Key Findings

- Wastewater surveillance (WWS) for COVID-19 involves testing community wastewater for the presence of SARS-CoV-2 shed from infected individuals. Viral genetic fragments are detected using molecular methods to quantify the amount of virus in the sample which, when tracked over time, results in a “wastewater signal” for a monitored community or area.

- WWS for COVID-19 has developed rapidly throughout the pandemic period and is now occurring in many countries. In Canada, approximately 60% of the population is monitored through a mix of federal, provincial and territorial WWS programs. Approximately 75% of Ontarians are also represented through WWS programs with exceptions such as rural communities not connected to centralized wastewater treatment systems.

- WWS data are being assessed with a key aim of identifying trends in SARS-CoV-2 levels within wastewater systems, as a proxy for changes in the prevalence of recent infection within the community. Unlike clinical testing, WWS testing is not reliant on healthcare encounters; for example, likelihood of, or eligibility for, having PCR testing. At present, signals cannot
confidently be used to predict numbers of cases, however they are being used to forecast trends in clinical outcomes.

- Based on a Public Health Ontario survey, Public Health Units (PHUs) find WWS data to be a useful component of COVID-19 surveillance, particularly for monitoring population-level trends over time and as an early indicator of trends in the community. However, some PHUs noted delays in WWS data reporting, and several would like improved test turnaround times, as well as support with the interpretation of WWS data and the determination of appropriate public health actions.

- As wastewater is an environmental sample, WWS data are best interpreted in the context of environmental factors that can affect the signal. These differences, as well as assessment of goals, strengths and gaps of existing WWS programs can help inform future improvements.

- In most cases, WWS data are likely best interpreted in the context of temporal trends (rather than discrete points in time or individual readings) and as a complement to existing COVID-19 surveillance information, which together may inform specific public health actions. However, qualitative testing, testing for presence or absence of virus, is used to monitor communities or institutions with no known cases, if and when, detecting any occurrence would trigger action.

- As WWS is further refined for public health purposes, the science, methods and best practices will continue to evolve, even in the short term. Establishing a guiding framework to facilitate agreement on clear objectives, set roles and responsibilities for different partners and scope both the research/exploratory in addition to the operational work is critical to the development of a WWS system that is fit for purpose and to best organize public resources for this multidisciplinary/multi-agency work.

Introduction

The purpose of this review is to provide an update to the April 2021 Public Health Ontario FOCUS ON – Wastewater Surveillance of COVID-19 (available upon request) to summarize recent application of wastewater surveillance (WWS) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

This overview is informed by recent peer-review articles, as well as grey literature published by Ontario Public Health Units (PHUs) and major public health agencies. We also summarize the results of Public Health Ontario’s (PHO) survey of PHUs and consider the findings in view of the purpose of public health surveillance.1

Other applications of WWS such as profiling illicit drug use, antimicrobial resistance, food borne pathogens, and other viral infections (enteric and respiratory) such as polio and influenza are out of scope for this review. Also out of scope here is consideration of ethical questions that may arise as technology and surveillance evolve.2

Background

Individuals with COVID-19 shed the virus SARS-CoV-2 in respiratory fluids, saliva, urine, and stool to toilets, sinks and showers.3 Genetic fragments of the virus can be detected and quantified in wastewater collected at the wastewater treatment plant (WWTP) or at sites throughout the sewershed (Figure 1).4 The quantity, or level, of virus detected in the wastewater (i.e., wastewater signal) can be tracked and
Contributions upstream of the sampling sites determine who and what are captured and may include several communities, cities, regions, and health units as well as institutional, commercial and industrial contributions. By sampling upstream of a WWTP, different areas in the sewershed can be tested to enable a more targeted surveillance of communities. For example, Figure 1 shows three sampling sites within one large sewershed. Site 2 provides information on city C; site 4 on city A; site 3 on urban centre B; and site 1 includes all cities, centres and their institutions.

