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Wastewater-Based Public Health Surveillance for Emerging Infectious Diseases and Antimicrobial Resistance

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Abstract

Wastewater based public health surveillance has emerged as a complementary approach for monitoring infectious diseases and antimicrobial resistance (AMR) at the population level. Unlike conventional clinical surveillance, wastewater analysis can provide community wide information without relying on individual testing or healthcare attendance. This review examines the application of wastewater based epidemiology for monitoring antimicrobial resistance. Current approaches include culture-based methods, PCR, metagenomic sequencing, and bioinformatic analysis for identifying pathogens. Wastewater surveillance can support early detection of pathogen circulation that may remain underrecognized by clinical surveillance. Recent advances in wastewater genomics and machine learning have further expanded opportunities for pathogen detection. However, differences in sampling strategies, wastewater composition, and interpretation of resistance markers remain important challenges. Detection of antimicrobial resistance genes does not always indicate viable resistant pathogens or clinically relevant resistance. Standardized protocols and ethical governance are therefore required to improve reliability and public health utility. Integrating wastewater surveillance with clinical and genomic data within a One Health framework could strengthen early warning systems and improve preparedness for emerging infectious diseases.

1. Introduction

The emergence and rapid spread of infectious diseases have exposed important limitations in conventional public health surveillance. Clinical surveillance remains the primary approach for identifying infections, estimating disease burden, and guiding patient management. However, clinical systems depend on individuals seeking healthcare and being correctly diagnosed. Mild, asymptomatic, undiagnosed, and geographically isolated infections can therefore remain outside routine surveillance. These limitations become particularly important during the early phase of an outbreak, when the number of clinically recognized cases may still be small. Wastewater-based surveillance has emerged as a complementary approach that can provide population-level information without requiring individual diagnostic testing. Wastewater contains biological material continuously released by a population. Viral RNA, bacterial DNA, antimicrobial resistance genes, and metabolites can enter sewage through wastewater. Sampling wastewater therefore provides an opportunity to examine a pooled biological signal generated by a community. The approach is particularly useful for pathogens that are shed into wastewater before or during clinical infection (Mkilima, 2025; Singh et al., 2024). Poliovirus surveillance has used sewage sampling for decades, particularly because infected individuals can shed virus without developing recognized paralytic disease. The approach became an important component of the Global Polio Eradication Initiative and demonstrated that environmental samples could identify viral circulation in populations (HOVI et al., 2012). The same principle was subsequently adapted to other pathogens.

The COVID-19 pandemic substantially expanded the application of wastewater surveillance. SARS-CoV-2 RNA could be detected in wastewater and monitored over time,

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allowing population-level changes in viral circulation to be assessed independently of individual diagnostic testing. A global review of wastewater-based epidemiology reported considerable variation in the lead time between wastewater signals and clinical case increases. Geographic region, sampling frequency, seasonality, and clinical testing practices influenced the observed relationship (Kumar et al., 2022). The findings established an important principle for wastewater surveillance. A wastewater signal should be interpreted as a complementary epidemiological indicator rather than as a direct substitute for clinical case counts. The application has now expanded beyond SARS-CoV-2. Several bacterial pathogens have been investigated through wastewater surveillance (Pramanik et al., 2025). Antimicrobial resistance has become another major area of interest because wastewater contains antibiotic-resistant bacteria and antimicrobial resistance genes (ARGs) (Foxman et al., 2025; Punch et al., 2025). Recent developments in sequencing and metagenomics have further changed the scope of wastewater surveillance. Conventional PCR can detect predefined targets with high sensitivity, whereas sequencing can provide information about pathogen variants. Machine learning is also being incorporated into wastewater analysis to identify temporal patterns and support interpretation of complex genomic datasets (Zhuang et al., 2025). The current direction of wastewater surveillance is therefore moving from single-pathogen detection toward integrated public health intelligence. A single wastewater sample can potentially provide information about multiple infectious diseases and antimicrobial resistance simultaneously. The combination of molecular diagnostics, epidemiological modelling, and artificial intelligence could establish wastewater as a continuous population-level monitoring platform. The major challenge is now to convert biological signals into reliable and actionable public health information.

