CORPORATE

ECDC framework to guide the integration of wastewater-based surveillance into infectious disease surveillance at the EU/EEA level

December 2025

Summary

This ECDC framework serves to guide the integration of wastewater-based surveillance (WBS) into infectious disease surveillance and public health decision-making at the EU/EEA level. It outlines how ECDC will strategically and scientifically lead this integration and provides contextual information for policymakers at the national level and in EU institutions, public health stakeholders at the national level, and country actors in the relevant sectors. Key European initiatives promoting WBS and the role of WBS in infectious disease surveillance are also described, including important considerations and challenges.

WBS can provide useful data for monitoring polioviruses, SARS-CoV-2, influenza viruses and other pathogens. It can also offer informative infectious disease surveillance data for risk assessments and public health decision-making, and can complement data from other established indicator- and event-based surveillance. WBS data should be interpreted and communicated in relation to data collected by these other surveillance systems. WBS systems for public health need careful planning and should address public health needs.

Changes in EU legislation in early 2025 have made wastewater sampling for multiple purposes mandatory in EU Member States. The recast Urban Wastewater Treatment Directive requires Member States to establish enhanced stakeholder collaboration, identify pathogens for WBS, perform WBS routinely and in public health emergencies, and report WBS data to the European Commission. The Directive also introduces a requirement to conduct monitoring of antimicrobial resistance in wastewater.

Effective integration of WBS into infectious disease surveillance requires automated real-time access to electronic WBS data. At present, WBS data collection and integration at the EU/EEA level is insufficient; as the new Directive includes data reporting obligations, this will require continued investments at national and EU/EEA levels. Ensuring the sustainability of human resource and operational investments done thus far – at both EU and national levels – is paramount to build solid structures now and in the years to come.

To coordinate the consolidation and further integration of WBS as a complementary surveillance system for infectious disease at the EU/EEA level, ECDC will:

- **Action 1** – Form a WBS network within ECDC's Coordinating Competent Bodies structure to support the development of a sustainable and flexible EU/EEA-level WBS system.
- **Action 2** – Offer laboratory support activities for the WBS network.
- **Action 3** – Identify candidate pathogens and related health issues for routine WBS for infectious disease through a combination of stakeholder consultation and ECDC expert assessment.
- **Action 4** – Coordinate and support the integration of WBS data into infectious disease surveillance, considering key challenges (see Annex) and potential needs for new surveillance standards.
- **Action 5** – Identify the most suitable database and reporting options for EU/EEA-level WBS data; in particular, EpiPulse will be explored for WBS data collection and integration.

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1. Introduction

Background

Wastewater-based surveillance (WBS) refers to the use of data originating from wastewater testing for surveillance activities. It is a subset of the broader term 'wastewater and environmental surveillance' (WES) – or simply 'environmental surveillance' – which may include samples from air, soil, the sea, rivers or lakes in addition to wastewater.

For the purposes of this document, 'wastewater' refers to sewered urban (municipal) wastewater (sewage and greywater), which comprises liquid waste discharged from domestic (e.g. housing, offices, schools, nursery homes) and non-domestic (e.g. hospitals, other medical facilities, industries, commercial establishments) sources. Influent wastewater – the dirty water entering a treatment plant – is where samples are most commonly taken to support infectious disease surveillance; however, sampling could occur at any point of the system.

WBS is not a new technology; it has been used for decades to monitor various surveillance objectives, including environmental surveillance of polioviruses [1]. Municipal wastewater samples are collected, processed and analysed using a range of laboratory methods. These include culture-based techniques for isolating bacterial or viral strains, and PCR methods for detecting specific DNA or RNA sequences. Additionally, tailored metagenomic approaches are gaining popularity for their broader detection capabilities.

Unlike clinical testing that tracks individual cases, WBS provides aggregate data from an entire community or at a more granular local level within a community, detecting the presence and concentration of a pathogen. To enhance the comparability of quantitative data across sampling sites, a thorough understanding of the methodologies used and the data normalisation techniques applied is essential.

In recent years, interest in WBS has increased in the European Union (EU) and the European Commission has launched several initiatives to explore and strengthen this approach (see Section 3). The recast Urban Wastewater Treatment Directive that entered into force on 1 January 2025 also sets new requirements for EU Member States to implement systems for routine WBS data collection that can also be used during health emergencies [2].

The level of implementation of WBS systems and the capacity of these systems to inform public health action has been heterogenous across EU/EEA countries since 2023 [3]. Likely due to a lack of EU/EEA-level coordination and guidance, WBS systems have included varying population coverages, sampling frequencies, pathogens and levels of sustainability, and have often been time-limited projects and initiatives. Since 2023, progress towards harmonisation has been made within the Health Emergency Preparedness and Response (HERA)-funded Joint Action EU-Wastewater Integrated Surveillance for Public Health (EU-WISH). Now is the time to take stock of the experience and learnings gathered thus far and build sustainable systems that can effectively inform public health decision-making. ECDC intends to pave the way in leading such efforts.

Purpose and audience

This ECDC framework serves to guide the integration of WBS into infectious disease surveillance and public health decision-making at the EU/EEA level. It outlines how ECDC will lead this integration and provides contextual information for policymakers at the national level and in EU institutions, public health stakeholders at the national level and country actors in the relevant sectors engaged in wastewater surveillance. It is structured as follows:

- **Section 2** – ECDC framework to guide the integration of WBS into infectious disease surveillance at the EU/EEA level;
- **Section 3** – Key European initiatives promoting WBS;
- **Section 4** – The role of WBS in infectious disease surveillance, including key considerations and challenges (summary table available in the Annex).

ECDC's role in infectious disease surveillance

ECDC is mandated to identify, assess and communicate current and emerging threats to human health from infectious disease in the EU/EEA. Within this scope, ECDC collects, validates, analyses and disseminates routine surveillance data on notifiable infectious disease from 30 EU/EEA countries [4,5]. EU/EEA-level surveillance is based on reporting from national systems and aims to promptly and effectively fight cross-border threats to public health from infectious disease.

Routine EU/EEA integrated surveillance outputs include the Surveillance Atlas of Infectious Diseases [6], the Annual Epidemiological Reports [7], daily and weekly communicable disease threat reports [8], a number of enhanced surveillance reports produced jointly with other EU agencies or the World Health Organization (WHO), weekly bulletins, online maps and peer-reviewed scientific journal articles.

ECDC works closely with EU/EEA countries and coordinates disease- or related health issue-specific networks for infectious disease surveillance in the region. The networks include nominated national disease experts and public health function experts with cross-cutting roles in areas such as surveillance, threat detection and microbiology.

