

The devil is in the details: emerging insights on the relevance of wastewater surveillance for SARS-CoV-2 to public health

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ABSTRACT

The severe health consequences and global spread of the COVID-19 pandemic have necessitated the rapid development of surveillance programs to inform public health responses. Efforts to support surveillance capacity have included an unprecedented global research response into the use of genetic signals of SARS-CoV-2 in wastewater following the initial demonstration of the virus' detectability in wastewater in early 2020. The confirmation of fecal shedding of SARS-CoV-2 from asymptomatic, infected and recovering individuals further supports the potential for wastewater analysis to augment public health conventional surveillance techniques based on clinical testing of symptomatic individuals. We have reviewed possible capabilities projected for wastewater surveillance to support pandemic management, including independent, objective and cost-effective data generation that complements and addresses attendant limitations of clinical surveillance, early detection (i.e., prior to clinical reporting) of infection, estimation of disease prevalence, tracking of trends as possible indicators of success or failure of public health measures (mask mandates, lockdowns, vaccination, etc.), informing and engaging the public about pandemic trends, an application within sewer networks to identify infection hotspots, monitoring for presence or changes in infections from institutions (e.g., long-term care facilities, prisons, educational institutions and vulnerable industrial plants) and tracking of appearance/progression of viral variants of concern.

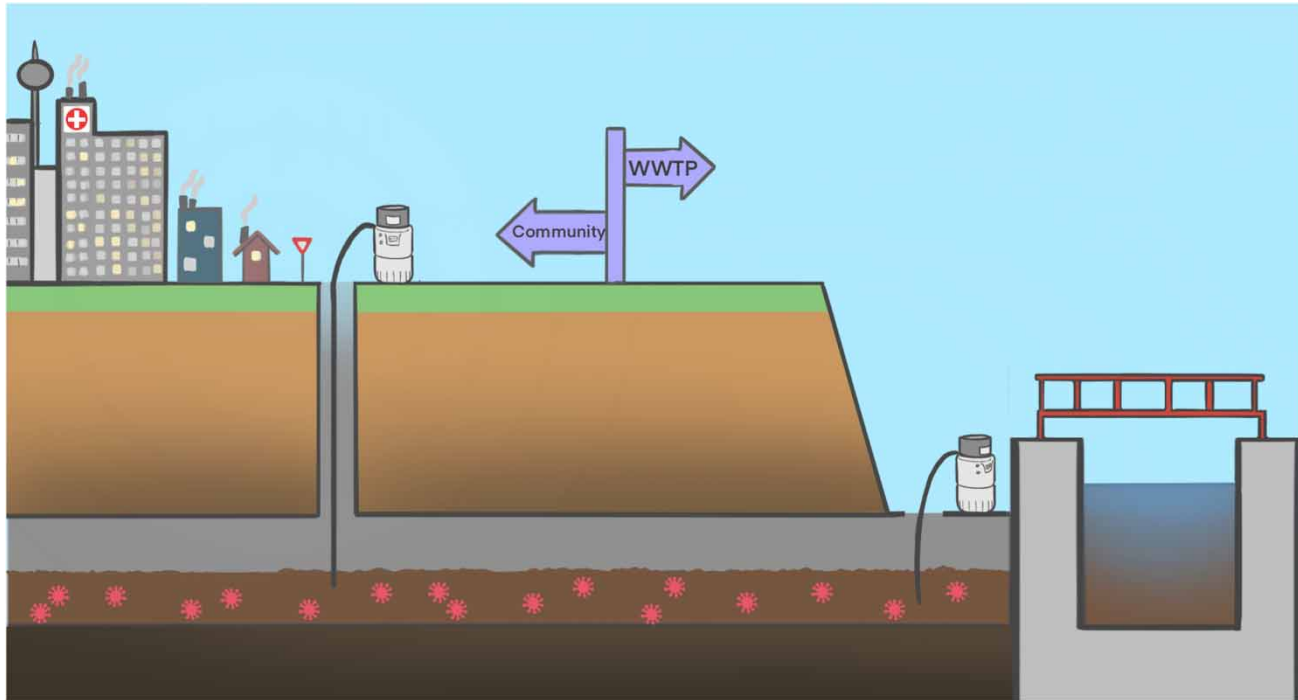
Key words: COVID-19, critical assessment, pandemic surveillance, public health, sewage

HIGHLIGHTS

- Wastewater-based surveillance (WBS) for the pathogen SARS-CoV-2 has experienced phenomenal growth in application across much of the world.
- Widespread adoption of WBS is relevant and useful where it can provide evidence that can reliably inform public health responses.
- The potential for WBS to provide early warning of the emergence of COVID-19 infection in relation to clinical testing is attractive but capability depends on specific details of WBS logistics.
- WBS data offers the advantage of being independent of extraneous factors such as policies governing clinical testing and data can be regarded as objective provided adequate QA/QC programs are provided to ensure validity of genetic signals of SARS-CoV-2 that are measured in wastewater.
- The potential for WBS to track the appearance and progression of viral variants of concern (VOC) in a sewer population has assumed increasing importance as the emergence of VOCs has proven challenging for managing the COVID-19 pandemic particularly as rapidly rising case counts pose a challenge for clinical testing.

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GRAPHICAL ABSTRACT



INTRODUCTION

The COVID-19 pandemic has been the most serious, acute public health epidemic of the past century in terms of infections, illness, deaths, economic and social impacts. On January 13, 2020, the World Health Organization (WHO; WHO 2021a) distributed the reverse transcription-polymerase chain reaction (RT-PCR) laboratory method for detecting genetic fragments of SARS-CoV-2, the virus that causes COVID-19, which has formed the backbone of clinical testing to identify if individuals were infected.

Based on experience with facilitating wastewater SARS-CoV-2 surveillance initiatives, collaboration and methods assessment through Canadian Water Network, combined with careful examination of international experience, we undertook a high-level review of the demonstrated strengths, limitations and future promise of wastewater surveillance, including the particular and practical challenges of ensuring surveillance results are relevant to informing meaningful public health measures.

A few experienced and insightful water researchers recognized early in the pandemic the opportunity to apply this highly sensitive, very specific analytical method to monitor wastewater for SARS-CoV-2. Several had submitted proof-of-concept results for refereed publication by April to early June 2020 (Ahmed *et al.* 2020; Gonzalez *et al.* 2020; LaRosa *et al.* 2020; Lodder & deRoda Huisman 2020; Medema *et al.* 2020a; Peccia *et al.* 2020; Randazzo *et al.* 2020a, 2020b; Sherchan *et al.* 2020; Wurtzer *et al.* 2020). This remarkably rapid dissemination of water and public health research led to rapid research applications evaluating this approach for tracking the pandemic around the world. The resulting research activity that arose has been unprecedented, but there needs to be a fair assessment of what wastewater surveillance for SARS-CoV-2 (WBS¹) can and cannot be relied upon to achieve.

¹ Prior practice in this field has been described as wastewater-based epidemiology but we find this term to be inaccurate in its reference to epidemiology. According to the Centres for Disease Control and Prevention: 'Epidemiology is the method used to find the causes of health outcomes and diseases in populations. In epidemiology, the patient is the community and individuals are viewed collectively. By definition, epidemiology is the study (scientific, systematic, and data-driven) of the distribution (frequency, pattern) and determinants (causes, risk factors) of health-related states and events (not just diseases) in specified populations (neighborhood, school, city, state, country, global).' <https://www.cdc.gov/careerpaths/k12teacherroadmap/epidemiology.html>. Surveillance is an accurate description of what we are reviewing. Surveillance is an element of epidemiology, so we have chosen to refer to wastewater-based surveillance (WBS): of the pathogen SARS-CoV-2.

However, the rapid development of the techniques applied to WBS for SARS-CoV-2 has led to inevitable uncertainties in dependability and meaning of the results that determine its value for use in public health decisions. In addition, the challenges and competition for time and resources allocated to pandemic response have led to difficulty in securing sufficient capacity and support to rapidly conduct needed pilot work to address uncertainties over very short timelines. In our experience with the Canadian Water Network (CWN) Wastewater Coalition, much of the initial work and advancements that were viewed and promoted with optimism by the research community and locally engaged public health partners have been met with varying degrees of skepticism and even indifference among public health decision-makers. Understandably, public health authorities are looking for more certainty and confidence in the meaning of WBS data, prior to investing time and effort into supplementing conventional clinical testing data with wastewater-based data as a guide to decision-making.

