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Wastewater surveillance for public health:

Wastewater contains information on pathogen spread, evolution, and outbreak risk

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Abstract

A University of California San Diego researcher collects wastewater on campus for SARS-CoV-2 analysis and sequencing.

Graphical Abstract

Dating back to the origins of modern epidemiology, wastewater surveillance has predominantly been used to track pathogens spread by fecal-oral transmission such as those that cause cholera and polio. However, more than just these “enteric” pathogens are shed via the gut, as highlighted by the success of severe acute respiratory syndrome coronavirus

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2 (SARS-CoV-2) wastewater surveillance (1, 2), recent work on tracking influenza virus (3) and monkeypox virus (4), and observations of extensive pathogen diversity in stool (5, 6). Wastewater is now a core component of infectious disease monitoring, providing a variant-specific, community-representative picture of public health trends that captures previously undetected spread and pathogen transmission links. Building on recent laboratory and analytical advances to identify the diverse pathogens present in sewage will be essential to ongoing efforts to understand disease risks and will transform infectious disease surveillance.

Pathogen surveillance typically involves sampling of infected individuals, requiring extensive specimen acquisition, clinical testing, and sequencing coordinated across different sites and laboratories. This type of clinical surveillance is expensive, time-consuming, and subject to bias owing to disparities in public participation and frequency of testing and sequencing, which may limit outbreak preparedness and response by public health organizations, especially in underserved communities (7). Although clinical surveillance will remain fundamental to infectious disease response, wastewater-based approaches enable fast and cost-effective surveillance, even in current blind spots.

Wastewater monitoring enables rapid pathogen detection and community prevalence quantification. At sites where wastewater from the population collects and mixes, so too do a diverse array of microbes shed from individuals. The resulting mixture is representative of local infections, and with now-standard laboratory processing and quantitative polymerase chain reaction (qPCR), sensitive concentration measurements of specific pathogens can be obtained affordably and in only a few hours. Pathogen concentrations accurately estimate prevalence (the number of current infections in the population), and given that wastewater trends often precede corresponding clinical detections, they may allow for early detection (8).

Wastewater can be used to track infectious disease dynamics from the community level to building level, and from sources ranging from sewers and wastewater treatment plants (WWTPs) to surface waters and point sources (e.g., natural pooling sites) (9). At large WWTPs and other sites of converging wastewater flows, a single sample can capture the community-wide pathogen landscape, even for cities with millions of inhabitants (10). Such approaches are cost-effective and time-efficient and maximize the number of individuals covered by surveillance. In many countries, WWTPs already collect composite wastewater samples (taken regularly throughout the day) that can be analyzed to yield essential information on local pathogen prevalence, which enables timely mobilization of public health interventions. In areas lacking centralized sewer infrastructure, similar methods can be used to study samples from surface waters and point sources, although additional considerations of topography, water pooling, and flow are needed to maximize catchment size (11).

At a focused spatial scale, wastewater can be used for monitoring at the level of individual or small clusters of buildings to enable reliable detection of even a single infected person (10, 12). Such monitoring is of particular importance to vulnerable communities and high–

population density sites, such as health care (e.g., nursing homes) or educational facilities (12), as well as airports, where detection can be acted on to contain pathogen spread.

Understanding the determinants of pathogen incidence, however, including mutational changes, variant introductions, or emerging pathogens, requires genomic sequencing. Unlike qPCR, sequencing is effective regardless of pathogen variant and identifies mutation frequencies across the entire genome, even for mutations that exist at low levels in the population. During the COVID-19 pandemic, wastewater sequencing–based analyses have enabled early detection of emerging SARS-CoV-2 variants, estimation of variant prevalence (10, 13), identification of the impact of specific mutations on pathogen fitness, and characterization of the mutational processes that lead to variants of concern (14). Sequencing of building-level wastewater samples has enabled extraction of single genomes that have been used to identify new mutations and variants; reveal cryptic, or undetected, spread (especially at sites that are not well covered by clinical surveillance); and elucidate local transmission networks (10). The ease of sharing sequencing data enables collaborative analyses of pathogen trends around the world, enhancing preparedness and informing public health guidance.

For sequencing of a specific pathogen, targeted amplicon-based approaches are needed to selectively amplify and sequence pathogens of interest from wastewater. Although this approach presents technical challenges and requires ongoing development, amplicon sequencing can provide similar sensitivity to qPCR, excellent sequencing coverage and depth, and effectiveness regardless of pathogen variant. As a result, almost all clinical and wastewater sequencing of SARS-CoV-2 has used this targeted approach. Initiating the collection of high-quality pathogen-specific sequencing data requires only minimal training, aided by community efforts such as ARTIC that provide open-source amplicon scheme designs and protocols.