Specifically for laboratory aspects, Regulation (EU) 2022/2370 mandates ECDC to 'foster the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' [5]. Under this mandate, ECDC coordinates EU/EEA-wide networks of microbiology laboratories embedded in disease-specific networks. They primarily contribute to integrated epidemiological and microbiological surveillance of EU-notifiable infectious diseases, as well as to the detection of emerging diseases. To strengthen capacity within these networks, ECDC has offered such support activities as EU/EEA-wide laboratory network coordination, external quality assessments (EQA), laboratory staff training, establishment of reference microbial strain collections, supranational reference services, method harmonisation and development of standard procedures. These activities are currently being transferred to an EU reference laboratory (EURL) model, following updated legislation for infectious disease surveillance and control [4].

2. ECDC framework to guide the integration of wastewater-based surveillance into infectious disease surveillance at the EU/EEA level

As the EU agency responsible for infectious disease surveillance, threat detection, assessment and communication at the EU/EEA level, ECDC is well positioned to lead the consolidation, further development and coordination of national WBS systems for EU/EEA-wide use, with the aim of addressing data gaps and limitations in current surveillance systems.

Over the years, ECDC has evaluated and integrated various surveillance systems and data sources into EU/EEA-level surveillance. For example, consolidating individual disease surveillance networks into The European Surveillance System (TESSy) from 2007 to 2011, integrating genomic typing into TESSy since 2012, and merging TESSy and additional surveillance systems into the European surveillance portal for infectious diseases (EpiPulse) in 2023. Integration of WBS into infectious disease surveillance at ECDC should follow the standard procedures ECDC has applied previously to integrate surveillance systems.

Public health needs and specific objectives should be the main drivers for integrating WBS for EU/EEA-level surveillance, and significant coordination and support will be needed to ensure sufficient data quality and public health impact. To this end, ECDC will coordinate and collaborate with relevant actors, including EU/EEA countries, WHO and European Commission services and agencies, including the Directorate-General for Health and Food Safety, HERA, the Joint Research Centre (JRC) and the Directorate-General for Environment, as well as the Joint Action EU-WISH.

Importantly, the pioneering work of the JRC and HERA in EU wastewater and environmental surveillance – as well as the work of EU-WISH – will be fundamental and form the basis of the next steps ECDC will take to strategically integrate WBS into EU/EEA-level surveillance of infectious disease. At the same time, to ensure that this vision translates into effective systems leveraging WBS, continued funding at both the EU and national levels – now and in the years to come – is critical to ensuring solid and sustainable action.

Action 1. Form an EU/EEA wastewater-based surveillance network

ECDC will form an EU/EEA WBS network of officially nominated national experts for WBS following the ECDC Coordinating Competent Bodies (CCB) structure [9]. National WBS representatives, including National Focal Points and/or laboratory operational contact points, should represent the national public health sector and preferably include or be connected to the environmental national competent body, as outlined in the recast of the Urban Wastewater Treatment Directive [2]. Coordination with other European Commission services will be required to ensure such a network is well-embedded into the European context of similar networks for other WBS sectors.

The role of the network will be the overall coordination of wastewater activities for public health at the EU/EEA-level, including:

- Data reporting;
- Sharing experiences and best practice;
- Agreeing on interpretation criteria and the best public health use of WBS data;
- Identifying methodological or scientific gaps (including needs for ongoing applied research);
- Building flexibility into the WBS system in case new pathogens or related health issues need to be included in outbreak or pandemic situations to stimulate timely and complete data reporting;
- Highlighting needs for legal and ethical considerations.

Action 2. Offer laboratory support activities

ECDC will offer laboratory support activities for the WBS network following standard ECDC procedures. This support will be needs-based but could include, for example:

- External quality assessments;
- Trainings;
- Reference material distribution;
- Reference services;
- Method harmonisation;
  Development of standard procedures;
- Support for continuous applied research to further methodology and applications.

ECDC may draw on existing initiatives for this laboratory support, including the super-site sentinel network procured by HERA and the JRC [14].

Action 3. Identify priority pathogens and related health issues suitable for routine wastewater-based surveillance for infectious disease at the EU/EEA level

To develop a list of candidate pathogens and related health issues (e.g. diseases or antimicrobial resistance (AMR)) suitable for routine EU/EEA-level WBS, ECDC will organise stakeholder consultations to critically review relevant considerations, including:

- Technical and operational feasibility;
- The current situation or condition of knowledge, research or understandings in relation to proposed surveillance objectives (i.e. 'state of play');
- Possible public health actions that could be taken in response to signals (to support evidence-based decision-making at the EU/EEA level);
- Interpretation criteria and integration into standard surveillance outputs.

The consulted stakeholders will include country representatives with strong backgrounds in WBS and/or epidemiology who are using this type of data for surveillance and public health decision-making at the national level.

ECDC will employ a two-step approach to conduct this review so that priority pathogens and related health issues can be selected. First, the scientific and operational validity of including the candidate pathogens and related health issues in WBS will be appraised. Then, consultation with ECDC disease experts and relevant disease networks to assess which specific public health needs can be addressed by including these in WBS and how. Consultations with the disease networks will follow the formats laid out by the Coordinating Competent Bodies and can include ad hoc or permanent working groups, task forces or subnetworks [9].

Economies of scale may be an important factor in EU/EEA-level WBS, if multiple tests can provide more informative data from the same wastewater samples; however, country-specific surveillance needs may differ across the region, leading to varying priorities. Therefore, the formulation of EU/EEA level implementation requirements needs to consider these national differences. A structured yet adaptable approach to WBS implementation will support an evidence-based approach that is aligned with public health needs.

Selection criteria for candidate pathogens and related health issues

ECDC will considers two main criteria to determine whether candidate pathogens and related health issues are suitable for routine WBS and integration into EU/EEA-level infectious disease surveillance [10-12]:

- Analytical and operational feasibility for WBS;
- Public health usefulness at the EU/EEA level (i.e. the 'information for action' principle).

Analytical and operational feasibility

Analytical and operational feasibility for WBS includes aspects such as:

- Evidence that biomarkers are sufficiently stable and unambiguously detectable in wastewater;
- Agreed methods for sampling, concentration and detection of the candidate pathogen;
- Confirmed correlation between WBS data and clinical or epidemiological observations indicating the WBS data reflects human health data in the catchment area;
- Possibility for data standardisation and comparability between sampling sites;
- Identification of any knowledge gaps and/or research needs related to relevant methodologies or approaches that need to be addressed before a target can be considered.

Public health usefulness

Public health usefulness can be assessed based on whether an activity fills gaps in or improves existing surveillance for a given pathogen or related health issue, for example by:

- Increasing the sensitivity of the surveillance system for a specific pathogen or disease;
- Improving epidemiological trend monitoring and the ability to detect rare or emerging diseases;
- Facilitating early outbreak detection;
- Tracking the emergence and spread of pathogen variants or genetic determinants.

ECDC will assess how and to what level WBS data can complement other surveillance data collected by, for example:

- Expanding the population under surveillance;
- Improving timeliness or provide early warning signals;
- Enhancing interpretations of other data streams;
- Helping to determine relevant public health actions.

