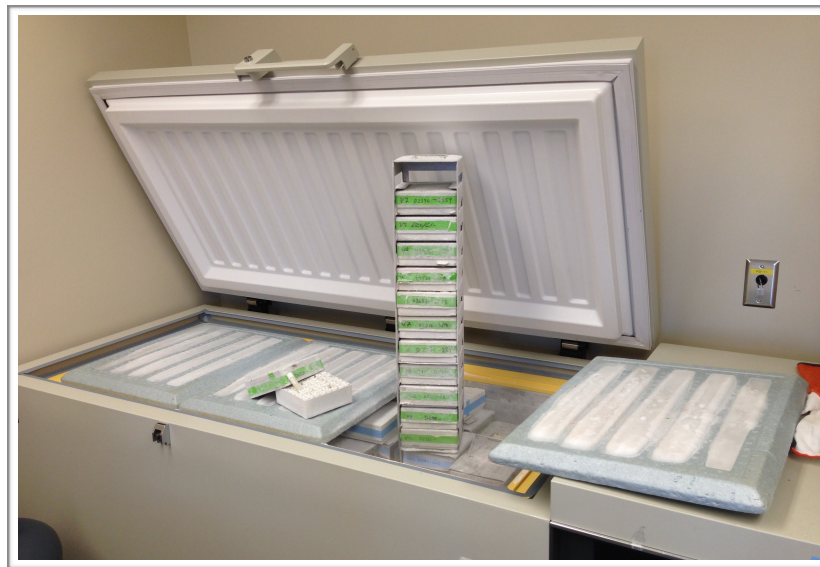


Canadian *Burkholderia cepacia* complex research and referral repository



2019 Annual Report: August 1st 2018 to July 31st 2019



a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

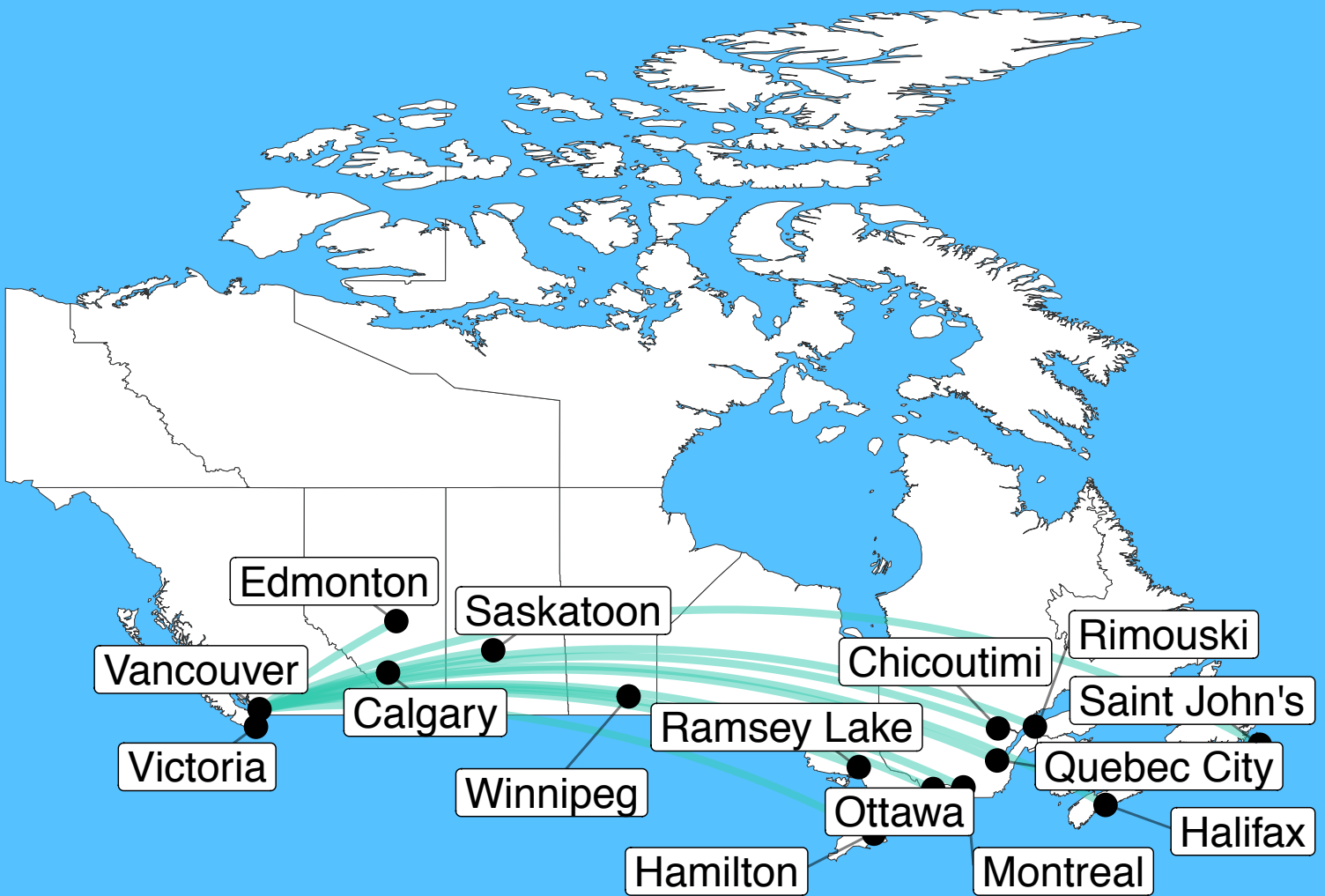


2018/19 Activity

137 Isolates identified

= 44 % increase on previous year

19 ^{served} Hospitals
across
8 Provinces



90 CF Patients

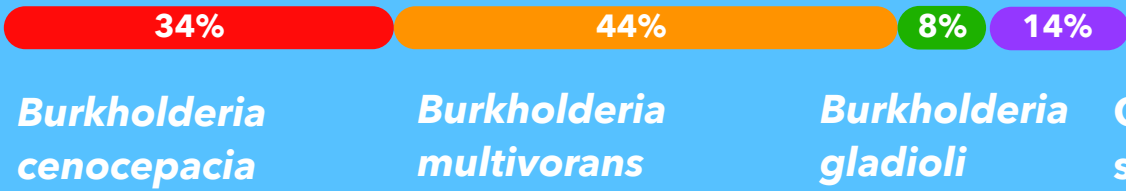


Table of Contents

Table of Contents	2
Report Summary	3
Isolate Summary 2018/19	4
Report Discussion	5
Strain-typing of Burkholderia samples 2015-18	9
CBCRRR Operations Update	11
The CBCRRR for Researchers	14