2. From Environmental Surveillance to Wastewater-Based Public Health Intelligence

Poliovirus is one of the examples for environmental surveillance. Infection can occur without recognizable clinical symptoms, while infected individuals can shed poliovirus in feces. Sewage sampling can therefore identify viral circulation that may not be apparent from acute flaccid paralysis surveillance alone (Kishore et al., 2024). An analysis of poliovirus surveillance in Pakistan between 2011 and 2015 demonstrated the potential value of environmental sampling. The estimated average population sensitivity of a single environmental sample was 59.4%, whereas the average monthly sensitivity of acute flaccid paralysis surveillance in a district and neighbouring districts was approximately 30%. Environmental sensitivity varied between sampling locations with median estimates ranging from 33.3% to 79.2%. The results also demonstrated that wastewater surveillance is not uniformly sensitive. Sampling-site selection and local epidemiological conditions strongly influence performance. Environmental surveillance continued to contribute to global poliovirus monitoring during 2022–2023. The integration of environmental sampling with clinical surveillance remained important for identifying poliovirus circulation and monitoring transmission in settings where clinical disease was not sufficient to define the extent of transmission (O'Reilly et al., 2018). The polio experience established several principles that remain relevant to modern wastewater surveillance. Sampling locations should represent an appropriate population. Sampling frequency should be sufficient to detect temporal changes. Laboratory methods require high sensitivity and quality control. Genomic characterization is particularly valuable when the objective is to determine whether detected viruses represent persistent transmission, importation, or emergence of a new lineage.

SARS-CoV-2 transformed wastewater surveillance from a specialized environmental technique into a widely used public health surveillance approach. Viral RNA released by infected individuals can enter sewage, allowing community-level monitoring without testing each person. This became particularly useful when clinical testing capacity was limited or when testing behaviour changed during different phases of the pandemic. Longitudinal studies demonstrated that wastewater measurements could follow increases and decreases in viral circulation. A global assessment of COVID-19 wastewater studies showed that the lead time between wastewater signals and reported clinical cases varied considerably between locations (Kumar et al., 2022). The variation emphasizes that wastewater surveillance performance depends on sampling frequency, wastewater composition, and the sensitivity of clinical surveillance. An important development was the expansion from quantitative viral detection to genomic surveillance. A study from Hong Kong analysed wastewater samples representing more than six million people and used sequencing to monitor the replacement of Delta by Omicron and the emergence of Omicron sublineages (Xu et al., 2023). Wastewater genomic signals preceded some corresponding clinical trends, demonstrating that sewage can provide not only information about the quantity of viral material but also information about the genetic composition of the circulating viral population. A two-year longitudinal study conducted in Pune monitored 11 sewer sheds between January 2022 and December 2023. RT-qPCR was used to detect SARS-CoV-2, influenza A H1N1, influenza A H3N2, and RSV. Respiratory viral RNA was detected in wastewater before corresponding clinical cases were

reported in several instances (Pramanik et al., 2025). The study is particularly relevant because it demonstrates that wastewater surveillance can be adapted to multiplex respiratory virus monitoring rather than remaining focused on a single pathogen. Wastewater sequencing has also been used to detect emerging SARS-CoV-2 lineages. A study involving wastewater samples collected in Mumbai between August 2022 and June 2023 identified emerging lineage-associated signals before their clinical recognition. The study demonstrated the potential of combining wastewater sequencing with genomic surveillance to identify changes in viral populations at an early stage (Kadam et al., 2025). These observations indicate a major transition in wastewater epidemiology.

