that we either receive the isolates or a summary of the species identifications. It is also likely that because of the previous fee-for-service funding formula that we previously received only the first isolate for many patients. In order to look at the usage of the CBCRRR, we present below an analysis of the numbers of isolates and numbers of unique patients from whom we have seen isolates: i) since our provision of service free to clinics; ii) in the past five years iii) since CF Canada began funding us in 2001 and iv) since Dr. Speert first began this service in 1994.

	Since 1994		Since 2001		Since 2011		Since April 2015	
	Isolates	Patients	Isolates	Patients	Isolates	Patients	Isolates	Patients
Total* received	2211	940	1338	517	567	234	163	96
Average per year	98	42	89	34	102	42	123	72
Formal reports issued**	N/A	N/A	735	456	311	200	94	73
Average per year	N/A	N/A	49	30	56	36	71	55

* = Total number of isolates received by the CBCRRR, including the full collection from the Vancouver clinics. ** formal reports are issued upon receipt of a requisition form from a hospital or clinic and so this represents 'use' of the CBCRRR by clinics/hospitals. It should also be noted that prior to 2011 we received some isolates with codes attached, rather than patient names, therefore we may have seen some patients more than once.

Total data is also presented in the Table below broken down by province.

	Since 1994		Since 2001		Since 2011		Since April 2015	
	Isolates	Patients	Isolates	Patients	Isolates	Patients	Isolates	Patients
AB	167	99	111	55	73	36	27	20
BC	1059	196	748	146	300	72	82	34
MB/SK	50	48	28	26	3	3	0	0
ON	449	362	152	111	32	18	4	3
QC	270	148	220	124	126	77	43	32
NL/NB	24	13	8	7	5	5	5	5
NS	192	81	71	56	28	25	2	2

Notes: Patient numbers in columns in this table may not add up to the numbers in the table describing CBCCRRR use nationally on the previous page because some patients may move from Province to Province. BC numbers include all isolates submitted to us through the Vancouver clinics, which also include a limited number of isolates of other related species such as *Pandoraea*

These data highlight:

- We have seen more isolates from more patients on average per year since the new funding - this is based on the number of formal reports issued (55 patients per year vs 36 per year over the last five years and 30 per year of the course of CF Canada funding of the CBCCRRR). This metric is the most appropriate one to assess for pro-active use of the CBCCRRR because it requires a request from a clinic and so does not include the isolates we routinely collect from Vancouver clinics.
- While we have not received isolates from all people culturing *Burkholderia* in Canada, looking at the last 5 years we have still seen isolates from 200 unique patients. In total, we have identified isolates from over 450 CF patients since CF Canada began funding the CBCCRRR in 2001.
- The province level data confirms that we receive isolates from almost all provinces. Of the larger provinces by population, we have good submission levels from AB, BC and QC, while only some clinics in ON use our service.

Strain-typing

While species identification provides an important piece of information to clinicians, the history of *Burkholderia cepacia* complex infections in people with cystic fibrosis (with well documented cases of transmission) means it is important to go beyond a species level identification. Strain typing information about isolates of BCC that have been cultured from people with CF enables more detailed information to be provided to people with CF, their physicians and the community. Specifically strain level data will tell us:

1. **Whether or not the strain that has been cultured is the same as strain(s) that have been cultured from other patients in the same centre or other centres across Canada.**
2. **When someone with CF re-cultures a BCC is it the same infecting strain as the previous culture?**

We have chosen to employ a combination two other methodologies for strain-typing: RAPD (one developed in our lab previously) and MLST. Importantly, these allow us to generate strain identifications, in the form of MLST ‘sequence-type’ #s, that are easy to understand by both physicians and any scientist in the world.

RAPD Typing

1. Uses a PCR primer to amplify parts of the bacterial genome
2. Produces a ‘fingerprint’ for the bacterial isolates
3. **Advantages:** fast and cheap. We can get a comparison in as little as 2-days. Good for comparison
4. **Disadvantages:** more difficult to use for a large number of isolates and between lab comparison due to problems inherent in gel-based techniques.