The application of WBS for SARS-CoV-2 has expanded dramatically over the past 21 months, along with increased interest in the potential to inform public health concerns generally, including by the media and the public. In addition to a multitude of individual local applications, national and regional wastewater surveillance programs are now either in development or underway in many countries as a result (Gawlik *et al.* 2021). The rapid expansion of knowledge, research and application over such a short timeframe has significantly challenged the ability to vet processes using conventional peer-reviewed publications and research study and evaluation approaches. However, WBS for SARS-CoV-2 now has an initially established body of peer-reviewed literature supporting this young, but rapidly evolving area. This development is occurring in parallel with the ongoing and changing needs of the pandemic management, as the very demographics and characteristics of the disease itself continue to change. There is a strong need to view this research and experience in the context of the significance of the key findings and uncertainties to ongoing public health decision needs. The research community will continue to refine understanding and identify gaps and uncertainties in response to an expressed ongoing need. But to serve pandemic management and further engage the public health community, *the devil will always be in the details* in terms of the relevance of the different knowns and unknowns to informing public health practitioners. By use of this idiom here and in our title, we assert that although the meaning of evidence gathered by this approach may appear straightforward, there are many subtle but important details that may well justify conclusions that differ from those apparent upon first consideration.

METHODS

The CWN COVID-19 Wastewater Coalition (CWN 2021a) was established to pursue WBS for SARS-CoV-2 among capable Canadian investigators, wastewater utilities and public health authorities with the express intent of ensuring from the outset of the work that such an undertaking was driven by a goal of determining if or how the technique could serve the needs of public health authorities. In addition, CWN participated as a member of the Global Water Research Coalition (GWRC 2021), providing an important window on relevant initiatives among research agencies in the nine-member countries and expanded inputs from invited participants from other countries, together with interactions involving the International Water Association (IWA) and the WHO. These collaborations provided us with an early window on which groups were pursuing WBS and the literature that was emerging and how and where the approach was being supported and adopted in Canada. We have followed this targeted head start with a focused literature review to provide a more comprehensive perspective on the documented experience with WBS. We obtained a potentially relevant list of references by searching Web of Science (all databases) covering the period from March 2020 to mid-July 2021 with a topic search strategy of (SARS-CoV-2 OR Covid-19) AND (wastewater) AND (monitoring OR surveillance). From this list of 251 references, we considered those that were directly relevant to the WBS applications being pursued by the CWN coalition (surveillance at influent to community wastewater treatment plants (WWTPs) or at targeted locations in sewer systems). We supplemented references from this approach with those that were cited or otherwise brought to our attention by mid-2021 but we did not attempt to perform a formal systematic review. Because of the rapidly emerging nature of this topic, we chose to include a consideration of 23 preprints that had not been refereed. Readers need to recognize the caution included in these preprints about relying on them because they have not been refereed. We have found that four of these were subsequently published so that we have cited the refereed published versions.

Through the foregoing approach, we have collected publications that describe the implementation of WBS, and we have reviewed that published experience in the context of our own experience in seeking to enable accelerated collective

action and progress for WBS in Canada. As such, we reviewed this evolving literature for what it suggests to us about the strengths and limitations of WBS for SARS-CoV-2 toward the goal of ensuring findings that are relevant to public health needs.

RESULTS

The literature has experienced an overwhelming proliferation of publications over the past year on a variety of research studies that are relevant to WBS. This paper does not attempt to provide an exhaustive review of the practice of WBS, nor a comprehensive review of all the publications relevant to WBS. Rather we seek to provide a high-level critical perspective about the strengths and limitations of WBS from our extensive involvement and assessment over more than a year, using a summary of what we deemed, based on our experience with the CWN Wastewater Coalition, to be a significant and relevant selection of some of the most applicable literature for that assessment.

Geographic distribution of WBS activity

Applications of WBS have not only been rapidly implemented, but they have also been global in scope. WBS papers had already been published² by investigators from 36 countries, including Argentina, Australia, Bangladesh, Brazil, Canada, Chile, China, Czech Republic, Ecuador, Finland, France, Germany, Greece, Hungary, India, Iran, Israel, Italy, Japan, Mexico, Nepal, the Netherlands, Pakistan, Portugal, Qatar, Saudi Arabia, Singapore, South Africa, Spain, Sweden, Switzerland, Thailand, Turkey, United Arab Emirates, United Kingdom and the United States (U.S.). The global coverage of these publication locations is illustrated and cited in [Figure 1](#).

These publications must be understood as representing only a very small fraction of actual WBS activity that has and continues to occur. Given the speed of development of this research over such a short time (less than 1.5 years), the ability of publication systems to keep up and advance knowledge in a timely fashion has been a major challenge. In our experience with the CWN Wastewater Coalition, this challenge is highlighted in the resulting disparity between those anxious that the public health community embraces WBS and those reluctant to invest time and resources in such a challenging and rapidly evolving approach until there is a proven record of success.

To give a sense of the much greater activity currently ongoing, relative to published results, the University of California Merced website lists over 3,145 sites (as of November 30, 2021; [Naughton 2021](#); [Naughton et al. 2021](#)). The CWN is aware of WBS activity at over 80 locations in Canada ([CWN 2021b](#)). EU countries are widely adopting WBS ([Gawlik et al. 2021](#)), including the Netherlands, which began monitoring WWTPs in February 2020 and has now been monitoring all 352 WWTPs in the country daily since June 2021 as well as 250 WWTPs in Spain since mid-2020 ([Randazzo et al. 2020b](#)).

Early assessment of WBS

The rapid advancement of WBS has been driven by the demands of the severe public health challenges posed by the pandemic and has taken place, while procedures for WBS have been developed and assessed in progress. [Medema et al. \(2020b\)](#) provided an early, very detailed review and summary of the potential benefits and constraints for WBS being able to generate evidence that can be useful for informing public health decision-making. This overview was founded on understanding the successful history of wastewater surveillance for polio, which provides some similar characteristics to COVID-19 for detection in wastewater, and a review of 21 WBS publications (11 peer-reviewed and 10 preprints). Several concerns were elaborated, including how well wastewater surveillance can be understood in terms of how it represents the cumulative human sources of virus, how quantitative results are reported to be meaningful and how wastewater SARS-CoV-2 results can be understood in relation to community COVID-19 prevalence and data limitations that restrict the ability of wastewater results to accurately estimate community COVID-19 prevalence.

Occupational and environmental risk of COVID-19 transmission

Although much of the research and applications have been focused on WBS and its potential and benefits, once evidence was released that SARS-CoV-2 was found in clinical samples of faces and urine from hospitalized cases ([Lo et al. 2020](#)), a primary question arose whether there was a substantial occupational risk to sewer and wastewater treatment plant

² As of July 31, 2021.



