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Wastewater surveillance for SARS-CoV-2 to support return to campus: Methodological considerations and data interpretation

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Abstract

The COVID-19 pandemic has been challenging for various institutions such as school systems due to widespread closures. As schools re-open their campuses to in-person education, there is a need for frequent screening and monitoring of the virus to ensure the safety of students and staff and to limit risk to the surrounding community. Wastewater surveillance (WWS) of SARS-CoV-2 is a rapid and economical approach to determine the extent of COVID-19 in the community. The focus of this review is on the emergence of WWS as a tool for safe return to school campuses, taking into account methodological considerations such as site selection, sample collection and processing, SARS-CoV-2 quantification, and data interpretation. Recently published studies on the implementation of COVID-19 WWS on school and college campuses were reviewed. While there are several logistical and technical challenges, WWS can be used to inform decision-making at the school campus and/or building level.

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Wastewater-based epidemiology, COVID-19, SARS-CoV-2, Schools, Surveillance.

Introduction

The COVID-19 pandemic brought by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has claimed more than five million lives across the globe until the end of year 2021. The pandemic has been challenging for various institutions, such as school systems and universities, due to widespread closures and subsequent challenges in limiting the transmission upon re-opening their campuses [1,2]. School closures at all levels, from daycares through post-secondary institutions, have been utilized as a community mitigation measure or non-pharmaceutical intervention to limit the spread of SARS-CoV-2 within the community [1,3]. The potential for disease transmission among school-aged children and the known risks to staff and educators led to widespread closures of schools beginning in the spring of 2020. School closures have had unintended but detrimental side-effects on health, well-being, and educational development of students [1,4,5]. As schools re-open their campuses to in-person education, there is a need for frequent screening and monitoring of the virus to ensure the safety of students, staff, and the surrounding community.

Although individual diagnostic testing campaigns are commonly utilized to screen staff and students, these efforts are complicated by logistical difficulties, high cost, and long-term sustainability. Wastewater surveillance (WWS), also referred to as wastewater-based epidemiology (WBE), is a rapid and economical approach for obtaining comprehensive health data on a local, regional, national, and even global scale [6–9]. Because infected individuals shed the SARS-CoV-2 virus and viral fragments in their feces, genetic traces of SARS-CoV-2 can be detected in sewage and wastewater [10]. Analysis of a wastewater sample for the presence of SARS-CoV-2 genes can be an effective and efficient way to test defined catchment areas. Wastewater-based testing can be used alongside clinical testing to provide cost-effective and objective measures of the presence of COVID-19 in the community [11,12]. WWS affords a potential advantage that infected individuals contribute to the signal independent of health-seeking behavior or access to healthcare. Because of the ability to observe viral shedding prior to the onset of symptoms or even in the absence of symptoms, in some applications, WWS has provided an early warning to a local or regional surge in COVID-19 cases.

Prior to the SARS-CoV-2 pandemic, WWS had been implemented at school and college campuses to detect illicit drugs in various settings [13—15]. As schools began planning for the 2020—2021 school year, many applied WWS as a tool (Figure 1) to provide situational awareness and actionable information in support of COVID-19 mitigation efforts.

