FOCUS ON
Wastewater Surveillance of COVID-19

Key Findings

- Wastewater surveillance (WWS) for COVID-19 involves the testing of sewage for the presence of SARS-CoV-2 virus ribonucleic acid (RNA), which, if positive, suggests some level of COVID-19 presence in a source population.

- A key advantage of WWS is that a single test represents an independent signal from the entire population contributing to the sampled wastewater stream regardless of health status (symptomatic, asymptomatic, or recovered) or access/utilization of clinical testing.

- Limitations include that there are no standard methods for sampling or testing of wastewater and the data generated must be interpreted within the context of both the sites and sources being sampled, the testing methods and laboratories performing the testing and the clinical epidemiology of the catchment area being sampled. This requires significant collaboration between parties involved, e.g., utilities, laboratories, public health.

- WWS for COVID-19, when combined with corresponding clinical testing and epidemiology data, may have potential for tracking or anticipating COVID-19 disease trends but is not yet recommended as a means to estimate prevalence. It is a rapidly evolving area of research and operationalization, and as an adjunct to clinical testing for assessing infection rates in a community.
Introduction

The purpose of this review is to: provide a summary of global WWS activities and to provide public health practitioners with pragmatic considerations for assessing and interpreting WWS data, including potential uses and limitations for public health purposes. Technical aspects of WWS, such as specific sampling or analytical techniques are outside the scope of this document.

Background

What is Wastewater Surveillance (WWS)?

Wastewater (also referred to as sewage) is water mixed with other waste material collected from household (e.g., toilets and sinks), industrial (e.g., process effluents), and in many locations, seasonally varying levels of natural sources (e.g., rain and drainage water). Wastewater surveillance (WWS) is the sampling and analysis of wastewater to monitor the prevalence, or occurrence, of diseases like polio and measles, illicit drug use, and as an early warning indicator for hepatitis A and norovirus outbreaks within communities.¹,²

One of the earliest reports on WWS for COVID-19 was by Medema et al who surveyed sewage samples from six cities throughout the Netherlands and the Amsterdam airport between February and March, 2020. A correlation between measured increases of viral RNA in wastewater and increases in the number of COVID-19 cases was observed.³

WWS for COVID-19 is now active in over 1,000 sites in nearly 50 countries around the world.⁴ Many studies have observed a correlation between the quantity of viral RNA in wastewater and the prevalence of COVID-19 in the population¹ Globally, various research groups are working to understand how WWS of COVID-19 might be used by public health agencies to complement existing COVID-19 surveillance tools (e.g., clinical testing, hospitalization data).¹,⁵

WWS for COVID-19 is still considered nascent in its development and use.¹ While viral RNA can be detected in wastewater, challenges remain with respect to sampling, sample preparation, analytical techniques, understanding the dynamics of viral shedding, the development of harmonized monitoring programs and translating viral RNA wastewater data into indicators of active infection, making interpretation challenging.¹,⁶,⁷,⁸ Whether and how to action the data needs to be informed by an understanding of how the data are generated and their limitations, and remains an area of ongoing enquiry in science and practice.
Methods

PHO Library Services conducted a literature search of MEDLINE, EMBASE and Scopus databases for English-language articles published from 1946 to date. Search terms included, among others: corona virus*, COVID-19, variant of concern, sewer*, biosolid*, wastewater, municipal water, environmental monitoring, surveillance, monitor*and wastewater-based epidemiological monitoring. The literature search identified a total of 312 unique records (243 from Medline and 69 from the EMBASE/Scopus databases) which were screened for relevance.

A grey literature search was also conducted including several custom Google search engines tailored to Canadian health departments and agencies, Ontario public health units and international (outside of Canada) public health resources and a general google query without site domain restrictions were run. Up to the first 100 results of each query applied to each search were screened for relevance.

This review was not systematic, and reflects the state of the science at the date the report was written. Studies published after the date of the literature searches (conducted over a period of February 09 to February 17, 2021) will not have been captured. The search methodologies and results from the literature and grey literature searches are available upon request.

Results

How is COVID-19 Detected in Wastewater?