Figure 1 | Geographic distribution of available publications on WBS (A1: Iglesias *et al.* 2021; A2: Ahmed *et al.* 2020; Black *et al.* 2021; B1: Ahmed *et al.* 2021a; B2a: Fongaro *et al.* 2021; B2b: Prado *et al.* 2021; B2c: Acosta *et al.* 2021; C1a: Acosta *et al.* 2021a, 2021b; C1b: D'Aoust *et al.* 2021a, 2021b; C2: Ampuero *et al.* 2020; C3a: Zhang *et al.* 2020a; C3b: Zhao *et al.* 2021; C3hk: Xu *et al.* 2021; C4: Mlejnkova *et al.* 2020; E: Guerrero-Latorre *et al.* 2020; F1: Hokajarvi *et al.* 2021; F2a: Trottier *et al.* 2020; F2b: Wurtzer *et al.* 2020; G1a: Agrawal *et al.* 2021; G1b: Westhaus *et al.* 2021; G2: Pechlivanis *et al.* 2021; H: Roka *et al.* 2021; I1a: Arora *et al.* 2020; I1b: Hemalatha *et al.* 2021; I1c: Kumar *et al.* 2021; I2: Nasser *et al.* 2021; I3: Bar Or *et al.* 2020, 2021; I4a: Rimoldi *et al.* 2020; I4b: LaRosa *et al.* 2020, 2021; J: Haramoto *et al.* 2020; Hata *et al.* 2021; Kitamura *et al.* 2021; Miura *et al.* 2021; M: Carillo-Reyes *et al.* 2021; N1: Napit *et al.* 2021; N2: Medema *et al.* 2020a; P1a: Sharif *et al.* 2021; P1b: Yaqub *et al.* 2021; P2: Tomasino *et al.* 2021; Q: Saththasivam *et al.* 2021; S1: Hong *et al.* 2021; S2: Wong *et al.* 2021; S3: Johnson *et al.* 2021; Street *et al.* 2020; S4a: Balboa *et al.* 2021; S4b: Chavarria-Miró *et al.* 2021; Fernandez-Cassi *et al.* 2021; Rusinol *et al.* 2021; S4c: Randazzo *et al.* 2020a, 2020b; S5: Saguti *et al.* 2021; S6: Goncalves Cabecinhas *et al.* 2021; T1: Wannigama *et al.* 2021; T2: Alpaslan Kocamemi *et al.* 2020; Kocamemia *et al.* 2020; UAE: Albastaki *et al.* 2021; Hamouda *et al.* 2021; UK: Hillary *et al.* 2021; USAz: Betancourt *et al.* 2021; USCa: Graham *et al.* 2021; USCo: Reeves *et al.* 2021; USCT: Peccia *et al.* 2020; USFI: Gering *et al.* 2021; USGa: Liu *et al.* 2020; USHi: Li *et al.* 2021; USIn: Bivins *et al.* 2021; USKy: Fuqua *et al.* 2021; USLa: Sherchan *et al.* 2020; USMa: Wu *et al.* 2020; USMi: Miyani *et al.* 2020; USMn: Melvin *et al.* 2021; USMt: Nemudryi *et al.* 2020; USNv: Gerrity *et al.* 2021; USNy: Green *et al.* 2020; Wilder *et al.* 2021; USNc: Gibas *et al.* 2021; USTx: Stadler *et al.* 2020; USUt: Weidhaas *et al.* 2021; USVa: Curtis *et al.* 2020; Gonzalez *et al.* 2020; USWi: Feng *et al.* 2021).

workers and others to environmental exposures via the water route (Lodder & de Rosa Huisman 2020). Jones *et al.* (2020) performed a detailed review of available evidence about those possibilities. They reported the abundance of detected gene copies³ (gc) signaling the presence of SARS-CoV-2 in urine ($\sim 10^2$ – 10^5 gc/mL) and feces ($\sim 10^2$ – 10^7 gc/mL), which are both lower than the virus count in nasopharyngeal fluids ($\sim 10^5$ – 10^{11} gc/mL). Domestic sewage will contain all three of these excretions because it is not limited to toilet drainage, but also includes bath and laundry wastewaters. Jones *et al.* (2020) concluded that other than direct person-to-person contact, sewage is very unlikely to transmit COVID-19 in the environment, although Kang *et al.* (2020) provided probable evidence for sewage aerosol transmission in an apartment building. Giacobbo *et al.* (2021) agreed that extensive aquatic environmental transmission of COVID-19 was not likely and noted that as of the submission date of their review (February 1, 2021), there had been no case of COVID-19 attributed to SARS-CoV-2-contaminated water or wastewater among the more than 100 million cases worldwide (262 million cases as of November 30, 2021). Ahmed *et al.* (2021b) reviewed the critical distinction between possible and probable wastewater transmission of COVID-19. Buonerba *et al.* (2021), in a wide-ranging review about the hazards associated

³ Gene copies that can be detected by PCR need not and most likely are not from intact, viable virus particles, meaning that those detections likely do not reflect infectious risk.

with SARS-CoV-2, reported that conventional water disinfection inactivated this virus and this finding was also verified by Greaves *et al.* (2021).

Dada & Gyawali (2021) performed a quantitative microbial risk assessment (QMRA) of occupational exposures to wastewater containing SARS-CoV-2 under three levels of assumptions of community COVID-19 infection and judged the occupational risks to be low. Zaneti *et al.* (2021) also performed QMRA for moderate, aggressive and extreme exposure scenarios, causing worker ingestion of wastewater, and estimated that the two higher scenarios exceeded the WHO population infection risk criteria based on Disability-Adjusted Life Years. Regardless, both QMRAs had to rely on limited relevant knowledge about SARS-CoV-2, notably for estimating the critical dose–response relationship using an estimate for SARS-CoV-1 and no direct evidence to estimate worker exposure to SARS-CoV-2. There has been, as yet (as of November 30, 2021), no epidemiological evidence of occupational risk of COVID-19 among wastewater personnel. In any case, domestic wastewater is known to pose an infection risk from a wide variety of enteric pathogens, making it essential that anyone occupationally exposed to untreated wastewater be provided with and effectively use appropriate personal protective equipment. Independent of these quantitative risk predictions, it should be clear that sampling for WBS must be conducted under established occupational health and safety practices for any exposure to wastewater.

Variability of methods used for detecting SARS-CoV-2 in wastewater

The majority of WBS has relied on detecting and quantifying the presence of genetic fragments of SARS-CoV-2 in wastewater using reverse transcription-quantitative polymerase chain reaction (RT-qPCR, described in detail along with other analytical options by Feng *et al.* 2020), as the foundational method for the detection of genetic signals of SARS-CoV-2 in wastewater. However, the dominant focus of much of the research and discussions on the strengths and limitations of the PCR analysis methods, while clearly critical to the validity of WBS, can serve to oversimplify the reality that there are multiple steps involved in sample collection and preparation required to process and concentrate wastewater samples. Each of these steps can introduce variability, errors or uncertainty to the utility and ultimate interpretation at many points in the process, prior to running the ultimate PCR analysis (Kumblathan *et al.* 2021).

Analytical techniques for the detection of SARS-CoV-2 to support WBS rapidly evolved over 2020 and early 2021 in parallel across many research laboratories. Rather than a single standard technique or protocol, the result has been the development of a variety of different, but apparently effective approaches. Hamouda *et al.* (2021) and Zhou *et al.* (2021) provided overviews of the different methods involved in surveillance of SARS-CoV-2 in wastewater considering: sample preliminary treatment, virus concentration, virus extraction, virus analysis and data analysis/interpretation. Philo *et al.* (2021) reviewed the wastewater concentration processes and evaluated a few of them by comparing the recovery of a spiked surrogate human betacoronavirus (OC43). They found that the best-performing concentration method evaluated had an average recovery of 24.3% (95% CI: 23.8–24.9%). Gerrity *et al.* (2021) found greater variability in recovery of bovine coronavirus for four concentration methods with recoveries ranging from only $2.1 \pm 0.87\%$ up to $55 \pm 38\%$. Such studies face the challenge that the behavior of spike additions necessary to perform recovery evaluations will be influenced to an unknown degree by the partitioning of the spike between the solid and liquid phase, compared with the partitioning behavior of native SARS-CoV-2 in the authentic wastewater.

Methods seeking to detect and quantify genetic markers for SARS-CoV-2 from wastewater samples, which present a complex matrix for analysis compared to either clinical or clean water samples, face recognized challenges from inhibition of the PCR step due to complexities of the sample matrix that occur to an unpredictable degree from one wastewater sample to another. Yet, in a survey of published results for SARS-CoV-2 in wastewater or sludge, Buonerba *et al.* (2021) found only 3 out of 43 publications specifically reported treating samples for inhibitor removal.

Taken together, the results from these and other studies about the range of different methods and protocols currently in active use suggest that, with WBS for SARS-CoV-2, there is unlikely to be a single standardized methodology established in the near future. In lieu of the development of a single standardized method for WBS for SARS-CoV-2, there is a clear need for the development of standardized quality assurance and quality control (QA/QC) requirements that can streamline the approach to the evaluation of the various methods, improving confidence in the reliability of the analytical results from the different methods and making more useful and reliable any comparisons between different sites or laboratories.

Gawlik *et al.* (2021) have called for developing a QA/QC framework, noted that the EU is committed to implementing a proficiency test and called for more interlaboratory testing such as reported by Chik *et al.* (2021). The establishment of

standardized QA/QC requirements should significantly improve the basis for greater confidence in the use of the analytical data for public health applications going forward. Ultimately, there is a need to seek an approximation of proficiency testing that is normally done by public health laboratories for diagnostic analyses. Currently, given the variety of actual techniques in use and the variability of site conditions, although confidence in the consistency of results over time for a given location and lab is likely to be improved, these various sources of uncertainty are likely to make it a major challenge to compare results across different laboratories using different methods.