It is important to note that wastewater collection systems have site specific characteristics that influence the wastewater signal for a given outbreak and complicate comparisons of data between systems. In addition to contributions from toilets, sinks, and showers, wastewater will often also include discharges from institutional, industrial and/or commercial enterprises, as well as from rainfall and snowmelt in locations or circumstances where storm and sewer collection systems combine; as well as potential groundwater infiltration where pipes are cracked.

Figure 1: Wastewater Surveillance, Shedding, Sampling in the Sewershed, Laboratory Analysis, Alignment with Clinical Data and Interpretation.


The occurrence of SARS-CoV-2 gene fragments in wastewater is unrelated to healthcare seeking behaviours unlike clinical testing or the likelihood of, or eligibility for getting tested. As well, people can shed virus to wastewater from feces, urine, saliva and/or sputum, regardless of disease state (e.g., presymptomatic, symptomatic or asymptomatic; active infection or convalescent phase). The time course of shedding, symptoms, and clinical outcomes in Figure 2 illustrates the potential of WWS to provide advanced notice of new trends for COVID-19 in the community. However, shedding is variable (e.g., viral load shed over time or gene copies/gram of feces) and may be affected by factors such as immunity, vaccination, stage of illness, the variant of SARS-CoV-2, etc. In Ontario, the wastewater data
have been compared to clinical data from the same area as the sewershed, including COVID-19 cases and related hospitalizations, to determine correlation and identify deviations.\textsuperscript{5,9}

Figure 2: Viral shedding, stages of symptoms and clinical outcomes demonstrates the window of opportunity for regular WWS to provide early detection (World Bank, 2022). \textsuperscript{10}


Methods

On February 14, 2022, PHO Library Services conducted the search for peer-reviewed articles and reviews in Ovid MEDLINE using a combination of Medical Subject Headings (MeSH) and keywords related to WWS of COVID-19. The search was adapted to Ovid Embase and the results were combined and de-duplicated. The search was restricted to English language review articles published since February 2021 to capture the overall discussion of WWS from the time of the initial primary literature search conducted for the April 2021 FOCUS ON (available upon request). The search also included primary literature from November 2021 to February 2022 (the time of the update) to capture the emergence of the Omicron variant. The literature search identified 151 unique records which were screened for relevance. The search strategy is available upon request.

A grey literature search, using custom Google search engines, was also conducted from February 2021 onward using a search strategy similar to that used for the peer-reviewed articles tailored to Canadian health departments and agencies, Ontario PHUs and international (outside of Canada) public health resources. General Google queries without site domain restrictions were also run. The search strategy for grey literature can be provided upon request. Up to the first 100 results of each custom query were screened for relevance.

An additional search was conducted for publications by Canadian authors known to be active in WWS research through national meetings hosted by Public Health Agency of Canada (PHAC). This search was limited to literature published since February 2021 and is available upon request.
To assess the value, use and needs related to Ontario’s WWS from PHU perspectives, an online survey was open to all 34 PHUs from April 14, 2022 to May 3, 2022.

Results

Applications of wastewater surveillance for COVID-19

There were four main applications of wastewater surveillance identified in our review: trends and advanced notice, detecting emerging virus, detecting or trending SARS-CoV-2 variants and mathematical modelling.

Trends and advanced notice

WWS data have been used for assessing COVID-19 trends and may provide an early indication of COVID-19 emergence within a population. Adequate sampling frequency and timely sample processing are critical for data integrity and trend analysis, as well as for the implementation of subsequent public health actions. Trending of viral concentrations (up or down) have been reported to lead trends in reported cases and hospitalization by days and up to a week within a sewershed, in many, but not all circumstances.

Detecting emerging virus

This section discussed qualitative testing when a virus or variant of concern is newly introduced to a population.

The earliest reports where SARS-CoV-2 was detected in wastewater occurred in the Netherlands when both clinical and wastewater testing were newly introduced and both infrequent. The acute detection of virus in the wastewater of a community that was previously virus free, was indicative of disease emergence and significant both in terms of surveillance and public health action.