3. Wastewater Surveillance for Emerging and Re-emerging Infectious Diseases

3.1 Respiratory viruses

Respiratory viruses represent an important target group because several pathogens can circulate simultaneously and produce overlapping clinical symptoms. Influenza, RSV, and SARS-CoV-2 can therefore be monitored through a common wastewater surveillance framework. The two-year Pune study demonstrated the feasibility of simultaneous surveillance for influenza A, RSV, and SARS-CoV-2 using wastewater RT-qPCR (Pramanik et al., 2025). The detection of respiratory viral RNA before clinical cases increase supports the potential use of wastewater as an early population-level signal. Similar surveillance programmes have been developed in other countries, including studies that monitor influenza A, influenza B, RSV, and SARS-CoV-2 together. Congregate settings provide another useful application. Long-term care facilities and other high-density environments can experience rapid pathogen transmission. Wastewater monitoring in such locations may provide an additional signal without requiring continuous individual testing. The approach is particularly relevant to populations at increased risk of severe respiratory disease. The interpretation of respiratory virus signals still requires caution. Viral shedding varies among individuals, and wastewater concentrations can be affected by dilution and changes in population size. Wastewater detection therefore provides evidence of viral material entering the sewer system rather than a direct measurement of the number of infected individuals.

3.2 Variant and genomic surveillance

Quantitative PCR provides rapid information about pathogen abundance, but it cannot normally determine the genetic composition of the circulating population. Sequencing adds this information and can identify variant proportions, mutations, and temporal changes. Wastewater sequencing successfully tracked the transition from Delta to Omicron in Finland. A longitudinal analysis of wastewater samples collected during 2021–2022 showed that the wastewater variant profile was consistent with clinical genomic observations from the same population (Lipponen et al., 2024). The finding supports the use of wastewater as a complementary genomic surveillance system. Nanopore sequencing has also been investigated for rapid wastewater genomic surveillance. The principal advantage is the possibility of generating sequence information with relatively short turnaround times. However, wastewater genomes are often fragmented because samples contain genetic material from multiple individuals at different stages of infection. Low target concentrations can also reduce genome coverage. These limitations require appropriate enrichment, sequencing depth, and bioinformatic quality control (Vigil et al., 2024). Machine learning provides another emerging approach. A 2025 study analysed 3,659 wastewater samples from urban and rural locations in Southern Nevada and combined genome sequencing with machine-learning approaches to identify SARS-CoV-2 variant-associated mutation patterns (Zhuang et al., 2025). The study illustrates how genomic surveillance can move beyond simple variant identification toward recognition of complex mutation patterns. The combination of quantitative molecular detection and sequencing is therefore becoming increasingly important. PCR can provide rapid information about abundance, while sequencing can characterize genetic diversity. Integration of both approaches can improve the ability to identify emerging pathogen lineages.

3.3 Enteric and other emerging pathogens

Enteric pathogens are particularly suitable for wastewater surveillance because gastrointestinal shedding can result in substantial pathogen concentrations in sewage. Poliovirus remains the most established example. Other targets include hepatitis A and E viruses, norovirus, cholera-associated organisms, typhoid and paratyphoid organisms, and other enteric pathogens. Mpox represents an example of a relatively recent application. Mpox virus DNA can be shed through skin lesions, urine, and stool, allowing environmental detection in wastewater. An analysis conducted by the U.S. Centers for Disease Control and Prevention included 3,492 wastewater samples collected from 89 sites in 26 counties and compared results with 281 reported mpox cases (Adams et al., 2024). Weekly wastewater surveillance had a sensitivity of 32% for detecting one or more persons shedding virus, increasing to 49% when at least five persons were shedding and 77% when at least 15 persons were shedding. The findings demonstrate that wastewater sensitivity depends strongly on the number of infected persons contributing to the sampled catchment. Fungal pathogens

provide another emerging application. Candida auris is an important healthcare-associated pathogen because of antifungal resistance, environmental persistence, and the potential for transmission within healthcare facilities. A Southern Nevada study collected 91 wastewater samples from seven sewersheds and detected C. auris in 72 samples, corresponding to 79% positivity (Barber et al., 2023). Detection was higher in sewersheds serving healthcare facilities involved in outbreaks than in other sewersheds. The findings suggest that wastewater could provide an additional environmental signal for monitoring healthcare-associated pathogens. Such applications expand the concept of wastewater surveillance beyond common viral diseases. Emerging bacterial, fungal, and viral pathogens can potentially be incorporated into surveillance systems when sufficient biological shedding and reliable molecular targets are available (Table 1).

