

Wastewater surveillance of pathogens can inform public health responses

Wastewater monitoring has been used to identify SARS-CoV-2 outbreaks and track new variants. This sentinel system should be expanded to monitor other pathogens and boost public health preparedness.

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Since the late 1930s, scientists have known that infectious poliovirus was present in the sewage of cities experiencing outbreaks¹. As a result, wastewater surveillance has played an important part in the polio-eradication campaign². Building from the work on poliovirus, data from wastewater have complemented clinical surveillance during the COVID-19 pandemic by offering a comprehensive view of infection burden and transmission — both symptomatic and asymptomatic — and information on which SARS-CoV-2 variants are circulating in a community^{3,4} (including so-called cryptic variants that have never been detected in clinical samples⁵). Yet despite the established value of wastewater surveillance for monitoring poliovirus and its emerging importance during the COVID-19 pandemic, most global public health surveillance systems still rely heavily on medically attended case data⁶. This needs to change to enable effective pandemic preparedness and response.

Sewage science

Wastewater monitoring involves the collection of pooled samples from community- or institution-level sewage systems. For community-level surveillance, the sewers are a catch-all, aggregating human waste into centralized locations. However, sampling can be done at much more localized scales and in communities that lack centralized sewage collection and treatment systems. Sampling from centralized locations can provide rich insight into pathogenic viruses, bacteria and protozoa present in the community⁷ (Fig. 1). Of these, pathogens that are stable in wastewater and/or are consistently shed in fecal material or urine are strong candidates for wastewater monitoring.

From a public health perspective, diseases that are under-reported by traditional

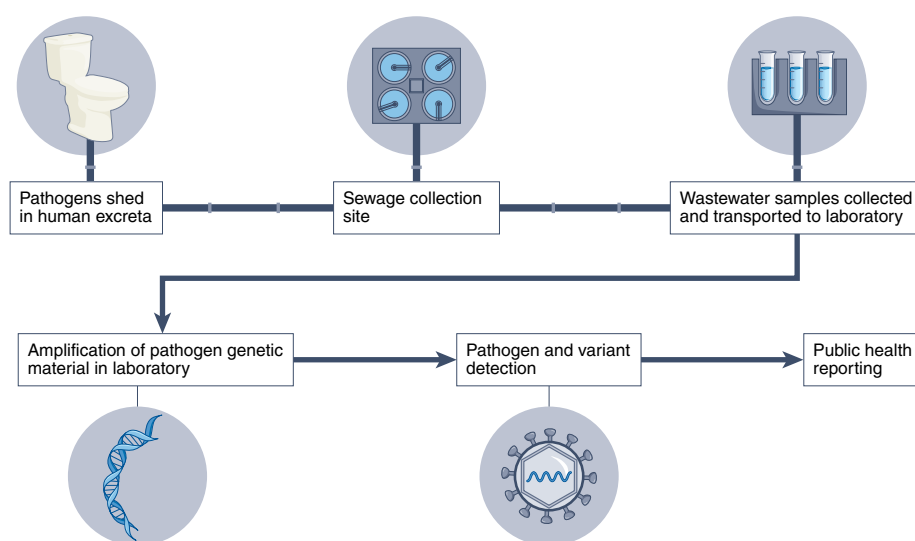


Fig. 1 | Wastewater-monitoring pathway. After SARS-CoV-2 is shed in human feces, the sewage may travel to centralized locations, such as wastewater treatment plants. Depending on the goals and resources for wastewater monitoring, a variety of methods can be used to collect samples of the sewage, which is routinely done between twice weekly and once monthly. The samples are first processed in a lab to concentrate and amplify genetic evidence (RNA or DNA, depending on the pathogen). Genetic material is then tested to detect and quantify pathogens of interest, with PCR-based methods or NGS used for variant detection. Results are then reported to public health departments to improve situational awareness and inform action.

surveillance systems and lead to widespread poor health outcomes should be prioritized in wastewater-monitoring programs. These include diseases with nonspecific symptoms, such as polio, influenza and viral gastroenteritis, or those for which patients do not seek care until symptoms are serious. There is also a strong use case for conducting wastewater monitoring for diseases for which current diagnostic tests are inadequate, have limited accessibility and/or are expensive and for which the data could help guide vaccine campaigns or other interventions that can improve health outcomes.

