May 2024

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

PROVINCIAL LABORATORY MEDICINE SERVICES
Provincial Health Services Authority
BCCDC Public Health Lab

LABORATORY TRENDS

A Report from the BCCDC Public Health Laboratory

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Sequencing for identification, resistance characterization and genotyping of mycobacteria

Since summer of 2023, the BCCDC Public Health Laboratory (PHL) Mycobacteriology/TB Laboratory has been whole genome sequencing (WGS) *Mycobacterium* spp. organisms to provide information on genotyping, drug resistance markers, and identification of TB complex subspecies. This has replaced the previous mycobacterial interspersed repetitive units-variable-number tandem repeats (MIRU-VNTR) method. The following changes are to be expected:

Antimicrobial resistance

One cultured *M. tuberculosis* complex organism per patient per anatomic site will have genotypic prediction for antimicrobial resistance reported. Testing will be repeated on a new isolate if the patient remains culture-positive 3 months post-diagnosis. Genotypic predictions will be available for a subset of antimicrobial agents which have been validated to have high sensitivity and provide a high positive predictive value.

Phenotypic susceptibility testing remains the gold standard for M. tuberculosis *and will continue to be performed on all cultured isolates. Depending on the rate of growth of* M. tuberculosis *isolates, genotypic predictions may be made available to clinicians in advance of phenotypic susceptibility results.*

Identification

Every cultured *M. tuberculosis* complex organism will have identification provided to sub-species level. Previously this information was only provided for extra-pulmonary TB cases. Appearance of novel sub-species types among pulmonary cases at this time would be reflective of a change in laboratory practice, rather than TB epidemiology.

Relatedness

Every cultured *M. tuberculosis* complex organism will be compared via WGS for relatedness against other isolates in BC, both currently and historically circulating. Public Health practitioners will be provided with cluster reports of any identified clusters of related organisms to prospectively assist with contact tracing and follow up. Any healthcare practitioner can request a report on relatedness of an organism of interest by submitting the **Cluster Investigation form** to the laboratory.

In fall of 2023, BCCDC PHL TB Laboratory has introduced a next-generation sequencing (NGS)–based amplicon sequencing to provide both identification of non-tuberculous mycobacteria (NTMs), prediction of inducible macrolide resistance for *M. abscessus* complex members, as well as direct-from-specimen genotypic resistance predictions for a number of antimicrobial drugs on specimens with sufficient microbial load. Amplicon NGS resistance testing is done for the following drugs: isoniazid, rifampin, pyrazinamide, fluoroquinolones. As a result of this development, you **can request***:

1. Direct-from-specimen genotypic resistance prediction testing on samples that are AFB smear-positive, have been identified as TB-positive by PCR and come from patients with risk factors for drug-resistant TB infection.

2. Direct-from-specimen NTM identification on samples that are AFB smear-positive, have failed to grow in culture or have clinical indications for rapid identification testing.

*Testing needs to be requested by contacting the BCCDC PHL medical microbiologist on call (604-661-7033; BCCDC_MicroOncall@bccdc.ca). Testing is available twice per week and takes 2-3 business days to complete once the sample is at the BCCDC PHL TB Laboratory.

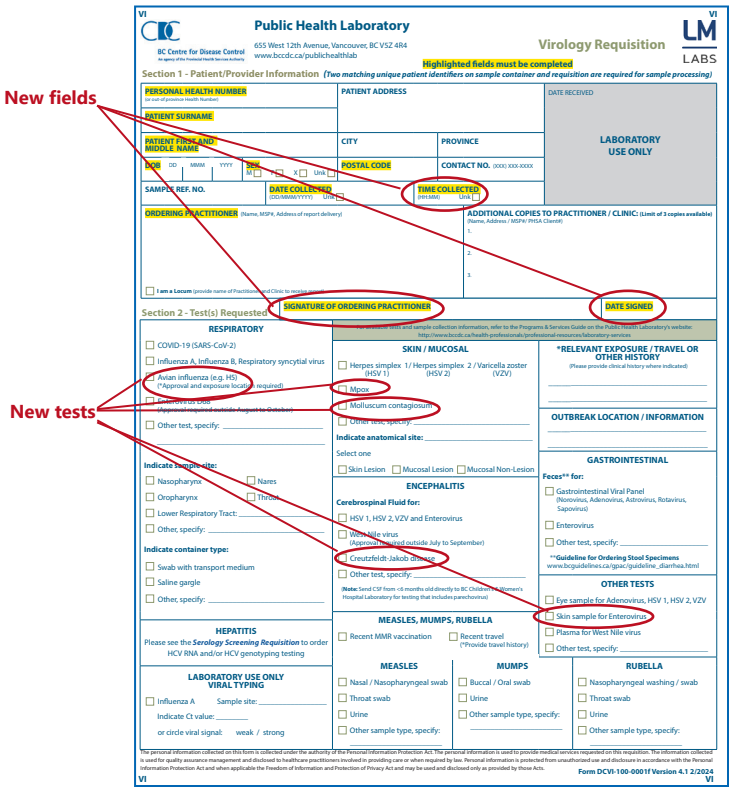
Expect to see inducible macrolide resistance genotypic prediction reported for *M. abscessus* complex organisms that have been requested to have phenotypic susceptibility testing performed. Phenotypic susceptibility testing continues to be performed at the National Microbiology Laboratory (NML) and susceptibility testing requests need to be submitted as usual, via faxing to BCCDC PHL TB Laboratory the **NML request forms**.

