

Wastewater-Based Epidemiology for Early Detection of Emerging Viral and Bacterial Threats

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Abstract: Background: Wastewater-based epidemiology (WBE) uses human sewage and other wastewater-impacted environmental samples to assess population-level exposure, infection and health-related biomarkers. Long used in poliovirus surveillance, WBE expanded rapidly during the COVID-19 pandemic because it could detect SARS-CoV-2 shed by symptomatic, presymptomatic and asymptomatic individuals without relying on health-care attendance or clinical testing. **Objective:** This narrative review critically examines the principles, applications and limitations of WBE for early detection of emerging viral and bacterial threats, with emphasis on recent technological advances and the Indian public health context. **Key findings:** Wastewater signals can precede clinically reported trends when testing or reporting is delayed and can reveal transmission during periods of limited clinical surveillance. Established or emerging applications include poliovirus, SARS-CoV-2, influenza, respiratory syncytial virus, mpox, enteric viruses, antimicrobial-resistant bacteria and resistance genes. Genomic sequencing can identify circulating variants and previously unrecognized lineages, while metagenomic approaches offer the possibility of agnostic pathogen discovery. However, pathogen shedding, sewer connectivity, wastewater flow, rainfall, industrial discharge, sample processing and laboratory platforms introduce substantial uncertainty. Detection of nucleic acid does not necessarily indicate viable organisms, active transmission or clinical risk. Evidence for bacterial early-warning applications remains less mature than that for selected viruses. **Conclusion:** WBE is a powerful but indirect surveillance approach that should be integrated with clinical, laboratory, demographic and environmental data. Sustainable implementation requires standardized protocols, representative sampling, quality assurance, pre-agreed action thresholds, ethical safeguards and close communication between laboratories and public health authorities. India can build on its poliovirus and COVID-19 experience, but systems must accommodate open drains, decentralized sanitation and marked inequalities in sewer access.

Keywords: Wastewater-based epidemiology; environmental surveillance; emerging infections; genomic surveillance; antimicrobial resistance; India

INTRODUCTION

Infectious-disease surveillance traditionally depends on symptomatic individuals seeking care, clinicians recognizing disease, specimens being collected and laboratories reporting results. Each step introduces delay and selection. Mild or asymptomatic infections may remain invisible, while testing access, reporting practices and health-seeking behaviour can change over time. Wastewater-based epidemiology offers a complementary view by aggregating biological material excreted or washed from many people into a shared environmental sample.

Wastewater and environmental surveillance refers to testing sewage, wastewater or waters affected by human waste for pathogens, genetic material or other health-related markers. The underlying principle is straightforward: infected people may shed viruses, bacteria or microbial genes in faeces, urine, sputum, saliva, skin debris or other bodily material that enters wastewater. A sample collected from a treatment

plant, pumping station, sewer, institutional outlet or open drain may therefore reflect infection in part of the contributing population.

Environmental surveillance has supported the Global Polio Eradication Initiative for decades. The COVID-19 pandemic then transformed WBE from a specialized eradication tool into a widely recognized component of public health surveillance. Early studies demonstrated that SARS-CoV-2 RNA concentrations in sewage broadly tracked community infection dynamics and could provide earlier signals than reported case data when clinical reporting was delayed.[1,2]

The scope has since expanded. Public health programmes are investigating influenza, respiratory syncytial virus, mpox, measles, enteric pathogens, antimicrobial resistance and zoonotic threats. WHO now promotes a pathogen-agnostic framework in which wastewater surveillance is prioritized according to public health objectives, feasibility and the availability of an actionable response.[3]

Enthusiasm must nevertheless be balanced by methodological caution. Wastewater is a complex and changing mixture, not a direct population specimen. WBE rarely identifies infected individuals, cannot consistently estimate exact case numbers and may generate signals without clear public health meaning. Its value depends on whether results can inform decisions that clinical surveillance alone would not support as quickly or equitably.

The WBE Surveillance Pathway

A functional WBE system consists of linked epidemiological, environmental and laboratory components. The first step is defining the surveillance question. A programme designed to detect reintroduction of poliovirus requires different sampling frequency, laboratory sensitivity and action thresholds from one intended to monitor seasonal influenza trends.

