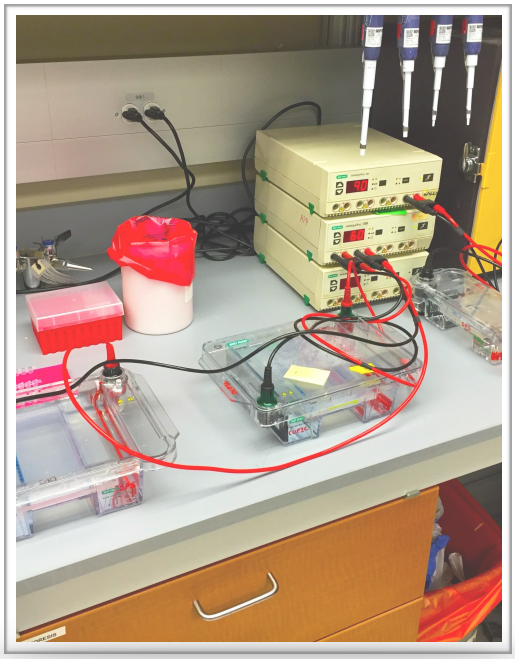
# Canadian *Burkholderia cepacia* complex research and referral repository



*2017 Annual Report: August 1st 2016 to July 31st 2017*

Cystic Fibrosis Canada

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

BC Children's Hospital Research Institute

# Summary

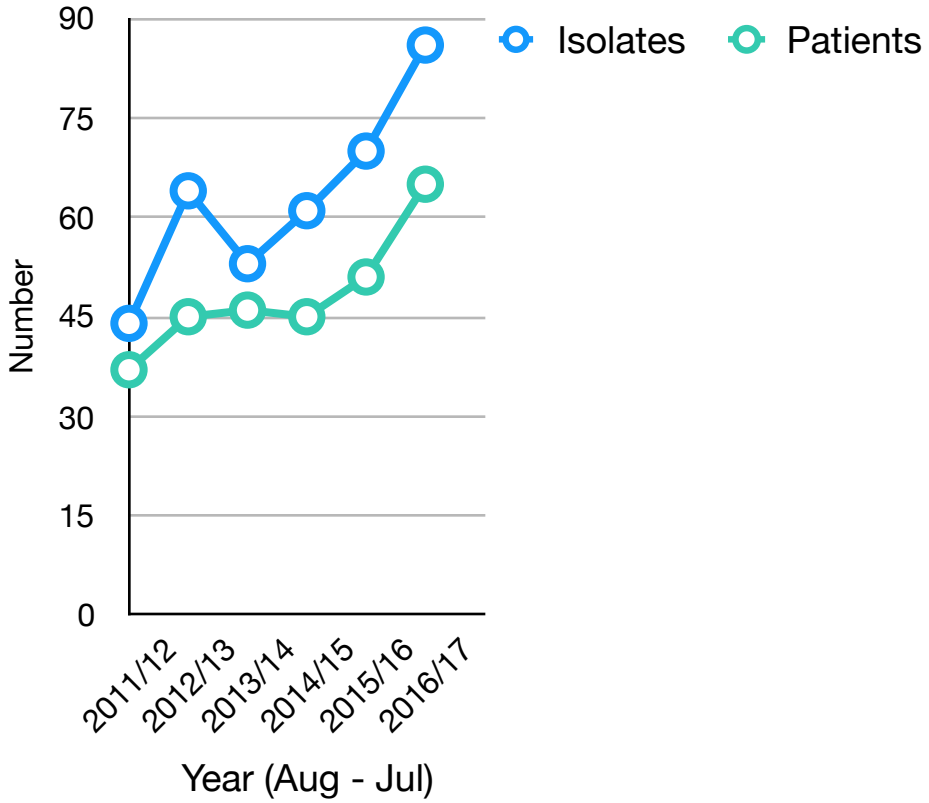
We are delighted to present the 2016/17 Canadian *Burkholderia cepacia* complex research and referral repository (CBCCRRR) annual report. Thanks to an enhanced grant received from Cystic Fibrosis Canada, as of 1st April 2015 we have been providing *Burkholderia* identifications **free of charge** to all Canadian CF clinics.