New Virology requisition

An updated Virology requisition is now in circulation and available for download.

Section 1 – Patient/Provider Information has been reorganized to improve the flow for data entry. New fields for patient telephone number and practitioner signature and date (as per Ministry of Health mandate) have been included. Mandatory fields have also been highlighted.

Under **Section 2 – Test(s) Requested**, new options include Avian influenza, Mpox, Molluscum contagiosum, Creutzfeldt-jakob disease and Skin sample for Enterovirus. The organization of tests are also improved under general headings.



Tracking SARS-CoV-2 immunity in antenatal samples*

Citlali Marquez, Sarah Mansour, Janice Andrade, Elisabeth McClymont, Agatha Jassem, Deborah Money, Inna Sekirov

The COVID-19 antenatal serosurveillance project is a collaboration between UBC researchers working at the Women's Health Research Institute and the BCCDC PHL to better understand the SARS-CoV-2 serostatus of the BC population. A representative sample set of residual clinical samples collected for first trimester screening is tested for antibodies against SARS-CoV-2. Antenatal samples are ideal given that over 90% of pregnant individuals in BC are tested during the first trimester. This ongoing project has collected between 100-300 samples weekly since November 2022.

The data collected is used as a proxy to the BC population with the objective of tracking the progression of SARS-CoV-2 immunity due to infection and vaccination within the province. For this, we use a multiplex electrochemiluminescent assay that can detect multiple SARS-CoV-2 antigens and that can help us differentiate between:

- Individuals that are seropositive against nucleocapsid (Np) + spike (S) and/or RBD and that are likely to have gone through infection, with or without vaccination.
- Individuals who are seropositive against S and RBD, but not Np and are likely to be vaccinated and uninfected; other possible scenarios are vaccination with breakthrough infection that did not elicit an anti-Np response or a remote infection with anti-Np response that has waned.
- Individuals seropositive for ≤ 1SARS-CoV-2 targets, who are likely to be unexposed.

Results so far have shown a quick rise of infections during the first months of 2022 when the SARS-CoV-2 Omicron variant surged, followed by a stabilization period that lasted until August 2023 when a new uptick on infections was detected, coinciding with the 2023-2024 respiratory season (Figure 1). A year-to-year comparison of S and Np antibody levels has shown that although there is some decline in the S antibody concentration compared to 2022, these are still well above our reactivity cut-offs and reveal sustained immunity. Additionally, we see a progressive increase in Np antibodies, suggesting an increase of infections over time (Figure 2). Overall, the use of antenatal samples has shown to be a fitting mechanism to better understand SARS-CoV-2 immunity. The continued tracking of the immunity against SARS-CoV-2 in BC can provide an important insight into variations on infection rates and vaccine effectiveness.

Figure 1. BC antenatal samples tested for IgG spike/RBD (S) and nucleocapsid (Np) proteins to determine natural infection and vaccination status. Data represents prenatal samples collected from individuals residing in Vancouver Coastal, Fraser, Interior, Northern and Island Health Authorities between November 2021 and March 2024. Black line tracks the proportion of S+Np+ individuals per month.