Example gel showing shared patterns:

Mahenthiralingam et al 1996

Multi-locus Sequence Typing (MLST)

1. Extract DNA
2. PCR and sequencing of house keeping genes and comparison of DNA sequence with a international database
3. **Advantages:** allows large scale comparisons and produces an easy to understand number - e.g. ST-32.
4. **Disadvantages:** cost - also does not provide information about the whole genome.

Housekeeping genes:

atpD
gltB
gyrB
recA
lepA
phaC
trpB

Sequence:

...CCTGA... ...GGCAT... ...GATTC... ...TTACG... ...CAGGT... ...AATCC... ...TAACG...

Query BCC PubMLST database (<http://pubmlst.org/bcc/>)

Assign allele type:

16 11 10 14 11 6 79

↓ ↓ ↓ ↓ ↓ ↓ ↓

ST-32

Produces a sequence type:

39 records returned (1 - 25 displayed). Click the hyperlinks for detailed information.

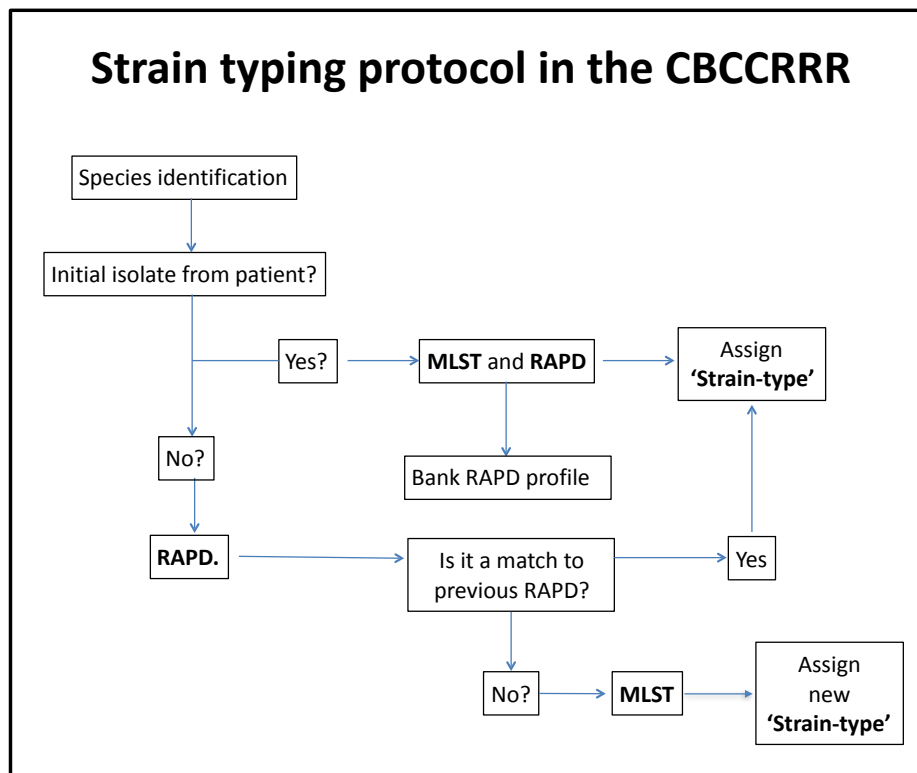
Page: 1 2 > Last

id	isolate	aliases	species	genomovar	LMG	country	source
38	POPR8		B. cenocepacia	IIIa		Mexico	ENV
132	C7929		B. cenocepacia	IIIa		Canada	CF
180	5-457		B. cenocepacia	IIIa		Czech Republic	
181	R-16597	M076	B. cenocepacia	IIIa		Belgium	CF
182	BCC1118	13479	B. cenocepacia	IIIa		UK	NON
450	74-X127		B. cenocepacia	IIIa		Czech	CF
451	150-1238		B. cenocepacia	IIIa		Czech	CF
452	7-460		B. cenocepacia	IIIa		Czech	CF
453	40-817		B. cenocepacia	IIIa		Czech	CF
454	9-483		B. cenocepacia	IIIa		Czech	CF
455	CEP0156		B. cenocepacia	IIIa		UK	CF