Representativeness of samples and analytical results

In addition to potential sources of variability in quantifying the genetic signal present in collected samples, the use of WBS data to inform public health analyses must also consider the representativeness of the samples themselves with respect to actual SARS-CoV-2 inputs to the system, including the variability in samples relative to sewer system conditions. For example, the interpretation of concentration results for SARS-CoV-2 in wastewater is subject to variable dilution of sanitary sewage (household wastewater) by groundwater infiltration into sewers and mixing with stormwater. The variable nature of wastewater, the fecal strength of wastewater and the proportion of SARS-CoV-2 contribution to ultimate samples will also differ between and within North America and Europe/Asia/Africa, e.g., because of different per capita domestic water usages and different system designs (Jones *et al.* 2021). The use of fecal indicator substances as part of the sample analysis has become a common means to attempt to 'normalize' wastewater results for variation in sewage fecal strength, with common choices being pepper mild mottle virus (PMMoV) and crAssphage (Wilder *et al.* 2021; Zhu *et al.* 2021). Such normalization techniques are continuing to be assessed to determine whether they will be more useful for informing public health decisions than normalizing by flow.

Under the conditions of rapid uptake and implementation of emerging sampling and analytical techniques, there have been relatively limited opportunities to standardize and validate techniques while seeking to respond to urgent needs for rapid results. Although there is general agreement about the desirability of having standardized sampling and analytical protocols, it has not been possible to implement such standardization while simultaneously performing WBS during the course of the pandemic. Chik *et al.* (2021) reported on an interlaboratory study among eight laboratories organized by the CWN. Wastewater from the City of Winnipeg, at a time when it had only 85 known cases in a total population of 700,000 (1.2 cases per 10,000), was provided as a blank. This was spiked at low and high levels with Gamma-irradiated inactivated SARS-CoV-2 and human coronavirus strain 229E at low (18 ± 2 gc/mL and 10 infectious units per mL, respectively) and high ($1,800 \pm 200$ gc/mL and 1,000 infectious units per mL, respectively) by Canada's National Microbiology Laboratory (Public Health Agency of Canada). The samples were then distributed to the participating labs which were blinded to sample content. All laboratories distinguished the spikes within a total range of $1.34 \log_{10}$ on the high spike.

The Water Research Foundation funded an interlaboratory study involving 32 U.S. laboratories using a total of 36 specific standard operating procedures representing eight groups of methods (Pecson *et al.* 2021). Each participating laboratory received two raw wastewater samples believed to contain native SARS-CoV-2. Recoveries of a spike of OC43 showed a $7 \log_{10}$ range, indicating that reported results needed to be corrected for recovery, thereby showing 80% of recovery-corrected results were within a band of $\pm 1.15 \log_{10}$.

Detection of low levels of SARS-CoV-2

The lowest concentration of SARS-CoV-2 that can be reliably detected in wastewater using an analytical method has been a key consideration in the usefulness of WBS ever since several early studies established that it could be realistically detected at relatively low levels (Ahmed *et al.* 2020; Gonzalez *et al.* 2020; LaRosa *et al.* 2020; Lodder & deRoda Huisman 2020; Medema *et al.* 2020a; Randazzo *et al.* 2020a, 2020b; Sherchan *et al.* 2020; Wurtzer *et al.* 2020). The application of the technique in a surveillance capacity to provide early warning of the presence of the pathogen, prior to its recognition through clinical testing, requires confidence in the ability to reliably detect the presence of the pathogen at very low levels when there are few cases contributing to wastewater.

The lowest concentration of gc that is consistently detectable by a method is commonly referred to in the WBS literature as 'sensitivity' because that use of the term is common in the environmental and analytical sciences. However, medical sciences and epidemiology ascribe a different meaning to the term sensitivity: the conditional probability that, given that the analyte is present, how likely it is that it will be detected (Rizak & Hrudey 2006). Therefore, we will use the

expression minimum detectability level to avoid that potential confusion. Regardless of the term used, a consideration of how well WBS can detect the presence of genetic indicators of SARS-CoV-2 at very low levels in wastewater is an important assessment to be made when considering the findings of different labs and studies. The minimum detectability levels that are too high will allow for false negatives with WBS for SARS-CoV-2. In contrast, other than the possibility of cross-contamination during sample handling in the field or the lab, contamination of reagents or poor specificity of target genes (Ahmed *et al.* 2022), false positives with WBS are generally a less likely concern. False positives would be a concern in low COVID-19 prevalence areas where detection in wastewater may not be expected, thereby making any detection a matter of interest.

A summary of reported detection levels was provided by Medema *et al.* (2020b), reporting that for inlet wastewater to WWTPs the lowest value was 19–120 gc/L (Ahmed *et al.* 2020). However, it is difficult to assess levels of detection across studies with confidence based on the comparison of published results to date because of the differences in the way that they are interpreted or reported. Ahmed *et al.* (2021b) seek to better clarify this point on the relevant elements of minimum detectability level in their paper by providing a distinction between assay and method sensitivity/precision.

Monitoring for SARS-CoV-2 in wastewater and capability for early warning

Monitoring at WWTPs and early warning

From the outset, a common reason for promoting public health use of WBS as a contributing data source for COVID-19 management has been the potential for it to provide an ‘early warning’ of the presence of COVID-19 in the population contributing to that sewer system. As discussed below, assessing this potential for practical application to public health decisions based on reported results must consider the actual timelines. The ability to detect the genetic signature of SARS-CoV-2 in wastewater samples that were collected, but not reported, prior to dates of reported COVID-19 detection from clinical samples must be distinguished with sufficient confidence from real-time detection and WBS reporting prior to clinical reporting being able to support decisions about public health measures.

The possibility of the quantitative WBS signals providing early warning of the appearance of the virus in community wastewater, prior to detection in clinical samples, or the possibility of showing trends in community prevalence either to predict or to confirm changes in community prevalence of COVID-19 has been a common theme of published studies.

A summary of 17 studies that provide suggestive evidence that WBS might be able to deliver these predictive capabilities is provided in Table 1, but the devil is truly in the details. Fongaro *et al.* (2021) reported advanced wastewater SARS-CoV-2 detections: 107, 93, 22 and 9 days before the first clinically reported clinical COVID-19 case on March 13, 2020 in Florianopolis, Brazil. Chavarria-Miró *et al.* (2021) reported the same 41 days before the first reported COVID-19 case on January 15, 2020 in Barcelona, Spain. LaRosa *et al.* (2021) reported SARS-CoV-2 detections in Milan and Turin 65 days and Bologna 23 days before the first reported COVID-19 case on February 21, 2020. All these reports were clearly based on the analysis of archived wastewater samples dating back to 2019 before there was any awareness of COVID-19 and clinical testing at those locations. As reported earlier and widely cited, Medema *et al.* 2020a analyzed contemporary wastewater samples that were reported to provide a SARS-CoV-2 signal 6 days before the first COVID case was reported in Amersfoort, The Netherlands. Peccia *et al.* (2020) reported a SARS-CoV-2 signal in primary sludge 0–2 days ahead of clinical test results, according to date of collection, and 6–8 days ahead by date of clinical reporting for New Haven, CT, USA. Nemudryi *et al.* (2020) in Bozeman, MT, USA, D’Aoust *et al.* (2021a) in Ottawa, Canada, Roka *et al.* (2021) in Budapest, Hungary and Kumar *et al.* (2021) in Gandhinagar, India all inferred advanced wastewater signals from 2 days to up to 2 weeks compared with clinical COVID-19 case reports.