Report Summary

We are delighted to present the 2018/19 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 137 isolates from referring hospitals, a 44% increase from the previous years total of 95. Moreover, we have seen year-on-year increases in numbers of isolates received every year since 2013/14, in that time annual isolate numbers submitted to the repository have more than **trebled** from 2011.

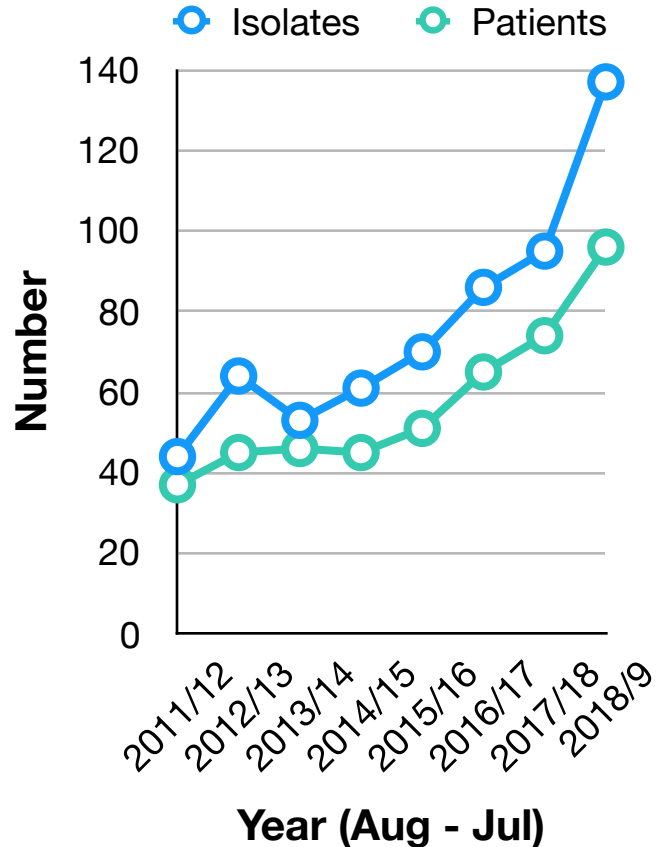
The 137 isolates were from 97 unique patients. Of the 137 isolates, 130 were from 90 people with CF. We also assisted hospitals with identification of 7 isolates from 7 non-CF patients.