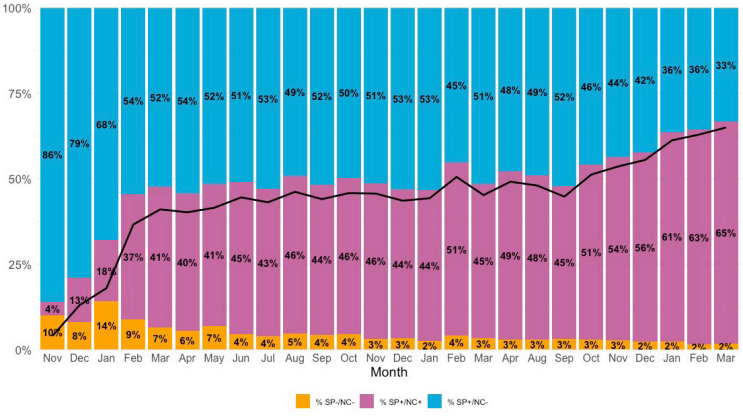
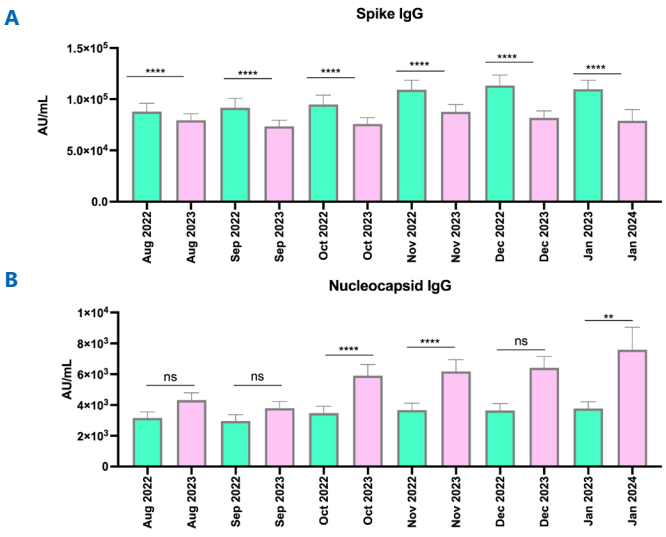


Figure 2. Year to year antibody concentration comparison. A-B) Anti-S and anti-Np concentration levels between 2022-2023/2024. Statistical significance was determined using a two-tailed unpaired t-test (** p ≤ 0.01; *** p ≤ 0.001; ****, p < 0.0001 , ns = not significant).



*Prepared by Citlali Marquez, Research Projects Leader

A multiplex qRT-PCR assay for detection of Influenza A and H5 subtype targeting new SNPs present in high pathogenicity avian influenza Canadian 2022 outbreak strains

Tracy D. Lee, Frankie Tsang, Kathleen Kolehmainen, Natalie A. Prystajecky, Agatha N. Jassem, John R. Tyson

One of the core functions of the public health laboratory is being prepared to respond to outbreaks and to ensure that our assays can detect circulating strains. In 2022 when the highly pathogenic avian influenza subtype H5N1 was circulating in both wild and commercial flocks it was imperative that the single nucleotide polymorphisms (SNPs) detected be incorporated into our existing qRT-PCR H5 subtyping assay. The ensuing revised assay incorporates two different hemagglutinin H5 targets and a third matrix (M) gene target. The validation results are summarized in Table 1. This assay is used to subtype influenza A positives in avian samples but is also available for suspect human cases.

Table 1. Summary of validation results. CoV = coefficient of variation.

Validation Metric		Summary of Results
Analytical Specificity	Specificity	58/59 (1 discordant)
	Cross-reactivity	0/13
Analytical Sensitivity		1 copy/µL for Flu-A M and 10 copies/µL for H5 (Ct≤36)
Precision (CoV)		0.04-2.87%
Accuracy		98.3% for Flu-A M, 100% for H5-P3, and 100% for H5-P4

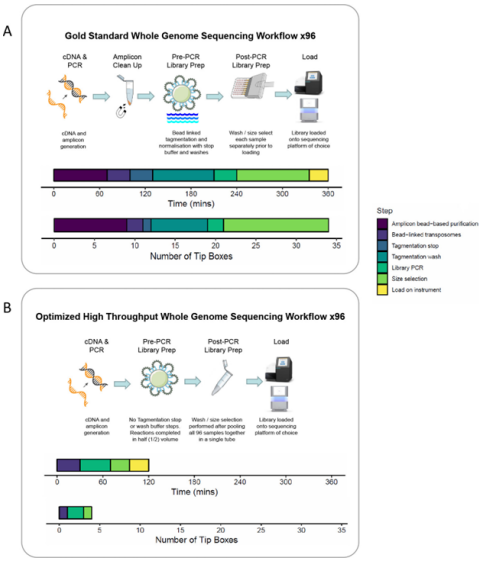
Available from: https://doi.org/10.1101/2023.12.13.23298992
Accessed on March 13, 2024