SARS-CoV-2 (the virus that causes COVID-19) is shed in feces and to a lesser extent, urine of a proportion of infected individuals who are either asymptomatic, pre-symptomatic, symptomatic, or recovering from COVID-19, for periods ranging from a few days to several weeks.\(^9,10\) In those who shed the virus fecally (estimated at 30-60%), the viral load is highly variable with an estimated range of \(10^4\) to \(10^8\) copies/L (of stool) depending on the course of infection; however, peak viral shedding occurs just before the onset of symptoms.\(^11,12\) Viral shedding in feces also appears to be independent of the presence of the COVID-19 virus in the respiratory tract.\(^1,2,5,6,12\) Infected individuals will also discharge both nasal and respiratory secretions to sewage.\(^11\)

Wastewater surveillance involves the collection of untreated wastewater and/or primary sewage sludge samples from a wastewater treatment plant; however, samples have also been obtained from the sewer network, pumping stations, hospital or institutional settings and maintenance holes. Once collected, wastewater samples undergo processing in order to concentrate and ultimately extract the viral RNA prior to performing polymerase chain reaction (PCR) amplification for virus detection.\(^1,5,8,13\) Since COVID-19 RNA is shed in feces of some infected individuals, surveillance of wastewater and/or sewage sludge for viral RNA provides population-level data regarding COVID-19 infections (i.e., symptomatic, asymptomatic, pre-symptomatic, and resolved) within a given community.\(^5,14\)

Thus, wastewater surveillance may provide a non-invasive, anonymous and scalable method (single facilities/institutions to large cities) of obtaining pooled samples from a population within a geographic area at a point in time, with the catchment generally referred to as a “sewershed,” and independent of clinical testing.\(^1\) Compared to pooling clinical testing data, testing a population of interest through a single sample is also highly cost-effective.
How has wastewater surveillance for COVID-19 been used so far?

There are many regions throughout North America (e.g., North West Territories, Burlington Vermont, Michigan, Utah, Alaska, New York City and Ohio) and in Ontario currently engaged in nascent wastewater surveillance programs. These programs are being used alongside clinical testing data to identify the presence of COVID-19 in a community, observe trends in the direction of the epidemic, as a potential early indicator of increases in COVID-19 infections (as viral RNA has been detected in wastewater 3-7 days prior to increasing clinical case counts) and to determine the amount of virus that are variants of concern (VOC) relative to total viral load within a WWS. For the majority of these, it remains unclear, from the literature reviewed, if/how the data have been actioned.

A notable example occurred at the University of Arizona, where a WWS program was successful at providing an early indication of the presence of COVID-19 in a dormitory that had not previously reported cases of COVID-19. After detecting viral RNA in wastewater from the dormitory, subsequent testing of all students identified three asymptomatic individuals who tested positive for COVID-19, helping to inform interventions to prevent further spread.

A well-publicized and extensive COVID-19 WWS program is in the Netherlands, which was established early in the pandemic, and involves sampling 300 individual sewage treatment plants throughout the country. The data appear to be used to monitor trends over time and to provide comparisons between different regions. Researchers there, however, have thus far been unable to establish a clear threshold or signal value in wastewater that could inform action. Thus, the level of coordination and scale of current projects vary widely.

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Through the Canadian Water Network, the COVID-19 Wastewater Coalition (a national collaboration of municipal utilities, public health agencies and government), has been coordinating the testing of wastewater for COVID-19 in over twenty cities across Canada. In Ontario, the Ministry of Environment, Conservation and Parks (MECP) is coordinating and funding university-led efforts by centralizing and merging sewage analysis, geospatial and clinical data to study the potential role of WWS for COVID-19 in various locations. As a result, Ontario’s public health units may have opportunity to receive and potentially use WWS data to inform public health strategies/decision making alongside other available data.

An Ontario example is the City of Ottawa’s COVID-19 WWS program. The city’s wastewater treatment facility collects and treats the wastewater from approximately 92% of the city’s population, providing one centralized location where representative wastewater samples can be obtained. Ottawa was one of the first communities in North America to collect wastewater samples, test for SARS-CoV-2 RNA and report the results on a daily basis. Ottawa communicated the steep increase in the wastewater signal and an increase in clinical test positivity through Twitter to reinforce the importance of public health measures in December 2020.

What are the potential uses for covid-19 wastewater surveillance?

The US CDC recommends that jurisdictions that seek to explore WWS for COVID-19 in their communities consider the following steps:
• Develop and articulate a clear understanding of the needs of the community with respect to ongoing management of COVID-19, and how WWS may help address them.

• Develop a sampling strategy that considers all relevant factors (location, frequency, transport, testing method, normalization methods, data processing, reporting, etc.).