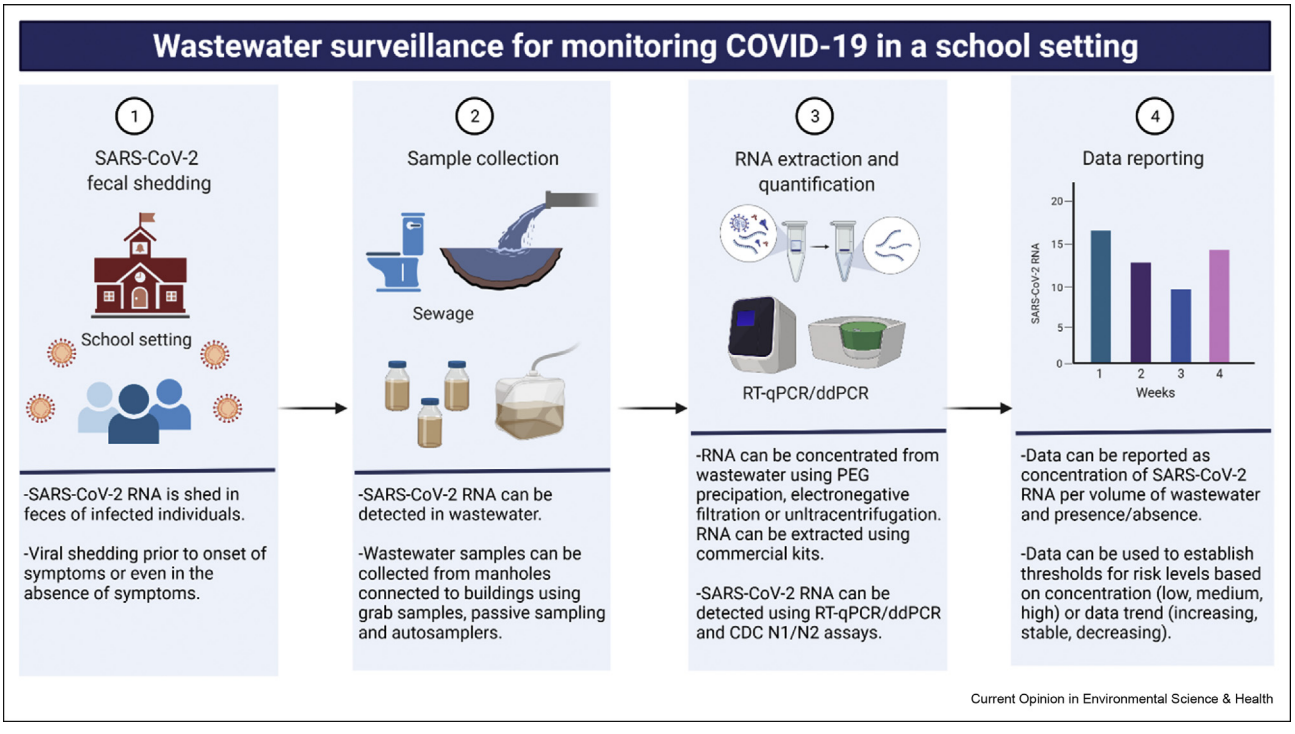
Emergence of WWS as a tool for safe return to school campuses

Near-source tracking (NST) is the process of WWS conducted in sewer drains and manholes that serve individuals or groups of buildings and allow for the detection of small numbers of infected individuals who are contributing to the waste stream [16]. NST, when combined with targeted clinical testing, has shown value in managing outbreaks and is now being widely used across various facilities [11,17—21]. Compared to the sampling of larger catchment areas within the sewershed (i.e., wastewater treatment plant influent), NST has the potential to be more sensitive in observing newly infected individuals, due to numerous factors including, but not limited to, the size of the contributing population, dilution of signal, and travel time (and associated viral degradation) of sewage from toilets to sample collection points. The concept of NST has been applied to school and university campuses, including the isolation of college dorms by collecting samples from sewer lines exiting specific buildings prior to their mixing with other sewage networks.

Following decisions to re-open campuses to in-person education for the 2020—2021 academic year, many researchers began monitoring sewage for the presence of SARS-CoV-2 to inform decision-making by health officials and campus administrators [22]. We describe studies in the scientific literature published in 2020—2021 that document the use of WWS within school campuses, including primary, secondary, and institutions of higher education.

Recently published research on the implementation of COVID-19 wastewater monitoring in school and college campuses is summarized in Table 1. Two recent studies [23,24] describe WWS as a tool that can support the mitigation of SARS-CoV-2 transmission for primary and secondary schools. Four additional preprints and published studies describe the implementation of WWS on institutions of higher education such as college campuses [11,20,25,26]. Additionally, a survey [22] of WWS on 25 US colleges and universities describe methods used to monitor campus wastewater for SARS-CoV-2 during the Fall 2020 semester. The authors discuss the challenges of implementing WWS on college campuses and describe a WWS framework that includes the information needs of decision-makers, wastewater

Figure 1



Technical framework for using wastewater surveillance for monitoring COVID-19 in a school setting.