Qualitative testing (for presence or absence of virus) may be useful when monitoring higher-risk communities or institutions with no known COVID-19 cases, if this would trigger further specific public health action. Once a case is known to be present, presence/absence analysis is not helpful to detect additional infections within the community unless coupled with quantification and trend analysis.

Building-level WWS may have allowed for the early detection of asymptomatic COVID-19 cases in congregate settings such as dormitories, correctional facilities and long-term care homes, though it was unable to distinguish new infections from persistent convalescent viral shedding. WWS has also detected SARS-CoV-2 in the sewage of a community with no recent known cases, highlighting the potential for early warning of disease within a population.

Detecting or trending SARS-CoV-2 variants

Researchers in Ontario and elsewhere have developed wastewater specific methods to estimate the relative proportions of viral lineages within a given system, or community, with the aim of tracking new variants as they arrive and potentially displace previously dominant lineages. This type of information has led in some cases to decisions about public health measures and recommended precautions.
The National Microbiology Laboratory (NML) applies whole genome sequencing and metagenomics to sewage samples to understand and provide lineage apportionment estimates to several communities across Ontario. While the techniques are being developed, initial examinations of both national wastewater and clinical data have revealed consistent findings. This approach is dependent on regular updates to international repositories of clinical sequences and data pipelines such as Pangolin. Several academic laboratories in Ontario are now also sequencing sewage samples using similar methodologies.

**Mathematical Modelling**

WWS data has been applied to modelling to estimate reproduction rates and forecasting trends in cases and/or hospitalization.

At this time, concentrations of SARS-CoV-2 virus in wastewater cannot be used to reliably estimate the number or percentage of infected individuals within a community, although approaches are being developed and showing results comparable to models built on clinical data. To reduce uncertainty, models must incorporate functions that relate the concentration of virus in wastewater to the number of infected individuals in a community. However, shedding rates vary and have been reported to vary by three orders of magnitude Further, with new variants, vaccination, acquired immunity, and various therapeutics, these uncertainties are even greater.

The Ontario Science Advisory Table and others. These \( R_e \) estimates are presented on a publicly available dashboard alongside \( R_e \) estimates developed using clinical data (confirmed cases, hospitalizations and/or deaths). The models. Assumptions in wastewater modeling include degradation rate of the SARS-CoV-2 genetic signal in the sewage; reliability and standardization of data quality from laboratories; and comparable shedding across persons and populations regardless of age, immune status, and level of illness.

**Programs by Jurisdiction**

**GLOBAL**

At this time, wastewater testing is occurring at over 3,000 sites and in more than 60 countries. Countries such as Finland, France, the Netherlands and the United Kingdom have established national or regional wastewater monitoring programs for SARS-CoV-2. As of March, 2022, there are approximately 1,370 wastewater treatment plants across the European Union that are sampled as part of a WWS program.

An extensive SARS-CoV-2 WWS program in the Netherlands was established early in the pandemic and now samples over 300 individual sewage treatment plants, serving more than 17 million people country-wide. The aim of the WWS program is to facilitate early detection in the event of localized outbreaks and to identify relative proportions of variants. Similarly, Finland, the UK Health Security Agency and the region of Victoria in Australia use wastewater monitoring data to assess trends in different cities or regions within the country, and quantify proportions of different VOCs. Wastewater data have been used alongside clinical testing data, case data, hospitalization rates and other data to inform the pandemic response. The observations from WWS have supported decisions to issue public health advisories, or encourage those living in a specific area to stay home if feeling sick and/or encourage increased clinical testing. This has led some WWS programs to increase the frequency and extent of wastewater testing in specific areas. It remains to be seen how programs may change as these countries shift away from the acute pandemic response.
In the United States (US), the Centers for Disease Control and Prevention (CDC) launched the National Wastewater Surveillance System (NWSS) in September, 2020 to track the presence of SARS-CoV-2 in wastewater throughout the US and to build capacity for other applications of sewage surveillance. The NWSS works with regional health departments with the goal of contributing to the facilitation of timely action and limit the spread of COVID-19.\textsuperscript{4} WWS data have predominantly been used by health departments to: i) provide an early indication of possible infection within a community; ii) track trends in infections in a community; and iii) screen for indicators of the spread of COVID-19 at a specific site (e.g., dormitory, correctional facility or other building/institution) in order to initiate additional testing and/or mitigation measures.\textsuperscript{13}