Among the wide range of biomarkers and chemicals that can be measured in sewage to assess health and well-being⁸, one of the most promising has been viruses. Additionally, a growing body of evidence indicates that wastewater may be an ideal environment for the detection of antimicrobial-resistant pathogens — a rising global public health threat⁹. Other applications include assessing community use of illicit and therapeutic drugs¹⁰; monitoring consumption of everyday compounds, including caffeine, nicotine and alcohol¹¹; and measuring exposure to environmental

Table 1 | Comparison of RT-PCR versus NGS approaches for wastewater monitoring

Method	Region measured	Quantitative	Tolerance to mutations	Novel variant discovery	Turnaround time	Target-specific development time	Cost	Interpretation	Limitations
RT-PCR	Small amplicons (hundreds of bases)	Yes	Depends on location	No	Day	Weeks	Low	Easy	Provides only presumptive evidence, as it relies on mutation presence or absence as a proxy for variant presence or absence; inconsistent performance over time, especially if novel mutations emerge in regions targeted by assay primers
NGS	Whole genome (30,000 bases)	No	Yes	Yes	Days to weeks	None	High	Difficult	Dominant variants may overpower signal from emerging variants; requires initial enrichment step using viral probes and targeted amplicon, which increases costs and turnaround times

contaminants such as pesticides and plasticizers¹².

Numerous studies over the past 30 years have shown that wastewater testing provides an accurate population-level view of disease¹³, and recent research has shown that trends in wastewater concentrations of SARS-CoV-2 are strongly predictive of clinical case counts^{14,15}. Although the lead time may diminish as the pandemic progresses¹⁶, the value of wastewater as an early indicator has increased as diagnostics have shifted from the health system to at-home testing. As the rate of at-home diagnostic testing increases, the reliability of clinical testing data for public health surveillance may further diminish¹⁷. A warning of increased cases from wastewater surveillance can provide health departments with critical lead time for making decisions about resource allocation and preventive measures¹⁸. Furthermore, unlike testing of individual people, wastewater testing provides insights into the entire population within a catchment area, which enables communities without robust syndromic surveillance systems to have a cost-effective, efficient tool for monitoring population health.

Current wastewater-monitoring practices are not a panacea. Given the vast quantity of human and animal microbes that end up in wastewater, the data are inherently noisy, and more work is needed to reliably distinguish signal from noise in biomarker concentrations measured in wastewater samples. There is also a lack of consensus on wastewater-monitoring techniques and

how and if the data should be standardized, such as using human fecal biomarkers like the pepper mild mottle virus, which is common but diet dependent. Although wastewater data have been recognized as a valuable complementary data source to clinical case counts during the COVID-19 pandemic, confusion exists about how to integrate these data with existing data sources, such as hospital bed capacity or local sales of in-home diagnostic tests, to provide public health officials with a more holistic measure of risk in their community. More work is also needed to expand wastewater-surveillance capacity in rural areas and unsewered settings. Thus, complexities remain to be solved as health departments around the world rapidly adopt this tool¹⁹. A recent survey in the United States found a gap between public health officials' recognition of the value of wastewater monitoring and their use of it for pandemic management²⁰.

Variant detection

The genetic code of viruses, including SARS-CoV-2, continually mutates, giving rise to novel variants that can have different transmissibility, severity and other disease characteristics. The role of variant detection within wastewater monitoring remains largely underexplored scientifically and underutilized in public health and clinical diagnostics. In fall 2021, The Rockefeller Foundation launched the Wastewater Action Group to study various approaches to translating wastewater data into public health action. When the Omicron variant of

SARS-CoV-2 was identified in South Africa, the Wastewater Action Group mobilized to determine how wastewater monitoring could be combined with rapid identification of different genetic variants to characterize the changing nature of the pandemic.