The goal in generating strain-typing information about isolates submitted to us is:

- i) to provide clinician's with easy to interpret information (in this case in the form of an MLST ST #) about the strains circulating in their centre
- ii) to provide both clinician's and the wider research community both in Canada and abroad with data relating to strain-level epidemiology.

For the past year, all isolates have been typed using the following scheme:

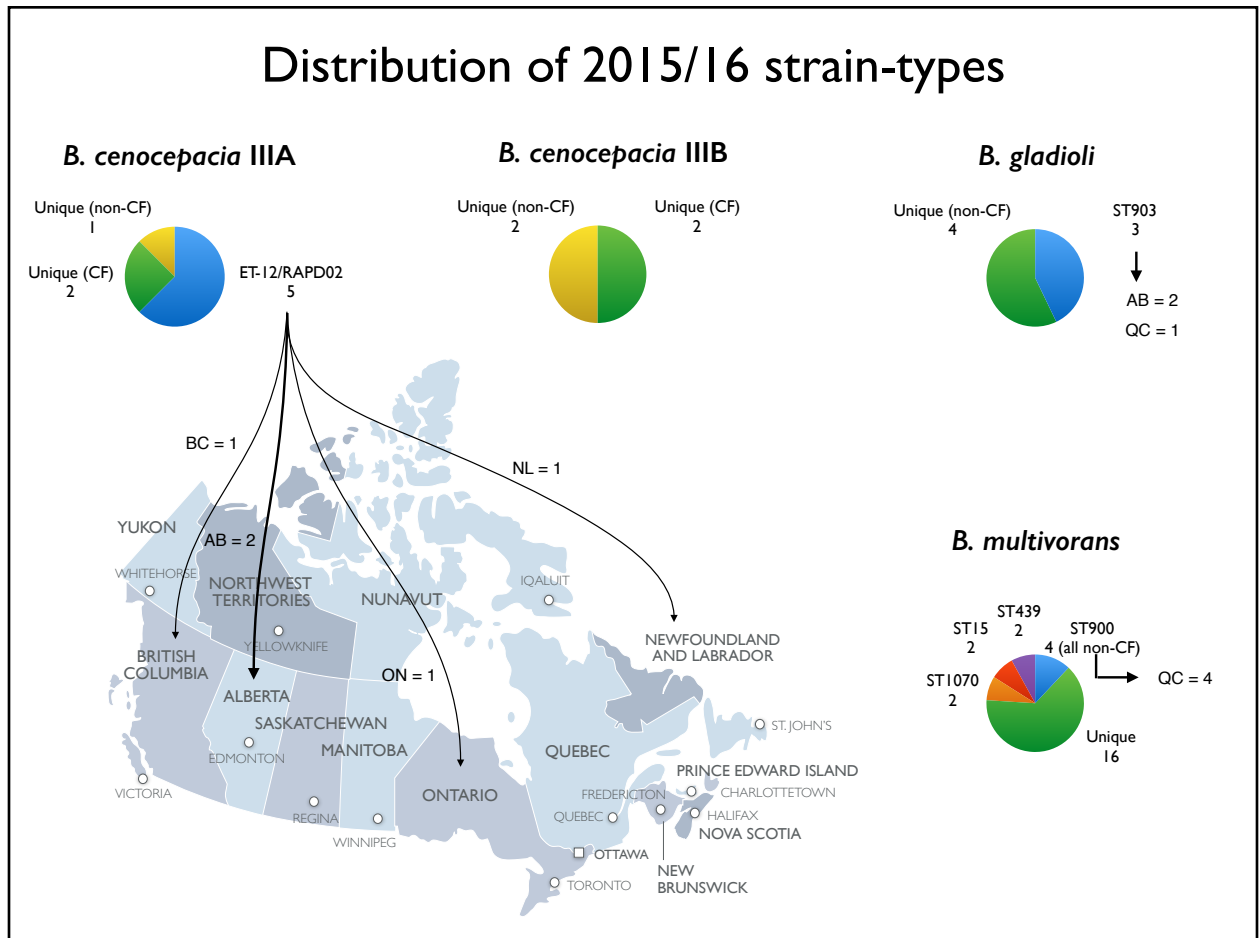


It is necessary to batch the isolates for MLST ST assignment. Currently this is done 3 times a year, so we have not currently been reporting this information with the species identification. As we build our databank then it will often be possible to provide this information on the report form for isolates where we have previous isolates from that particular person with CF.

Did you know: you can now request strain-level data about patients in your centre at any time by contacting us directly.

To discuss your needs contact Dr. James Zlosnik: jzlosnik@cfri.ca

Strain-typing data 2015/16