**CANADA**

The Public Health Agency of Canada (PHAC) reports approximately 60% of the Canadian population’s sewersheds are a part of WWS for COVID-19.\textsuperscript{47–49} This is achieved through a mix of 65 federal WWS testing locations across Canada and separate provincial programs, to bring the total number of monitored sites to approximately 250. Sites in British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Prince Edward Island, Newfoundland and Labrador, Nova Scotia, Nunavut, and the Northwest Territories are sampled with population coverage between 27% and 79%.\textsuperscript{49}

Across Canada, academic groups and agencies participate in four working groups hosted by PHAC to share regional updates, research findings, and technical challenges and solutions related to 1) identifying variants, 2) surveillance, 3) modelling and epidemiological interpretation, and 4) laboratory methods.\textsuperscript{48} Many Ontario municipal authorities and local public health units were early adopters in the use of WWS.\textsuperscript{50–64} While there remains questions on how best to sample, analyze and interpret wastewater data, the reduced availability of clinical testing data associated with the arrival of Omicron in Ontario increased interest and reliance on WWS as a pandemic indicator.

Ontario’s Wastewater Surveillance Initiative is led by the Ministry of Environment, Conservation and Parks (MECP) and currently facilitates collection of wastewater data from approximately 170 different sampling sites through 13 academic laboratories.\textsuperscript{28,29,65} It is estimated that the initiative covers over 75% of the Ontario population.\textsuperscript{9} Many of these sampling sites occur within monitored sewersheds to provide information on specific subsets or institutions which allows comparison with corresponding clinical data. The sampling sites include municipal wastewater pumping stations, treatment plants, long-term care homes, university campuses, correctional facilities, hospitals, homeless shelters, and retirement homes. Geographic representation is broad, with sites from Ottawa to Windsor, and Northern Ontario. The Ontario Science Advisory Table Dashboard takes these data and generates provincially and regionally aggregated trends in the wastewater signal.\textsuperscript{36}

The NML also independently conducts analysis on several locations in Ontario using PCR and genome sequencing\textsuperscript{32}, some of which are also sampled as part of Ontario’s Wastewater Surveillance Initiative. The NML performs testing for some municipal wastewater sites, as well as correctional facilities in the City of Toronto and a few First Nations communities.\textsuperscript{32,66,67}

**ONTARIO PUBLIC HEALTH UNIT SURVEY**

An online survey was administered by PHO to all 34 Ontario PHUs to gather information and perspectives on the local value, use, and needs of PHUs related to wastewater surveillance for SARS-CoV-2. A detailed report of these findings is available on the PHO website\textsuperscript{68}. Briefly, all PHUs that responded to the survey (n=33) reported using WWS data for COVID-19 surveillance in their region, particularly after changes to clinical testing guidelines. The most common uses included monitoring population-level trends over time, as an indicator of the presence of COVID-19 in the community, and as
an early indicator of trends in the community. However, some PHUs noted delays in WWS data reporting and several would like improved test turnaround times as well as more sampling sites in their region to increase population coverage. PHUs also reported needing more support with WWS data interpretation and guidance on appropriate public health actions. In addition, PHUs expressed interest in expanding the use of WWS data for the surveillance of other public health issues (e.g., influenza, norovirus, opioid use).