We are also delighted to report that the CBCCRRR secured renewed funding from CF Canada to permit operation from 2017-2020. Additionally, we are also happy to report that the CBCCRRR is continuing operation after the retirement of its founder and director Dr. David Speert. Day-to-day operations will continue to be managed by Dr. Zlosnik, who will also co-direct the repository. Dr. Zlosnik will be joined in leadership of the repository by Dr. Manish Sadarangani and Dr. Mark Chilvers. Dr. Sadarangani is an Assistant Professor in the Division of Infectious Diseases, Department of Pediatrics, UBC and is an expert in bacterial molecular genetics and vaccine development. Additionally, Dr. Sadarangani is director of the Vaccine Evaluation Centre at UBC. Dr. Chilvers is a Clinical Associate Professor in the Division of Respiratory Medicine, Department of Pediatrics and Director of the paediatric cystic fibrosis clinic at BC Children's Hospital and will help provide oversight and direction for the repository.

This year we have received 86 isolates from referring hospitals, a 23% increase from the previous years total of 70. Of these 81 isolates were from 65 CF patients. We also assisted hospitals with identification of BCC for 5 other samples from non-CF patients. For new infections, the mean age (where indicated on the requisition form) was 24.5 years, median was 20.0 years with a range of 5.6 to 56.5 years. Several trends continue from previous years:

- **We continue to see an increase in the number of isolates sent to us for identification.**
- ***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.**

## CBCCRRR Submissions since 2011




**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 CBCCRRR 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@bcchr.ca

# Isolate Summary 2016/17

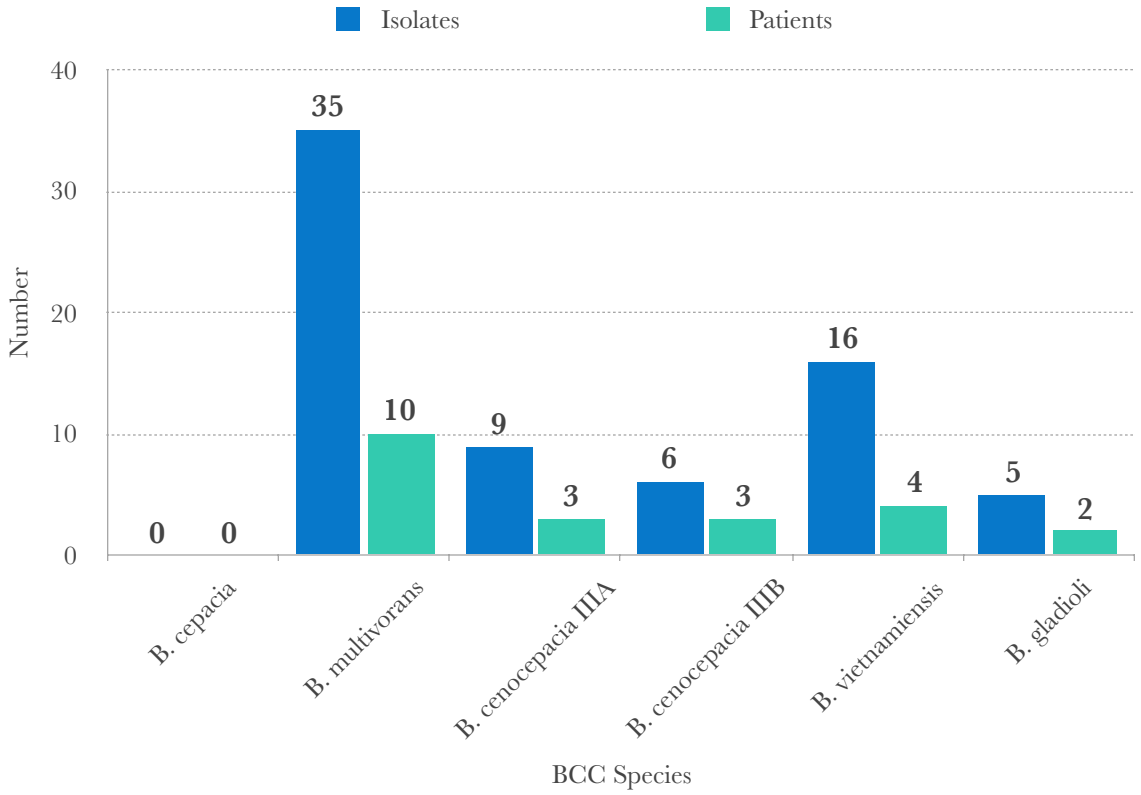
| Province | # samples | # patients [# w.CF] (# new w.CF) | Isolates ID'd as *B. cenocepacia* [# pt w. CF] (# new CF cases) | Isolates ID'd as *B. multivorans* [# pt. w. CF] (# new CF cases) | Other Isolates ID's [#pt w.CF] (# new CF cases) |
|---|---|---|---|---|---|
| **AB** | 17 | 14 [14] (4) | 3 [2] (0) | 9 [8] (3) | *B. gladioli*: 3 [2] (0)<br>*B. vietnamiensis:* 1 [1] (1)<br>*Pandoraea spp.* 1 [1] (0) |
| **BC** | 22 | 14 [14*] (4**) | 7 [4] (2) | 13 [10] (1) | *B. gladioli:* 1 [1] (1)<br>*Staphylococcus* spp. 1 [1] (-) |
| **NL** | 6 | 6 [5] (3) | 3 [3] (0) | 1 [1] (1) | *B. vietnamiensis* 1 [1] (1)<br>*Achromobacter dolens* 1 [1] (1) |
| **NS** | 2 | 2 [2] (1) | 2 [2] (1) | - | - |
| **ON** | 3 | 3 [3] (3) | - | 3 [3] (3) | - |
| **QC** | 36 | 30 [27] (7) | 8 [6] (3) | 18 [16] (3) | *B. cepacia:* 3 [1] (0)<br>*Burkholderia spp.:* 1 [1] (0)<br>*B. vietnamiensis:* 4 [3] (0)<br>*B. diffusa:* 1 [1] (0)<br>*Delftia spp.* 1 [1] (1) |
| **Total** | 86 | 69 [65] (22) | 23 [17] (6) | 44 [38] (11) | 19 [15] (5) |