For the new isolates from CF patients (those from patients not previously received by the CBCRRR, the median age was 22 years, with the youngest being 5 and the oldest being 46.

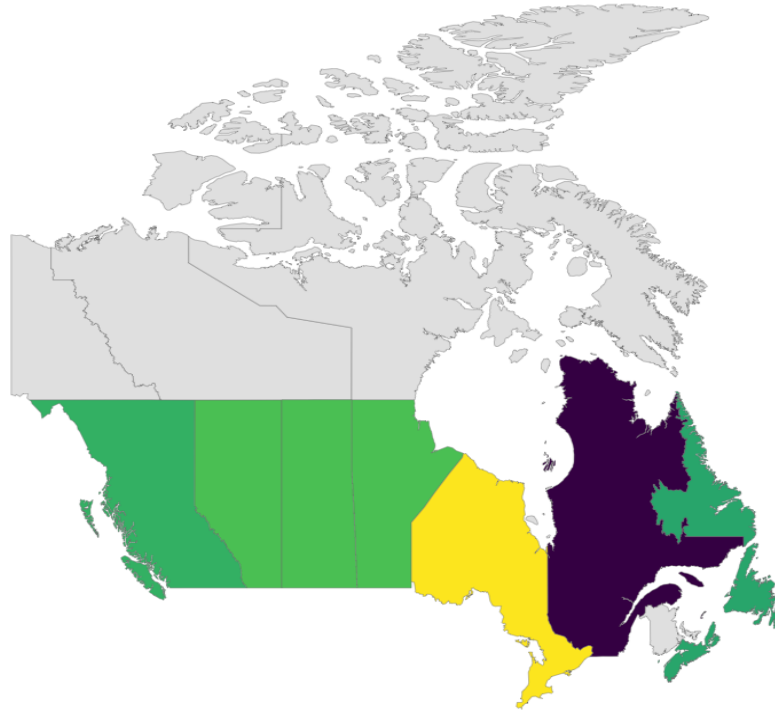
Annual samples:



44% over last year
94% over last renewal
209% over 2011



Isolate Summary 2018/19



Note, AB, MB and SK were merged as were NL and NS due to low numbers in one or more of those provinces.

Numbers of isolates submitted to the CBCRRR during 2018/19 by province

Province	# isolates	# patients [# w.CF] (# new w.CF)	Isolates ID'd as <i>B. cenocepacia</i> [# pt w. CF] (# new CF cases)	Isolates ID'd as <i>B. multivorans</i> [# pt. w. CF] (# new CF cases)	Other Isolates ID's [#pt w.CF] (# new CF cases)
AB & MB & SK	21	16[14](3)	6[4](0)	9[6](1)	<i>B. contaminans</i> 22 <i>B. gladioli</i> 2[2](0) <i>B. graminis</i> 11 <i>B. vietnamiensis</i> 10
BC	24	13[13](6)	14[8](6)	9[5](1)	<i>Pandoraea apista</i> 11
NL, NS	26	17[17](2)	23[14](1)	11	<i>B. dolosa</i> 1[1](0) <i>B. vietnamiensis</i> 1[1](0)
ON	8	6[6](3)	2[1](0)	4[3](2)	<i>B. ambifaria</i> 1[1](0) <i>B. cepacia</i> 11
QC	58	46[41](14)	7[4](3)	32[26](8)	Bcc other species 11 <i>B. anthina</i> 1[1](0) <i>B. cepacia</i> 10 <i>B. diffusa</i> 1[1](0) <i>B. fungorum</i> 10 <i>B. gladioli</i> 10[5](2) <i>B. plantarii</i> 10 <i>B. vietnamiensis</i> 3[3](0)
Total	137	97[90](28)	52 [31] (10)	55[40](13)	30(21)(8)

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 98 isolates from 28 people with CF. Included in these numbers are those listed on the table on p.4. We currently do not have complete information on the species designations for these as they are processed in batches (samples with requests for identification from the CF clinic are processed immediately). In total the Vancouver collection comprises over **1,100** isolates from **170** people with CF.