• Develop a reporting and analytic framework with a minimum dataset including, for example, sewershed characteristics, sampling methods, testing methods, and analytic methods.

• Develop a method to calculate relevant trends. Using the data to estimate population point prevalence is currently not recommended, because the current level of uncertainty in estimates are too great to be useful.

• Develop a plan to share the data and its interpretation.

Building on the CDC’s consideration for exploring WWS for COVID-19, two main areas for potential WWS data were found in the literature: population-level surveillance/research and an ‘early warning system’ or leading indicator.

**POPULATION-LEVEL SURVEILLANCE/RESEARCH:**

WWS of COVID-19 provides a unique dataset that may be used to observe spatial and temporal trends vis-à-vis other measurable related metrics, e.g., confirmed cases, hospitalizations, deaths. As with such established disease prevalence data, WWS data could be correlated to weather, seasonality, population activities (e.g., school openings/closures, mass events, tourism), public health interventions (e.g., lockdowns and re-openings, travel bans), and other potentially relevant information which may inform our understanding of COVID-19 transmission.¹,⁵

WWS might also potentially be used to evaluate the effectiveness of public health measures. For example, lockdowns, restrictions for certain businesses, or mask mandates could be appraised using WWS data (and other surveillance streams) over time before and after implementation.²⁸

In larger communities or sewersheds, WWS may be used to monitor general trends.¹,⁵ Measuring changes in WWS post-vaccination (or where vaccination is underused) could also be an area of enquiry.²⁹

In general, observation of trends appears to be the main use for WWS in various jurisdictions currently.

**AS ‘EARLY WARNING SYSTEM’ OR LEADING INDICATOR**

A sustained and significant increase in COVID-19 RNA in sewage (where prevalence is low) might act as a potential “early warning system” in a given area, with some studies observing a signal 2-14 days prior to clinical detection or hospitalization¹,²,³⁰ Depending on the precision of defining the catchment area that samples represent (e.g., within a single institution such as hospital, correctional facility, or long-term care home, or for remote communities), options for intervening could be considered, such as targeted restrictions, increased testing, or communication of a potential for increased risk to the affected population.¹,³⁰ WWS may provide an additional tool to bolster surveillance efforts where testing rates are low or access to resources is limited, such as in remote or underserved areas, noting that technical and logistical challenges may need to be overcome.³¹ Screening for COVID-19 variants of concern (VOCs) could also be done in this context, and has been reported in Ontario.³²,³³
Considerations when assessing wastewater data on COVID-19

Given the relatively nascent nature of COVID-19 WWS, several considerations may be useful when assessing wastewater data on COVID-19 including:

A CURRENT LACK OF STANDARDIZED TESTING METHODOLOGY:

- There is currently significant variability in WWS methodologies including sampling parameters, testing and analytic methods.\(^1\) A recent investigation indicated that primary clarified sludge from wastewater treatment facilities may provide a more sensitive measure of the viral RNA signal compared to influent wastewater.\(^34\) Additionally, investigations have explored normalizing COVID-19 viral copies in wastewater to the number of copies of the pepper mild mottle virus (PMMV) in an effort to account for variations in fecal matter concentrations i.e., as an indicator of the fraction of the stream that is of human-diet origin. Any interpretation would need to account for these variables of sampling and testing unique to individual WWS efforts.

- Sampling methods (e.g., sample frequency and timing; location along the path from source to treatment facility; wastewater versus sewage sludge; grab sample versus composite sample; wastewater flow rate during sample collection; catchment population; laboratory analysis) will affect data collected, and would also have to be considered in analysis and interpretation.

- Neither the Ct value (i.e., the number of nucleic acid amplification cycles needed to achieve a positive signal, which is generally lower where there are higher concentrations of RNA present) nor genetic units by weight or volume, are likely to provide reliable estimates for community rates of infection, given the variability noted above.\(^35\) In fact, there appears to be no single accepted “threshold” for quantification of COVID-19 RNA in wastewater.\(^13\) Currently, the CDC and WHO caution against using the data to make point estimates of population prevalence of active disease.\(^36,31\)

SHEDDING DYNAMICS AND POTENTIAL FOR UNDER-REPORTING/OVER-REPORTING:

Although many (but not all) individuals with current or recent COVID-19 infection shed viral RNA in the stool and urine, there is variability in both the concentration (at least 1 order of magnitude) and the duration of shedding (from a few days prior to onset of symptoms up to several weeks after recovery where transmission is no longer occurring) among those infected.\(^6,37\) Based on this, it is estimated that up to 60% of positive sewage samples could be attributable to resolved (non-infectious) cases.\(^38\) Additional considerations include:

- Low community prevalence may result in lower viral load in the wastewater system, potentially below the level of detection of the assays, such that the virus is not detected when samples are tested in spite of cases within the community.