* = 2 patients had more than one species

* * = 1 patient had more than one species

# 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 78 isolates (6 of which were accompanied with requisitions and included in the data on page 4.) from 22 people, 3 of which appear to be new acquisitions (all different species: one each of *B. cenocepacia* IIIA, *B. multivorans* and *B. gladioli*). Below is a summary of these isolates (note, 7 are awaiting identification):




**Did you know: the CBCCRRR 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@bcchr.ca

# Report Discussion

- This year continues the now well established trend of *Burkholderia multivorans* being the most commonly identified species in samples sent to us, accounting for approximately 50% of isolates sent to us (both in terms of isolates and individual people with CF).

- *B. cenocepacia* was the second most identified species - and continues to be sent to us from patients we have not seen previously seen (6 new patients with *B. cenocepacia* vs. 11 new patients with *B. multivorans*), possibly indicating new infections. For the most part this does not appear to be patient to patient transmission, based on different recA/gyrB allele types, however we have noted a novel MLST clone from at least four patients (ST-1074) across three geographically separated locations and we will continue to monitor new isolates for this. Irrespective of infection control, while *B. multivorans* is the dominant BCC species in CF, *B. cenocepacia* continues to cause a significant number of infections.

- 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 identifications such as *Achromobacter*, *Delftia* etc. With the exception of the *S. aureus* (which was presumably a incidental contaminant) the organisms that did not ID as BCC are ones that can be confused with BCC due to similar biochemical characteristics. We are not clear how these were originally identified or whether MALDI-TOF (a newer technology employed in microbiology laboratories) was involved in the original identification.

- 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.

- 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.

- Last year we reported that we may be unable to continue operations after David Speert's retirement in June 2017. Thanks to the support of Cystic Fibrosis Canada, we will now be able to continue providing this facility to the CF community for at least the 3 years beginning in April 2017 under new leadership (see Repository update p. 8).

# 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 of two other methodologies for strain-typing: A DNA fingerprinting method known as RAPD (developed in our lab previously) and DNA sequence method known as MLST. MLST offers the advantage that it allows 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.

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.**

It is necessary to batch the isolates for MLST ST assignment. Currently this is done 2 or 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. We aim to begin doing this within the next year.