While these selection criteria are important to ensure a sustainable and impactful WBS system, it is also critical that systems are flexible and can be rapidly adapted during emergencies or pandemic situations. Therefore, less rigid criteria may be applied when novel priority pathogens or related health issues need to be addressed due to a significant emerging threat.

ECDC will also take stock of efforts made to review WBS approaches for public health use, including outcomes from specific work packages in the Joint Action EU-WISH.

Action 4. Coordinate and support the integration of WBS data into infectious disease surveillance

ECDC will integrate WBS as a complementary surveillance tool at the EU/EEA level and will define relevant surveillance objectives and standards deemed technically and operationally feasible and relevant to fulfil public health needs. The Regulation (EU) 2022/2371 [4] on serious cross-border threats to health mandates ECDC to develop surveillance standards that encompass the full range of EU/EEA-level surveillance activities.

Therefore, ECDC may include WBS in surveillance standards when required to support integration of WBS data into infectious disease surveillance. Surveillance standards could include:

- Specific surveillance objectives;
- Surveillance methods;
- Basic surveillance system descriptors;
- Indicators to be monitored;
- Mandatory key variables;
- Minimum national reporting completeness required for each key variable to enable meaningful data analysis at the EU/EEA level.

Whenever relevant, ECDC will propose WBS as a surveillance data source and engage the relevant disease networks accordingly. In such cases, WBS will be an integral part of surveillance standards for achieving the various surveillance objectives of a given pathogen. Furthermore, Member State compliance to such standards will be monitored and reported to the Commission and Member States on an annual basis.

Action 5. Identify the most suitable database and reporting options for EU/EEA-level wastewater-based surveillance data

To effectively integrate WBS into infectious disease surveillance, ECDC requires automated real-time access to electronic WBS data. At present, WBS data collection and integration at the EU/EEA level is insufficient; as the recast Urban Wastewater Treatment Directive includes data reporting obligations, this will require continued investments at national and EU/EEA levels. Moving forward, ECDC will identify the most suitable option for data sharing and analysis at the EU/EEA level, and will consider previously developed tools such as the European Dashboard operated by the JRC [13].

For country-level data submission, the Urban Wastewater Treatment Directive [2] requires EU Member States to establish national systems that facilitate timely communication of WBS data to the European Commission.

For EU/EEA-level data submission, a platform needs to be available and accompanied by reporting protocols and guidance for participating countries. Centralised EU/EEA-level WBS data collection can be performed using existing ECDC reporting tools. ECDC plans to explore using the EpiPulse platform for this purpose, as it allows reporting of structured indicator based-data, as well as ad hoc event-based data.

Wherever possible, data collection should be done through automated procedures to maximise efficiency and timeliness. Double-reporting should be avoided (referred to as the 'Once Only Principle').

Data analysis, assessment and regular scientific outputs

ECDC will set up pipelines for the management, analysis, integration and visualisation of WBS data. Data standardisation and analysis should be clearly described to facilitate data interpretation. Relevant statistical algorithms for trend analysis and alert will be used. The key principle for analysis is that the WBS data should be presented for interpretation in the context of other relevant available clinical and epidemiological information. Results from integrated analyses will be included and communicated in the standard ECDC outputs, dashboards and assessments.

3. Key European Union initiatives promoting wastewater-based surveillance

A series of EU initiatives have been launched by the European Commission – in particular by HERA and the Directorate-General for Environment in collaboration with the JRC – to facilitate and evaluate the use of WBS for infectious disease surveillance in the EU and beyond, and ultimately institutionalise WBS for public health. These initiatives have played a fundamental role in setting the groundwork for the activities described in this framework. ECDC intends to significantly build on the investments made thus far, with a view to establishing a public health-driven agenda in the field and adequate integration into EU/EEA surveillance systems.

Key EU projects and networks promoting WBS are described below. Additional initiatives include:

- Recommendations from the European Commission for a common approach to WBS for SARS-CoV-2 during the COVID-19 pandemic and tasking HERA to strengthen genome sequencing and environmental monitoring, including WBS [14,15], which were bolstered by WHO guidance [16,17];
- The 2020 grants that the Directorate-General for Environment provided to 25 Member States to establish national systems and physical and digital infrastructure for monitoring COVID-19 and its variants in wastewater;
- An EU umbrella study for SARS-CoV-2 sewer surveillance [18];
- An expert consultation by WHO and the European Commission;
- A call for tender funded by HERA and launched through the EU4Health programme to procure such services as measuring emerging pathogens and pollutants in untreated wastewater at strategic locations and forming a network of sentinel super-sites within the EU/EEA [19].

The recast Urban Wastewater Treatment Directive

The recast Urban Wastewater Treatment Directive entered into force on 1 January 2025 [2]. It regulates the collection, treatment and discharge of urban wastewater to protect the environment and human health. This new version of the Directive includes a new article (Article 17) on the surveillance of urban wastewater for public health parameters, including infectious disease and AMR.

Under the Directive, Member States shall set up national systems for cooperation and coordination between competent authorities responsible for public health and those responsible for urban wastewater treatment. Candidate pathogens and related health issues to consider for national WBS include SARS-CoV-2, polioviruses, influenza viruses, emerging pathogens and any other public health parameters that Member States' competent authorities consider relevant, considering recommendations from ECDC, HERA, WHO and others. Where a public health emergency is declared, relevant public health parameters are to be monitored.

In addition, Member States are to establish monitoring for AMR in wastewater. Recital 35 of the Directive specifies that AMR surveillance aims to further develop scientific knowledge on the main sources of AMR so that adequate action can be taken in the future (details such as sampling frequency and methods will be specified in implementing acts by July 2026). Member States need to ensure appropriate and timely communication and reporting of WBS results to relevant authorities and EU platforms. The European Commission is empowered to adopt implementing acts specifying the format of the information to be provided.

European Union Wastewater Observatory for Public Health – Digital European Exchange Platform (EU4S-DEEP)

The EU Observatory for Public Health – Digital European Exchange Platform (EU4S-DEEP) interacts with a global network of national contact points and organises regular implementation meetings to improve harmonisation and enable exchange of best practice [20]. It links to national WBS resources and produces monthly SARS-CoV-2 WBS bulletins for the EU/EEA and selected countries globally. It also coordinates ad hoc exercises, such as the 2023 airport and aircraft surveillance of SARS-CoV-2 [21], and bulletins on avian influenza virus and monkeypox virus (MPXV) detected in wastewater in the EU/EEA [22]. EU4S-DEEP facilitates interaction between the JRC, HERA, other European Commission services, EU/EEA countries and third countries.

European Union Joint Actions

The Joint Action EU-Wastewater Integrated Surveillance for Public Health (EU-WISH) [23], funded by HERA through the EU4Health programme, combines expertise from 25 EU/EEA countries and Ukraine to strengthen national capacities for WBS for public health. The consortium consists mainly of representatives from national public health bodies. It maps priority pathogens and suggested surveillance objectives for WBS and defines, harmonises and expands WBS strategies, technical procedures and relevant operational approaches including effective, easily interpretable, and actionable communication of WBS parameters.