Sampling sites determine population coverage and spatial resolution. Wastewater-treatment-plant influent can represent hundreds of thousands of residents efficiently but may conceal neighbourhood variation. Upstream sampling from pumping stations, manholes, open drains, hospitals, schools or prisons provides greater localization but covers smaller populations and raises stronger ethical concerns. Twenty-four-hour composite samples generally capture temporal variation better than single grab samples, but automated collection may be costly or technically difficult. Passive samplers can accumulate target material over time and may be useful in low-flow or non-sewered settings, although recovery differs by pathogen and device.

Samples undergo concentration, nucleic-acid extraction and pathogen detection, commonly using quantitative reverse-transcription polymerase chain reaction, digital PCR or targeted sequencing. Culture remains important for some bacterial pathogens and poliovirus because it can demonstrate viable organisms. Metagenomic sequencing can characterize broad microbial communities, resistance genes and unexpected agents but requires advanced laboratory and bioinformatic capacity.

Results must then be interpreted alongside catchment population, flow, rainfall, temperature, travel time and indicators of faecal strength. Normalization using wastewater flow, ammonia, crAssphage, pepper mild mottle virus or other markers may reduce variability, but no normalizer performs uniformly across all settings. The final and most frequently neglected step is translation into action: intensified clinical testing, vaccination, outbreak investigation, hospital preparedness or targeted risk communication.

Early Detection of Viral Threats

Poliovirus

Poliovirus remains the most mature application of environmental surveillance. Infected persons can shed virus in faeces without developing paralysis; consequently, acute flaccid paralysis surveillance detects only a small fraction of infections. Systematic sewage sampling can demonstrate silent circulation, document geographic spread and verify interruption of transmission.

Environmental detection does not automatically indicate a clinical case. Programme response depends on virus type, genetic characteristics, previous detections, population immunity and sampling context. Repeated detection of genetically related virus may indicate sustained circulation and trigger vaccination or intensified field investigation.

Polio also illustrates the limitations of WBE. Areas without connected sewerage may be poorly represented, while environmental isolates require high-quality culture, molecular typing and strict biosafety. Even a highly sensitive system cannot replace acute flaccid paralysis surveillance and clinical investigation.

SARS-CoV-2

SARS-CoV-2 established the operational value of WBE at unprecedented scale. In the Netherlands, viral RNA was detected in sewage during the early epidemic and broadly correlated with reported COVID-19 prevalence.[1] In Connecticut, sewage-sludge concentrations tracked community cases and hospital admissions and preceded reported test results by several days when reporting delays were present.[2]

The magnitude of the “lead time” is not fixed. Wastewater may precede case notifications when people delay testing or reporting is slow, but it may coincide with or follow clinical signals when testing is rapid. Claims that WBE universally predicts outbreaks weeks in advance are therefore inaccurate.

Wastewater sequencing also enabled variant surveillance when individual genomic testing declined. National-scale programmes demonstrated that variant frequencies in sewage could reproduce major clinical trends and identify emerging variants.[4] Mixed wastewater samples, however, contain viral material from many infections. Reconstructing lineages is difficult when genomes are fragmented or when variants share mutations.

A further insight was the detection of “cryptic” SARS-CoV-2 lineages not well represented in clinical databases. Such sequences may reflect prolonged infections, unobserved local transmission or non-human sources. They are useful signals for investigation but should not automatically be interpreted as newly circulating human variants.

Influenza and respiratory viruses

Influenza A, influenza B and respiratory syncytial virus can be detected in wastewater despite being primarily respiratory pathogens. Material may enter sewage through sputum, nasal secretions, faecal shedding, handwashing and bathing. Emerging evidence shows correlations between wastewater concentrations and clinical activity, particularly when the solids fraction is examined.

Respiratory-virus WBE may be valuable when testing is concentrated among hospitalized patients or when community testing declines. It may support decisions about hospital readiness, laboratory testing and public communication. Interpretation is more difficult than for enteric viruses because shedding pathways and quantities are less consistent.

Influenza A surveillance has additional One Health potential. Detection of viral material may originate from infected humans, animals, food-processing waste or animal products. Wastewater evidence alone cannot determine the host species or prove community transmission. Subtyping and linkage with veterinary and clinical surveillance are therefore essential.

Mpox and other emerging viruses

Mpox viral DNA has been detected in wastewater during outbreaks. US evaluation found relatively low sensitivity for a single reported case but substantially better detection as case numbers increased.[5] This demonstrates an important general principle: WBE performance depends on shedding intensity, disease prevalence and the size of the contributing population.

Wastewater surveillance may reduce dependence on care seeking for stigmatized infections, but localized sampling can also risk identifying small communities. Positive detection should not lead to stigmatizing assumptions about particular neighbourhoods or population groups.