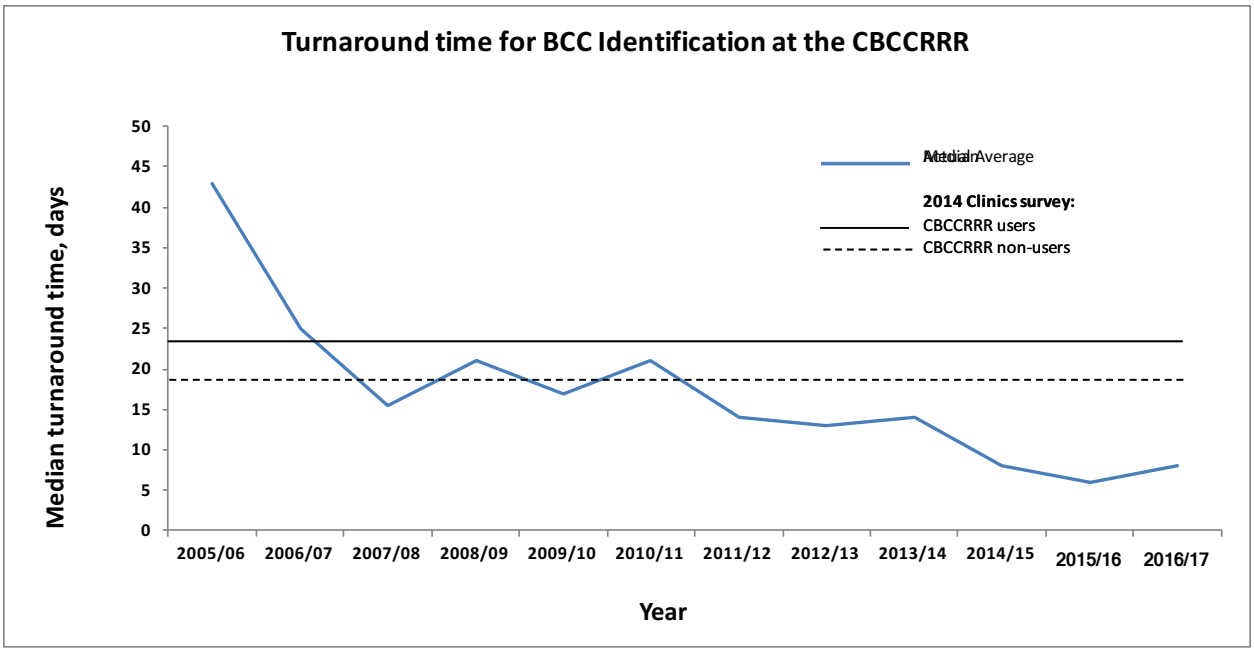
We expect to be presenting a much fuller analysis of strain types in Canada in the next annual report - as well we will be submitting that information for publication

# CBCCRRR Update

The CBCCRRR continues to be operated and managed on a day to day basis by Dr. James Zlosnik. During this year, Ms. Rebecca Hickman left for another position at the BC Centre for Disease Control and a new technologist, Ms. Adriana Cabrera, joined the laboratory.

Turn around times are slightly increased from our previous record level in 2015/16, however at a median of 8 days they were still the second lowest since we started recording. With staff turnover we expect that there may be some increase in the next year, however we are still at less than half the average requested time by clinics. Indeed in the last 6 years we have cut the time taken to identify and report back by more than two-thirds.

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.



# CBCCRRR Publications in 2016/17

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

**The description of a novel Burkholderia species:**

- Vandamme P, Peeters C, De Smet B, Price EP, Sarovich DS, **Henry DA, Hird TJ, Zlosnik JEA**, Mayo M, Warner J, Baker A, Currie BJ and Carlier, A. Comparative genomics of *Burkholderia singularis* sp. nov., a low G+C content, free-living bacterium that defies taxonomic dissection of the genus *Burkholderia*. *Frontiers in Microbiology*

**A large-scale whole genome study of *Burkholderia cenocepacia* from people with CF**

- Lee AH, Flibotte S, Sinha S, Paiero A, Ehlrich R, Balashov S, Ehilrich GD, **Zlosnik JEA**, Mell J, Nislow C. Phenotypic diversity and genotypic flexibility of *Burkholderia cenocepacia* during long-term chronic infection of cystic fibrosis lungs. *Genome Research*, Apr; 27(4); 650-662.

**A description of poor outcome by infections of *Ralstonia mannitolytica*, at least some of which were clonal suggesting possible patient-to-patient transmission.**

- Bilodeau L, Lavoie A, Carricart M, Tremblay F, **Zlosnik JEA**, Berthaumie Y. *Ralstonia mannitolilytica* in cystic fibrosis: a new predictor of worse outcomes. *Respiratory Medicine Case Reports.* 20; 48-50

**The above study underscores the role the CBCCRRR can play in helping CF clinics with outbreaks of organisms similar to BCC bacteria.**

**We are able to help clinics who may be worried about patient-to-patient transmission of any Gram-negative organism. By sending us isolates that may be of concern, we can store them for future analysis, ensuring they are not lost.**

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

A number of further studies are currently underway and we expect will lead to publication.

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

Lafayette S, Casgrain PA, Zealy C, Houle D, Radzioch D, Perinet S, Smalley N, Dandekar A, Heirali A, Parkins M, **Zlosnik J**, Rousseau S, Nguyen D. Protease deficient *Pseudomonas aeruginosa* isolates are common Cystic Fibrosis infections and cause exaggerated pulmonary inflammation. Submitted to North American CF Conference, Indianapolis, US November 2017.