Two complementary methods for tracking novel variants, reverse transcription-polymerase chain reaction (RT-PCR) and next-generation genomic sequencing (NGS), can be integrated into wastewater-monitoring programs to inform pandemic responses. Each approach has advantages and limitations (Table 1).

From the original Wuhan strain of SARS-CoV-2 through each new variant, researchers around the globe have quickly made full genome sequences publicly available, enabled by inexpensive and relatively fast RT-PCR assays²¹. This sharing of viral genomes opened the door to tracking SARS-CoV-2 variants not only in clinical samples but also in community wastewater.

Although RT-PCR has been used in clinical testing for more than three decades²², its use in wastewater testing is less common, although some prior work has been done to characterize bacterial variants in wastewater, including *Escherichia coli* variants²³ and antibiotic-resistant strains²⁴. During the COVID-19 pandemic, RT-PCR has become an essential tool for detecting SARS-CoV-2 variants in wastewater. The method is highly sensitive, has a quick turnaround time²⁵ and is cost-effective, since one wastewater sample can yield information on thousands of viral genomes

Table 2 | Case studies for wastewater surveillance

Location	Method	Approach	Benefits
Oklahoma	RT-PCR	Since October 2020, several municipalities across Oklahoma have implemented wastewater monitoring for SARS-CoV-2 to provide public health officials with an early warning of increasing SARS-CoV-2 prevalence in the community. The results are communicated weekly to Tulsa's public health leaders. When Omicron was first detected in the state, Tulsa adapted its wastewater testing to assess the relative contributions of the Delta and Omicron variants, using allele-specific RT-PCR.	Testing revealed distinct patterns across different racial or ethnic groups ²⁹ , provided an early warning of Oklahoma's transition from Delta to Omicron, and signaled to local physicians and hospitals that a surge in COVID-19 cases was imminent. As Omicron infections can be resistant to the antibody treatments used for Delta infections, the variant information from wastewater monitoring also enabled local physicians to target antibody treatments to patients on the basis of the relative abundance of Omicron in their community's wastewater.
Houston	NGS	In May 2020, the Houston Health Department collaborated with Rice University to establish a city-wide wastewater epidemiology program to monitor SARS-CoV-2 among the 2.3 million Houston residents. The system provides weekly measurements and targeted amplicon sequencing of the SARS-CoV-2 genome at 39 centralized wastewater treatment plants. By the time the first Omicron case was identified in the Houston area in December 2021, the Houston Health Department had already implemented a rapid wastewater variant-screening pipeline, collecting wastewater samples each Tuesday and summarizing the variant sequencing and other wastewater results in reports generated each Friday.	Researchers were able to detect Omicron in the wastewater days before the first clinical case was reported in Houston. The health department quickly incorporated wastewater detection of Omicron into ZIP-code-level metrics that enabled them to prioritize resources. The city's public health authority also provided local infectious-disease doctors and hospital leaders with wastewater-based data on Omicron spread, which they used to inform staffing and personal protective equipment needs.
Southwestern tribal nations	Variant detection without sequencing	The 5.4 million American Indians and Alaska Natives in the United States encompass 574 distinct tribes with more than 130 languages. Cultural preferences and, in some cases, past ethical breaches in research on tribal communities have led some tribal nations to prohibit genetic sequencing of wastewater samples during the COVID-19 pandemic. When two or more tribes use a single wastewater-collection system, genetic sequencing cannot be ethically performed if any one of their communities or villages voices objections. In such situations, an alternative approach is to analyze the gene products, such as virus-specific proteins and peptides, using mass spectrometry.	This approach not only circumvents concerns associated with genetic sequencing but also overcomes a key challenge with wastewater sequencing: degradation of viral RNA in the sewer system. Wastewater-borne viral proteins may be more stable than viral RNA in the sewer system. As a result, sequencing of high-copy-number proteins and peptides in wastewater may be another promising alternative for detection of a persistent signal of the public health threat.

in a population, which has made it the workhorse of public health response.