Methods are being developed for measles, norovirus, hepatitis viruses, enteroviruses and emerging zoonotic pathogens. Before routine adoption, programmes must establish whether the target is reliably shed, whether the assay is specific and whether detection leads to an actionable intervention.

Detection of Bacterial Threats and Antimicrobial Resistance

Bacterial WBE presents distinct opportunities and difficulties. Sewage contains a vast background microbiome, including harmless commensals, environmental bacteria and clinically important pathogens. Detection of a species does not necessarily indicate disease because some organisms colonize healthy individuals or persist and multiply in wastewater.

Targeted surveillance may detect *Salmonella*, *Shigella*, pathogenic *Escherichia coli*, *Vibrio cholerae*, *Campylobacter*, *Clostridioides difficile* and other enteric pathogens. Culture can provide isolates for antimicrobial-susceptibility testing and genomic comparison, but many organisms lose viability during sewer transport or are outnumbered by background flora.

For emerging bacterial outbreaks, WBE is most useful when linked with clinical surveillance. A rise in a pathogen-specific marker may guide active case finding or food and water investigations, but it rarely identifies the source by itself.

Antimicrobial resistance is a particularly important application. Metagenomic analysis of urban sewage from 60 countries demonstrated that wastewater can characterize differences in the abundance and diversity of resistance genes across populations.[6] A systematic review subsequently found that wastewater surveillance can detect antibiotic-resistant bacterial pathogens, although methods and reporting remain highly heterogeneous.[7]

The “resistome”—the total collection of resistance genes in a sample—is not equivalent to the burden of clinically resistant infection. Resistance genes may occur in non-pathogenic organisms, extracellular DNA or dead bacteria. Metagenomic detection often cannot establish which bacterium carries a gene or whether that organism can cause human disease. Culture and long-read or linked-read approaches can improve assignment but add cost and complexity.

Hospital effluent, pharmaceutical-manufacturing discharge, livestock waste and domestic sewage may carry different resistance profiles. WBE can help identify environmental hotspots and monitor trends, but causal attribution requires source-specific sampling and chemical data on antimicrobial residues.

Table 1. Priority Applications of Wastewater-Based Epidemiology

Pathogen or threat	Principal surveillance objective	Major advantages	Important limitations	Potential public health response
Poliovirus	Detect silent transmission and verify elimination	Established methods; detects infection without paralysis	Incomplete coverage in non-sewered populations; intensive culture and typing required	Vaccination response, acute flaccid paralysis search and expanded environmental sampling
SARS-CoV-2	Monitor community trends and variants	Captures symptomatic and asymptomatic shedding; independent of test-seeking behaviour	Variable shedding, rainfall and sewer effects; exact case estimation unreliable	Hospital preparedness, clinical testing, vaccination messaging and variant investigation
Influenza and RSV	Track seasonal respiratory-virus activity	Supplements declining community testing; may provide population-wide trends	Respiratory shedding into sewage is incompletely characterized	Adjust diagnostic readiness, hospital capacity and public communication
Mpox	Detect community circulation where clinical presentation may be stigmatized or missed	Non-invasive and potentially less dependent on care seeking	Low sensitivity at very low case numbers; ethical concerns with small catchments	Targeted clinical awareness, testing access and vaccination where indicated
Measles and other vaccine-preventable viruses	Detect potential transmission before widespread recognition	May identify shedding during incubation or missed cases	Assays and interpretation remain under development; vaccine strains may complicate results	Active case finding, immunization review and outbreak preparedness
Enteric viruses	Detect norovirus, hepatitis A/E and other faecally shed agents	Strong biological rationale and often high shedding	High background circulation may reduce outbreak specificity	Food and water investigation, institutional alerts and hygiene

				measures
Enteric bacteria	Monitor <i>Salmonella</i> , <i>Shigella</i> , pathogenic <i>E. coli</i> , cholera and others	Can reveal population circulation beyond diagnosed cases	Background flora, poor viability and difficulty distinguishing colonization from disease	Culture confirmation, case finding and food-water source investigation
Antimicrobial resistance	Track resistant organisms and resistance genes	Captures community-wide resistome; supports One Health surveillance	Genes may be in non-pathogens or non-viable organisms; poor comparability between methods	Investigate hotspots, support stewardship and strengthen wastewater control
Novel or unexpected pathogens	Pathogen-agnostic detection through metagenomics	Potential to identify unanticipated agents or lineages	High cost, contamination, uncertain clinical meaning and bioinformatic complexity	Verification through targeted assays and risk-based epidemiological investigation
Institutional surveillance	Monitor hospitals, prisons, dormitories or schools	Greater spatial resolution and rapid cluster detection	Privacy and stigmatization concerns; small denominator variability	Local testing, infection-control measures and voluntary public health support

Public Health Significance

WBE offers three major public health advantages. First, it is less dependent on access to health care and diagnostic testing. One sample can aggregate contributions from a large population, including people with mild or asymptomatic infection.