All of these reports are subject to the variability in clinical testing programs that were being implemented to varying degrees as the pandemic emerged in early 2020. Multiple factors were involved, such as the availability of testing, timing of reporting relative to test date and policies about who could be tested for whatever reasons (e.g., persons who are asymptomatic, symptomatic, under active treatment or exposed to active cases). Even without these challenges, most of these reports about early warning signals are based on retrospective analysis. This distinction is critical because an expectation, based on retrospective, not ‘real-time’, analysis, that WBS can provide ‘real-time’ advance warning, is subject to important details of timelines even given the potential advantages of WBS (can respond to asymptomatic cases, is not subject to inevitable clinical testing limitations about patient choice, policies about who can get tested, availability of testing and time delays in reporting clinical testing). As an illustration of the variability of the clinical testing numbers, Gonzalez *et al.* (2020) reported that for a WBS

Table 1 | Publications reporting possible early warning from sampling WWTPs

Location	Sampling	Monitoring period 2019/2020	WW detection vs. relevant clinical case	Comments	References
Brazil, Santa Catarina, Florianópolis	Sewer manhole 24 h composites	Frozen samples Oct 30, 2019 to Mar 4, 2020	WW detections Nov 27, Dec 11 archived, Feb 20, Mar 4	First confirmed case on Mar 13 in region, WW detection in frozen -0 °C, archived samples	Fongaro <i>et al.</i> (2021)
Spain, Barcelona	Two WWTPs, 24 h composites	Archived samples back to Dec 2019 until May June 2020	First detection in archived WW sample, 41 days before the first case reported 15 Jan	Clearly, a retroactive determination based on an archived WW sample. Later WW samples tracked reported cases	Chavarria-Miró <i>et al.</i> (2021)
USA, LA, southern Louisiana	Influent WW, 2 WWTPs, 9–24 h composites, 6 grabs	Jan 13 to Apr 29 4 sample rounds	Detected in last 2 rounds Apr 8 and Apr 29 when cumulative cases were already ~4,000 and ~6,200	No WW signal when no cases were known, but WW signal was clear when cases were documented. No insight was provided on early warning	Sherchan <i>et al.</i> (2020)
Italy, Milan, Turin, Bologna	Influent WW, 5 WWTPs, 24 h composites	Oct 9, 2019 to Feb 28, 2020	First detections in archived WW were Dec 18, 2019 in Milan & Turin and Jan 29, 2020 in Bologna	First clinical case reported on Feb 21, 2020, so Milan & Turin results were 65 days earlier and Bologna was 23 days earlier	LaRosa <i>et al.</i> (2021)
The Netherlands, Amsterdam, Amersfoort, Apeldoorn, Den Haag, Schipol, Tillburg, Utrecht	Influent WW, 7 WWTPs, 24 h composites	4 sample rounds over 48 days Feb 5 to Mar 25	Gene copies per mL of wastewater correlated (log-log) with cumulative clinical cases	First WW signal detected in Amersfoort on Mar 5, 6 days before the first cases were reported, the WW lead time was established retroactively	Medema <i>et al.</i> (2020a)
Sweden, Gothenburg	Influent WW, 1 WWTP 24 h composites	Feb 10 to Jul 5 weekly samples over 20 weeks	WW showed 4 peaks over the 20 weeks. No data on cases, only hospitalizations	Limited granularity of data, but the claim was that WW data provide 3–4 week warning of hospitalization peaks	Saguti <i>et al.</i> (2021)
Australia, QLD, Brisbane (SEQ)	Influent WW, 2 WWTPs, composites	Feb 24 to Apr 4; 8 sample days over 39 days	1st WW 'weak' detection on Mar 26, when total cases in prior 28 days were ~120 cases, 2nd WW 'weak' detection on Apr 4 with ~250 cases in prior 28 days	WW signals presented as proof of concept for detecting SARS-CoV-2 signals in WW, but this paper does not provide evidence for meaningful WW early warning of cases	Ahmed <i>et al.</i> (2020)
USA, NV, Las Vegas, South Nevada	Primary effluent WW, 2 WWTPs, 1 large, 1 small grab at daily low flow, some 24 h composites	Mar 2 to May 27, 29 daily samples over 61 days	First WW detection occurred on Mar 09 when clinical cases had first been reported to health. Now, archival data show Mar 9 symptom onset date had a 7-day running avg=12.4 cases/days for ~2 million population	WW detection was sensitive to detect a signal of SARS-CoV-2 for a WWTP serving a population of ~1 million (not entire ~2 million), but WW sampling started after case onsets and the first WW sample on Mar 2 was not positive. Does not support early warning for a large WW system	Gerrity <i>et al.</i> (2021)
France, Paris region	Influent WW 3 WWTPs, composites	Mar 5 to Apr 23	Mar 05 WW positive vs. Mar 4 consultation about COVID symptoms	The WW results do not clearly support advanced early detection in WW of clinical cases	Wurtzer <i>et al.</i> (2020)
Japan, Ishikawa preft., Toyama preft.	Influent WW, 5 WWTPs, morning grabs	Mar 5 to May 29	Mar 19 detection in Ishikawa WW when there were 4 clinical cases (0.3/100,000), Apr 3 detection in Toyama WW when there were 8 cases (0.8/100,000)	The first WW detections happened when the reported clinical cases were very low, but the WW signal cannot be claimed to be found in advance of clinical cases being reported and do not account for under-reporting of cases	Hata <i>et al.</i> (2021)
USA, VA Southeast Virginia	Influent WW 9 major WWTPs, composites	Mar 9 to Jul 28 weekly	Mar 9 first WW positive, Mar 9 first clinical cases confirmed		Gonzalez <i>et al.</i> 2020

				The clinical case numbers were limited by testing which covered only 69 patients on Mar 9, rose to 1.18 M by end of 21 weeks	
Spain, Murcia region <i>Murcia, Cartagena, Molina de Segura, Lorca, Cieza, Totana</i>	Influent WW 6 WWTPs, morning grabs	Mar 12 to Apr 14 approximately every 5th day	WW detection Mar 12 in Lorca, Mar 16 in Murcia and Cieza, first clinical case, Mar 8. Cases prevalent in Murcia Mar 20, all 3 by Mar 25	The data for detected cases vs. detection in wastewater provide no firm basis for claiming early warning because data are not readily comparable	Randazzo <i>et al.</i> (2020a)
USA, CT New Haven metro Hamden, East Haven, Woodbridge	Primary sludge, 1 WWTP morning grabs	Mar 19 to Jun 1 daily	RNA in sludge were 0–2 days ahead of clinical test results by date of collection, 6–8 days ahead of positive test results by the reporting date	Sludge results were not a leading indicator vs. positive test results by date of specimen collection. WW led lagging indicator – hospitalizations by 1–4 days and test results by clinical result reporting date by ~1 week.	Peccia <i>et al.</i> (2020)
USA, MT, Bozeman	Influent WW, 1 WWTP	Mar 30 to Jun 12, 17 daily samples over 74 day period	WW detections near detection limit and time in a decline of the first wave and a rise of the second wave. Limited data, paper inferred WW could precede clinical reports by 2–4 days	Based on retrospective interviews, clinical testing for COVID-19 typically occurs 3–9 days after symptom onset and may vary, depending on multiple policy factors. WW testing can detect signals during this window before reporting clinical testing	Nemudryi <i>et al.</i> (2020)
Canada, ON, Ottawa	Primary sludge 1 WWTP, 24 h manual (4×6 h) composites	Every 2nd day, Jun 20 to Aug 4	WW signals correlated with case signals: 1. new daily cases, 2. % positivity, 3. hospitalization	Extensive WW testing program provided credible evidence of WW signal preceding case increases by 48 h, clinical testing at 81/100 k population	D'Aoust <i>et al.</i> (2021a)
Hungary, Budapest	Influent WW, morning grabs at 2 WWTPs, composites at 1	Weekly for 44 weeks, Jun 5 to Nov 3	WW signal $r^2=0.72$ ($p<0.001$) with daily new cases during 17 week rising case period, 6–333 new daily cases	Clear, significant correlations of WW signal with daily new cases and active cases. Possible ~1 week advance WW signal rise apparent.	Roka <i>et al.</i> (2021)
India, Gandhinagar	Influent WW, 4 WWTPs, grab samples at 1	Bi-weekly, then weekly Aug 7 to Sep 30	Based on inference from patterns for infrequent WW sampling claim 1–2 week WW advance warning	No information provided on the timeliness of COVID case reporting and infrequent data make the claims of 1–2 week advance warning unpersuasive	Kumar <i>et al.</i> (2021)

sampling program that ran from early March to the end of July, 2020, the number of individuals who had been clinically tested over that period increased from 69 to 1,180,000.

Bibby *et al.* (2021) provided an insightful analysis of the capability of WBS to provide early warning by considering estimates for the time lag for fecal shedding of SARS-CoV-2 before or after the appearance of symptoms (a major driver for individual clinical testing) and the time delays in reporting clinical results before WBS results. They concluded that WBS could, at best, normally be expected to provide about 4 days of early warning for circumstances where clinical testing is readily available and reported rapidly.