- More comprehensive data regarding fecal shedding in the population, including by age, gender, level of illness or symptoms, viral strain, vaccination and/or recovered infection status and other variables, would enhance the ability to correlate wastewater estimates with clinical findings.

- Certain communities, institutions, and geographic areas do not use centralized wastewater treatment (e.g., areas that use septic tank systems), and therefore would be excluded from datasets (up to 14% in Canada).\(^39\)
• Geospatial alignment between individuals contributing to a given sewershed and the population being monitored through clinical and other available surveillance data, will enable interpretation and potential actions.

• Uncertainty consequent to the emergence of variants that may not be detected, depending on the assay used.

**CHALLENGES IN TEST INTERPRETATION:**

• Temporal trends in COVID-19 WWS data have been reported to be widely variable over short periods within a single testing site, posing a challenge to leveraging data for use in early detection.\(^1\) Deciding what level of change in a given sewershed is relevant, and how to action that finding, would need to be established based on how that sewershed represents the local public health unit, incorporating the various intricacies noted.

• Though the Arizona State University example offers insight into the potential for WWS to be actioned in a congregate living-type setting, WWS of more distributed populations (e.g., municipalities, public health units, neighbourhood wastewater catchments in dense urban areas) in reducing the population burden of COVID-19 has not yet been clearly demonstrated.\(^40\) Additional considerations include technical and logistical feasibility, cost, and resource allocation.\(^14\)

**Discussion**

**Application of WWS Data to Public Health Decision Making**

While several potential uses have been proposed, the following are key considerations in public health’s interpretation and use of WWS data:

• WWS does not replace established methods of surveillance, and therefore the nature of its complementary role should be considered as it is implemented,

• Establishing a validated and reliable testing strategy, including sampling methodology, laboratory processing and analysis, and data analysis, would be needed if WWS is to inform public health action,

• Interpretation and application of wastewater surveillance data by local public health units requires an understanding of the technical intricacies and limitations of a given WWS program. This requires close collaboration with key partners (e.g., utilities, laboratories) who could inform the public health unit on potentially relevant technical aspects of the program, as discussed above, and

• There remains limited experience and understanding of both the interpretation and subsequent public health actions based on WWS data in jurisdictions where it is currently in use.

In most sewersheds, WWS results are likely best interpreted in the context of temporal trends (rather than discrete points in time or individual readings) and as complementary data to already established COVID-19 surveillance information, which together may inform specific action(s).\(^10,16,17,21\) For example,
should WWS results trend upward over time within a particular catchment area, correlation with other surveillance signals, epidemiology, clinical data and other public health information for that area would be critical to the appropriate interpretation and use of the WWS data. Depending on this assessment, public health actions that could be considered have been proposed by others.\textsuperscript{17} Discussion with other local and provincial public health partners could be helpful, especially as WWS signals are first being incorporated into other surveillance streams. Actions could include\textsuperscript{17}:

- Communication about a potential increase in cases.
- Increasing communication and outreach efforts in communities where increased virus shedding has been found.
- Increasing human clinical and/or wastewater testing in affected communities and adding additional testing locations, if necessary.
- Continuing to evaluate and monitor clinical case data.
- Recommending additional public health restrictions or infection control measures, as appropriate.

Conclusion

As knowledge and testing techniques are refined, WWS for COVID-19 may become part of the public health toolkit. While it cannot replace existing surveillance mechanisms for COVID-19 it may be able to complement them.

At this time, WWS may have the potential to provide a cost-effective early indication of the presence of COVID-19 and/or tracking of trends in a community. When used alongside existing COVID-19 indicators, WWS data may help inform the need for increased testing, public health measures and/or communications in affected communities; however, it is currently not recommended to be used to estimate the prevalence of active infections.\textsuperscript{41} Current use is mainly in large community based sewersheds to track trends. In certain smaller sewersheds (e.g., single institution such as a correctional facility, hospital) there is greater potential to use WWS as a supplementary tool to detect and act upon possible introduction of infection before wider spread occurs, but evidence to support this remains limited.
References


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