Table 1

Recently published studies using COVID-19 wastewater monitoring in a school or college setting.

Locations of schools/ colleges	Sampling volume, method, and frequency	Virus concentration method	RNA extraction method	SARS-CoV-2 quantification method	Reference
Sixteen schools in England (ten primary, five secondary, and one post-secondary)	A total of 5 L of composite samples (7 h time-weighted) were collected twice a week initially (Tuesday and Thursday); then four times a week (Monday to Thursday) over a period of nine weeks (October–December 2020) using Aquacell P2-COMPACT (Aquamatic) autosamplers	Wastewater samples were centrifuged (30 min at 3,000×*g* at 4 °C), and supernatants were spiked with an extraction control murine norovirus before concentration using the poly-ethylene glycol (PEG) precipitation method with an overnight incubation	NUCLISENS® RNA extraction kit on a MINIMAG® (BioMérieux, France) following the manufacturer's instructions	RT-qPCR using the RNA UltraSense™ One-Step Quantitative RT-PCR System (ThermoFisher, UK) targeting the nucleoprotein (N), N1 fragment, and envelope protein (E) gene using a QuantStudio™ 7 Pro Real-Time PCR System (ThermoFisher, UK)	[23]
Three schools in Omaha Public School district in Nebraska, USA (two middle schools and one high school in a medically underserved area)	A total of 250 mL of wastewater grab samples were collected twice a week in sterile polypropylene containers from manholes adjacent to the buildings on school grounds over a period of five weeks (November 9–December 11, 2020)	A total of 70 mL of well-mixed wastewater was divided into two 50 mL conical tubes and centrifuged at 3,500 *g* for 20 min to concentrate solids; the supernatant was removed, and the remaining pellet was resuspended in up to 2 mL of water	Qiagen RNeasy PowerSoil Total RNA kit following the manufacturer's instructions	RT-qPCR using the IDT 2019-nCOV RUO kit. RUO kits include all published assays for the nucleocapsid genes N1 and N2 developed by the Centers for Disease Control and Prevention	[24]
Thirteen dorms at University of Arizona in Arizona, USA	Grab samples (1 L) were collected from manholes specific to each dorm using a pole/dipper and submerging a sterile Nalgene bottle into the flowing wastewater until it was full (August 24 to November 20, 2020)	Stepwise vacuum filtration of 70 mL aliquots through membrane filters of 0.8, 0.65, 0.45, and 0.22 μm pore sizes followed by centrifugal ultrafiltration of the filtrate using the CentriconPlus-70 filter, 100 kDa cutoff	Qiagen QIAmp Viral Mini Kit following the manufacturer's instructions	RT-qPCR using the IDT 2019-nCOV RUO kit performed using the LightCycler® 480 Instrument II (Roche Diagnostics, Indianapolis, IN)	[11]
Nineteen on-campus sites at UNC Charlotte were chosen to cover 17 dormitories as well as the University's Greek Village	Samples from dormitory sites were collected three times each week during the period of September 28, 2020–November 23, 2020; ISCO GLS compact and Hach AS950 portable autosamplers were used to collect composite samples (24-h time-weighted) from manholes or cleanout	A total of 40 mL of aliquot was taken from each sample, and the pH was adjusted to 3.5–4.0 using concentrated HCl, followed by the addition of $MgCl_2$ hexahydrate and electronegative filtration using 0.45 μm membrane	Qiagen QIAmp Viral Mini Kit following the manufacturer's instructions	RT-qPCR using the IDT 2019-nCOV RUO kit performed using a CFX96 qPCR thermocycler (Bio-Rad laboratories, Hercules, CA)	[25]
One manhole location near a campus apartment complex at Kenyon College in Ohio, USA, along with parallel sampling at Gambier Wastewater Treatment Plant	Grab samples initially followed by 24-h composite samples from manhole twice a week using a YSI PM-12 autosampler (November 2020 to January 2021)	Sample was centrifuged as well as filtered using a negatively charged HA filter membrane. Both filtrate and centrifuged pellet were extracted for RNA	Samples shipped to LuminUltra, Florida for analyses	RT-qPCR using the CDC primer set N1	[26]
Dormitories, community-use buildings, and library at		A total of 200 mL of samples were mixed with 8% (w/vol) PEG 8000		RT-qPCR using the CDC primer sets N1 and N2 performed using	[20]