Recognising the value of these activities, ECDC works closely with EU-WISH, HERA and the JRC to ensure the results inform ECDC-driven approaches to integrate WBS for public health into EU/EEA infectious disease surveillance. Another Joint Action, the second edition of the European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI2), is focused on the appropriate use of antimicrobials in humans and animals, as well as the impact of their use on environmental contamination, and includes WBS of AMR in their scope.

Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON)

Since 2022, HERA has worked with the JRC to develop a global wastewater sentinel system for early warning and pandemic preparedness. This joint work, which included the involvement of ECDC and other Commission services, resulted in the creation of the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON) [24]. The Consortium was launched in March 2024, in collaboration with several EU/EEA countries and stakeholders, as well as international ones, such as the Gates Foundation and WHO.

GLOWACON aims to strengthen international collaborations for capacity building, research and development, technical guidance and trust-building. The overarching objective is to develop a global sentinel system for pandemic preparedness, while closely engaging with EU Member States and international stakeholders.

Collaboration with the World Health Organization (WHO)

WHO is a key collaborator for EU services, providing long-standing guidance on environmental surveillance of poliovirus surveillance and WBS for other pathogens. For example, WHO identified WBS as a promising COVID-19 surveillance tool and issued relevant guidance [16,17]. Most recently, a decision tool to facilitate multi-pathogen approaches for WBS was introduced [12]. In light of the recognised partnership between the European Commission and WHO, HERA is supporting WHO in the development of activities to advance WBS at the global level, including the development of a pilot platform for data sharing.

4. The role of wastewater-based surveillance in infectious disease surveillance

WBS has been identified as a complementary surveillance tool capable of providing data for infectious disease surveillance. Scientific evidence on its application has substantially increased in the wake of the COVID-19 pandemic – in particular, in providing useful population-level surveillance data where clinical testing data is insufficient and transmission may go undetected. It is being applied more frequently to address policy priorities, as well as to monitor diseases that are rare, unexpected or present with only sub-clinical/mild symptoms, which can lead to less clinical suspicion or reduced healthcare-seeking behaviour. WBS also enables countries to ensure operational readiness and vigilance from a pandemic preparedness perspective, as they can implement additional priority pathogens or related health issues when needed.

WBS offers valuable insight into disease transmission dynamics and – together with other surveillance data – can support decision-making for public health. While some studies describe the influence of WBS data on public health decision-making, these are mostly from a non-European context. An analysis by Benedetti et al. [3] indicates that many countries regarded WBS for SARS-CoV-2 as useful in supporting other surveillance data, but the WBS activities did not independently prompt public health actions. For most pathogens, WBS data should be considered complementary to other epidemiological data and be interpreted in that context in order to influence public health decision-making and public health actions.

At present, instances where WBS data on its own would prompt public health actions are reserved for diseases with potentially high impact but insufficient options for clinical surveillance, such as poliovirus. Public health actions that could be taken in response to WBS data include:

- Raising community awareness;
- Increasing outreach to populations at greater risk;
- Adapting testing strategies or implementing local testing;
- Enhancing or implementing specific surveillance activities;
- Improving preparedness of the healthcare system;
- Conducting local vaccination campaigns;
- Generating hypotheses for operational research and epidemiological modelling;
- Developing surveillance and preparedness strategies.

While WBS comes with its own limitations and challenges (summary table available Annex), it also circumvents several biases present in clinical and epidemiological surveillance. It provides a non-invasive and anonymous method for regularly tracking the presence of pathogens within a community, regardless of the availability or uptake of clinical testing or the population's healthcare-seeking behaviour. WBS is largely symptom agnostic if both asymptomatic and mildly symptomatic individuals shed pathogen-containing material and it enters the wastewater collection system. However, there are important epidemiological studies – including studying risk factors or assessing severity of disease – that cannot be addressed by WBS, since it delivers population-level data, not individual data; for such epidemiological studies, data collected through clinical testing is required.

Once sample collection and processing are established, the presence of multiple pathogens can be detected from a single sample, increasing cost-effectiveness if pathogen-specific WBS systems sufficiently align in terms of their testing frequencies and the laboratory algorithms they apply. The population under surveillance can also be adjusted based on the wastewater treatment plant's catchment area or through using specific sampling points. Detection assays such as PCR allow a high flexibility to quickly adapt WBS systems to include additional or emerging pathogens. Both bio-banked and prospectively collected samples can be helpful in studying relevant public health questions – such as spread and distribution of pathogens or virulence factors and resistances over time – or to improve detection methods.

While this framework focuses only on WBS for infectious disease and related public health issues, it is noteworthy that WBS can also be used to monitor industrial chemicals, pharmaceuticals, illicit drugs or other narcotics.

Wastewater-based surveillance as a complement to routine infectious disease surveillance

A primary example of the long-standing and successful application of environmental surveillance including WBS is that of polioviruses, where WHO – through the Global Polio Eradication Initiative – has advocated for and supported its implementation globally [25]. WHO has also developed and published important guidance on prioritisation, implementation and integration of wastewater-based and environmental surveillance for pathogens [12].

The public health value of WBS has been demonstrated for a number of applications, in particular:

- **Monitoring and early warning of disease trends** – Community or sentinel WBS systems can provide insight into infection trends, making them a potentially valuable complement to other surveillance data, especially in situations of low clinical testing or for diseases with mostly sub-clinical or mild symptoms. Examples include national monitoring of the spread of SARS-CoV-2 in many EU/EEA countries [13]. Such data often correlate with clinical testing data and can provide warning signals for increases in clinical cases or hospitalisations [26-28]. However, viral evolution can lead to modified viral properties, such as shorter incubation time, that reduce or diminish the early warning time window; for example, data from the later stages of the COVID-19 pandemic showed that the SARS-CoV-2 virus had evolved. This highlights the need for continuous evaluation of WBS data [29]. Likewise, WBS of influenza virus has been useful for national [30,31] and regional [32,33] monitoring. WBS data can also serve as a basis for disease modelling approaches; a recent example showed how integrating WBS data with clinical hospitalisation data improved COVID-19 hospitalisation forecasts in Denmark [34].
- **Detection of unusual events and outbreaks** – Routine WBS helps to establish baseline data to detect unusual events and potential outbreaks. Examples are a city-wide influenza outbreak in Ottawa in 2022 [31] or the detection of circulating vaccine-derived poliovirus in London wastewater in early 2022 (but no cases of paralytic polio), which prompted increased immunisation efforts and may have prevented an outbreak [35,36]. In July 2024, the presence of circulating vaccine-derived poliovirus (cVDPV) was confirmed in wastewater in the Gaza Strip and prompted an immunisation campaign [37]. Four EU/EEA countries and the UK also reported cVDPV2 in wastewater between September and December 2024 [38].
- **Genomic surveillance** – WBS has the potential to support genomic surveillance of selected pathogens, such as SARS-CoV-2 or influenza virus, or health issues such as AMR. For example, variants of SARS-CoV-2 have been successfully tracked through WBS in many places, including Rotterdam [39], and WBS data can serve as an early warning signal for the introduction of a known variant [40]. Influenza virus type and subtype distributions can also be monitored throughout an influenza season; for example, in the city-wide influenza outbreak in Ottawa [31], influenza virus from wastewater samples was typed and influenza A virus was subtyped. For AMR surveillance, WBS can include the objective to detect drug-resistant pathogens that may be affecting the population under surveillance or to increase knowledge of the main sources of antimicrobial resistance in the environment [41,42]. Analyses could seek to detect resistance genes or investigate bug-drug combinations (i.e. the particular resistances to drugs detected in specific pathogens) by cultivating and characterising resistant bacterial isolates from wastewater [41,42].
- **Evaluation of public health interventions** – WBS can support assessment of the effectiveness of public health measures by tracking changes in pathogen levels in wastewater, where such changes would be expected, thereby informing policy decisions. For example, WBS of SARS-CoV-2 could indicate whether current vaccination campaigns are sufficient – if an early warning signal or pockets of community transmission are detected, this could prompt re-evaluation of vaccination campaigns [43]. Another example is WBS of poliovirus to support verification of absence of disease in the context of eradication programmes [44].