It is important to interpret these data with caution - they represent only a single year of submissions to the repository. Some will represent new infections and some represent pre-existing infections. As we collect more data, we will be able to form a clearer picture of the epidemiology of BCC in Canada. Of note from these data:

- The data in the above Figure represents people the bacteria were cultured from, rather than #s of isolates.
- The vast majority of *B. multivorans* cases are caused by isolates with unique MLST STs - this supports previous data we have published from Vancouver (and others have published in other centres) suggesting the advent of infection control has been very successful in preventing patient-to-patient spread of BCC.
- For *B. cenocepacia* we have classified isolates by their traditional RAPD type designation, where this is known. This showed a cluster of ET-12/RAPD02 isolates. However, these were spread between provinces and also are in fact several different MLST ST#s (ET-12 is known

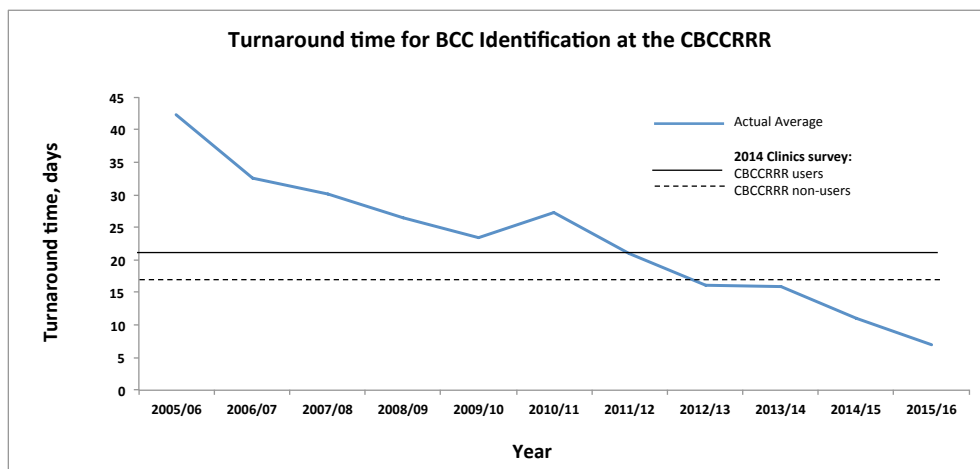
to comprise several different MLST subgroups) and so is not currently suggestive of any patient-to-patient spread.