(*continued on next page*)

Table 1. (*continued*)

Locations of schools/ colleges	Sampling volume, method, and frequency	Virus concentration method	RNA extraction method	SARS-CoV-2 quantification method	Reference
the Tulane University in New Orleans, Louisiana, USA	A total of 500 mL of grab samples from manholes were collected weekly (August 19 to December 1, 2020)	(Promega Corporation, Madison WI) and 0.2 M NaCl (w/v) followed by overnight incubation; samples were centrifuged at 4700×*g* for 45 min at 4 °C, and the pellet was resuspended in the remaining liquid, approximately 2–4 mL	Qiagen QIAmp Viral Mini Kit following the manufacturer's instructions	a StepOne Plus™ real-time PCR sequence detector (Applied Biosystems, Foster City, CA)	
One location at the University of Notre Dame	A total of 1200 mL of composite sample (24 h time-based) was collected daily from manhole using Avalanche multibottle, multifunction sampler (Teledyne ISCO, Lincoln, NE) from April 8, 2021 to May 26, 2021	A total of 50 mL wastewater subsample was centrifuged at 10000 g for 10 min at 4 °C. The pellet was then resuspended using 1 mL of a PBS/ Tween 20 solution (10 mM sodium phosphate, 0.15 M NaCl, and 0.05% Tween 20) by pipet mixing and vortexing	AllPrep PowerViral DNA/ RNA kit (Qiagen) following the manufacturer's instructions	RT-ddPCR using the CDC N1 assay performed using Bio-Rad QX200 Droplet Digital PCR System (Hercules, CA)	[56]

infrastructure, sampling plan, wastewater analysis, data interpretation, communication of results, and adaptations made to programs over time.

Methods for near-source tracking of SARS-CoV-2 RNA in wastewater

Below we have summarized a few methodological considerations when developing a wastewater-based monitoring program for schools and/or colleges.

Site selection

The surveillance program carried out by Gutierrez et al. [23] in England involved 16 schools located across neighborhoods of various income levels and with diverse school populations. While the demographics were described as diverse by the authors, no criteria or methodology was provided for site selection. Crowe et al. [24] conducted WWS in three urban schools within the Omaha Public School system. The authors indicated that sites were selected in medically underserved areas of the city to maximize value of the pilot. Demographics were not influential factors in site selection for college campuses. Instead, factors influencing site selection on college campuses were logistical ease of sampling, ability to associate findings with individual buildings with emphasis on dormitories, and cost. Some college campuses, as reported by Barich et al. [26] and Harris Lovett et al. [22], elected to monitor wastewater collected from treatment facilities serving their campuses or surrounding communities.

Before initiating a wastewater monitoring campaign in a school setting, several factors should be considered to establish the technical feasibility of routine monitoring for SARS-CoV-2 in wastewater [27]. The locations of the schools should be ideally in areas served by the municipal sanitary sewer system and sewer lines with free-flowing wastewater should be accessible through manholes and/or pumping stations. For manhole locations, sewer lines should be no more than 25 feet below the street level due to sampling equipment limitations [28]. The study design should also include such contextual information as sanitary sewer maps for the school buildings, flow rate and temperature of wastewater, and water usage bills. Lastly, coordination with school facilities departments helps to provide field human resources for routine collection of wastewater samples.