Table 1: Applications of wastewater surveillance for emerging infectious diseases

Infectious Disease/ Pathogen	Surveillance Target	Detection Approach	Public Health Relevance	Reference
SARS-CoV-2	Viral RNA and variant lineages	RT-qPCR, digital PCR, sequencing	Wastewater concentrations can provide population-level signals of SARS-CoV-2 circulation and can support early identification of changes in community transmission.	(Zhuang et al., 2025)
Influenza A	Influenza A RNA	RT-qPCR	Longitudinal wastewater monitoring can detect influenza circulation and complement clinical respiratory-virus surveillance.	(Pramanik et al., 2025)
Respiratory syncytial virus	RSV RNA	RT-qPCR	Wastewater detection provides an additional population-level indicator for respiratory-virus activity.	(Pramanik et al., 2025)
Mpox	Mpox viral DNA	qPCR	Wastewater surveillance detected mpox signals during community transmission and provided a complementary surveillance approach.	(Adams et al., 2024)
Candida auris	C. auris DNA	qPCR / molecular detection	Community-scale wastewater monitoring demonstrated the potential to identify circulation of an emerging fungal pathogen during an outbreak.	(Barber et al., 2023)
Poliovirus	Poliovirus RNA	Environmental sampling and molecular surveillance	Environmental surveillance can detect poliovirus circulation in populations where clinical acute flaccid paralysis surveillance may miss infections.	(O'Reilly et al., 2018)

4. Wastewater Surveillance of Antimicrobial Resistance

4.1 Antibiotic resistance genes and resistant bacteria

Antimicrobial resistance presents a more complex surveillance challenge than single-pathogen detection. Resistance involves multiple bacterial species, ARGs, mobile genetic elements, antimicrobial exposure, and environmental selection. Wastewater receives microorganisms and resistance determinants from households, hospitals, healthcare facilities, agriculture, food production, pharmaceutical manufacturing, and other sources. Wastewater can therefore provide a population-level view of the resistome. Frequently investigated resistance determinants include genes associated with β-lactam, carbapenem, aminoglycoside, macrolide, tetracycline, sulfonamide, and quinolone resistance. A global metagenomic study demonstrated substantial geographical differences in wastewater resistomes. ARG relative abundance was higher in some densely populated settings, including Hong Kong and India, than in Sweden and Switzerland in the analysed dataset. Associations between antibiotic resistance and antibiotic concentrations, metal resistance genes, and environmental factors were also identified (Prieto Riquelme et al., 2022). Such observations demonstrate that wastewater AMR surveillance can provide information about ecological drivers of resistance in addition to simply documenting resistant bacteria. Hospital wastewater is particularly important because healthcare facilities can contain high concentrations of clinically relevant resistant organisms. Metagenomic analyses have identified numerous ARGs

in hospital wastewater, including resistance determinants associated with clinically important Gram-negative pathogens. A national-scale comparison of high-throughput qPCR and metagenomic approaches further demonstrated the value of combining targeted and untargeted methods (Alhazmi et al., 2024). High-throughput qPCR can sensitively quantify predefined resistance genes, while metagenomics provides broader information and can identify resistance determinants that were not included in a predefined assay panel. Culture-based methods remain useful because they permit recovery of viable resistant organisms and allow subsequent antimicrobial susceptibility testing. Molecular methods provide greater sensitivity for detecting low-abundance genes, while metagenomics can characterize multiple resistance determinants and microbial hosts. No single method captures all dimensions of environmental AMR.