Rapid, high-throughput, cost-effective whole-genome sequencing of SARS-CoV-2 using a condensed library preparation of the Illumina DNA Prep kit

Rebecca Hickman, Jason Nguyen, Tracy D Lee, John R Tyson, Robert Azana, Frankie Tsang, Linda Hoang, Natalie A Prystajecky

The COVID-19 pandemic changed the landscape of what was expected of the public health laboratory. With the availability and utility of whole genome sequencing in the response, the laboratory had to optimize the workflow to accommodate increasing samples positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To also address shortages in laboratory reagents and consumables and the need for rapid turnaround, the laboratory optimized and condensed an Illumina DNA library preparation method that was compatible with a liquid handler. The new protocol eliminated some steps and enabled pooling post-PCR rather than each sample. This achieved a much quicker (by 4 hrs) workflow (Figure 3B) that cost 55% less, using 85% fewer pipet tips while not compromising on the quality of sequencing. This condensed method has enabled the generation of over 250,000 SARS-CoV-2 genomes and is being applied across a host of other organisms that are currently sequenced.

Figure 3. Comparison of step-wise workflow, total time to complete, and tip box usage between the (A) original whole genome sequencing method and the (B) new method per plate of 96 samples.



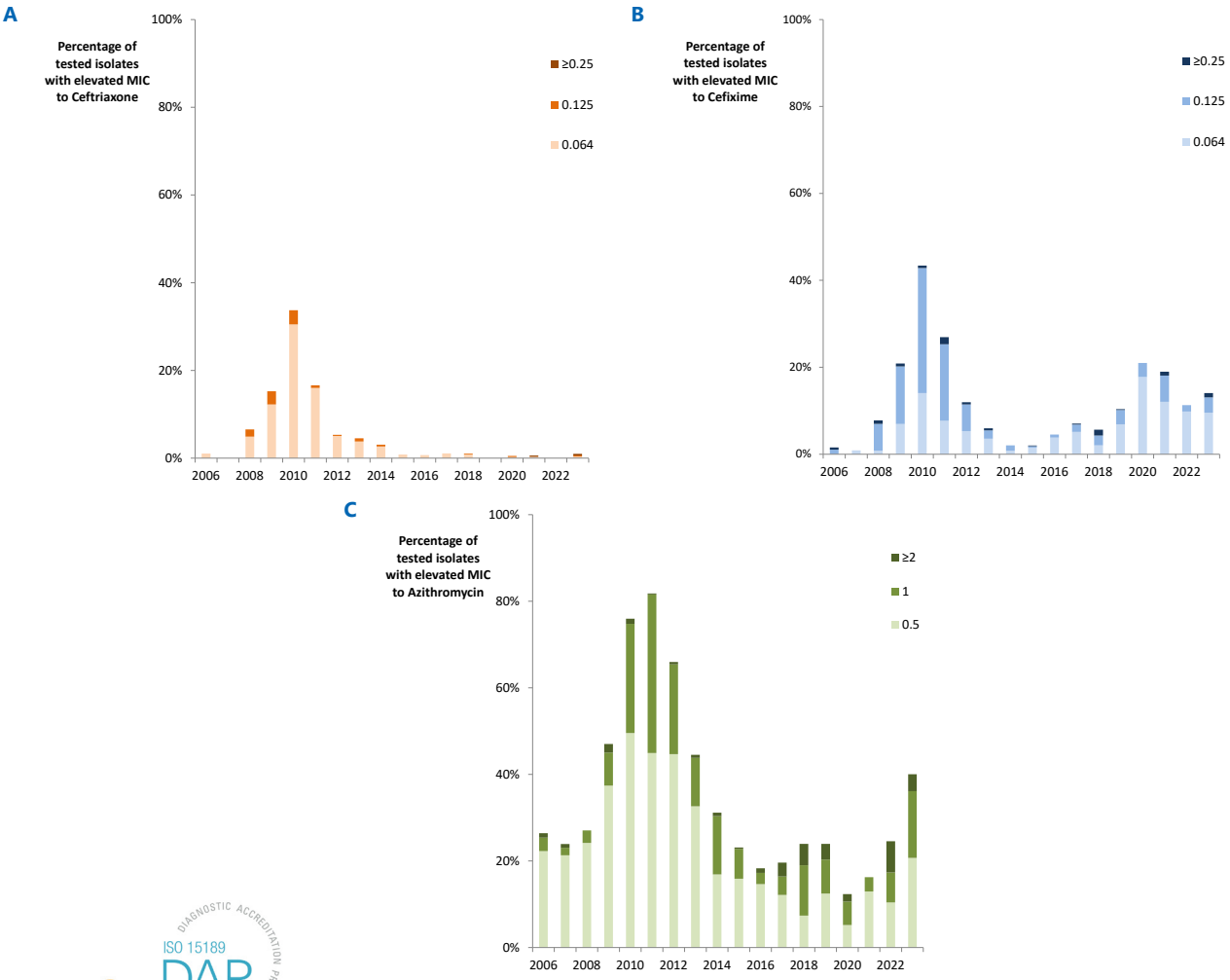
Available from: https://pubmed.ncbi.nlm.nih.gov/38315007/
Accessed on March 13, 2024