- Our typing strategy did successfully detect an outbreak in a non-CF population - where 4 isolates of *B. multivorans* from 4 different patients were sent to us from a single centre. These were identical by RAPD and MLST and follow-up discussions with the hospital confirmed they represented a small outbreak.
- Currently there are not sufficient numbers of isolates and patients to draw any further conclusions about prevalence. We are currently expanding our analysis back to April 1st 2015 and so by the end of March 2017 we will have two-full years of data to report upon.
- Because MLST data is generated after we have sent the formal species identification to the clinics, we are still working on the best way to provide this information. However, clinics should note that they can request this at any time for one or all of their patients. They can also request lists of species identifications going back as far as we have records. They should not hesitate to contact us and we will be happy to provide any information we have at the time.

CBCRRR Update

The CBCRRR continues to be operated and managed on a day to day basis by Dr. James Zlosnik, with Dr. David Speert as the laboratory director. Ms Rebecca Hickman is the current CBCRRR technologist. Overall, our operations are running both effectively and efficiently. We are very pleased we have managed a continued improvement in our turnaround time, which averaged a record 7 days in the past year. Indeed in the last 5 years we have cut the time taken to identify and report back by more than two-thirds. We were delighted by this years' further improvement as it exceeded our expectations of what could realistically be achieved with our resources (we operate only on business days and not weekends). In addition, it puts our reporting time to well below that indicated as desirable by CF clinics during the 2014 survey on our services.

Overall, turnaround time has been dramatically improved since 2005. There are a number of reasons for this, primary among which is technological advancement brought about by our ability to obtain species identification through sequencing of the *recA* and *gyrB* MLST alleles. Prior to this it was necessary to conduct a range of biochemical and genetic tests which had to be pieced together to arrive at a final species identification. Additionally, we would like to acknowledge the vital role played by our technologists (Ms. Deborah Henry, Mr. Trevor Hird and Ms. Rebecca Hickman) during this period and we are very grateful for their hard work.



CBCRRR Publications in 2015/16

We have published or contributed to a number of peer-reviewed studies in the past year, including:

- A description of changes seen in the genome of isolate of *B. multivorans* from a chronic infection in cystic fibrosis:

Silva IN, Santos PM, Santos MR, **Zlosnik JEA**, Speert DP, Buskirk SW, Buger EL, Waters C, Cooper VS, Moreira LM. Long-term evolution of *Burkholderia multivorans* during a chronic cystic fibrosis infection reveals shifting forces of selection. *mSystems*. 1(3): e00029-16.

- A whole-genome analysis of three isolates (which demonstrate mucoid phenotype variation) from a chronic *B. cenocepacia* infection:

Miller R, Hird TJ, Tang P, **Zlosnik JEA**. Whole-genome sequencing of sequential clinical mucoid/nonmucoid isolates of *Burkholderia cenocepacia* from a cystic fibrosis lung infection. *PLoS One*. 24;10(11):e0143472.

- A study investigating tobramycin activity against clinical isolates of *B. cepacia* complex grown as biofilms (biofilm growth is thought to be the likely mode of growth in the CF lung)

Kennedy S, Beaudoin T, Yau YCW, Caraher E, **Zlosnik JEA**, Speert DP, LiPuma JJ, Tullis E, Waters V. Activity of tobramycin against cystic fibrosis isolates of *Burkholderia cepacia* complex grown as biofilms. *Antimicrob. Agents Chemother.* 60(1), 348-55.

Additionally, we contributed to a case report of *B. dolosa* infection in a person with cystic fibrosis in a Canadian centre:

Lam G, Salgado J, Kanji J, **Zlosnik JE**, Brown N, Leung W. An unusual case of *Burkholderia dolosa* infection in a cystic fibrosis patient but not his sister: A case report. *Clinical Microbiology Newsletter*. in-press.

We have also contributed to several further studies that either have been submitted or will be submitted for peer-review and publication in the near future. These include: the description by a Canadian centre of invasive *Ralstonia* species bacteria; whole-genome sequencing of a large collection of *Burkholderia cenocepacia* clinical isolates; isolation of *Burkholderia multivorans* from a paediatric liver transplant patient (see abstract below); a study examining the efficacy of novel antimicrobials against *Burkholderia*.