4.2 Linking environmental resistance to clinical risk

The major challenge in AMR wastewater surveillance is interpretation. Detection of an ARG does not automatically indicate the presence of a clinically important resistant pathogen. Resistance genes may originate from environmental bacteria, non-pathogenic organisms, and transient microbial populations. Risk interpretation becomes stronger when an ARG is associated with a clinically important bacterial host. Metagenomic approaches can provide information about these relationships, although host assignment and mobility assessment remain technically challenging. A systematic review of wastewater surveillance for antibiotic-resistant bacterial pathogens identified 48 relevant studies comparing wastewater findings with clinical evidence. Escherichia coli, Enterococcus spp., and Enterobacteriaceae were among the most frequently investigated organisms (Tiwari et al., 2022). The review also emphasized that relating wastewater resistance to clinical cases is difficult because wastewater can contain resistant organisms originating from asymptomatic individuals, animals, and environmental sources. A 2025 analysis further emphasized the potential and limitations of wastewater surveillance for public health action. ARGs and resistant bacteria can be detected in wastewater and may provide early information about emerging resistance. Meanwhile, the current methods remain too variable for some quantitative comparisons across space and time. Recent reviews have consequently shifted attention toward standardization and integration. A global One Health review of wastewater AMR surveillance from 2014 to 2024 identified substantial growth in the field but also considerable methodological heterogeneity (Foxman et al., 2025; Punch et al., 2025). Another recent review emphasized the need for longitudinal studies across geographically diverse populations and population-level drivers of AMR (Malcom & Bowes, 2025). Wastewater surveillance should therefore be considered complementary to clinical antimicrobial susceptibility testing. Its major value lies in providing a population-level environmental signal that can identify resistance patterns that may not yet be visible through conventional clinical reporting. (Table 2).

Table 2: Wastewater surveillance for antimicrobial resistance

AMR target	Surveillance approach	Major observations	Public-health application	Reference
Antibiotic-resistant bacteria	Culture-based detection	Wastewater can capture resistant bacterial populations circulating within connected communities.	Community-level AMR monitoring	(Tiwari et al., 2022)
Antibiotic-resistance genes	qPCR / metagenomics	ARG profiles in wastewater can provide information on the community resistome and its temporal variation.	Monitoring resistance burden and emerging resistance determinants	(Chau et al., 2022)
ESBL-producing bacteria	Culture and molecular detection	ESBL-producing organisms have been repeatedly detected in wastewater, supporting wastewater as a complementary AMR surveillance matrix.	Early identification of resistant bacterial populations	(Tiwari et al., 2022)
Carbapenem-resistant Enterobacteriaceae	Culture-based and molecular methods	Carbapenem-resistant organisms have been detected in community and hospital wastewater.	Surveillance of clinically important resistance	(Tiwari et al., 2022)

Vancomycin-resistant Enterococcus	Culture-based surveillance	Wastewater can identify VRE circulation at population and healthcare-associated levels.	Tracking resistant enterococci	(Punch et al., 2025)
Multidrug-resistant organisms	Metagenomic / culture-based approaches	Combined genomic and culture approaches provide broader information on resistant organisms and resistance determinants.	One Health AMR surveillance	(Punch et al., 2025)
Community resistome	Shotgun metagenomics	Wastewater metagenomics can characterize diverse resistance genes without restricting surveillance to predefined organisms.	Population-level resistome monitoring	(Prieto Riquelme et al., 2022)

5. Sampling, Laboratory Detection and Wastewater Genomics

Reliable wastewater surveillance begins with appropriate sampling. A grab sample represents wastewater at a single point in time. Composite sampling collects wastewater over a defined period and can reduce short-term variation. Flow-weighted composite sampling can further account for changes in wastewater volume. Sampling location determines the population represented by the surveillance system. Large municipal treatment plants can represent hundreds of thousands of people, whereas hospital sewers and residential neighbourhoods provide more localized information. Small catchments may be particularly useful for outbreak investigation, while large catchments provide broader population-level surveillance. Wastewater characteristics can influence pathogen recovery. Viral RNA and bacterial DNA can also partition differently between the liquid and solid fractions. For this reason, some surveillance programmes analyse wastewater solids rather than only liquid samples. The analytical workflow generally includes sample collection, concentration, nucleic acid extraction, inhibitor removal, molecular detection, normalization, and interpretation. RT-qPCR remains widely used for targeted viral RNA detection because it is sensitive and relatively rapid. Digital PCR provides precise quantification at low target concentrations. Metagenomic sequencing provides a broader approach by characterizing multiple organisms and genetic determinants simultaneously.