Neisseria gonorrhoeae Susceptibility Trends

The spread of antimicrobial-resistant *Neisseria gonorrhoeae* and emergence of increasing resistance to third generation cephalosporins is a global public health issue, being closely monitored by BCCDC. The Bacteriology & Mycology Laboratory performs *N. gonorrhoeae* antimicrobial susceptibility testing on culture-positive isolates to cephalosporins as well as alternative antimicrobials including azithromycin, ciprofloxacin, penicillin, spectinomycin, and tetracycline.

There were peaks in resistance to commonly used antibiotics between 2008-2012 (Figure 4). Since then, decreased susceptibility to ceftriaxone (MIC ≥0.125 µg/mL) has remained rare (Figure 4A). The number of isolates with elevated MIC to cefixime started increasing again in 2016, peaking in 2020 (21%) (Figure 4B) and now fluctuating between 14 and 19%. In contrast, isolates with elevated MIC to azithromycin continues to increase following a low in 2020 (Figure 4C).

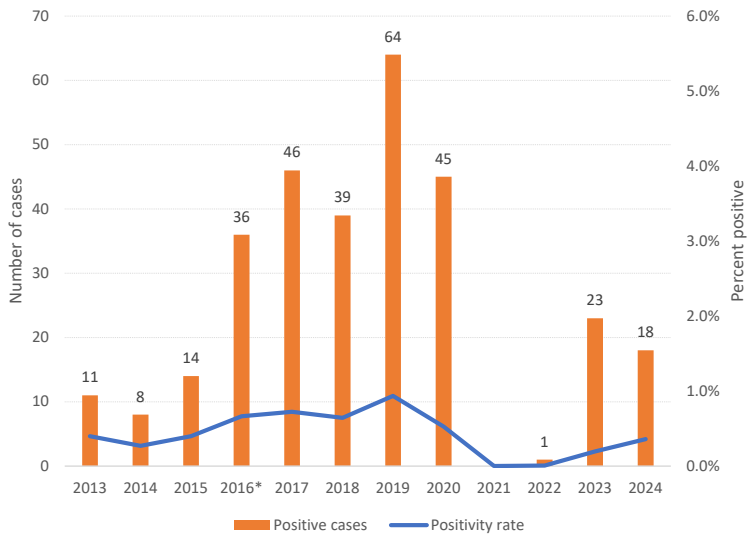
Figure 4. Percentage of tested *N. gonorrhoeae* isolates with elevated minimum inhibitory concentrations (MICs) to cefixime (A), ceftriaxone (B), and azithromycin (C) from 2006-2023, Bacteriology & Mycology Laboratory, BCCDC PHL. MIC units are in µg/mL.



Mycoplasma pneumoniae Trends

Mycoplasma pneumoniae is one cause of atypical bacterial pneumonia. At the BCCDC PHL, testing was previously performed by request only using a laboratory-developed multiplex NAT targeting *M. pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila.* In February 2016, testing using the NxTAG Respiratory Pathogen Panel from Luminex® was adopted, an assay which detects 20 respiratory pathogens including these bacterial targets. This meant that atypical bacteria could be detected without clinicians having to request a specific test when presented with a probable respiratory infection. The result of this change in practice is reflected in the increased number of atypical bacteria cases, including inceased detection of *M. pneumoniae* from 2016-2020 (Figure 5). Following an absence of *M. pneumoniae* detections in 2021 and only one detection in 2022, cases were again being detected in 2023 with 23 cases. After the first quarter of 2024, 18 cases have already been detected, suggesting that detections will return to levels seen pre-pandemic.