**WWS as a Surveillance Strategy**

Public health surveillance is the ongoing, systematic collection, analysis, interpretation, and dissemination of data regarding a health-related event for use in public health action to reduce morbidity and mortality and to improve health. A formal evaluation of a public health surveillance system includes assessing whether the surveillance system is meeting its objectives of monitoring a health outcome and contributing to public health actions.

The key difference from most other public health surveillance systems is that WWS involves sampling the environment and making an inference about a health-related outcome at the population level, rather than directly measuring a COVID-19 indicator such as the associated number of disease cases, deaths, or hospitalizations. In addition to people shedding the virus at different rates, the SARS-CoV-2 wastewater signal is affected by variables such as environmental contamination, degradation, testing methodologies, sampling strategies, dilution and concentration. The environmental nature of WWS means that interpretation for action may require more understanding about the context of sampling than is typical in other public health surveillance systems where consistent principles are applied to data interpretation (e.g., test eligibility, test characteristics, case definition) and which are typically stable across time.

It is also important to note that in Ontario, WWS was set up rapidly over the course of the pandemic, and did not come about initially as a formal public health surveillance program. WWS started with academic laboratories, participating local public health units and then MECP working to centralize the effort and expand coverage.

Based on the environmental nature of WWS and the PHU survey, and considering the intended purpose of a public health surveillance system, the following are some overall strengths and limitations to help inform further refinement of WWS in Ontario:

- A WWS program design should consider major differences between WWS and most public health surveillance systems in order to set it up for success and to meet the needs of public health, i.e., the different disciplines, expertise, and coordination that may be required to realize a program. However, wastewater testing for public health purposes still needs to be based on ongoing, systematic collection, analysis, interpretation, and dissemination of data.

- Two strengths of the system in Ontario include the partnerships developed by MECP with academic institutions in order to provide wastewater testing and data thus far and the independence of the data from individual health care encounters and clinical testing guidelines, which makes it a more consistent source of information on population trends. However, further work is needed to validate WWS data in the absence of more representative clinical testing.

- While the frequency of sampling was consistent with sampling recommendations from the US CDC, according to the PHU survey the timeliness of the data reported required improvement for use by PHUs. The CDC recommends samples should be processed within 24 hours to allow data use.
• Gaps reported by Ontario PHUs through the PHO survey include the lack of standardization of testing, variable strategies for data analysis, lack of guidelines for data interpretation and public health action, among others.

• Further engagement with PHUs and lessons learned from other jurisdictions and their WWS initiatives will likely be needed as the pandemic and information needs evolve. In addition, feedback from other partners involved in the current program such as the participating laboratories, utilities and national agencies should be considered given the rapid pace of development in this technology.

Discussion and Conclusions

WWS has emerged as a unique tool with potential to support the COVID-19 response. WWS provides a means to test entire facility and community populations regardless of clinical testing or health care encounters. As such WWS can provide an indication of the community’s health status while reducing the impacts of barriers to health care and testing (e.g., time off work; remoteness, access to rapid tests, language).

Jurisdictions globally, nationally and across Ontario are using WWS data as part of their current approach to COVID-19 surveillance. In Ontario, the two most common uses for WWS data are monitoring trends over time and as an early indicator of trends in the community. Improving turnaround times from sampling to data reporting, data interpretation support and best practices for response were noted by many PHUs as areas needing improvement or development.

In most cases, WWS data are likely best interpreted in the context of temporal trends (rather than discrete points in time or individual readings) and as a complement to existing COVID-19 surveillance information, which together may inform specific public health actions. The US CDC and others also recommend that WWS not be used in isolation and that interpretation of WWS should come with caveats (in the absence of other surveillance indicators) if informing public health action, or providing public awareness. The reasons include lack of standardization, knowledge gaps, risk of environmental contamination or degradation, dilution or concentration, and the day to day signal variability. However, qualitative testing, testing for presence or absence of virus, has been used to monitor communities or institutions with no known cases when detecting any occurrence would trigger action.