Targeted and untargeted methods have different strengths. A surveillance programme monitoring a known pathogen can use qPCR or digital PCR for rapid and cost-effective detection. Emerging-pathogen surveillance requires broader sequencing because the biological target may not be known in advance. Genomic surveillance has already demonstrated the additional information obtained from sequencing. A coordinated workflow combining droplet digital PCR with nanopore sequencing showed that quantitative PCR can provide rapid estimates of SARS-CoV-2 concentration while sequencing provides information about circulating variants and mutation patterns (Vigil et al., 2024). Normalization is essential for interpreting quantitative wastewater measurements. Raw gene copies per litre cannot always be compared directly between samples because wastewater dilution and population contributions vary. Human fecal markers, population estimates, and chemical biomarkers can be used to account for some of this variation. No single normalization approach is universally applicable. The appropriate method depends on the pathogen, wastewater characteristics, and intended interpretation. Consistent use of normalization methods becomes particularly important when data from multiple treatment plants or geographical regions are compared. Quality control must also cover the molecular and sequencing workflow. Extraction controls, amplification controls, inhibition controls, recovery controls, negative controls, sequencing controls, and reference materials are needed to distinguish genuine biological signals from laboratory contamination and analytical artefacts. Standardization remains a major research need. Differences in sampling frequency, concentration methods, extraction protocols, primer design, normalization, sequencing platforms, and bioinformatic pipelines can produce different results even when the same pathogen is being studied. Establishing common protocols will therefore be essential for integrating wastewater data across regions.

6. Data Integration, One Health and Public Health Decision-Making

Wastewater data become more informative when integrated with clinical, epidemiological, and demographic information. A wastewater signal generally indicates that biological material associated with a pathogen is entering the sewer system. The signal does not automatically provide the number of infected individuals or indicate disease severity. Clinical surveillance can help determine whether an increasing wastewater signal corresponds with increasing diagnosed infections. Hospital admission data can provide information about clinical severity. Vaccination coverage can influence the relationship between infection and hospitalization. Mobility data can explain changes in the population contributing to a catchment. Rainfall and wastewater flow can help identify dilution-related changes.

The COVID-19 experience demonstrated the importance of this integrated approach. Wastewater concentrations were most useful when evaluated as longitudinal trends rather than isolated measurements (Kumar et al., 2022). Genomic sequencing added another layer by allowing changes in viral lineages to be identified. Artificial intelligence and machine learning may further improve interpretation. Time-series models can combine wastewater concentrations with clinical cases, population characteristics, and genomic information. Such models may help identify unusual patterns that are difficult to recognize from individual datasets. Recent wastewater research has already demonstrated the use of machine learning for identifying emerging SARS-CoV-2 genomic patterns (Zhuang et al., 2025). The One Health framework is particularly relevant because wastewater represents an interface between human health, and microbial evolution. Human wastewater can contain organisms and genes originating from households, healthcare facilities, food systems, agriculture, and industrial sources. AMR surveillance therefore benefits from an approach that considers the movement of resistance across these interconnected systems (Punch et al., 2025). Hospital wastewater represents an important component of this framework. Healthcare facilities can act as reservoirs for multidrug-resistant organisms and clinically important ARGs. Community wastewater provides information about broader population-level resistance. Comparison between the two can help identify differences in resistance patterns and potential sources. The public health value of wastewater surveillance ultimately depends on whether surveillance results lead to action. An increasing viral signal may trigger additional clinical testing, communication with healthcare facilities, or targeted genomic sequencing. Detection of a clinically important resistance determinant may support further investigation of healthcare or environmental sources. A 2025 scoping review illustrates an important gap between surveillance and action. Among 974 wastewater surveillance studies, only 84 described public health action and only 28 incorporated strategies to facilitate action within their study design (van der Drift et al., 2025). Most studies that resulted in public health action involved SARS-CoV-2 and poliovirus, while more recent applications included influenza, RSV, hepatitis A, and mpox. This finding indicates that future wastewater studies should define the intended public health response before surveillance begins. A technically sensitive assay has limited practical value if the resulting signal cannot be interpreted or connected to an appropriate intervention.