Authentic early warning with WBS is inevitably subject to the frequency of wastewater sampling, i.e., sampling only once a week could not guarantee advance warning of less than a week regardless of the inherent meaning of the WBS signal. Likewise, sample shipping, processing, analysis and result reporting time lags will determine how much 'real-time' early warning could actually be achieved for any particular WBS program.

Sampling wastewater in sewers to identify community hot spots or institutions

The potential for sampling wastewater directly from sewers serving known populations offers a number of potential advantages, including less dilution of viral load and greater potential to detect relative increases in viral load, e.g., from non-detectable to readily detectable and an ability to target wastewater results to possible remedial actions. Unavoidable or potential disadvantages include a smaller contributing population to provide a viral signal, samples from sewers that are not routinely available, thereby requiring additional targeted sampling, sewer sampling being inherently more dangerous than obtaining samples from a WWTP and requiring professionally trained personnel. There will be greater uncertainty about how representative a sample can be that is taken from a sewer with varying depth of flow. Some success has been reported with the use of Moore swabs (Rafiee *et al.* 2021) and purpose-designed passive samplers (Schang *et al.* 2021) for obtaining what are effectively composite samples from sewers.

Table 2 summarizes results from nine selected studies that made use of in-sewer sampling for the detection of SARS-CoV-2 in wastewater. Chavarria-Miró *et al.* (2021) were monitoring both wastewater influents to WWTPs and grab samples from urban sewers in Barcelona, Spain and when the WWTP samples declined to non-detectable levels, sewer monitoring identified COVID-19 hot spots that facilitated the adoption of local mitigation measures. Wong *et al.* (2021) and Xu *et al.* (2021) used sewer sampling in Singapore and Hong Kong, respectively, to identify hot spots in apartment buildings that guided clinical testing and quarantining requirements. Acosta *et al.* (2021) sampled sewers that served acute care hospitals in Calgary, Canada, finding that the viral signals indicated the occurrence of hospital-acquired infections. Betancourt *et al.* (2021) and Gibas *et al.* (2021) used on-campus sewer sampling of student residences to identify the need for targeted clinical testing of students that allowed identification and quarantining of infected students before outbreaks occurred. Gibas *et al.* (2021) discussed the practical realities and logistics of operating sewer sampling such that it would provide results in a manner timely enough to allow for effective public health interventions. Harris-Lovett *et al.* (2021) reported on a consortium of 25 U.S. colleges that applied WBS in campus sewer systems. Black *et al.* (2021) identified the presence of COVID-19 cases residing in regions of the sewer network. This study was facilitated by a comprehensive sewer sampling program combined with an effective case identification and quarantine program that documented the geographic locations of cases. They found that it was possible, though not likely, that a single infected person in a sewer catchment could be detected, and that virus detection in sewer samples justified further investigation in an area of low or no COVID-19 prevalence.

The sewer monitoring applications mentioned above provide clear illustrations of the need for appropriate ethics guidance over WBS that was suggested for CWN by Hrudehy *et al.* (2021). Gawlik *et al.* (2021) noted that the European Commission has adopted this ethics guidance for its proposed sentinel sewage surveillance system for Europe.

Estimating COVID-19 prevalence, incidence and measures of epidemic spread

The possibility of being able to estimate the prevalence of COVID-19 in a community by means of WBS has been a particularly attractive aspiration. The logistical and practical limits of clinical testing to establish disease prevalence are clearly problematic in a pandemic when cases undergo waves of exponential growth, including providing effective access to clinical testing for a large and rapidly expanding population of interest, initial periods of no or mild symptoms among infected individuals, individual reluctance to be tested until medical care becomes necessary, uncertainty about quantitative levels of virus

Table 2 | Publications reporting sampling from sewer networks to identify specific locations

Location	Sampling	Monitoring time frame – 2020	WW detection with respect to relevant clinical case	Comments	References
United Arab Emirates, <i>Dubai</i>	9 pump stations grabs, weekly 49 sewer regions grabs, bi-weekly	Apr 22 to May 4 then May 7 to Jul 7	Monitoring period did not allow for the assessment of possible early detection	Overall decline of sewage signal with overall decline of clinical cases. Did show geographic distribution of sewage signal, but not related to known cases	Albastaki et al. (2021)
Spain, <i>Barcelona</i>	Grab, sewer sampling after prior WWTP sampling	May 18, May 25	WWTPs had declined to not detectable, but sewer samples showed detections	WWTP detections recurred June 2–8, followed by an outbreak (300 cases) declared in early July	Chavarria-Miró et al. (2021)
Singapore	Hourly composites, 2 diurnal periods from building sewer	Jul 4 to Jul 20, post: Jun 23 cluster of cases in building	WW was positive with spikes from Jul 4 to Jul 9 led to identification of 2 cases	1 case identified by WW was asymptomatic, 1 case had diarrhea explaining the observed WW spikes	Wong et al. (2021)
Canada, AB <i>Calgary</i>	Sewers – 3 acute care hospitals, 24 h composite, 2 per week	Aug to Dec	Spikes in cases were attributed to hospital-acquired infections	Patients being treated for severe COVID-19 are not using toilet facilities therefore not appearing in WW was recognized	Acosta et al. (2021)
USA, AZ <i>Tuscon</i>	Sewer manholes focussed on student residences, morning grabs, initially daily, then 2 per week,	Pilot program Aug 18 to Aug 31 then throughout fall semester Aug 24 to Nov 20	During 2 week pilot, positive WW samples led to clinical testing that detected positives the next day for 3 cases that were removed	WW testing provided early warning of 2 asymptomatic and 1 symptomatic students who were tested because of positive WW results for the residence, likely preventing an outbreak	Betancourt et al. (2021)
Australia, VIC <i>Melbourne & southern Victoria</i>	Sewer manhole sampling at 46 catchments, mostly weekly, composites and grabs	Aug 25 to Oct 27	Determined odds ratios – change in odds of detecting virus in WW if one or more COVID cases are in the catchment.	486,193 person-location days were recorded – 72.8% were within –2 to +55 days of illness onset. WW is the best at detecting cases within the first 2–3 weeks of illness. Positive WW justifies a search for cases	Black et al. (2021)
USA, NC <i>Charlotte</i>	19 sewer sites covering 17 student residences grabs 3 per week	Sep 28 to Nov 23 for fall semester	WW results were reported 26–30 h after sample collection, 40 positives for 332 samples analyzed	Sep 30 sample, analyzed Oct 1 positive, lockdown of 151 students Oct 2, testing revealed 1 positive isolated Oct 3. Over period, 8 positive WW identified 13 cases	Gibas et al. (2021)
USA, 17 states	25 campuses surveyed, 1–7 per week, 18/25 were composite samples	Aug–Sep (one in May) throughout fall academic session	WW program widely variable. Used experience to develop a framework for surveillance program design	Some programs definitely prevented outbreaks by WW detection. Some programs with extensive clinical testing were skeptical of the value of WW	Harris-Lovett et al. (2021)
China, Hong Kong	3 manholes, 3 h composites	Jun 8 to Sep 29	MH samples positive Jul 26, start clinical testing Jul 27	Cases identified in buildings Jul 29, giving 2 day lead time in WW detection	Xu et al. (2021)

shedding of individuals and challenges of timely data collection, reporting and analysis across the population of interest. In contrast, wastewater can represent viral signal contributions from an entire sewered population with a single daily composite sample. As attractive as this prospect for WBS appears, there are major challenges to delivering meaningful estimates of COVID-19 prevalence in the community based on WBS.