Middleton MA, Layeghifard M, Klingel M, Stanojevic S, Yau Y, **Zlosnik JEA**, Speert DP, Coriati A, Ratjen F, Tullis E, Stephenson A, Wilcox P, Freitag A, Chilvers M, McKinney M, Lavoie A, Wang PW, Guttman D, Waters V. Distribution and clinical impact of clonal *Pseudomonas aeruginosa* infection in the Canadian cystic fibrosis population. Submitted to North American CF Conference, Indianapolis, US November 2017.

Alyazidi R, Hickman B, Li LM, Osowicki J, Goldfarb D, Tilley P, Speert DP, **Zlosnik JEA**. Evaluation of the Performance of Randomly Amplified Polymorphic DNA (RAPD) Method for Microbiological Typing in an Invasive *Serratia marcescens* NICU Outbreak. International Meeting on Emerging Disease and Surveillance, Vienna, Austria. 2017.

# FUNDING OF THE CBCCRRR HAS ALSO PERMITTED THE FOLLOWING:

The funds provided by CF Canada for the operation of the CBCCRRR have permitted other synergies for CF. In the year 2016/17 these have included:

**Grant applications:**

Operating grant applications to Genome BC and Cystic Fibrosis Canada were made during 2016/17. Both were for research studies which propose implementing cutting-edge technologies into clinical care for people with CF. We expect to also submit an operating grant funding proposal to the BC Lung Association focussed on Burkholderia cepacia complex infections in CF.

**Symposium**:

An international symposium, organized by Dr. Zlosnik, was organized and held in Vancouver in honour of the research career of Dr. David Speert - the founder of the CBCCRRR. This symposium contained a full session on CF microbiology and was attended by a range of experts in CF microbiology. The talks from the sessions were recorded and are available online at: http://mediasite.phsa.ca/Mediasite/Showcase/DPS

**Studentship**

Through his involvement in the CBCCRRR, Dr. Chilvers has been able to fund a co-op student for two terms in the repository (approximately $5,000 per term). This studentship will allow us to leverage the potential in the culture collections to generate publishable data which will be of use to both the research and clinical communities.

**Collaborations**

Dr. Zlosnik has been able to continue to establish new research collaborations and build upon existing ones that will leverage the repository for the benefit of the CF community.

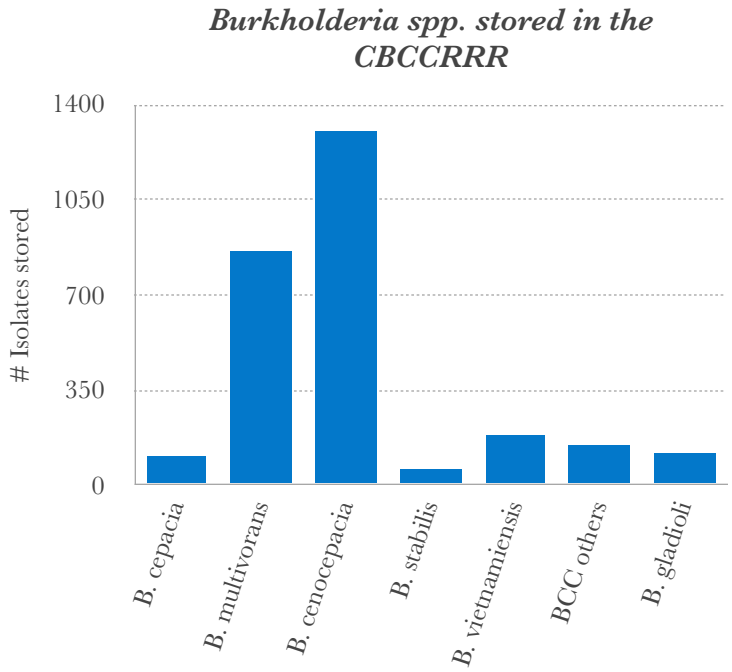
Through working with co-director Dr. Sadarangani, Dr. Zlosnik is building expertise in bacterial whole-genome sequencing. Through working with Dr. Chilvers, closer

interactions between the BC Children's Hospital CF clinic and researchers in the BC Children's Hospital Research Institute are being forged.

Externally, through grant applications Dr. Zlosnik has continued his collaboration with Dr. Corey Nislow (co-director of the genome sequencing facility at UBC) and worked with Professor Fiona Brinkman at Simon Fraser University to propose projects that will apply cutting-edge genomics and bioinformatics to dealing with BCC infections in CF (this project would also bring in Dr. Esh Mahenthiralingam from Cardiff University). Additionally, Dr. Zlosnik continues to collaborate with a range of other investigators across Canada and the world to contribute to our understanding of BCC infections in CF.

# The CBCCRRR for Researchers

The CBCCRRR 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 CBCCRRR 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@bcchr.ca