Intensified and focused wastewater-based surveillance

Settings with established WBS can intensify or tailor their system to respond to specific signals or events to fulfil national (or other level) surveillance objectives and respond to evolving needs in the short term. This could include such adjustments as increasing sampling frequency, fast-tracking analyses and reporting workflows, increasing or changing population coverage, or modifying or adding genomic surveillance aspects. These actions serve as time-limited responses that can provide value to integrated disease surveillance.

The following are examples where WBS was adapted for specific situations:

- **Mass-gathering events** – WBS can provide cost-effective, anonymous, non-invasive and convenient additional surveillance data during mass-gathering events such as large sporting events. For example, the French National Public Health Agency identified priority pathogens that were relevant and feasible for monitoring during the Olympic and Paralympic Games in Paris in 2024. They presented a model framework for identifying context-specific WBS objectives for mass gathering events [45].

- **Monitoring of populations at greater risk** – By focusing on specific sites like hospitals, long-term care facilities, correctional facilities and schools, WBS can offer valuable insights into disease burden or transmission within groups or facilities that are more vulnerable to infections or outbreaks, supporting timely intervention. A small study in nursing homes in Spain in 2021 found 5–19 days of lead time for detecting SARS-CoV-2 RNA in wastewater before spotting the first clinical cases [46]. Similarly, a Canadian study from 2024 observed WBS signals ahead of clinical cases in the majority of participating long-term care facilities [47]. WBS can also support AMR surveillance, both when following a population over time or taking a snapshot of a priority population or setting, e.g. a hospital [48].

Pandemic and emerging disease preparedness

WBS has the potential to strengthen preparedness against emerging infectious diseases and known pathogens. A flexible WBS system capable of integrating new tests and adapting population coverage and sampling frequency can play a critical role in timely outbreak detection. WBS can support preparedness in various ways, including:

- **Early warning, outbreak detection and containment** – In the situation of a new pandemic or emerging disease, WBS can contribute to early warning signals to support containment in an alert phase. Studies from the Netherlands and several states in the United States (US) demonstrated that WBS of MPXV provided early identification of high-infection areas before clinical testing infrastructure could be put in place or when uptake of clinical testing was limited due to fear of stigmatisation [49,50]. In 2022, following a confirmed paralytic polio case in Rockland County, the state of New York implemented WBS that helped detect poliovirus across multiple counties and emphasised the need for vaccination campaigns to support containment [51,52].
- **Monitoring of trends and mitigation** – During a pandemic, WBS can be useful to track disease spread and support mitigation. For example, following highly pathogenic avian influenza A(H5N1) outbreaks at poultry and dairy cattle farms in the US, enhanced A(H5N1) testing was implemented in wastewater samples from treatment plants across multiple states. This provided a tool to support geographical delineation of the outbreak and results could largely be confirmed with data from other surveillance systems [53-55]. However, to fully assess the significance of these findings, it would be crucial to determine whether the H5N1 detected in WBS originated from animal or human sources.
- **Point of entry surveillance** – Testing wastewater at points of entry into a country can be useful to track introductions of a pathogen or variant of interest. During the COVID-19 pandemic, aircraft wastewater monitoring in New York City, US [56] and Darwin, Australia [57], among others, were used as a complementary early warning system for detecting SARS-CoV-2 variants of concern. At Italy's largest airport in Rome, MPXV was quickly added to the list of pathogens monitored via WBS after the emergence of mpox in 2022 [58]. A study from Sweden investigated SARS-CoV-2 levels and variants in aircraft and airport wastewater compared with local population data and urban wastewater from treatment plants [59]. The study found that variants initially detected only in aircraft and airport wastewater did not spread widely during the study period. Additionally, it demonstrated that wastewater monitoring was more effective than clinical testing for the early detection of specific variants, revealing notable delays in clinical surveillance.

Considerations and challenges in wastewater-based surveillance

WBS systems should consider several factors at the design stage to ensure they support public health needs and provide useful data – such as filling a surveillance gap or providing added value in the context of other existing surveillance systems. Integrating WBS with traditional surveillance systems is crucial, as WBS data need to be calibrated, contextualised and interpreted together with other epidemiological data. Resource requirements – including the cost of sustainable and representative sampling and logistics, such as the need for prospective sample storage (biobanking) – should be considered and balanced against public health benefits. Additional considerations include ensuring legal and regulatory compliance (including for biosafety and biosecurity) and addressing privacy and ethical aspects (e.g. WBS systems should be under the oversight of ethics boards to avoid misuse of sample and data sources).

A table summarising the key challenges and considerations related to WBS is provided in the Annex. The HERA-funded Joint Action EU-WISH brings together competences from 26 European countries and will address and clarify many of these challenges. It will be fundamental to draw on the lessons learned by EU-WISH to further develop WBS for Europe.

The need for cross-sectoral coordination

One of the most innovative and complex aspects of WBS for the surveillance of infectious disease from a public health perspective is that samples are taken from the environmental sector. This has various implications in terms of required coordination, namely:

- Public health agencies and authorities do not necessarily have the competence and resources to access wastewater sampling sites, and to collect and process wastewater samples;
- Results obtained from environmental samples may not only reflect the health status of the serviced human population, but could also reflect nearby trade, industrial or economic activities or the health of local animal populations;
- Detailed knowledge of collecting systems infrastructure and how they operate is needed to identify and select entry points for sampling when locations other than wastewater treatment plants are chosen;
- WBS can be relevant for the surveillance of additional threats – not only infectious diseases – such as chemical contamination or the consumption of pharmaceuticals, illicit drugs or narcotics.