Hart & Halden (2020) provided an early proposal for modeling COVID-19 prevalence from WBS in promoting the merits of widespread adoption of WBS. The latter has clearly happened, but the analysis of WBS results to demonstrate the validity of resulting estimates of community COVID-19 prevalence remains to be shown. Farkas *et al.* (2020) noted that: '*translating the viral titres from wastewater into the actual number of cases within a community is highly challenging, if not impossible*' because of the number and difficulty of verifying assumptions such as the quantitative and temporal dynamics of viral shedding in feces at various stages of infection and the persistence of the viral signal in wastewater as a function of sewer travel time and environmental conditions. Cao & Francis (2021) retrospectively analyzed 9.5 months of WBS and clinical case data from a WWTP in Pennsylvania, applying a multivariate, time-series model to conclude, retroactively, that WBS data could predict the clinical case data. Fernandez-Cassi *et al.* (2021) provided a retrospective analysis of wastewater monitoring in Switzerland during the first wave of the pandemic in March 2020 and determined that WBS and confirmed clinical case numbers are useful and independent measures of the incidence⁴ of COVID-19 infection. They concluded that WBS provided a better estimate of the rise of incidence while confirmed case numbers provided a better estimate of the decline of incidence following the peak. Zhu *et al.* (2021) reported that understanding the viral shedding dynamics of infected individuals was the main limitation of translating WBS data into estimates of COVID-19 prevalence. Li *et al.* (2021) considered viral shedding dynamics among what they believed were five major elements contributing to uncertainty (virus excretion rates, virus decay in sewers, sampling location, preservation of samples and analytical detection) for estimating prevalence. They concluded that the analytical uncertainty of PCR detection of SARS-CoV-2 was the largest factor contributing to uncertainty in estimating prevalence. In making that judgment, Li *et al.* (2021) may not have sufficiently weighed that analytical accuracy is much more amenable to control by investigators than the highly variable and largely unknown virus excretion rates among individual cases over the course of infection during the rise and fall of the epidemiological curve.

The reproductive rate, R_0 , is an estimate of the number of new cases created for each incident case and, as such, R_0 provides critical insight for public health decision-making about the need for interventions to reduce the spread of the disease. Zhang *et al.* (2020b) estimated the reproductive rate R_0 for the COVID-19 outbreak on the Diamond Princess cruise ship as a median of 2.28 (95% CI: 2.06–2.52) using the clinical case data. This high R_0 demonstrates the COVID-19 transmission risk posed by the cruise ship environment. Huisman *et al.* (2021) estimated the *effective* reproductive rate, R_e , using longitudinal WBS data in Zurich, Switzerland and San Jose, CA, USA finding their R_e estimates to be as similar to those from case report data as were the R_0 estimates based on clinical data (observed cases, hospitalizations and deaths) to each other.

The proportion of clinical tests found to be positive (positivity rate) is another public health indicator relied upon to judge the status of an epidemic. Stadler *et al.* (2020) analyzed 22 weeks of WBS data for Houston, USA to determine that they could achieve a strong predictive indicator of clinical testing positivity rates 2 weeks in advance, providing evidence that proved useful for making real-time public health responses such as deploying clinical testing resources.

Detecting genetic variants of SARS-CoV-2

As the COVID-19 pandemic has persisted internationally since being declared in March 2020, the emergence of mutations of the original native strain of SARS-CoV-2, so-called variants of concern (VoCs), has frustrated efforts to control the pandemic. Even with the benefit of having remarkably effective vaccines, VoCs that are proving more transmissible than the native strain are causing new waves of infection primarily among unvaccinated populations. Consequently, the literature concerning the detection of VoCs has been growing extremely rapidly in recent months. As of the end of November 2021, WHO (2021b) has

⁴ '*Incidence* refers to the occurrence of new cases of disease or injury in a population over a specified period of time. Although some epidemiologists use incidence to mean the number of new cases in a community, others use incidence to mean the number of new cases per unit of population.... Prevalence differs from incidence in that prevalence includes all cases, both new and pre-existing, in the population at the specified time, whereas incidence is limited to new cases only.' <https://www.cdc.gov/csels/dsepd/ss1978/lesson3/section2.html>.

designated five mutated strains to be VoC: alpha, beta, gamma, delta and omicron (added on November 26, 2021). Delta cases have dominated and rose rapidly in many countries during 2021. WHO has also declared two variants of interest, lambda and mu, and seven variants being monitored for evidence of behavior that may justify concern. These variants reflect the reality that viruses will inevitably mutate during a pandemic.

WBS is proving to be a valuable approach to aiding in the tracking of VoCs. [Jahn et al. \(2021\)](#) used WBS in Switzerland to monitor for the alpha and beta variants between July and December 2020, finding the alpha variant in wastewater from a Swiss ski resort 2 weeks before it was first verified in a clinical sample. [Bar-Or et al. \(2021\)](#), [Goncalves Cabecinhas et al. \(2021\)](#), [Hillary et al. \(2021\)](#), [LaRosa et al. \(2021\)](#), [Martin et al. \(2021\)](#), [Pechlivanis et al. \(2021\)](#), [Prado et al. \(2021\)](#) and [Yaniv et al. \(2021\)](#) have all reported a variety of promising applications for detecting and tracking the first appearance of VoCs in wastewater which creates a particularly valuable prospect for WBS to enhance our knowledge about the evolutionary dynamics and behavior of a virus during the course of a pandemic. These insights will inevitably have to come from retrospective analyses.

Cost-effectiveness of WBS

Determining costs for implementing WBS is a challenge because costs will be determined by local and regional circumstances as well as by whether the investigators have existing capabilities and capacity. An informative attempt to estimate costs for WBS has been provided by [Gawlik et al. \(2021\)](#) who surveyed a group of European laboratories engaged in WBS; 13 groups from 7 countries responded to a questionnaire with 17 practical questions concerning costs. They concluded that a WBS monitoring program involving twice weekly samples at a European WWTP would cost approximately €25,000 per year. [Weidhaas et al. \(2021\)](#) estimated WBS programs performed in Utah, USA, which include weekly sampling of WWTPs treating greater than 4 mL/day, would cost approximately US\$220 per sample and cover 79% of the Utah population (US\$0.005 per person/week).

Supporting public health decision-making

In making the case that WBS should be ethically governed as public health surveillance, [Hrudey et al. \(2021\)](#) note that the first guideline offered by the WHO (2017) guidance for public health surveillance included the statement that: ‘*Surveillance systems should have a clear purpose and a plan for data collection, analysis, use and dissemination based on relevant public health priorities.*’ Although this implied connection between WBS and public health decision-making may be self-evident to many, it is not clear to us from the WBS literature that all investigators do recognize that WBS should be first and foremost about informing public health decision-making.

[Medema et al. \(2020b\)](#) make the public health priority explicit and describe the factors bearing on the ability of WBS to provide the requisite support to public health decision-making, including the design of adequate sampling strategies, considering the spatial and temporal resolution of sampling, reliable sample storage, replicate sampling and analysis, and quality controls for the molecular methods used for the reliable quantification of SARS-CoV-2 in wastewater. [Prado et al. \(2021\)](#) describe how a combination of WWTP and sewer sampling in the state of Rio de Janeiro, Brazil was able to use geoprocessing of data to support weekly updates to a heat map to promote public awareness of the local epidemic situation and need for public health interventions. [Hillary et al. \(2021\)](#) demonstrated with a longitudinal analysis of wastewater at WWTPs for six major urban centers in northwest England and Wales that tracking variants in wastewater reflected findings in clinical samples and WBS were able to confirm the success of lockdown measures (restricted movements and social distancing). However, they also note the need for a better understanding of analytical quantification of the genetic signals for SARS-CoV-2 to improve confidence in applying WBS data to support public health decision-making. The latter observation is consistent with the conclusions of [Li et al. \(2021\)](#) concerning analytical variability being the largest factor affecting the estimation of COVID-19 prevalence from WBS data.

DISCUSSION

Our analysis of possible capabilities projected for wastewater surveillance of SARS-CoV-2 focused on what information can be provided by WBS results that would be useful for informing public health decision-making. Perhaps one of the enduring benefits from all of the WBS initiatives has been that it required and achieved extensive interdisciplinary collaboration between environmental scientists/engineers and public health professionals. The recognition of the engagement of these groups led to the initiation of our ethics guidance for this activity (CWN 2020; [Hrudey et al. 2021](#)). That guidance reported

that the first ethical obligation for any public health surveillance activity is to ensure having a clear and meaningful purpose that is explained through a plan that outlines how data will be collected, analyzed, used for decision-making and, ultimately, made publicly available.

Our review of what has been published or otherwise disseminated about programs of WBS for SARS-CoV-2 has identified 10 factors that are potential advantages for this approach as a means of providing supplementary evidence that can inform public health decision-makers in a meaningful way. The relative importance of some of these factors may change as effective vaccination becomes more common, new VoCs emerge and the dynamics of the pandemic change.