We have also been included as co-authors on the following abstracts that have been accepted for presentation at conferences.

Winsor GL, Dhillon BK, Bertalli C, **Zlosnik JEA**, Cardona ST, Brinkman F. The Burkholderia genome database: more genomes, more analyses, more plans. International Burkholderia cepacia Working Group, Columbus, Ohio. April 2016.

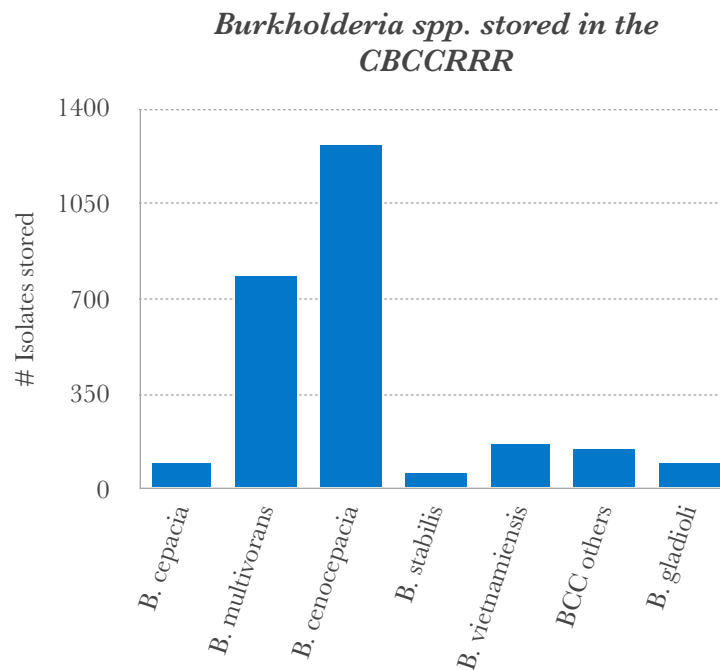
Ines SN, Santos PM, Santos MR, **Zlosnik JEA**, Speert DP, Buskirk SW, Bruger EL, Waters CM, Cooper VS, Moreira LM. Long-term evolution of *Burkholderia multivorans* during a chronic cystic fibrosis infection reveals shifting forces of selection. International Burkholderia cepacia Working Group, Columbus, Ohio. April 2016.

Ho SC, Nashid N, Otley A, Waters VJ, LiPuma JJ, **Zlosnik JEA**, Yau YCW, Binita KM. *Burkholderia multivorans* septicaemia in a pediatric liver transplant recipient. North American Society for Pediatric Gastroenterology, Hepatology and Nutrition, Washington DC, October 2015

Note that while we are pleased with our productivity, it is slightly below where we would normally expect it to be due to Dr. Zlosnik having been absent on parental leave during the first part of 2016.

The CBCRRR for Researchers

The CBCRRR is a resource for researchers as well as clinics and we welcome contact from researchers who wish to discuss their needs. All the isolates sent to us for identification are frozen and stored indefinitely and most are available to researchers de-linked from their clinical identifiers. Isolates are available either as part of a collaboration (to academic researchers - requiring minimal shipping charges) or on a cost recovery basis to both academic researchers and industry.



In addition to *Burkholderia*, researchers might like to note that we house a large collection of CF clinical isolates of other bacteria. This includes:

- More than 13,000 isolates of *Pseudomonas aeruginosa* from CF infections (including many sequential clonal isolates)
- Many other species of Gram-negative bacteria isolated from CF: *Achromobacter* spp., *Acinetobacter* spp., *Pandoraea* spp., *Ralstonia* spp., *Stenotrophomonas maltophilia* among others.

Did you know: the CBCRRR contains thousands of clinical samples of *Burkholderia* species bacteria as well as other CF pathogens.

To request isolates and discuss your needs contact Dr. James Zlosnik: jzlosnik@cfri.ca