Report Discussion

This year we would like to make the following noteworthy observations:

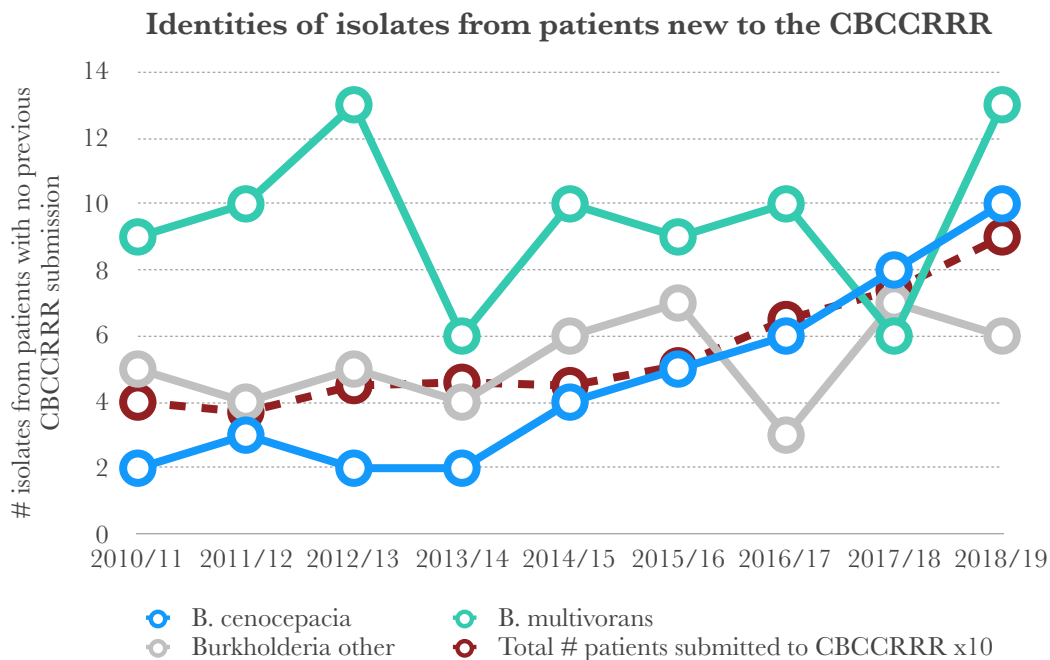
- The increasing numbers of isolates that we are receiving are very welcome and likely means that we are receiving isolates from the vast majority of CF centres with *Burkholderia* patients.
- ‘New’ isolates continue to be received, suggesting that infection by *Burkholderia* continues to be a risk in people with CF
 - Note in this context, a key finding in a study we have under revision at *Annals ATS* is that during the period 2000 - 2017 the incidence of *Burkholderia* infection in the CF population was unchanged, at approximately 6 new *Burkholderia* infections per 1000 people with CF. (note this finding was seen both in data derived from samples sent to us and in data deposited by the clinics in the Canadian CF Data Registry.
- *B. cenocepacia* and *B. multivorans* were the leading identifications in both the total number of isolates and in isolates that were new to us. This, again, underscores our previous observation that *B. cenocepacia* has not gone away in the CF population and still remains a leading cause of infections in people with CF. The well described epidemic potential of at least some strains of these species merits continued caution in regard of infection control.

- We identified an isolate of *Burkholderia graminis* from a patient with CF for the first time. It is likely this is the first such identification of *B. graminis* from CF in the world.
- We also identified one isolate of a potentially novel species of *Burkholderia cepacia* complex and sent this to the bacterial taxonomy laboratory in Belgium for further classification.
- This year we have again seen more isolates from more patients that at any time during the previous 8 years - we are grateful to the CF clinics and hospital microbiology laboratories for their submissions and we trust that the information we provide is useful.
- This year we saw only one isolate for which the identification was not a *Burkholderia spp.* - this was a *Pandoraea apista* and was sent to us as a presumptive *P. apista* for confirmation.
- The repository and the provision of identifications to CF clinics is functioning well despite staff turnover. We have a number of new initiatives that should improve the information CF physicians receive - notably we plan to change the report format to improve its interpretability and to provide comparative typing information on past isolates. We also now have a 'base' MLST type for each patient received since 2015 available from which to compare future isolates for both within patient strain-typing and broader clinic, province and nation-wide epidemiological surveillance.
- On both a provincial and national level, we see very little sharing of clones as defined by MLST.
- Clinics are encouraged to contact Dr. Zlosnik (jzlosnik@bcchr.ca) if they would like a summary of the strain-types identified from their clinics.