Second, WBE can remain informative when clinical surveillance changes. During COVID-19, home testing and declining laboratory reporting weakened case counts, whereas wastewater trends remained comparable over time within consistently monitored catchments.

Third, sewage can support multi-pathogen surveillance. Once collection, transport and laboratory systems are established, the same sample may be analysed for several targets. This creates potential efficiencies, but adding assays without a clear decision pathway can generate expensive data with little public health value.

WBE also has an equity dimension. It may reveal transmission in populations underserved by testing. Yet sewer-based systems can exclude informal settlements, rural communities, septic-tank users and populations without toilets. A system described as population-wide may therefore reproduce sanitation inequalities unless open drains, pumping stations and decentralized sanitation are intentionally incorporated.

Indian Perspective

India has extensive experience with environmental poliovirus surveillance, which contributed to the verification and maintenance of polio-free status. The COVID-19 pandemic stimulated WBE initiatives in Bengaluru, Hyderabad, Pune, Surat, Ahmedabad, Mumbai, Assam and other settings.

Bengaluru demonstrated the feasibility of combining sewage-treatment plants with open-drain sampling in a city where sewer connectivity is incomplete. This hybrid model is highly relevant for Indian cities because treatment-plant surveillance alone may omit populations living outside formal sewer networks.[8]

Indian studies generally found that SARS-CoV-2 wastewater trends corresponded with reported epidemic waves and could reveal continuing circulation during periods of low clinical testing. Genomic sequencing also contributed to variant monitoring. However, many initiatives remained research projects rather than permanently financed public health programmes.

India's sanitation diversity creates specific challenges. Wastewater may move through sewers, open drains, septic tanks, faecal-sludge systems and surface waters. Catchment populations are dynamic and

often poorly documented. Monsoon rainfall can dilute wastewater or cause overflow and mixing between drainage systems.

The country also has a compelling opportunity to integrate WBE with antimicrobial-resistance surveillance. Indian investigators have detected antibiotic-resistant bacteria across sewage-carrying drains in multiple cities, supporting concern that untreated wastewater and inadequate sanitation may serve as reservoirs and mixing environments for resistance.[9] Such findings should not be interpreted as direct estimates of resistant clinical infection, but they can guide environmental and One Health investigation.

A national platform could link the Integrated Health Information Platform, poliovirus networks, Indian Council of Medical Research laboratories, CSIR institutes, urban local bodies, sewage-treatment utilities and the National One Health Mission. The objective should be a coordinated surveillance service rather than disconnected pathogen-specific projects.

Recent Advances

Wastewater genomic surveillance has progressed from targeted mutation detection to deep sequencing and haplotype reconstruction. Improved bioinformatics can estimate the relative abundance of known variants and identify unusual mutation combinations. Global wastewater-genomics communities established through WHO's pathogen-surveillance networks are beginning to improve protocol exchange and interoperability.

Digital PCR offers greater tolerance of inhibitors and absolute target quantification without external calibration curves. Near-source biosensors and rapid concentration methods aim to shorten the interval between sample collection and public health reporting. Highly sensitive rapid platforms remain under validation, particularly for low-resource environments.

Metagenomic sequencing allows simultaneous detection of viral, bacterial and resistance targets. It is attractive for preparedness because it does not require prior selection of a single pathogen. However, untargeted sequencing is less sensitive for rare organisms, and identification depends on reference databases that remain incomplete.

Machine-learning and forecasting models are increasingly applied to relate wastewater signals to hospitalizations or reported cases. Their apparent accuracy can deteriorate when clinical testing, immunity, pathogen shedding or catchment composition changes. Models require continual recalibration and should support rather than automate public health decisions.

Airports, aircraft, ships and mass gatherings are emerging sampling environments. These sites may provide information about pathogen movement across borders, but they cannot reliably identify the country or individual source of a signal and require careful ethical governance.