Consequently, there is a clear need for national coordination, as well as coordination at the EU/EEA level. The development of WBS for infectious disease monitoring must be well-aligned with other initiatives led by the European Commission and its agencies. This coordination should include efforts related to other threats, including those affecting animal or environmental health (see the Annex). Dedicated national coordination structures may help foster mutual understanding and enhance collaboration and responsiveness across sectors.

Options for national, centralised laboratory testing should be explored to find the most cost-effective solutions, although it needs to be recognised that laboratory workflows for pathogens versus other chemical substances will likely require some degree of separation. Of note, in water microbiology, EN-ISO standards are being developed under the ISO/TC 147 Water quality, SC4 Microbiological methods, which includes WBS of SARS-CoV-2.

Importantly, stakeholders of related sectors – such as environmental agencies and wastewater and/or water utility providers – should be consulted and involved at an early stage.

Data comparability

A wide variability of factors can affect comparability of data across different time points at the same sampling site and across different sampling sites. Factors to consider for harmonisation include the sampling techniques and timings, as well as the laboratory assays and data normalisation, standardisation and analysis techniques used, all of which are critical to producing good-quality surveillance data.

Circumstantial factors – such as weather, moving populations, catchment area size or different types of wastewater treatment technology – can also have an impact on the comparability of data. Establishing guidelines, harmonising methods and participating in external quality assessments are vital.

Complexity of wastewater samples and data

WBS samples can often be complex, and validated methods for detection, typing, sequencing and culturing should be used to ensure reliable identification and characterisation of specific pathogens amidst contaminants. For novel pathogen detection, combining PCR methods with metagenomic sequencing can enhance pathogen identification; however, detecting low-abundance species and reconstructing entire genomes from complex wastewater samples remains challenging and can require enrichment steps, due to the higher level of uncertainties.

Data indicating the disease burden should be interpreted together with other surveillance data and the circumstantial factors mentioned previously. When assessing trends, analysis methods need to be robust and reliably detect significant changes in the data. Where possible, WBS data should be calibrated using other available surveillance data, such as prevalence studies. Similarly, as described previously, sequencing methods need to be validated to reliably deliver genomic characterisation and typing data on specific pathogens and their trends. Shedding and limits of detection are pathogen specific and need to be established. Knowledge of the sewer infrastructure is important as it can impact the time water takes to arrive at the treatment plant and the stability of pathogen material for detection in sewage becomes an important factor. Moreover, biomarker concentrations may vary during disease progression, and circumstantial factors could also affect detection accuracy and data interpretation.

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References

1. Kilaru P, Hill D, Anderson K, Collins MB, Green H, Kmush BL, et al. Wastewater Surveillance for Infectious Disease: A Systematic Review. Am J Epidemiol. 2023 Feb 1;192(2):305-22. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36227259

2. European Commission (EC). Directive (EU) 2024/3019 of the European Parliament and of the Council of 27 November 2024 concerning urban wastewater treatment (recast). Brussels: EC; 2024. Available at: http://data.europa.eu/eli/dir/2024/3019/oj

3. Benedetti G, Wulff Krogsgaard L, Maritschnik S, Stuger HP, Hutse V, Janssens R, et al. A survey of the representativeness and usefulness of wastewater-based surveillance systems in 10 countries across Europe in 2023. Euro Surveill. 2024 Aug;29(33):2400096. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.33.2400096

4. European Commission (EC). Regulation (EU) 2022/2371 of the European Parliament and of the Council of 23 November 2022 on serious cross-border threats to health and repealing Decision No 1082/2013/EU. Luxembourg: Official Journal of the European Union; 2022. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2371

5. European Commission (EC). Regulation (EU) 2022/2370 of the European Parliament and of the Council of 23 November 2022 amending Regulation (EC) No 851/2004 establishing a European centre for disease prevention and control. Luxembourg: Official Journal of the European Union; 2022. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2370

6. European Centre for Disease Prevention and Control (ECDC). Surveillance Atlas of Infectious Diseases. Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases

7. European Centre for Disease Prevention and Control (ECDC). Annual Epidemiological Report. Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/en/annual-epidemiological-reports

8. European Centre for Disease Prevention and Control (ECDC). Communicable Disease Threats Report. Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/en/publications-and-data/monitoring/weekly-threats-reports

9. European Centre for Disease Prevention and Control (ECDC). ECDC Coordinating Competent Bodies: Structures, Interactions and Terms of Reference. Stockholm: ECDC; 2023. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/Coordinating-Competent-Bodies-Structures-Terms-of-reference-and-interactions_2023.pdf

10. European Centre for Disease Prevention and Control (ECDC). Surveillance of communicable diseases in Europe – a concept to integrate molecular typing data into EU-level surveillance. Stockholm: ECDC; 2011. Available at: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/surveillance-concept-molecular%20typing-sept2011.pdf

11. National Academies of Sciences, Engineering, and Medicine. Wastewater-based Disease Surveillance for Public Health Action. Washington, DC: National Academies Press (US); 2023. Available at: https://www.ncbi.nlm.nih.gov/pubmed/37184191

12. World Health Organization (WHO). Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration. Geneva: WHO; 2024. Available at: https://cdn.who.int/media/docs/default-source/wash-documents/wash-related-diseases/wes-for-one-or-more-pathogens--guidance-on-prioritization--implementation-and-integration--pilot-version6dec2024.pdf?sfvrsn=6bbad2cd_3

13. Joint Research Centre (JRC). National SARS-CoV-2 WBS dashboards in the EU/EEA – EU Wastewater Observatory for Public Health. Brussels: JRC; 2023. Available at: https://arcgis.jrc.ec.europa.eu/portal/apps/dashboards/e296cdf0c0d042e6b60b07a351f2dc5c

14. European Commission (EC). COMMUNICATION FROM THE COMMISSION Introducing HERA, the European Health Emergency preparedness and Response Authority, the next step towards completing the European Health Union. Luxembourg: Official Journal of the European Unoin; 2021. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52021DC0576&qid=1641201161412

15. European Commission (EC). COMMISSION RECOMMENDATION (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. Brussels: EC; 2021. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021H0472

16. World Health Organization (WHO). Wastewater surveillance of SARS-CoV-2: questions and answers (Q&A). Geneva: WHO; 2022. Available at: https://www.who.int/europe/publications/i/item/WHO-EURO-2022-5274-45038-64164

17. World Health Organization (WHO). Environmental surveillance for SARS-CoV-2 to complement other public health surveillance. Geneva: WHO; 2023. Available at: https://iris.who.int/bitstream/handle/10665/372995/9789240080638-eng.pdf?sequence=1

18. Gawlik B, Tavazzi S, Mariani G, Skejo H, Sponar M, Higgins T, et al. SARS-CoV-2 Surveillance employing Sewage – Towards a Sentinel System. Brussels: Joint Research Centre; 2021. Available at: https://publications.jrc.ec.europa.eu/repository/handle/JRC125065

