Detecting Mycobacteria in Wastewater
Surveillance through Sewage

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Background

SURVEILLANCE AND WASTEWATER

Wastewater (WW) contains a lot more than just the water flushed through our pipes. In fact, disease monitoring through WW has been used in the past to detect poliovirus and SARS-CoV-2. With poliovirus, public health systems were able to track virus prevalence in high-risk communities to predict out-breaks and guide vaccinations. Wastewater surveillance (WWS) can provide near real-time data on bacterial or virus prevalence within a community without relying on patients to develop symptoms or seek medical advice. In the case of COVID-19 surveillance, the delayed onset of symptoms in COVID-19 causes a 2-week lag period when relying on diagnostic reports only. WWS provides near real-time data on how much virus is circulating in a given community.

MYCOBACTERIA AND DISEASE

Mycobacteria is a genus of organisms containing some of the causative agents behind diseases like: Tuberculosis disease (TB), Hansen disease (Leprosy), as well as many opportunistic pathogens, that can worsen disease in sick patients. The 7 organisms associated with causing TB are collectively referred to as Mycobacterium tuberculosis complex (MTBC), including *Mycobacterium tuberculosis* (MTB) which is the most common agent behind TB.

WASTEWATER SURVEILLANCE & MYCOBACTERIA

In 2019, TB was the leading *infectious* killer in the world, taking the lives of ~1.5 million people. As a preventable and curable disease the majority of TB cases come from developing nations or communities with little access to healthcare. The goal of this research is to develop a protocol that would allow for Mycobacterial surveillance through WW. Success in this project will provide public health systems with an affordable and accessible tool to monitor high-risk populations to aid in outbreak prevention and management, and guide preventative treatment efforts (antibiotics regimens or vaccine implementation).

Methods

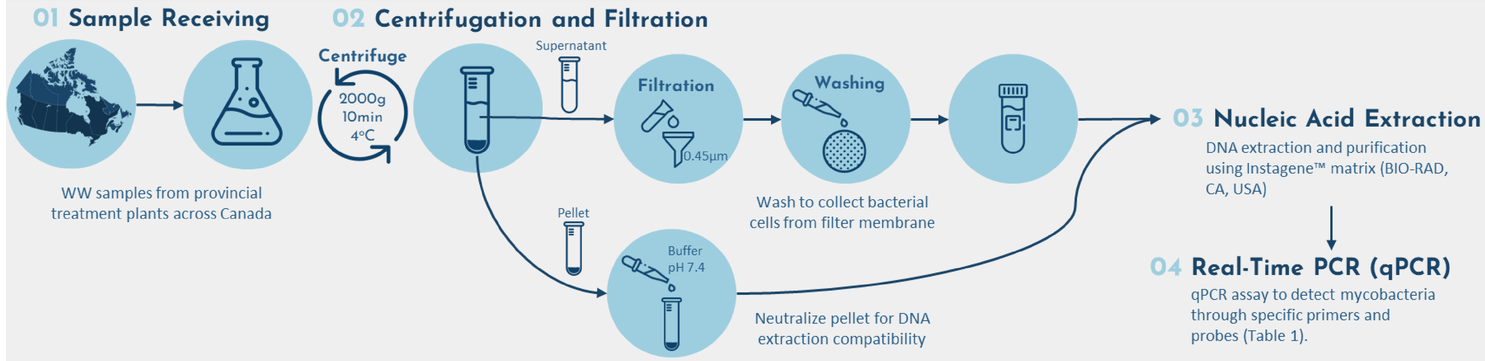

Figure 1. Overview of Mycobacteria WS protocol.

qPCR Targets	Description
MTBC	Detection of TB disease-causing mycobacteria: *M. tuberculosis, M. bovis, M. africanum, M. caprae, M. microti, M. pinnipedii.*
MTB	Identification of *M. tuberculosis.* **True MTB positive will also be positive for MTBC.**
MYCO	Detection of all *Mycobacteria* spp.

Table 1. Real-Time PCR targets.

Results

1) Concentrate mycobacterial DNA from wastewater into centrifugation supernatants.

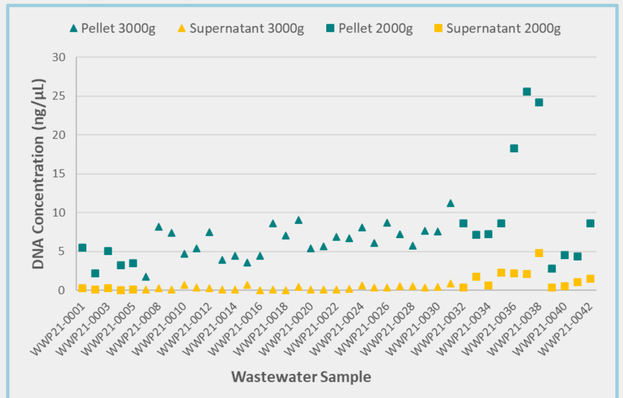

Figure 2. DNA concentrations from WW pellets and supernatants centrifuged at 3000g and 2000g. Pellets (green) consistently had higher DNA concentrations. Supernatants (yellow) that were separated by 2000g centrifugation (square) had higher DNA concentrations than supernatants centrifugated at 3000g (triangle).

