OPINION

Wastewater analysis can be a powerful public health tool–if it's done sensibly

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The coronavirus disease 2019 (COVID-19) pandemic sparked an explosion of interest in wastewater-based epidemiology (WBE; also known as wastewater monitoring or wastewater surveillance). Much has been said, in the scientific literature and popular press alike, about the public health value of tracking severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater. Emergence and spread of the omicron variant has recently pushed WBE for COVID-19 management back into headlines. Unfortunately, coverage of the potential of WBE is rarely balanced by a practical discussion of limitations and tradeoffs, especially when it comes to issues beyond technical challenges encountered in the lab.

We grapple with such issues frequently while managing a WBE program for Healthy Davis Together (HDT), a multi-pronged pandemic-response initiative in Davis, CA. Since launching in September 2020, the program has grown to include in-house analysis of wastewater collected on a weekly, triweekly, or daily basis from 70 sites distributed across the City of Davis and the University of California, Davis (UC Davis) campus sewer systems and from the influent of their wastewater treatment plants.

Sometimes wastewater-based epidemiology makes sense as a way to monitor disease outbreaks and other public health threats, and sometimes constraints argue for spending scarce resources elsewhere.

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We are glad that our wastewater data are informing local COVID-19 mitigation efforts. Results from wastewater collected from UC Davis dorm outflows are supporting the safe return of students to campus; results from wastewater collected from neighborhoods and broader city areas are helping public officials understand spatial changes in COVID-19 trends and react accordingly.

At the same time, launching and running a WBE campaign requires significant investments of time, money, labor, and expertise. Given that much information gleaned from wastewater is not directly actionable, and/or duplicates information from other sources, it is prudent to consider when these investments are worthwhile. Here, we offer insights based on our experience with HDT about when WBE makes sense and when constraints argue for spending scarce resources elsewhere.

A Brief History

The history of WBE has become a well-told story among practitioners (1). Although proposed as far back as the mid-1940s (2), WBE only began to gain traction as an epidemiological tool in the early 21st century. Applications of WBE in the 2000s and the 2010s were diverse—including monitoring use of pharmaceuticals (3) and illicit drugs (4), tracking flu prevalence (5), and, perhaps most notably, containing polio outbreak (6)—but remained known to only a relatively small group of specialists.

In 2020, the COVID-19 pandemic catapulted WBE into mainstream attention. Rapid disease spread coupled with global shortages of clinical tests drove attention to early reports (7) demonstrating the utility of WBE for tracking COVID-19. The following months saw colleges, cities, and states alike incorporate WBE into pandemic response. There are now hundreds of WBE programs, comprising thousands of sites, tracking COVID-19 worldwide (8). Such programs can provide—and are providing—meaningful public health benefits. But it is important to recognize their limitations.

Early Warning System

Individuals infected with SARS-CoV-2 typically begin excreting the virus several days before becoming symptomatic and hence several days before they are likely to seek COVID-19 testing. WBE can therefore help public health officials proactively identify "hotspots" of disease emergence and spread (9). The value of WBE as a leading indicator of infection was heralded early in the pandemic, especially amid prolonged delays in access to and delivery of diagnostic testing results.

But as Olesen and colleagues persuasively argue, WBE serves as a true early warning system only when background levels of COVID-19 are very low and clinical testing of the surveilled population is scarce or deficient (10). Otherwise, WBE can serve as an *independent* indicator of disease prevalence but not necessarily a *leading* indicator of outbreak potential. The extent to which sewage prevalence of SARS-CoV-2

leads community infection also depends on physical characteristics of the sewershed. Indeed, our comparisons of wastewater results with clinical results from HDT's (widely accessible and widely used) asymptomatic-testing program show good agreement between the two datasets but no consistent lead of one indicator over the other (11).

Unbiased Testing

Clinical testing programs only provide information on the subset of individuals who consent to testing. Estimations of COVID-19 prevalence from clinical data may therefore be biased as a result of factors such as health-seeking behavior, under-testing of asymptomatic cases, inequitable access to testing, and testing mandates that apply only to certain groups (e.g., educators). Conversely, WBE captures the pooled contributions of all individuals in a catchment area.