7. Current Challenges and limitations

Wastewater surveillance has several limitations that must be addressed before it can function as a fully standardized global surveillance system. The first challenge is biological variability. Pathogen shedding differs between individuals and changes across the course of infection. Viral shedding may occur through different biological routes. Bacterial abundance can be affected by colonization, antibiotic exposure, and environmental persistence. These differences make direct conversion of wastewater concentration into the number of infected people difficult. The second challenge is wastewater heterogeneity. Catchment populations vary in size and composition. Industrial discharge, rainfall, water consumption, sewer leakage, and intermittent flow can alter wastewater characteristics. Population mobility further complicates interpretation. Airports, universities, hospitals, tourist areas, and mass gatherings can contain populations that differ substantially from the permanent residential population. The third challenge concerns laboratory standardization. Different studies use different concentration methods, extraction protocols, molecular targets, controls, normalization markers, sequencing platforms, and bioinformatic pipelines. Comparisons between locations can therefore become difficult. The fourth challenge involves distinguishing molecular detection from infectiousness. PCR detects nucleic acids and does not necessarily demonstrate the presence of viable organisms. Detection of viral RNA or bacterial DNA therefore should not automatically be interpreted as evidence of infectious material or active transmission.

AMR introduces an additional interpretive problem. ARG detection does not establish clinical resistance, and the presence of a resistance gene does not necessarily indicate that the dominant wastewater organism is a human pathogen. More informative surveillance should therefore connect ARGs with their bacterial hosts, mobile genetic elements, abundance, persistence, and clinical importance. Emerging pathogens present another challenge. Targeted PCR is highly effective when the organism is already known, but a predefined assay cannot identify an unknown pathogen. Metagenomic sequencing provides a broader alternative, although low-abundance organisms, fragmented genomes, contamination, incomplete reference databases, and computational requirements remain important limitations. Ethical considerations are also becoming increasingly relevant. Wastewater surveillance is often described as anonymous because samples generally represent populations rather than individuals. However, small catchments can correspond to specific institutions or vulnerable communities. A positive signal from a school, prison, nursing facility, or small residential area could create stigma or unintended consequences. Responsible surveillance therefore requires careful consideration of privacy, data governance, communication, and proportionality. Another major research gap is the limited evidence linking wastewater signals directly to public health outcomes. Detection studies are abundant, but fewer investigations demonstrate how wastewater information changed clinical management,

outbreak control, antimicrobial stewardship, vaccination strategies, or allocation of public health resources (van der Drift et al., 2025). Future studies should therefore move toward prospective surveillance designs in which sampling, interpretation, thresholds, and response pathways are defined in advance. Such designs would allow the real-world public health value of wastewater surveillance to be measured rather than inferred from analytical performance alone. Low-resource settings also require greater attention. Wastewater surveillance could provide useful population-level information where clinical diagnostic capacity is limited. However, incomplete sewer networks, limited laboratory infrastructure, and financial constraints can restrict implementation. Low-cost concentration methods, portable molecular platforms, simplified sample preservation, and decentralized sequencing could help extend surveillance to underserved settings.

7. Conclusion

Wastewater-based surveillance has therefore evolved from an environmental monitoring technique into an emerging component of public health intelligence. Evidence from poliovirus, SARS-CoV-2, respiratory viruses, mpox, Candida auris, and antimicrobial resistance demonstrates its broad potential. At the same time, variation in pathogen shedding, wastewater composition, sampling strategies, analytical methods, and interpretation prevents wastewater measurements from being treated as direct substitutes for clinical data. Future progress will depend on standardization, genomic technologies, multiplex detection, One Health integration, artificial intelligence, and clearly defined public health response pathways. The central transition is therefore from simply detecting biological signals in wastewater to converting those signals into reliable, timely, and actionable information for infectious disease preparedness and antimicrobial resistance control.

Declarations

Ethics approval statement

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Data Availability Statement

The data are available from the corresponding author upon reasonable request

Competing Interests

The authors declare that they have no conflict of interest

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