Figure 5. Number of *Mycoplasma pneumoniae* positive cases from 2013-2024 Q1 and percent positivity. *Note that the NxTAG Respiratory Pathogen Panel replaced a laboratory-developed multiplex NAT in February 2016 for the detection of *M. pneumoniae*. Testing performed at the Bacteriology & Mycology Laboraotry (until 2016) followed by the Virology Laboratory, BCCDC PHL.



Shigella Trends

The number of monthly cases of *Shigella* detected by the BCCDC PHL in 2024 in comparison to historical months from 2013-2022 are depicted in Figure 6. There were four times the number of cases detected in January and February of 2024 compared to previous years. The number of cases detected in 2023 were also above historical average, particularly in November and December. From 2015-2022, excepting 2021, *S. sonnei* constituted more than half of the *Shigella* species detected (Figure 7). Outbreaks in 2022 and starting late 2023 into 2024, accounts for the continued dominance of *S. sonnei* over these years. From November 2023, 211 laboratory-confirmed cases of *S. sonnei* with the same WGS cluster code have been detected to date. The majority of these cases are linked to an underhoused population in Vancouver Coastal Health Authority and Fraser Health Authority. Smaller outbreaks of *S. flexneri* have also occurred in 2019 and starting the end of 2021.

The BCCDC PHL is partnering with the Public Health Agency of Canada and National Microbiology Laboratory in the Antimicrobial Resistance Network (AMRNet) Program to detect and monitor antimicrobial resistance (AMR), which represents a major threat to human health with significant global economic and security implications. AMRNet is a laboratory-based surveillance system that aims to capture data on antimicrobial susceptibility testing from bacterial and fungal pathogens across Canada. Data sharing agreements between BC Health Authorities/hospitals will enable data flow and ongoing national surveillance and will contribute to international comparisons. Surveillance is an essential tool to inform policies and for infection prevention and control responses. In the most recent *S. sonnei* outbreak, AMR data serves to guide empiric therapy for those who are difficult to follow up.

Figure 6. Number *Shigella* positive cases from 2013-2024 to date, Bacteriology & Mycology Laboratory, BCCDC PHL. The shaded area depicts the minimum and maximum number of monthly cases from 2013-2022.

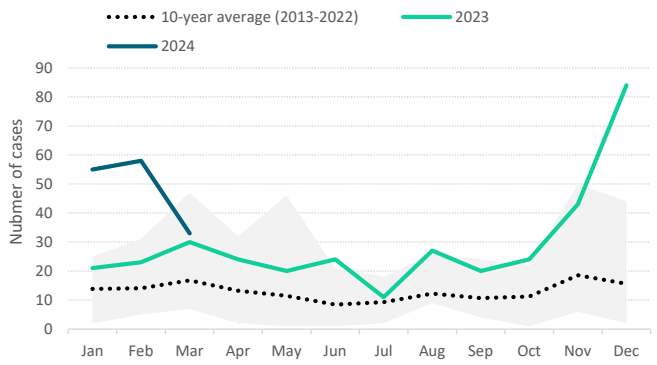
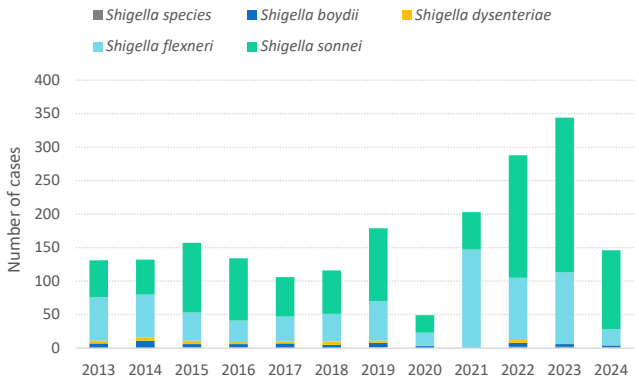


Figure 7. *Shigella* species detected from 2013-2024 to date, Bacteriology & Mycology Laboratory, BCCDC PHL.



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LM LABS

The Public Health Laboratory at the BC Centre for Disease Control (BCCDC) provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology laboratories and public health workers across the province and nationally. The BCCDC PHL is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions. The Provincial Toxicology Centre conducts toxicology testing and analysis for clinical patients, including therapeutic drug monitoring, drug screening tests and forensic toxicology analyses for the BC Coroners Service.

This report may be freely distributed to your colleagues. If you would like more specific information or would like to include any figures for other reporting purposes, please contact us.

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