Continued deployment of WBE in ways that take into account the needs of decision makers, and pragmatically weigh costs and benefits, will no doubt do much to help end the pandemic.

But acting on pooled wastewater results is challenging. In a clinical setting, individual contributions to a positive pooled sample can be retested to identify the source(s) of the positive; this is not so for wastewater samples. Although researchers have proposed in-sewer sensor networks that would isolate positive building outflows (12), such networks would require much cheaper and faster instrumentation and methods for detecting SARS-CoV-2 in wastewater. Moreover, the prospect of tracing genetic signals in wastewater back to individual sources amplifies privacy and ethical concerns surrounding WBE (13).

The actionability challenge leaves those seeking to incorporate WBE into active COVID-19 response with two options. Option one is to restrict WBE to settings where performing swift, directed interventions that include the entire population of interest is feasible. The efficacy of this approach has already been demonstrated at multiple college campuses (14, 15), where detection of SARS-CoV-2 in the outflow of residential dormitories may trigger testing of all dorm residents and isolation of residents testing positive. Other settings where WBE may be reasonably coupled with direct interventions include cruise ships, airplanes, nursing homes, and prisons.

Option 2 is to apply indirect interventions. In Davis, HDT geotargets text and email alerts to residents of a neighborhood where our team observes a sustained increase in wastewater SARS-CoV-2 levels. The alerts note that local virus levels are rising, emphasize good hygiene and social-distancing behaviors, and provide a link to sign up for clinical testing. HDT also occasionally distributes door hangers to residences in areas where wastewater SARS-

CoV-2 levels are especially concerning and where testing uptake is low. The hangers can be redeemed at HDT-run testing sites for small incentives (typically $5 gift cards to local businesses).

Cost-Effective Surveillance

WBE can be a cost-effective way to track disease trends. The median list price of a PCR-based clinical test for COVID-19 at a U.S. hospital is $148 (16). Multiply this by the hundreds or thousands of tests that must be conducted every week to obtain reliable data on COVID-19 trends in a community of any significant size and the tab quickly grows. By contrast, it costs our lab only about $300 to analyze a wastewater sample representing an entire population or subpopulation.* Strategically replacing some clinical testing with WBE at a national scale could save millions or billions of dollars without compromising surveillance accuracy (17).

But cost-effective is not the same as cost-free. We spent hundreds of thousands of dollars on equipment to establish the high-throughput sample-processing pipeline that our lab uses. Purchasing portable wastewater autosamplers costs tens of thousands of dollars more. HDT hired more than a dozen new staff to collect, process, and analyze samples, while we (along with our colleagues at the City of Davis and UC Davis) scaled down or abandoned other projects to focus on the WBE program. For us, the tradeoffs made sense. HDT funded program costs, and the program is scientifically important for us as well as important for the public health of our community. The calculus may be less favorable for other communities—at least for now. Creative integration of Moore swabs [gauze pads suspended in sewer flows to passively trap suspended particles (18)], loop-mediated isothermal amplification [a single-tube molecular detection technique that does not require the pricey thermocyclers used in PCR (19)], and other inexpensive techniques may soon shift the WBE cost–benefit ratio in a favorable direction.

Implications for End Users

With the above discussion in mind, we offer the following recommendations for end users seeking to incorporate WBE into COVID-19 response.

1) **Avoid redundancy between clinical testing and WBE.** Methods validation and/or quality control may require some parallel deployment of clinical testing and WBE. It is generally inefficient, however, to use both methods for the same scale of surveillance. WBE will add little at a hospital that mandates clinical testing of all patients, visitors, and staff. But WBE is far cheaper and less labor-intensive than mass diagnostic testing for tracking

broad disease trends. Well-designed COVID-19 response strategies will integrate the two surveillance approaches in ways that are complementary, not duplicative.

2) **Emphasize statistical thinking, data analysis, and data management.** Existing literature on WBE for SARS-CoV-2 focuses heavily on optimizing sample collection and processing. Comparatively little attention has been paid to thoughtful design of wastewater-sampling schemes [i.e., sampling so as to "maximize information gained relative to resources required for data collection and analysis" (20)]. Similarly, little attention has been paid to optimizing methods for pulling, organizing, analyzing, and presenting data, even though wastewater data can only support positive health outcomes when interpreted clearly and correctly. Our research demonstrates, for instance, that common methods of handling nondetects in quantitative PCR data can bias the recognition of trends in wastewater data (11). Better methods for imputing these "missing" data could enable more effective pandemic response. A strong WBE team will also include one or more data scientists tasked with synthesizing results (e.g., via an online "dashboard") for decision makers and the public.