Independence of WBS data from extraneous factors

Strengths

The measures of SARS-CoV-2 in wastewater are independent of policies and practices that inevitably govern the clinical testing of individual patients in the population. Policies for clinical testing have been variable between and within jurisdictions based on factors such as testing capacity, and economic, human and technical resource limitations. Wastewater is collected from individual residences, commercial and industrial buildings as well as community healthcare facilities or other institutions. Wastewater collection captures the fecal and household discharges from all persons using the sanitary facilities of those buildings. The nature of these characteristics is entirely independent of all clinical testing policies applied to individuals.

Limitations

The composition of the contributing population is a function of the sewer network design and in some areas, substantial populations are served by septic tank systems, not connected to the local sewer system, and septic tank pumpouts are only intermittently discharged to a WWTP. Knowledge of the population served by a sewer network can only be known in overall potential terms, with the actual contributing resident populations subject to unknown variation.

Objectivity of data

Strengths

The measures of signals of SARS-CoV-2, provided they are subject to effective QA/QC programs to ensure they provide an accurate representation of what is actually present in the wastewater, can be largely free from biases arising from individual behaviors in seeking clinical testing. All residents of sewered communities, except those who are incontinent, will contribute fecal and other bodily wastes to the wastewater system.

Limitations

The understanding of how SARS-CoV-2 in wastewater relates to important factors like incidence and prevalence of COVID-19 infection is likely to be site-specific and will be subject to effective data collection and interpretation of SARS-CoV-2 fecal shedding rates.

Cost-effective data generation

Strengths

Wastewater sampling whether at a WWTP or in a sewer provides a sample over a knowable or estimable time period that is the composite contribution of a knowable or estimable population that goes beyond individuals, meaning that analytical costs per individual should be very cost-effective in comparison with individual clinical testing.

Limitations

The costs of an individual wastewater analysis are higher than an individual clinical test because there is more sample preparation for wastewater analysis. Costs for sampling from sewers will be higher than sampling from WWTPs where composite sampling is often already available and only entails splitting of an existing sample.

Early detection of infection

Strengths

The potential for early detection is provided by the reality that asymptomatic and low symptom individuals who are less likely to be given clinical tests will still contribute SARS-CoV-2 to wastewater. Likewise, WBS is not dependent on the logistics of reporting individual clinical test data that may contribute to delays.

Limitations

Real-time early detection is governed by wastewater sampling frequency, sample delivery time, analytical processing time and reporting time, all of which can substantially reduce the capability of WBS to provide an early warning.

Tracking of trends**Strengths**

Quantitative WBS data so far have been shown to provide possible indicators of success or failure of public health measures (mask mandates, lockdowns and vaccination) as well as apparent changes in the prevalence of COVID-19 in the community.

Limitations

Real-time WBS trend data would be the most useful for informing public health decision-making, but how closely WBS data can approximate real-time data is subject to many practical limitations such as frequency of sampling, sample delivery time, analytical time and reporting time.

Estimation of disease prevalence**Strengths**

WBS data are not subject to the vagaries of virus shedding by asymptomatic individuals and arbitrary constraints that govern clinical testing such as policies (e.g., testing only individuals under medical treatment), personal choices to be tested and clinical testing capacity.

Limitations

WBS data cannot be translated into COVID-19 prevalence without site-specific knowledge about the quantitative and temporal dynamics of viral shedding in feces at various stages of infection, the persistence of the viral signal in wastewater as a function of sewer travel time and relevant environmental conditions.

Potential for informing and engaging the public**Strengths**

WBS data have the capability to provide, subject to the limitations noted in the discussion above and adequate confidence in the validity of the data, geographically specific and timely information for the public about the status of the pandemic in their community and the need for public health interventions.

Limitations

The utility of WBS for informing the public will be subject to the quality and extent of communication and explanation of the meaning of the WBS data and the resource requirements noted above for providing timely information.

Application within sewer networks to detect hotspots**Strengths**

There have been a number of reports of successful detection of locations with infected individuals who were then confirmed by clinical testing and who could be quarantined to reduce the risk of further spread of COVID-19.

Limitations

WBS within sewer networks is more resource-intensive than WWTP monitoring, requires knowledge about the nature and extent of the catchment served by any sewer location and, inevitably, invokes greater ethics concerns about handling the data collected.

Monitor infections within institutions**Strengths**

There have been reports of successful detection of infected university students in on-campus residences who were confirmed by clinical testing and who could be quarantined to reduce the risk of further spread of COVID-19. Other institutions such as nursing homes and prisons are prospective candidates for this approach.

Limitations

WBS within sewer networks is resource-intensive, and sewer locations to cover the target institution may be difficult to identify and to access for sampling. This approach may also entail ethical considerations that will be affected by the nature of the institution involved.

Tracking of appearance/progression of viral VoCs

Strengths

As the pandemic has progressed, the emergence of variants of SARS-CoV-2 has become a matter of increasing concern as some have proven to be much more transmissible and VoCs are perpetuating the pandemic causing infections even while extremely effective vaccines have been widely applied. WBS is adaptable and capable of tracking the emergence of such VoCs in a sewered population, but also has the capability, with the use of appropriate technology, of tracking the evolution of viral infection over time through tracking of less notable mutations.

Limitations

The capability of WBS to track VoCs requires adaptation of analytical methods, and the more general tracking of minor mutations requires more sophisticated analytical technology that is not as widely available as the basic RT-qPCR most commonly used in WBS.

CONCLUSIONS

Since the initial identifications of SARS-CoV-2 viral fragments in wastewater in March 2020, there has been an explosive growth of research and development related to WBS applications worldwide. The result has been a proliferation of different methods and approaches, unprecedented levels of peer exchange and the emergence of a significant and expanding research literature base.

WBS should first and foremost be aimed at providing meaningful contributions to informing public health decision-making. To achieve the intended contribution to pandemic management, WBS initiatives should be designed with a clear up-front recognition of how WBS results can best address the questions and needs of the public health community. The devil will inevitably be in the details for assessing the significance of new findings, unknowns and data limitations in this rapidly developing field. As the pandemic continues to evolve, the reality will be that assumptions must be continuously re-evaluated and valid generalizations will be few. A particularly strong potential for WBS is to complement clinical data by providing evidence that is independent of the biases introduced by policies, practices and performance of population clinical testing, thereby providing more objective indicators of community COVID-19 infections.

Reliance on WBS data by the public health community depends upon assurance that such data are valid. Such assurance is necessary to be confident in using WBS data for public communications. Given the rapidly evolving WBS activity, there is not likely to be convergence on a 'single' or 'standard' method in the immediate future. Consequently, there is a clear need to develop standard protocols and approaches to providing QA/QC of the different approaches to substitute for authentic proficiency testing that is normally done by public health laboratories.

The potential for WBS to provide early warning should not be oversold because that potential can currently only be realized by a substantial commitment to frequent sampling and rapid sample delivery, processing, analysis and reporting. WBS can provide particular potential value in surveillance for the emergence of COVID-19 in areas of low disease prevalence and for disease appearance or progression within institutions (e.g., schools and long-term care homes).

WBS can provide complementary evidence about disease trends within a population, which may become increasingly important if clinical testing rates decline. However, obtaining meaningful estimates of disease prevalence (total case numbers) by WBS alone would involve many unknowns at a local level that are not likely to be obtained without substantial, resource-intensive local research that undermines the cost-effective advantages otherwise offered by WBS.

Since early 2021, the significance of dominant genetic VoC of the SARS-CoV-2 virus to pandemic conditions and responses has become a major focus. Some WBS programs are already providing a valuable approach for tracking the spread of VoCs. On a longer-term basis, WBS applications involving genetic sequencing will have the potential to retroactively reveal the evolution of the pandemic within a specific community.

For the future, there exists good potential to build upon the base of compliance data collected at WWTPs, rethinking how routine regulatory monitoring may support public health surveillance. Government support, coordination and leadership for WBS are fundamental to exploiting the potential for WBS to contribute to public health protection. In any case, one of the clear benefits from WBS initiatives has been recognition of, and response to, the need for extensive interdisciplinary collaboration among environmental scientists/engineers and public health professionals. The value that WBS has demonstrated toward understanding the progression and dynamics of the COVID-19 pandemic should justify future applications of WBS toward public health surveillance of other pathogens excreted to wastewater.

Finally, addressing ethical considerations will become increasingly important and apparent as WBS is applied to smaller areas and individual buildings or institutions where public health goals and personal privacy issues are more likely to be more obvious and come into conflict.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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