Species identification in 'new' isolates

Last year we reported that *Burkholderia cenocepacia* was, for the first time in many years, the most common cause new infections in CF. This year, the most common species responsible for new infections people with CF is *B. multivorans* ($n = 13$). Nevertheless, the number of new cases of *B. cenocepacia* continued to climb ($n = 10$).

A note of caution with this years data is merited because we received a large number of samples from a single centre, which had not sent us any isolates in some years. A number of these were new to the repository but the requisition form indicated they were repeat isolates from patients. If those samples marked 'repeat' by the hospital are removed then the numbers change to new *B. cenocepacia* ($n = 9$) and new *B. multivorans* ($n = 7$). See also note below for additional information.



Note in clarification: unfortunately we are unable to be certain when we receive isolates from patients for whom we have not had a prior isolate, whether or not the isolate is the first from the patient - and hence a new infection. Our requisition form asks for this information, however this is not always filled out correctly and so the data provided 'new' is defined as a patient 'new' to the CBCCRRR. In the above graph we have excluded from 'new' isolates of *B. cenocepacia* from a clinic who started to send us isolates for the first time in 2013/14 and 2014/15 and for whom we contacted the clinic to find their *B. cenocepacia* isolates were all from long term infections.

B. cenocepacia is subdivided into four groups (IIIA - D), of which IIIA has historically been a major cause for concern in CF, while IIIC and D do not typically cause infections in CF. Of the new infections this year, 3 were IIIA and 7 IIIB. Notably, all of these new acquisitions were of different strain-types from each other. We previously reported concern over a cluster of new

cases belonging to MLST strain-type 1074. None of the isolates received in this year belonged to that strain-type.

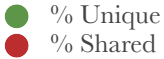






It is worthy of mention that we identified a cluster of four new infections of *B. cenocepacia* from a single centre. Following communication with the clinic and strain-typing it this does not appear to have been an issue of infection control within that centre and the only possible association that could have been thought of (by both ourselves and the clinic) was an unusual weather pattern around that time (high levels of air pollution caused by smoke from forest fires). Such events underscore the need for accurate strain-typing (in this case to rule out the source of infection as being something that the clinic might be able to control).

Strain-typing of *Burkholderia* samples 2015-18

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.

In this report we present a brief description of a survey of all the *Burkholderia* species isolated from people with CF and sent to us between January 2015 and December 2018. These data are **partially complete [note by next week I might have the new STs from the PubMLST database to complete them - I am including here what I have**

We obtained strain-level data for (normally) the first isolate sent to us for a given patient during that time - this was a total of 171 patients. We have applied MLST (multi-locus sequence typing) to obtain strain-level information about these isolates. MLST involves obtaining the DNA sequence of part of 7 genes, these sequences are then compared to an international database (PubMLST-BCC) to obtain strain-type numbers (STs). Isolates with the same ST may be related to each other, however strain-typing data should also be considered in an epidemiological context.

	Isolates	Unique patients	# Unique MLST strain-types	% Unique	 % Unique % Shared
Canada-wide	190	171	127	74.3	
Province					
British Columbia	57	50	46	92.0	
Maritimes	19	17	11	64.7	
Ontario	9	8	7	87.5	
Prairies	30	28	23	82.1	
Québec	75	68	62	91.2	

In the case of outbreaks - such as those seen in the past with *Burkholderia* - clusters of significant numbers of isolates with the same ST could indicate patient-to-patient transmission, particularly if they were in the same clinic. Outbreaks can also be caused by contaminated medical products or equipment. If there is no reason to suspect an outbreak then shared STs could indicate more common environmental clones.

Across Canada there were 9 MLSTs shared by more than 3 people:

ST	Species	# people	Provinces
15	<i>B. multivorans</i>	4	2
28	<i>B. cenocepacia</i> IIIA	7	3
234	<i>B. cenocepacia</i> IIIA	7	3
439	<i>B. multivorans</i>	4	3
620	<i>B. multivorans</i>	5	3
625	<i>B. multivorans</i>	4	3
900	<i>B. multivorans</i>	4	1
1071	<i>B. multivorans</i>	5	3
1074	<i>B. cenocepacia</i> IIIA	5	3

It is worth noting these data are preliminary and we will be completing a more thorough analysis shortly - including a national level analysis - which will be part of the manuscript we will submit for publication.