Although targeted approaches are excellent for monitoring known pathogens of concern, they examine only a tiny fraction of microbes in wastewater. For a broad survey of pathogens, untargeted metagenomic and metatranscriptomic shotgun sequencing approaches can be used to identify any circulating microbial DNA or RNA. A single sample can indicate the presence of viruses including monkeypox and influenza, identify pathogenic and antibiotic-resistant strains of bacteria (15), detect protist parasites including *Plasmodium falciparum* (which causes malaria), and search for new pathogens, although standard approaches may not provide sufficient sensitivity to detect rare pathogens. Unlike amplicon-based surveillance, which can identify pathogen variants shed by a handful of individuals within a population of millions (10, 14), untargeted methods require greater prevalence for reliable detection, although increasing sequencing depth can help improve the likelihood of pathogen detection. For more detailed profiling of a specific class of pathogens (e.g., viruses), probe-based enrichment methods can be used prior to shotgun sequencing for “semi-targeted” study of the group of interest and may be necessary to detect rare pathogens.

Designing an effective wastewater sequencing strategy gives rise to a fundamental trade-off between speed, cost-effectiveness, and local deployability. To keep costs down, most current sequencing is performed with hundreds to thousands of samples in parallel,

requiring expensive machinery, automation infrastructure (liquid-handling robots), and labor-intensive processing, with ~2- to 4-week turnaround times. However, because wastewater collections capture community-wide trends, samples can be prioritized for low-throughput, fast-turnaround sequencing at higher cost per sample for real-time tracking of pathogen dynamics. For example, highly automated fast-turnaround sequencers enable automation of the entire process from library preparation to analysis, require minimal technical expertise, and permit 24-hour sample receipt to data turnaround, but lead to a 10-fold increase in per-sample cost.

For deployment in low-resource settings, sequencing workflows need to be reconfigured in accordance with available instruments, reagents, funding, and facilities. Portable low-throughput sequencers can provide results much faster, are low cost, do not require automation infrastructure, and can be deployed almost anywhere. However, they generally have higher sequencing error rates and require additional laboratory and bioinformatics training. In low- and middle-income countries, where reagents generally need to be imported from abroad, transport is often slow and expensive, and reagents requiring refrigeration can degrade during shipping or at facilities with unstable electricity supply. Progressing toward more equitable and sustainable surveillance will require continued development of local, self-sustaining scientific ecosystems through laboratory and computational methods development and training, capacity building efforts, and financial support of domestic scientific enterprise.

Ongoing method development is key to further expanding the capabilities of wastewater surveillance. At present, untargeted metagenomic and metatranscriptomic methods suffer from bias toward a limited number of dominant bacterial species in wastewater and will require new laboratory techniques to ensure reliable detection of pathogens at low concentration. The latest enrichment methods for semi-targeted sequencing of pathogens from wastewater are specific to viruses and will need to be redesigned and tested for other types of pathogens. Current computational methods are still unable to identify new variants from community-wide mixtures and are just beginning to produce a wider range of informative readouts, including growth rates, which are essential to evaluate the potential size and speed of an emerging outbreak. As these methods evolve, there will also be a need for new standardized protocols and computational workflows that are open source and easily reproducible anywhere in the world.

The COVID-19 pandemic has laid bare the importance of pathogen surveillance to public health, and wastewater as one of our most powerful assets. National and regional wastewater surveillance programs are rapidly being established across the globe, allowing for continued analysis of SARS-CoV-2 variant dynamics despite limited clinical monitoring. Expansion of multipathogen and metagenomic wastewater sequencing efforts is enabling broad pathogen detection and genomic characterization, including environmental contamination by *Vibrio cholerae* (which causes cholera) in sites without effective sewage treatment, human and livestock-borne rotavirus (which causes gastroenteritis in children), and vaccine-derived polioviruses recently observed in London and New York. Beyond infectious pathogens, wastewater also has the potential to reveal changes in the human gut microbiome, which have been correlated with a wide range of health conditions and disease risks.

There is now an opportunity to build upon existing momentum to form the backbone of future surveillance capacity and scientific ecosystems across the world. Doing so will require global surveillance networks to encourage equitable technology distribution, data sharing, and collective exploration of microbial diversity, both human and zoonotic, to help pinpoint possible outbreaks and spillover risks. Close integration with local and international public health organizations will be essential to ensure timely, transparent, and effective intervention. Wastewater provides the means to identify, prepare for, and respond to future pandemic threats—what remains is to expand capacity to ensure readiness for the next potential outbreak.

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