Challenges and Limitations

The central analytical problem is variability. Pathogen concentrations reflect the number of infected people, quantity and duration of shedding, wastewater volume, sewer residence time, temperature, chemical inhibitors and laboratory recovery. Two communities with equal infection prevalence may generate different concentrations.

Shedding distributions are poorly defined for many pathogens. A small number of high shedders may dominate the signal, while some infected people contribute little. Back-calculating exact prevalence therefore requires uncertain assumptions.

Standardization is incomplete. Programmes differ in sample type, concentration method, target genes, controls, extraction, quantification, normalization and reporting units. Trends within one consistently operated site are often more reliable than direct comparison between laboratories.

Detection of nucleic acid does not demonstrate infectivity. Viral RNA or bacterial DNA can persist after organisms are no longer viable. Conversely, degradation may produce false-negative results even when transmission is occurring.

Action thresholds remain underdeveloped. A statistically significant rise is not automatically clinically meaningful, and a single positive result for a rare pathogen may represent contamination, travel-related shedding or assay error. Confirmatory sampling and pre-specified response algorithms are essential.

Ethical risks increase as sampling becomes more local. Monitoring a city treatment plant is broadly anonymous; monitoring a single building, prison wing or neighbourhood can create stigmatization or quasi-identification. Governance should define the smallest permissible catchment, data access and communication procedures.

Finally, WBE can divert resources if introduced without considering opportunity costs. A technically sophisticated programme is not justified when basic case reporting, laboratory diagnosis, sanitation or outbreak response remain unfunded.

Policy Implications

National WBE strategies should begin with explicit public health use cases. Pathogens should be prioritized according to disease severity, shedding evidence, assay performance, surveillance gaps and availability of an actionable response.

Quality-assurance systems should include process controls, recovery standards, negative and positive controls, proficiency testing and transparent metadata. Results should be reported with uncertainty and contextual information rather than as unqualified numerical estimates of infection.

Environmental data must be integrated with clinical cases, hospital admissions, laboratory results, vaccination, meteorology and sewer operations. Multidisciplinary interpretation teams should include epidemiologists, microbiologists, engineers, utility staff and public health decision-makers.

Communication protocols should specify what constitutes an alert, who receives it and what action follows. Public dashboards should avoid false precision and clearly explain that wastewater activity represents trends rather than confirmed individual cases.

Future Directions

Future research should define pathogen-specific shedding kinetics, environmental decay and relationships between wastewater concentrations and clinically important outcomes. Prospective multi-site studies are needed outside high-income sewered settings.

Methods must be developed for open drains, septic systems and faecal-sludge treatment. India and other low- and middle-income countries should lead this research rather than adopting protocols designed exclusively for centralized sewer networks.

For bacterial surveillance, culture, targeted sequencing and metagenomics should be combined to link resistance genes with clinically relevant organisms. Environmental signals should be compared with clinical antimicrobial-resistance data and antimicrobial use.

Economic evaluations should identify when multi-pathogen WBE provides better value than expanded clinical testing. Costs should include sampling, transport, laboratory platforms, data systems and the capacity to act on alerts.

Data standards and interoperable platforms are required so that results can be compared across regions and incorporated into international early-warning systems. Artificial intelligence should remain interpretable and subject to epidemiological review.

Most importantly, every surveillance target should have an agreed response. Detecting a threat without capacity for verification, communication or intervention creates information rather than preparedness.

CONCLUSION

Wastewater-based epidemiology has evolved from a specialized tool for poliovirus surveillance into a versatile platform for monitoring infectious threats. Its major strength is the ability to observe community-level biological signals without depending on individual testing or health-care attendance.

Evidence is strongest for poliovirus and SARS-CoV-2, while applications for influenza, RSV, mpox, enteric pathogens and antimicrobial resistance are expanding. Genomic and metagenomic methods create opportunities for variant tracking and early detection of unexpected threats, but they also introduce interpretive uncertainty.

WBE cannot identify every infected person, reliably calculate exact prevalence or prove that detected organisms are viable. Its greatest value lies in trends, early signals and surveillance of populations otherwise underrepresented in clinical data.

India can build a durable platform from its polio infrastructure and COVID-19 experience. Success will require hybrid sampling for sewered and non-sewered populations, permanent laboratory networks, data integration and clear public health action pathways.

Wastewater should be viewed as a pooled population specimen: informative, efficient and imperfect. When interpreted alongside clinical and environmental evidence, it can strengthen preparedness and provide earlier warning of emerging viral and bacterial threats. When used without standardized

methods or actionable objectives, it risks producing technically impressive signals with limited public health consequence.

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