CBCRRR Operations Update

The CBCRRR continues to be operated and managed on a day to day basis by Dr. James Zlosnik with support from Drs. Sadarangani and Chilvers. The CBCRRR technologist until December 2018 was Ms. Adriana Cabrera, who left to pursue graduate studies. We are grateful to Adriana for all her support during the time she filled this role. We are very happy to report that Ms. Cabrera's position has been filled by Mr. Tony Harn who now assists Dr. Zlosnik.

Despite staff turnover - which necessitates retraining - this year the median turnaround time for samples - from receipt to report generation - was again 14 days. We anticipate that this will be reduced further over the next year. As always, if clinics have urgent requests they are welcome to contact Dr. Zlosnik who ensure that their samples have priority at times of high sample processing.

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, Ms. Rebecca Hickman and Adriana Cabrera) during this period and we are very grateful for their hard work.

CBCRRR Publications in 2018/19

The follow manuscript is currently under revision at Annals ATS:

Zlosnik JEA, Henry DA, Hird TJ, Hickman BL, Cabrera A, Campbell M, Laino G, Chilvers MA, Sadarangani M. Epidemiology of Burkholderia infection infections in people with cystic fibrosis in Canada between 2000 and 2017. Manuscript under revision at Annals ATS.

Manuscripts submitted:

Saferali A; Tang AC; Strug LJ; Quon BS; **Zlosnik JEA**; Sandford AJ; Turvey SE. Immunomodulatory function of the cystic fibrosis modifier gene BPIFA1. Submitted to PLoS One.

This study is a previous collaboration by JZ.

Manuscripts under preparation:

Wong K, Dhaliwal S, Srigley J, Champagne S, Romney M, Tilley P, **Sadarangani M**, **Zlosnik J**, **Chilvers M**. MALDI-TOF for the accurate hospital-based identification of *Burkholderia cepacia* complex

Behroozian S, **Zlosnik JEA**, Davies J. Antibacterial activity of Kisameet Clary against *Burkholderia cepacia* complex and other bacterial pathogens isolated from patients with cystic fibrosis.

This study is a previous collaboration by JZ.

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. In November this year, Dr. Chilvers will be hosting a research day at BC Children's Hospital Research Institute, which will bring together the CF research expertise in British Columbia. Dr. Zlosnik has been able to contribute to this by organizing for external speakers and he will chair a sessions as well as deliver a presentation of the CBCRRR's work.

Externally, through grant applications, Dr. Zlosnik has continued his collaboration with Dr. Corey Nislow (co-director of the genome sequencing facility at UBC) and 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. Active collaborations, at various stages of completion, include:

- Professor Peter Vandamme (University of Ghent, Belgium) who is describing novel *Burkholderia* species
- Dr. Eric Deziel who is looking at bacterial communication.
- Dr. Jon Dennis - bacteriophage therapy against *Burkholderia*
- Professor Julian Davies and Dr. Shekoo Behroozian: novel antimicrobials against *Burkholderia*.
- Dr. Joseph Mougous: toxins from *Burkholderia*.
- Dr. Christopher Miller: inhaled nitric oxide activity against *Burkholderia*.
- Dr. Leonilde Moreira: Whole genome sequencing of *Burkholderia multivorans*