NGS of viral genetic material in wastewater, by contrast, can be used to indicate the relative abundance of viral mutations in wastewater, providing a broader view of genetic diversity than the limited presence-or-absence information that PCR methods provide. Because wastewater samples are pooled from an entire sewer-service population, the use of NGS with wastewater testing can provide information on viral mutations across many different infections. Routine sequencing of wastewater samples offers a foundation for studying how individual mutations change over time, and how variants of interest and variants of concern emerge and propagate.

Combining NGS (for a broad view of the full range of variants present) and targeted RT-PCR assays (for rapid and sensitive

quantification of known variants) is therefore the best approach. Ideally, these techniques can be used at different stages, with NGS being used infrequently at sentinel sites to scan for novel variants and PCR being used for more routine, cost-effective testing.

The emergence of the Omicron variant of SARS-CoV-2 was a defining moment for the role of wastewater monitoring in public health, as it revealed the practical value of this approach for detecting emerging variants in a community in real time²⁶, which were then used to inform decision making in Texas, Oklahoma, Kentucky and Arizona (Table 2).

Building acceptance

Considerable hurdles must be overcome at each phase of wastewater monitoring before it reaches its full potential as a

variant-surveillance approach. First, clinical sequences are often needed for the identification of novel variants and for guiding the development of primers for assays used to identify those variants in wastewater. Although this dependence can help hone focus on clinically and epidemiologically relevant variants, it also makes wastewater sequencing vulnerable to shifts in capacity for timely sequence information about current and emerging clinical strains.

In addition, many of the academic labs that have been the backbone of wastewater-sequencing efforts will pivot back to their usual research, which will compromise retainment of their expertise. A successful transition into long-term surveillance for public health will require continued cooperation in sharing of the research labs' deep expertise with their public and commercial partners.

Delays in wastewater reporting and ad hoc communication of findings can hamper the value and early-warning potential of this approach. Much of the power of these data comes from the ability to generate qualitative and quantitative situational awareness, through real-time data, about the spread of diseases across geographies. But wastewater data are rarely shared in real time, and when they are, relevant metadata, such as specification of primer sequences or sample type, as well as source population, are often incomplete. This missing information can impede the ability to detect outbreaks, including new variants, especially those circulating at low frequencies. Improvements in metadata standards and incentivizing a culture of data sharing, analogous to that created by platforms such as GISAID, will improve data access and data quality and provide broader returns to public health.

Researchers also need a shared platform that brings together multiple data sources to overcome the gaps and biases in any one source. When wastewater data are combined with clinical surveillance data, population mobility data and innovative proxies for disease, such as symptom surveys or digital-thermometer data, the added value becomes clear and can foster more buy-in and cross-agency cooperation.

Wastewater monitoring and sequencing require substantial will and collaboration among key stakeholders, including utilities, public health agencies and labs, which do not often work together and may have differing priorities. Even when sequencing capacity is available and there is sufficient buy-in, communities may not be accepting of this technology. Concerns about what information the sequences contain and how they will be used may be particularly seen among groups that have experienced health-related exploitation or mistreatment, including impoverished communities and Black, Indigenous or other people of color. Building and maintaining trust requires incorporation of community interests throughout the decision-making process; top-down implementation risks pushback by the communities monitored. In some cases, communication about the mechanisms and goals of wastewater surveillance from local leaders and experts may be enough to engender community buy-in. In other cases, as with some tribal nations in Arizona, trust must be built by co-design with community members, so that the technological approach and reporting can be modified to fit the needs of the community.

Maximizing value

Given the successes of wastewater monitoring for SARS-CoV-2, the next phase

of expansion should include multi-pathogen detection, to provide situational awareness of changes in the transmission or burden of pathogens circulating in the community, and early warning of novel or re-emerging threats. Dedicated wastewater monitoring, coupled with variant monitoring, is a valuable strategy for modernizing public health surveillance systems.