Sample collection and processing

Frequency, volume, and type of sample collected varied across all studies in school settings. Gutierrez et al. [23] reported sampling from school sites four times per week while Barch et al. [26] reported sample collection once per week. No studies reported less than weekly sample collection, including those that responded to the survey conducted by Harris Lovett et al. [22]. Sample volume collected from each sampling site ranged from 250 mL

to 5 L. While higher volumes may allow for detection at a lower limit of detection, processing, and concentration of higher volumes increase the time required. Strategies for sample collection included one-time "grab" samples, passive sample collection, and time-weighted composite sample collection using autosamplers. Gutierrez et al. [23] reported higher sample collection intervals corresponding with schools' lunchtimes when a higher flow rate (bathroom use) is expected.

Sampling design is a critical factor for detecting SARS-CoV-2 RNA in wastewater [29]. The concentration of SARS-CoV-2 RNA in free-flowing wastewater in manholes is expected to vary considerably, based on the usage of toilets by students and staff, as well as the sampling technique and frequency. Composite samples were collected using either time-weighted or flow-weighted sampling during school hours are better suited to capture the entire spectrum of wastewater generation [30]. This is typically achieved by installing portable autosamplers within the manholes and collecting composite samples during the school period. Grab sampling techniques may be used in situations where autosamplers are not available due to logistical and cost issues [31]; however, care should be taken to sample during peak fecal loading times such as between class periods and at lunch break.

It is recommended that samples be processed within 24–48 h after sampling for a timely turnaround of results. Various virus concentration methods, including ultracentrifugation, poly-ethylene glycol (PEG) precipitation, electronegative membrane filtration, adsorption-extraction, and ultrafiltration, have been used to concentrate SARS-CoV-2 in wastewater [32–34]. Methods based on electronegative membrane filtration and PEG precipitation have been widely adopted in school campus settings [11,20,22,23,25]. These methods do not depend on the availability of expensive equipment such as ultracentrifuges and can be easily performed in a BSL-2 laboratory with a standard membrane filtration apparatus and tabletop centrifuge. RNA can be directly extracted from the filters and/or pellets using commercially available RNA extraction kits. Overall, it is important to maintain a consistent workflow in order to compare results across samples.

SARS-CoV-2 quantification

Most WWS programs used commercial kits for the extraction of RNA that combine physical (e.g., bead-beating) with chemical (e.g., detergent) cell disruption methodologies. The most commonly employed molecular assay to measure SARS-CoV-2 RNA is reverse transcriptase – quantitative polymerase chain reaction (RT-qPCR) using the CDC recommended N1 and N2 primer/probe sets [35]. There are different platforms and reagents that may be used for RT-qPCR assays (Table 1). Wastewater contains a diverse range of PCR inhibitors, including fats, proteins, and humic/fulvic acids, which can cause problems later during downstream processing during PCR [36,37]. This results in challenges when making meaningful comparisons across different studies and in establishing spatial and temporal trends. However, advancements of molecular biology techniques offer new routes for the analysis of genetic material, including droplet digital PCR (ddPCR) [38–42]. In ddPCR, the absolute quantification of target genes is calculated using Poisson distribution statistics via the partitioning of template into tens and thousands of reaction wells. Owing to this partitioning effect, PCR inhibitory substances have been demonstrated to have less of an effect in environmental samples, including wastewater, when analyzed via ddPCR [43,44]. For the quality assurance/quality control, it is recommended to consult the published Minimum Information for Publication of Quantitative Real-time PCR (MIQE) [45] and the Digital MIQE guidelines [46].

Data interpretation

SARS-CoV-2 RNA quantification data can be reported in various ways, including reporting with absolute value data, e.g., the concentration of SARS-CoV-2 genes in wastewater (N1, N2, E, etc.). This approach establishes thresholds for risk levels based on concentration (e.g., low, medium, and high), data trend (e.g., increasing, stable, decreasing), and/or just the presence/absence of SARS-CoV-2 RNA in the wastewater samples. For reporting of results, several studies provided quantitative data on the number of gene copies per volume of wastewater, while some studies provided just presence/absence results. In many WWS research studies, sewer systems and/or samples are spiked with surrogate markers (e.g., bovine coronaviruses and pepper mild motile virus) to better estimate recovery efficiencies of different methods [47–49].