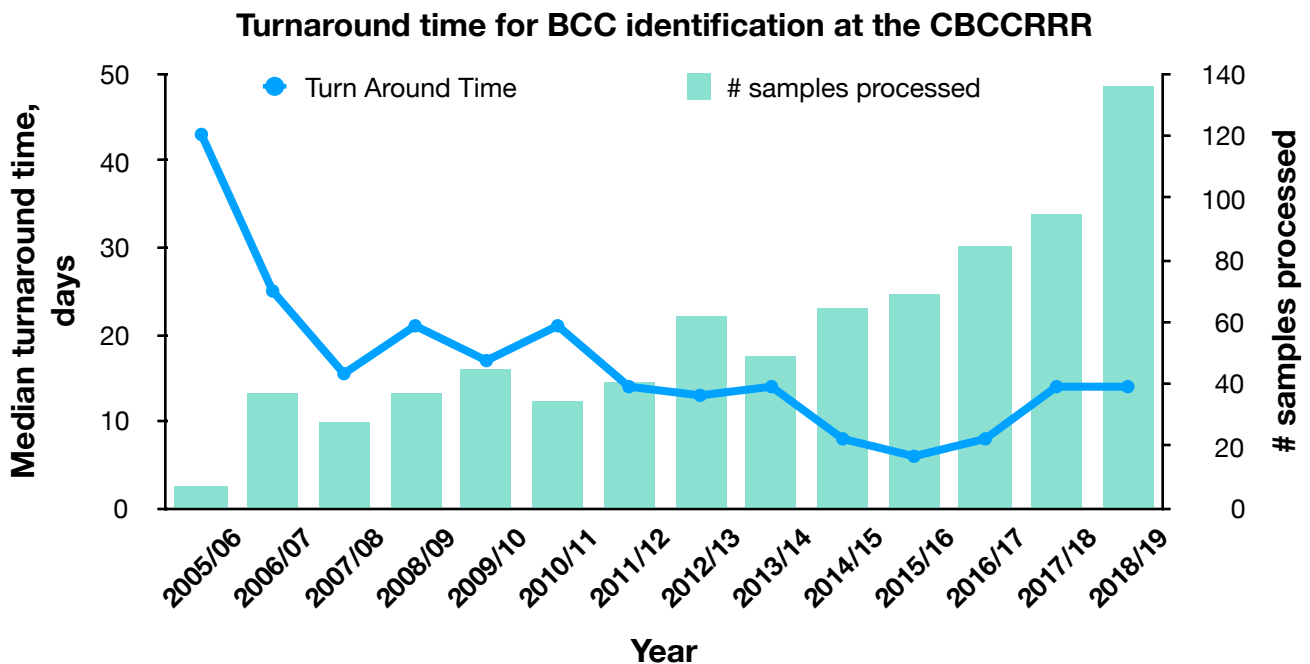
Provision of isolates to researchers

During the period of this report we provided a small number of isolates to other researchers. It may wish to be noted that immediately after the end period of this report we had requests for over 200 isolates from our collaborators.

As detailed throughout this report, we have many clinical isolates available and researchers are encouraged to contact us for further details if they wish to access any of these. We continue to offer to provide isolates on a cost-recovery basis to researchers. We have different rates available for researchers wishing to access these, depending on whether or not the researcher wishes to participate in a collaboration or would prefer to obtain isolates from us as a service. We have a separate rate for ‘for-profit’ organizations. We will be publishing this information to our website in the near future.

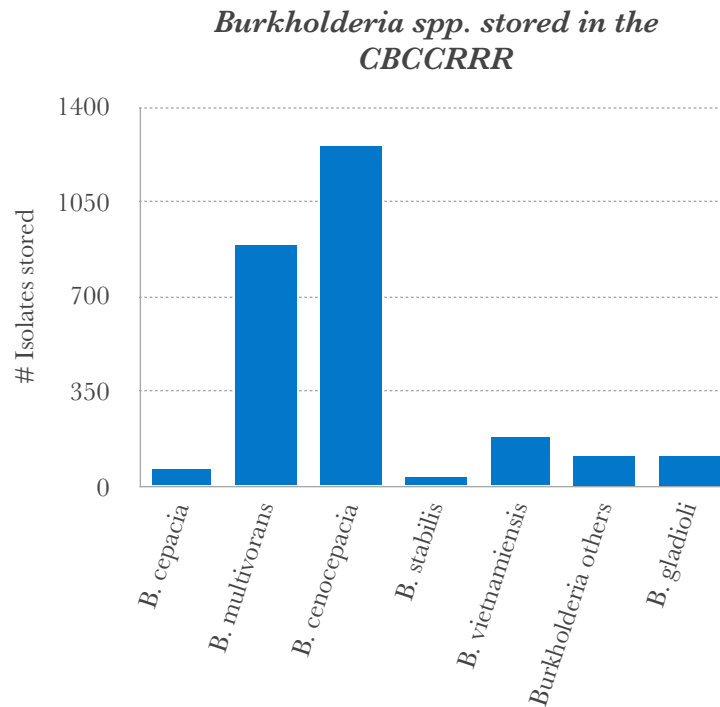
Provision of isolates and additional identification services to clinical microbiology laboratories.

This year, we also assisted one clinical microbiology laboratory with provision of 10 isolates for validation of their MALDI-TOF machines.



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@bcchr.ca