The development of successful wastewater-monitoring programs will require three critical components: funding to support the necessary wastewater-sampling equipment, lab testing supplies and personnel; researcher expertise for deriving new assays, developing risk metrics and communicating risk to non-technical audiences; and community engagement. The value of wastewater data is optimized when multiple stakeholders work closely together. Community leaders are crucial partners for the co-design of wastewater surveillance approaches and amplifying the benefits that monitoring provides.

Population growth and global warming are likely to increase human–pathogen interactions, which, combined with pathogen evolution²⁷, suggests a continuing need for technological advancements to keep pace with these changes and provide relevant surveillance²⁸. A global, adaptive early-warning system that includes wastewater variant monitoring will be critical for protecting the public and easing the strain on the healthcare system when the next novel threat emerges. □

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References

1. Paul, J. R. et al. *Science* **90**, 258–259 (1939).
2. Duintjer Tebbens, R. J. et al. *Food Environ. Virol.* **9**, 361–382 (2017).
3. Shah, S. et al. *Sci. Total Environ.* **804**, 150060 (2022).
4. Amman, F. et al. *Nat. Biotechnol.* <https://doi.org/10.1038/s41587-022-01387-y> (2022).
5. Karthikeyan, S. et al. *Nature* <https://doi.org/10.1038/s41586-022-05049-6> (2022).
6. Groseclose, S. L. & Buckeridge, D. L. *Annu. Rev. Public Health* **38**, 57–79 (2017).
7. Sinclair, R. G. et al. *Adv. Appl. Microbiol.* **65**, 249 (2008).
8. Venkatesan, A. K. & Halden, R. U. *Sci. Rep.* **4**, 1–7 (2014).
9. Fouz, N. et al. *Trop. Med. Infect. Dis.* **5**, 33 (2020).
10. Sulej-Suchomska, A. M. et al. *Sci. Rep.* **10**, 1–12 (2020).
11. Baz-Lomba, J. A. et al. *BMC Public Health* **16**, 1–11 (2016).
12. Rousis, N. I. et al. *Water Res.* **121**, 270–279 (2017).
13. Barras, C. *Nat. Med.* **24**, 1484–1488 (2018).
14. Feng, S. et al. *ACS Env. Sci. Technol. Water* **1**, 1955–1965 (2021).
15. Weidhaas, J. et al. *Sci. Total Environ.* **775**, 145790 (2021).
16. Xiao, A. et al. *Water Res.* <https://doi.org/10.1016/j.watres.2022.118070> (2022).
17. Rader, B. et al. *MMWR Morb. Mortal. Wkly. Rep.* **71**, 489 (2022).
18. Schmidt, C. *Nat. Biotechnol.* **38**, 917–921 (2020).
19. Naughton, C. C. et al. Preprint at [medRxiv https://doi.org/10.1101/2021.03.14.21253564](https://doi.org/10.1101/2021.03.14.21253564) (2021).
20. Keshaviah, A. et al. *The Rockefeller Foundation* <https://www.rockefellerfoundation.org/report/the-role-of-wastewater-data-in-pandemic-management/> (2022).
21. Wu, F. et al. *Nature* **579**, 265–269 (2020).
22. Deepak, S. A. et al. *Curr. Genomics* **8**, 234–251 (2007).
23. Franz, E. et al. *Sci. Rep.* **5**, 1–9 (2015).
24. Milobedzka, A. et al. *J. Hazard. Mater.* **424**, 127407 (2022).
25. Lou, E. G. et al. *Sci. Total Environ.* **833**, 155059 (2022).
26. Kirby, A. E. et al. *MMWR Morb. Mortal. Wkly. Rep.* **70**, 1242 (2021).
27. Mascola, J. R. et al. *J. Am. Med. Assoc.* **325**, 1261–1262 (2021).
28. Callaway, E. *Nature* <https://www.nature.com/articles/d41586-021-03619-8> (2021).
29. Kuhn, K. G. et al. *Sci. Total Environ.* **812**, 151431 (2022).

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M.B.D. and A.K. conceived of the project. M.B.D., A.K. and S.V.S. coordinated the work. All authors jointly drafted and revised the manuscript.

Competing interests

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