19. European Health and Digital Executive Agency (HADEA). Framework contracts to provide services and supplies to measure pathogens and pollutants of emerging concern in untreated wastewater samples as part of a global sentinel system. Brussels: HADEA; 2024. Available at: https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/tender-details/6e872914-29e5-43b7-bd94-6f21871a07b9-CN

20. Joint Research Centre (JRC). EU Wastewater Observatory for Public Health. Brussels: JRC; 2022. Available at: https://wastewater-observatory.jrc.ec.europa.eu

21. Deere D, Jones D, Ahmed W, Medema G, Kreuzinger N, Remmonay I, et al. Ad-hoc guidance: Wastewater sampling of aircraft for SARS-CoV-2 surveillance. Brussels: Joint Research Centre; 2023. Available at: https://wastewater-observatory.jrc.ec.europa.eu/media/guidance_files/Sampling_Aircrafts_JRC_2023.pdf

22. Joint Research Centre (JRC). Monthly reports. Brussels: JRC. Available at: https://wastewater-observatory.jrc.ec.europa.eu/#/content/monthly-chronicle

23. EU-Wastewater Integrated Surveillance for Public Health (EU-WISH). Homepage. Luxembourg: EU-WISH. Available at: https://www.eu-wish.eu

24. European Commission (EC). Launching GLOWACON: A global initiative for wastewater surveillance for public health. Brussels: EC; 2024. Available at: https://health.ec.europa.eu/latest-updates/launching-glowacon-global-initiative-wastewater-surveillance-public-health-2024-03-21_en

25. World Health Organization (WHO). Global Polio Surveillance Action Plan 2022–2024. Geneva: WHO; 2022. Available at: https://polioeradication.org/wp-content/uploads/2024/09/GPSAP-2022-2024-EN.pdf

26. Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci Technol Lett. 2020 Jul 14;7(7):511-6. Available at: https://www.ncbi.nlm.nih.gov/pubmed/37566285

27. Amato E, Hyllestad S, Heradstveit P, Langlete P, Moen LV, Rohringer A, et al. Evaluation of the pilot wastewater surveillance for SARS-CoV-2 in Norway, June 2022 – March 2023. BMC Public Health. 2023 Sep 4;23(1):1714. Available at: https://www.ncbi.nlm.nih.gov/pubmed/37667223

28. Rashid SA, Rajendiran S, Nazakat R, Mohammad Sham N, Khairul Hasni NA, Anasir MI, et al. A scoping review of global SARS-CoV-2 wastewater-based epidemiology in light of COVID-19 pandemic. Heliyon. 2024 May 15;10(9):e30600. Available at: https://www.ncbi.nlm.nih.gov/pubmed/38765075

29. Public Health Scotland (PHS). Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Technical Report. Edinburgh: PHS; 2024. Available at: https://www.publichealthscotland.scot/media/26992/evaluating-the-public-health-utility-of-wastewater-based-surveillance-of-sars-cov-2-in-scotland-final.pdf

30. Lehto KM, Lansivaara A, Hyder R, Luomala O, Lipponen A, Hokajarvi AM, et al. Wastewater-based surveillance is an efficient monitoring tool for tracking influenza A in the community. Water Res. 2024 Jun 15;257:121650. Available at: https://www.ncbi.nlm.nih.gov/pubmed/38692254

31. Mercier E, D'Aoust PM, Thakali O, Hegazy N, Jia JJ, Zhang Z, et al. Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. Sci Rep. 2022 Sep 22;12(1):15777. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36138059

32. Boehm AB, Hughes B, Duong D, Chan-Herur V, Buchman A, Wolfe MK, et al. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. Lancet Microbe. 2023 May;4(5):e340-e8. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36965504

33. Maida CM, Mazzucco W, Priano W, Palermo R, Graziano G, Costantino C, et al. Detection of influenza virus in urban wastewater during the season 2022/2023 in Sicily, Italy. Front Public Health. 2024;12:1383536. Available at: https://www.ncbi.nlm.nih.gov/pubmed/39109154

34. Gudde A, Krogsgaard LW, Benedetti G, Schierbech SK, Brokhattingen N, Petrovic K, et al. Predicting hospital admissions due to COVID-19 in Denmark using wastewater-based surveillance. Sci Total Environ. 2025 Feb 25;966:178674. Available at: https://www.ncbi.nlm.nih.gov/pubmed/39904216

35. Hill M, Pollard AJ. Detection of poliovirus in London highlights the value of sewage surveillance. Lancet. 2022 Oct 29;400(10362):1491-2. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36243023

36. Wise J. Poliovirus is detected in sewage from north and east London. BMJ. 2022 Jun 23;377:o1546. Available at: https://www.ncbi.nlm.nih.gov/pubmed/35738666

37. Global Polio Eradication Initiative (GPEI). Variant type 2 poliovirus isolated from sewage samples in Gaza. Geneva: GPEI; 2024. Available at: https://polioeradication.org/news/variant-type-2-poliovirus-isolated-from-sewage-samples-in-gaza/

38. European Centre for Disease Prevention and Control (ECDC). Detections of poliovirus in sewage samples require enhanced routine and catch-up vaccination and increased surveillance. Stockholm: ECDC; 2025. Available at: https://www.ecdc.europa.eu/en/news-events/detections-poliovirus-sewage-samples-require-enhanced-vaccination-surveillance

39. Izquierdo-Lara RW, Heijnen L, Oude Munnink BB, Schapendonk CME, Elsinga G, Langeveld J, et al. Rise and fall of SARS-CoV-2 variants in Rotterdam: Comparison of wastewater and clinical surveillance. Sci Total Environ. 2023 May 15;873:162209. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36796689

40. Espinosa-Gongora C, Berg C, Rehn M, Varg JE, Dillner L, Latorre-Margalef N, et al. Early detection of the emerging SARS-CoV-2 BA.2.86 lineage through integrated genomic surveillance of wastewater and COVID-19 cases in Sweden, weeks 31 to 38 2023. Euro Surveill. 2023 Nov;28(46):2300595. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2023.28.46.2300595

41. Huijbers PMC, Flach CF, Larsson DGJ. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ Int. 2019 Sep;130:104880. Available at: https://www.ncbi.nlm.nih.gov/pubmed/31220750

42. Larsson DGJ, Flach CF, Laxminarayan R. Sewage surveillance of antibiotic resistance holds both opportunities and challenges. Nat Rev Microbiol. 2023 Apr;21(4):213-4. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36470999

43. Smith T, Cassell G, Bhatnagar A. Wastewater Surveillance Can Have a Second Act in COVID-19 Vaccine Distribution. JAMA Health Forum. 2021 Jan 4;2(1):e201616. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36218425

44. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, et al. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014 Nov 1;210 Suppl 1(Suppl 1):S294-303. Available at: https://www.ncbi.nlm.nih.gov/pubmed/25316848