3) **Define action thresholds.** WBE is only worthwhile if practitioners make clear how the results will be used. In collaboration with HDT, we defined wastewater action thresholds that consider (for a given site) the number of positive replicates, the virus concentration in a sample, and the number of consecutive positive samples. Action thresholds are tailored to different settings. For UC Davis, a single positive sample from a previously negative dorm outflow may spur testing of all dorm residents. For the city, action thresholds are set higher owing to population mixing across sampling zones and greater resources needed for meaningful response. Geotargeted alerts are typically only issued after a sustained increase in wastewater virus levels over three consecutive dates for a given sampling zone.

4) **Monitor fewer sites more frequently.** A study conducted by Feng and colleagues in Wisconsin concluded that "a minimum of two samples collected per week [is] needed to maintain accuracy in trend analysis" (21). We have similarly observed that practitioners need high-frequency sampling (three times per week for most of our sites) to obtain reliable, actionable information on COVID-19 trends. Resource-constrained WBE practitioners should consider monitoring fewer sites more frequently, sacrificing some spatial granularity to achieve greater sampling frequency. One exception is the university (or similar residential) setting, where the purpose of WBE is less to track trends and more to flag individual buildings that could house infected individuals. Achieving universal coverage of all buildings included in such settings may be worth sacrificing sampling frequency.

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*This estimate factors in costs of operating instrumentation, overhead, and labor (although not costs of sample collection or initial equipment investments). When considering marginal materials costs alone, our per-sample outlay is closer to the $13 cited by a university lab using a similar workflow (15).

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5) **Build on existing infrastructure and programs.** WBE programs do not always need to start from scratch. Wastewater treatment plant operators routinely collect influent samples—and sometimes also samples from further up in the sewershed—to measure a suite of physical, chemical, and biological water quality indicators. Structuring WBE around sample collection that already occurs is an easy way to reduce startup costs and time. Jurisdictions can also pursue partnerships with local academic and/or private-sector labs possessing instrumentation, personnel, and expertise that could be leveraged for in-house analysis of SARS-CoV-2 in wastewater. Investing to augment local capacity may be cheaper and logistically simpler than outsourcing sample analysis. Finally, personnel involved in WBE programs need not all be full-time staff. Temporary part-time employees and undergraduate student assistants hired through HDT help us immensely in collecting samples, performing routine lab tasks, and organizing data.

6) **Be prepared to adapt.** Successful WBE programs will be as dynamic as the COVID-19 pandemic itself. We have had to respond creatively when construction rendered certain sampling sites inaccessible, instrument malfunctions caused losses of samples and data, and supply shortages prevented us from carrying out laboratory protocols exactly as written. Our experience speaks to the importance of designing workflows that can easily accommodate changes. Practitioners should similarly be prepared to adapt PCR protocols as new variants emerge.

7) **Keep an eye on the future**. In addition to providing information about the state of the pandemic today, wastewater data can also suggest how the pandemic may evolve down the line. Crits-Cristoph and colleagues demonstrated that genomic sequencing of wastewater samples "can provide evidence for recent introductions" of new viral strains in a region before those strains are detected by clinical sequencing (22). Wastewater sequencing in multiple countries has also revealed novel SARS-CoV-2 lineages not detected in human circulation but potentially relevant to human health (23, 24). Regular communication among WBE practitioners, epidemiologists, and public-health officials is needed to ensure 1) that important wastewater results like these inform broader policy responses and 2) that practitioners adjust scope and approach to align with immediate needs.

We have been pleased to see such multilateral communication occurring with respect to the omicron variant. In December, spikes in wastewater viral load in South Africa compelled experts to sound the alarm about omicron's transmissibility, while researchers around the world are rapidly modifying WBE programs to focus on omicron detection. Continued deployment of WBE in ways that take into account the needs of decision makers, and pragmatically weigh costs and benefits, will no doubt do much to help end the pandemic.

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