2) Limit of detection (LOD).

Preliminary experiments reveal that the LOD for this protocol falls between the range of 200-500 CFU/mL (*M. bovis* BCG).

3) Detecting Mycobacteria in WW through real-time PCR and reducing non-specific amplification of the MTB target.

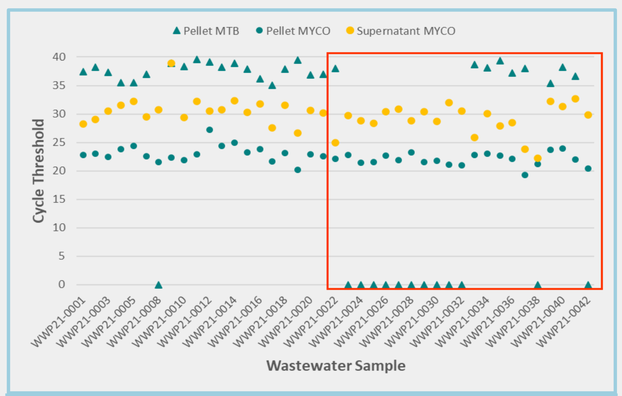

Figure 4. Real-time PCR Detection of MYCO and MTB in WW pellets and supernatants. Cycle threshold (CT) indicates how many DNA replication cycles occurred when a positive result was detected, higher concentrations of target DNA will have lower CTs. MYCO (circle) were detected in all samples. Low-level amplification of MTB (triangle) DNA occurred in 68% of pellets, however MTBC was not detected in any of the samples. Therefore, MTB CTs were considered false positives resulting from non-specific amplification. 5% DMSO was added to the MTB reactions to reduce non-specific amplification of MTB target, which it did with great success (red outline).

4) Screening for qPCR inhibitors.

qPCR uses fluorescence-labelled probes to signal the detection of specific DNA sequences. Inhibitors are chemicals that interfere with fluorescence and can lead to false negatives. No indication of inhibition was found when comparing DNA extraction controls to WW samples processed according to the finalized protocol.

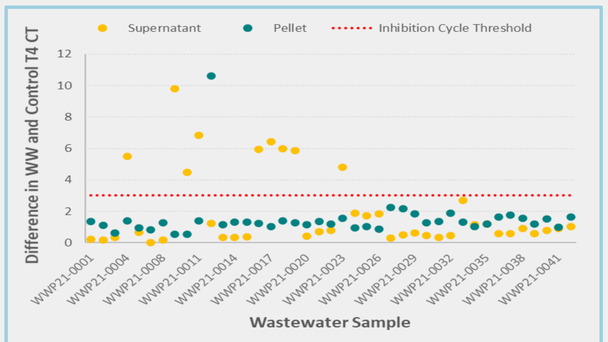

Figure 3. Difference in WW and extraction control T4 CT values. WW and controls were spiked with Bacteriophage T4 DNA prior to DNA extraction. A CT difference greater than 3 (red line) between WW and control indicates inhibition. All samples above the inhibition CT were processed prior to the finalization of the protocol and therefore do not indicate inhibition.

Conclusion

Mycobacteria WWS is in its early stages of development and there is still a lot of work to be done before this protocol can be put into practice: (i) determining a narrower limit of detection range; (ii) investigating bacterial contaminants and attempt to culture mycobacteria from WW; (iii) explore WS in conjunction with the mobile TB surveillance tool, GeneXpert MTB/RIF assay; (iv) finally, in the future, the NRCM hopes to process samples from northern Canadian communities with high TB prevalence, to better gauge the true impact this system can have on TB surveillance. With further developments to this protocol, WS has the potential to improve TB (and other mycobacterial disease) detection, coverage and ultimately aid in the WHO End TB Strategy.

Acknowledgements

World Health Organization. (2020a). Global Tuberculosis Report 2020. In *World Health Organization* (Vol. 66).
Larsen, D. A., & Wigginton, K. R. (2020). Tracking COVID-19 with wastewater. In *Nature Biotechnology* (Vol. 38, Issue 10, pp. 1151–1153). Nature Research. https://doi.org/10.1038/s41587-020-0690-1.

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