45. Toro L, de Valk H, Zanetti L, Huot C, Tarantola A, Fournet N, et al. Pathogen prioritisation for wastewater surveillance ahead of the Paris 2024 Olympic and Paralympic Games, France. Euro Surveill. 2024 Jul;29(28):2400231. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.28.2400231

46. Davo L, Segui R, Botija P, Beltran MJ, Albert E, Torres I, et al. Early detection of SARS-CoV-2 infection cases or outbreaks at nursing homes by targeted wastewater tracking. Clin Microbiol Infect. 2021 Jul;27(7):1061-3. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33601008

47. Pang X, Lee BE, Gao T, Rosychuk RJ, Immaraj L, Qiu JY, et al. Early warning COVID-19 outbreak in long-term care facilities using wastewater surveillance: correlation, prediction, and interaction with clinical and serological statuses. Lancet Microbe. 2024 Oct;5(10):100894. Available at: https://www.ncbi.nlm.nih.gov/pubmed/39182502

48. Flach CF, Hutinel M, Razavi M, Ahren C, Larsson DGJ. Monitoring of hospital sewage shows both promise and limitations as an early-warning system for carbapenemase-producing Enterobacterales in a low-prevalence setting. Water Res. 2021 Jul 15;200:117261. Available at: https://www.ncbi.nlm.nih.gov/pubmed/34082263

49. de Jonge EF, Peterse CM, Koelewijn JM, van der Drift AR, van der Beek R, Nagelkerke E, et al. The detection of monkeypox virus DNA in wastewater samples in the Netherlands. Sci Total Environ. 2022 Dec 15;852:158265. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36057309

50. Wolfe MK, Yu AT, Duong D, Rane MS, Hughes B, Chan-Herur V, et al. Use of Wastewater for Mpox Outbreak Surveillance in California. N Engl J Med. 2023 Feb 9;388(6):570-2. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36652340

51. Ryerson AB, Lang D, Alazawi MA, Neyra M, Hill DT, St George K, et al. Wastewater Testing and Detection of Poliovirus Type 2 Genetically Linked to Virus Isolated from a Paralytic Polio Case – New York, March 9-October 11,

2022. MMWR Morb Mortal Wkly Rep. 2022 Nov 4;71(44):1418-24. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36327157

52. Nelson B. What poo tells us: wastewater surveillance comes of age amid covid, monkeypox, and polio. BMJ. 2022 Jul 29;378:o1869. Available at: https://www.ncbi.nlm.nih.gov/pubmed/35905991

53. Wolfe MK, Duong D, Shelden B, Chan EMG, Chan-Herur V, Hilton S, et al. Detection of Hemagglutinin H5 Influenza A Virus Sequence in Municipal Wastewater Solids at Wastewater Treatment Plants with Increases in Influenza A in Spring, 2024. Environmental Science & Technology Letters. 2024;11(6):526-32. Available at: https://pubs.acs.org/doi/10.1021/acs.estlett.4c00331

54. United States Centres for Disease Prevention and Control (US CDC). Wastewater Data for Avian Influenza A(H5). Atlanta: US CDC; 2024. Available at: https://www.cdc.gov/nwss/rv/wwd-h5.html

55. Tisza MJ, Hanson BM, Clark JR, Wang L, Payne K, Ross MC, et al. Sequencing-Based Detection of Avian Influenza A(H5N1) Virus in Wastewater in Ten Cities. N Engl J Med. 2024 Sep 26;391(12):1157-9. Available at: https://www.ncbi.nlm.nih.gov/pubmed/39259887

56. Morfino RC, Bart SM, Franklin A, Rome BH, Rothstein AP, Aichele TWS, et al. Notes from the Field: Aircraft Wastewater Surveillance for Early Detection of SARS-CoV-2 Variants – John F. Kennedy International Airport, New York City, August-September 2022. MMWR Morb Mortal Wkly Rep. 2023 Feb 24;72(8):210-1. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36821716

57. Ahmed W, Bivins A, Smith WJM, Metcalfe S, Stephens M, Jennison AV, et al. Detection of the Omicron (B.1.1.529) variant of SARS-CoV-2 in aircraft wastewater. Sci Total Environ. 2022 May 10;820:153171. Available at: https://www.ncbi.nlm.nih.gov/pubmed/35051459

58. La Rosa G, Mancini P, Veneri C, Ferraro GB, Lucentini L, Iaconelli M, et al. Detection of Monkeypox Virus DNA in Airport Wastewater, Rome, Italy. Emerg Infect Dis. 2023 Jan;29(1):193-6. Available at: https://www.ncbi.nlm.nih.gov/pubmed/36476335

59. Perez-Zabaleta M, Berg C, Latorre-Margalef N, Owusu-Agyeman I, Kiyar A, Botnen H, et al. Wastewater surveillance of SARS-CoV-2 from aircraft to citywide monitoring. Nat Commun. 2025 Jun 2;16(1):5125. Available at: https://www.ncbi.nlm.nih.gov/pubmed/40456842

Annex. Summary of key challenges and considerations related to wastewater-based surveillance (WBS)

Topic	Challenges and considerations
Sampling and collection	• Inconsistent sampling methods across locations; • Variability in viral concentrations, e.g. due to environmental factors; • Degradation of target analytes (e.g. RNA) before analysis; • Need for optimised frequency and timing of sampling.
Laboratory analysis	• Performance (including sensitivity and specificity) of detection methods; • Contamination risks affecting sample integrity; • Standardisation of protocols for different pathogens; • Potential need for separate protocols for different pathogens; • Cost and labour of sequencing, PCR-based and isolation-based approaches; • Detection of low-abundance species and reconstruction of entire genomes.
Data analysis	• Production of robust and actionable indicators; • Normalisation and standardisation approaches; • Genomic analyses of next-generation sequencing or metagenomic workflows.
Data integration	• Linking and contextualising wastewater data with clinical and epidemiological data; • Lack of standardised metadata fields; • Differences in reporting formats across regions and countries in the EU/EEA.
Data interpretation	• Variability in viral shedding among infected individuals; • Impact of dilution and environmental degradation on viral load estimates; • Distinguishing between local/imported/mobile cases; • Distinguishing between human and other sources; • Data comparison between sites/countries.
Ethical and legal	• Privacy concerns regarding linking wastewater data to specific populations; • Regulatory challenges for data sharing and governance; • Public perception and potential stigma associated with findings; • Compliance with biosafety and biosecurity regulations; • Ethics board oversight to avoid misuse of systems and data.
Operational and logistical	• Coordination among multiple stakeholders (public health and environmental sector, utilities, researchers, etc.); • Need for specialised training and infrastructure; • Cost and resource requirements for sustained monitoring; • Scalability of WBS for different regions and settings; • Prospective sample storage (biobanking).
Public health action and communication	• Pathogen prioritisation to align with public health needs; • Defining thresholds and options for public health actions and response; • Effective translation of WBS findings into public health actions; • Risk of misinterpretation or overreliance on WBS data; • Ensuring timely response to emerging threats detected in wastewater; • Public engagement and acceptance of WBS.