As with other data reporting practices, public release of COVID-19 information should be mindful of the potential to stigmatize regions/communities. Addressing those unintended impacts starts with working alongside local groups to interpret and share the data. In addition, fields that have previously used WWS to understand community health may offer some lessons and recommendations on communicating and building public knowledge with this tool.

As programs and scientific methods continue to develop, best practices will emerge for laboratory methods, data analysis, and response, as well as program models. In this context and based on the findings discussed, public health use of WWS is ideally supported by a framework with clear objectives for intended use of the data (e.g., research, public health situational awareness and information for personal risk assessments, detecting signals in institutions), roles and responsibilities for all parties, and a plan for evaluation. Consideration should be given to establishing a framework that:
• articulates the purpose and goals of a WWS program that fulfills the current operational needs of PHUs as distinct from WWS for other audiences e.g., private, institutional, community, and broader regional levels through sentinel sampling sites
• Include tailored health communication plan to build community trust and articulate how data will be used to inform public health decision making and resource allocation
• includes the evaluation and documentation of the lessons learned with the objective of identifying best practices;
• is principled on prioritization and allocation of public resources to avoid duplication across jurisdictions and facilitates coordination of organizations from various sectors involved;
• steers the prioritization of what and how practice- and policy-relevant research on WWS continues given that WWS is also under study by academic, other public and private institutions for various COVID-19 and non-COVID-19 interests in parallel with public health;
• considers the costs and benefits of the program in the context of planned uses of the data, and the other complementary surveillance data streams available; and
• articulates the framework and goals in the context of other sources of data on SARS-CoV-2 and the likely need to shift to a broader surveillance strategy to monitor more generally for respiratory diseases and pathogens.

The framework would further support the operationalization of a wastewater program with a focus on the following components:

• Partnerships: timely coordination of sampling, laboratory testing, data analysis and dissemination to facilitate public health action involving sustainable partnerships
• Technical input and support regarding:
  • the locations and frequency of sampling, and processing turnaround based on the intended public health uses of WWS data.
  • the use of standardized methods to produce timely, consistent, high quality data.
  • support for interpretation of wastewater data, which requires consideration of the sampling strategy and context on an ongoing basis (and more coordination and resources than most passive public health surveillance systems).

While consensus is emerging on some of the applications of WWS for COVID-19, none are fully settled. With changes in technology, funding and capacity, ongoing assessment will be needed to determine public health surveillance information needs as well as potential long term uses of WWS. Changing data needs of users should inform program parameters such as where, how broadly and how often to sample, and refine questions relevant to policy and practice that require further evaluation.
References


50. Etches V. COVID-19 signal in our city’s wastewater is increasing more steeply again & Ottawa’s percent of tests coming back positive has jumped to 2%, signs that more of us could pass on the virus when in close contact with others. Please limit close contacts, seek testing if symptomatic @veraetches [Twitter]. 2020 [cited 2022 Aug 15]. Available from: https://twitter.com/VeraEtches/status/1343610702255239173


55. Durham Region Health Department. Durham region COVID-19 data tracker - status of COVID-19 cases in Durham Region [Internet]. Whitby, ON: Durham Region Health Department. 2022 [cited 2022 Aug 15]. Available from: https://app.powerbi.com/view?r=eyJrIjoiMjU2MmEzM2QtNDIiNS00ZmIxLWI5MzYtOTU0NT11Ym5MjQ2IiwidCI6IjUyZDdjOWMyLTk5MjUtNTAtNWI4LTMxOTMtNDc1MTc4YWI0NiJ9


58. Simcoe Muskoka District Health Unit. COVID-19 [Internet]. Barrie, ON: Simcoe Muskoka District Health Unit. 2022 [cited 2022 Aug 15]. Available from: https://www.simcoemuskokahealth.org/Topics/COVID-19#a427397d-b663-4da8-bea5-7e2ecbd5a02e