In addition to reporting the quantity of gene copies of SARS-CoV-2 within wastewater, reports typically include other metadata elements of system design including process, uncertainties, and wastewater quality parameters. The most effective programs involve school decision-makers in interpreting the data to support the modification of on-campus mitigation strategies. In some cases, decision-makers share results publicly via text message, email, or posts on their campus COVID-19 surveillance dashboard [50]. As reported by Barich et al. [26], the public reporting of WWS data helped generate public support for changes to mitigation measures on campus and increased public confidence in the school leadership's ability to manage risk on campus.

Challenges and opportunities in COVID-19 WBE in school settings

While conceptually, WWS offers advantages for monitoring public health, there are some critical challenges to be considered. For school settings, challenges can be categorized as logistical (supply chain and safety), technical (sample collection, concentration, RNA extraction, and RNA quantification), and data interpretation (correlation with clinical data and time to results).

Owing to increased demand on supply chains, the availability of autosampler pumps, centrifuge equipment, and RNA extraction kits has been limited over the course of the pandemic. In addition, as WWS for SARS-CoV-2 is an emerging area of research focus, further technical optimization of methodology (including concentration, RNA extraction, PCR, and data analysis) is needed. Interpretation and utilization of data by decision-makers are challenged by the time needed to obtain results and the ability to validate signals with other means of on-campus surveillance. Additional issues specific to school campuses include the paucity of data available on students' toilet use habits, obtaining representative samples in non-homogeneous wastewater and low-flow conditions, and collection system logistics.

Interpretation, communication, and use of wastewater results can be challenging because there is no standard or playbook established for universal application on campus. Harris-Lovett et al. [22] recommend WWS implementation as an adaptive process in lieu of following a single playbook. WWS programs should always consider privacy and ethics since the human waste from a school's wastewater contains a variety of information regarding behaviors and health status, often at the building level [51,52].

Using wastewater surveillance data to support public health decision-making

There have been several reports in which SARS-CoV-2 WWS was used to inform decision-making at the school campus and/or building level. For instance, in August 2020, on observing a positive signal in a dorm's wastewater sample, the University of Arizona quickly tested all 311 people who live and work there and found two asymptomatic positive individuals [11]. They also reported a correlation between SARS-CoV-2 prevalence in wastewater with the clinical COVID-19 detection cases in 13 dorms with a sensitivity of 76.0%, specificity of 90.7%, positive predictive value of 79.8%, and negative predictive value of 88.8%. In another case, Gibas et al. [25] reported that WWS was able to detect a single asymptomatic individual in a dormitory at the University of North Carolina at Charlotte. The building had a total resident population of 150−200. In both universities, WWS enabled the identification of COVID-19 cases, averting a potential disease transmission.

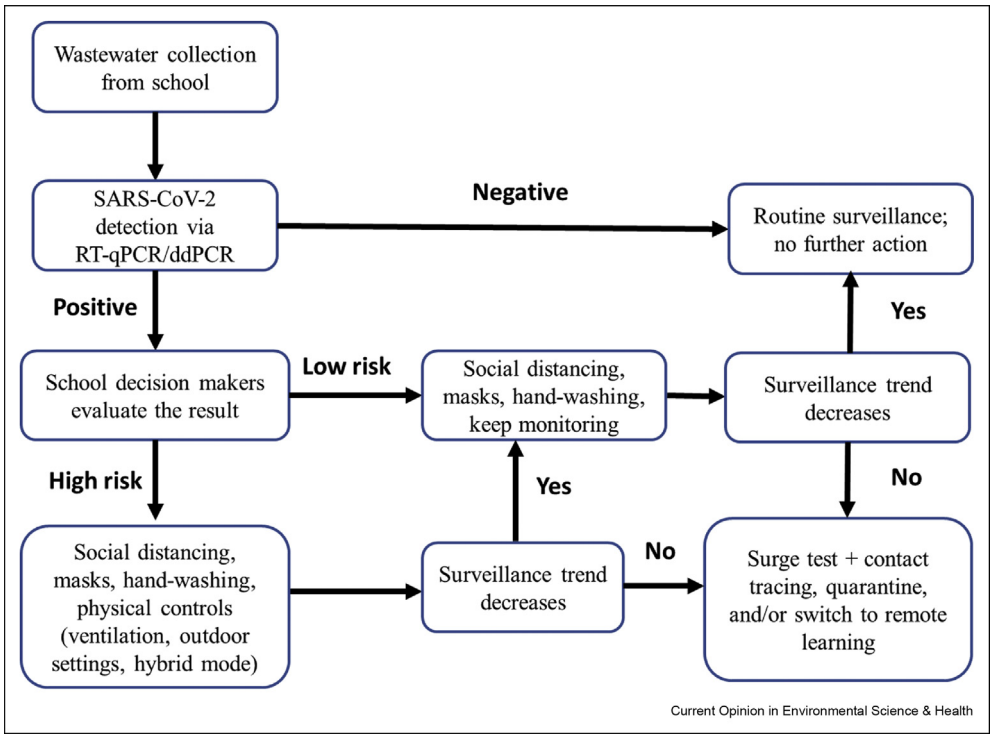
Some of the biggest advantages of using WWS for any pathogen or metabolite are that WWS does not rely on health-seeking behavior, is not limited by healthcare access issues, and in, the case of pathogens, might be useful in the pre-symptomatic and early symptomatic stages if shedding is occurring. Because of this, WWS can provide data for public health decision-making that is more robust and potentially timelier than current public health data streams (Figure 2).

Incorporating the WWS data stream into public health decision-making can be done several ways, depending on the use case. In the case of COVID-19, the data generated from WWS can be used in at least three important ways, as follows: (1) in a community, to monitor trends in SARS-CoV-2 infections and make public health decisions about the need for changes in mitigation measures; (2) in a closed population, to look for any detection of SARS-CoV-2 to make decisions about the deployment of clinical testing within the population and identify unknown COVID-19 cases; and (3) in a community or closed population, to monitor for the emergence of SARS-CoV-2 variants to make public health decisions about mitigation measures [53,54].

A day school environment has aspects of a community, as well a closed population and, as such, there are several ways in which WWS data could be used to support public health decision-making. First, if the school does not have any SARS-CoV-2 detections at baseline, the detection of SARS-CoV-2 in wastewater might be used to trigger the deployment of clinical diagnostic testing within the school population. Given that others external to the school population (e.g., an infected parent) might use the school restroom, it is possible that the clinical testing might not result in the detection of any COVID-19 cases. Regardless of whether school-wide diagnostic testing is done, the SARS-CoV-2 detection could also be used to trigger an increase in mitigation measures such as increasing the following: masking, outdoor time, ventilation of indoor spaces, filtration of indoor air, physical spacing, school-based vaccine clinics, and school communications campaigns. Next, if the school has SARS-CoV-2 circulating at baseline, with or without a school-wide diagnostic testing program, the above-mentioned mitigation measures could still be deployed. Finally, the wastewater samples coming from the school-based program can be tested for variants of SARS-CoV-2 to inform the national understanding of variant emergence and the need for the development of additional medical countermeasures (e.g., COVID-19 vaccine "booster") among other mitigation measures.

WWS can be used to supplement clinical testing to provide longitudinal assessments of a school population in addition to the point-in-time data obtained by clinical testing. In many instances, WWS provided an early warning signal of the spread of COVID-19 in

Figure 2



Public health decision-making based on wastewater surveillance data.

communities, particularly due to its ability to capture asymptomatic or presymptomatic individuals. When testing resources are unavailable or cost prohibitive, WWS can be used to obtain situational awareness and—when a positive signal is detected—help direct targeted individual testing on campus. As more teachers, staff, and students are vaccinated, a corresponding decrease in individual testing may be mitigated by the implementation of WWS to monitor for possible outbreaks, including vaccine breakthroughs, in the future [55].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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22. ** Harris-Lovett S, *et al.*: **Wastewater surveillance for SARS-CoV-2 on college campuses: initial efforts, lessons learned and research needs**. *Int J Environ Res Publ Health* 2021, **18**:4455.

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