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Analysis insights to support the use of wastewater and environmental surveillance data for infectious diseases and pandemic preparedness

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ABSTRACT

Wastewater-based epidemiology is the detection of pathogens from sewage systems and the interpretation of these data to improve public health. Its use has increased in scope since 2020, when it was demonstrated that SARS-CoV-2 RNA could be successfully extracted from the wastewater of affected populations. In this *Perspective* we provide an overview of recent advances in pathogen detection within wastewater, propose a framework for identifying the utility of wastewater sampling for pathogen detection and suggest areas where analytics require development. Ensuring that both data collection and analysis are tailored towards key questions at different stages of an epidemic will improve the inference made. For analyses to be useful we require methods to determine the absence of infection, early detection of infection, reliably estimate epidemic trajectories and prevalence, and detect novel variants without reliance on consensus sequences. This research area has included many innovations that have improved the interpretation of collected data and we are optimistic that innovation will continue in the future.

1. Introduction

Central to the field of infectious disease epidemiology has been the need to understand the extent of and reduce disease in a population. Surveillance for infectious diseases is a key source of information, and classically has focused on the detection of infected individuals. In many cases, estimation of the disease burden uses clinical surveillance, which is the reporting of disease diagnoses from hospitals and clinics. Clinical surveillance typically does much to inform disease burden, but direct estimates of disease burden from this data will be biased because of under reporting and under ascertainment as asymptomatic infection can precede outbreaks, under-estimate the true burden and hide sources of transmission throughout an epidemic. In certain scenarios, for example, antimicrobial resistance, there is a large burden of pathogen carriage that is poorly understood, and of which only a fraction will be reported through clinical disease notification. Randomised surveys such as cohort-based prevalence studies can be used instead, but these require very large and repeated tests to be distributed and conducted to detect relatively rare diseases at useful spatial or temporal resolutions. Estimates of incidence based on self-reporting metrics have also been used, but have strong issues with bias (Sah et al., 2021).

Environmental surveillance (ES) is a alternative to the above

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individual-based approaches that may be of use to public health decision-makers. This is characterised by the systematic collection of samples of environmental media (e.g., air, water, wastewater, soil) from selected locations, analysis of (typically) environmental) nucleic acids in the samples to detect, quantify, and characterise infectious diseaserelated pathogens, and collation of the data for the purpose of informing decisions (Leifels et al., 2022; Bass et al., 2023; Shaw et al., 2023). Wastewater (WW) surveillance may be regarded as a specific form of ES that is applied to sewer systems. While ES does not offer individual level data, and so has a lower limit on the resolution of insights, it requires much fewer tests and so incurs both a lower financial and logistical burden, especially at scale. The utility of WW surveillance for pandemic preparedness and response will likely depend on context, but some general principles for surveillance more broadly (Khan et al., 2018) should be considered: the utility of information, the timeliness of information, the reliability of situational awareness and guidance for actions. Here, we focus on the use of wastewater surveillance as a widely adopted approach for infectious disease surveillance, particularly within the context of the COVID-19 pandemic and the large body of research developed subsequently (Keshaviah et al., 2023).

Broadly speaking, wastewater-based epidemiology (WBE) emphasises the analysis and interpretation of pathogen data from water-based systems where human sewage is present, with the goal of improving public health. These (waste)water systems take a variety of forms, including convergent sewer networks that feed into treatment works, septic tanks, pit latrines, and disposal of untreated or incompletely treated sewage into surface waters, such as rivers, lakes and oceans. Additionally, surveillance can be focused on specific target populations including hospitals, prisons, refuge shelters, schools, and care homes by monitoring outflowing sewage at a building or compound level.

The collection and analysis of urban sewage for the detection of viruses can be traced back as far as 1939, where sewage samples tested positive for poliovirus during epidemics of poliomyelitis in affected countries (Trask et al., 1942). ES for poliovirus has since expanded considerably, (WHO, 2025) and can be considered as a 'use case' where ES data has provided evidence of circulation used to trigger a vaccination response, and the absence of poliovirus in ES data can help support a 'polio-free' status. It is possible to detect poliovirus in ES samples because the virus replicates in the gut of infected individuals and, consequently, a considerable amount of live virus is shed in faeces, allowing for the isolation of live virus through virus culture of environmental samples, as well as detection of ribonucleic acid (RNA) fragments via polymerase chain reaction (PCR) and genomic sequencing-based approaches (Klapsa et al., 2022). For pathogen detection more broadly, there have been broad methodological improvements for many pathogens, such as for the concentration and extraction of genetic material and removal of inhibitors, and improved PCR based technologies that can be used to detect many pathogens which reach the intestinal tract and shed in faeces, especially those that replicate in the gut (e.g. enteroviruses).

In this Perspective, we reflect on the progress made in recent years in WBE, focusing on the analytics and inference used to translate raw WWderived data into actionable information for public health, and especially with reference to pandemic preparedness. For recent reviews of the broader area of wastewater surveillance for infectious diseases we refer the reader to Shah et al. (Shah et al., 2022) for studies focussing on COVID-19 and Kilaru et al. (Kilaru et al., 2023) for studies of viruses (excluding SARS-CoV-2) and bacteria. This article is a forward-looking perspective, drawing upon previous research on analytics and modelling applied to wastewater data but also providing a commentary on the direction the field should take for improved inference and application. Analytics is essential in this field because intuitive metrics such as the total number of infected individuals are not directly outputted by analysis of WW samples, and because of the considerable innovation, and variability, in data. For epidemiological inference to be made, comparisons to clinical data and comparison across settings are

required, but present significant analytical challenges. We present progress made thus far and provide a perspective on future directions, discussed at a workshop held at the Alan Turing Institute (UK) in March 2023.

2. Advances in pathogen detection within wastewater

To date, quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) have been the gold standard for detection of viruses in environmental and clinical samples. qPCR is based on the principle that the amplification of a target sequence can be measured by tracking a fluorescent signal in real-time. Typically, the measured fluorescent signal correlates with the amount of target sequence in the sample. Absolute quantification can be deduced either by using a dilution series of standards incorporating the target sequence with known concentrations. These approaches are highly sensitive and accurate and can enable strain-level detection. In a systematic review of WW surveillance to infer COVID-19 transmission (Shah et al., 2022) a majority of included studies (n = 74) used RT-qPCR. Probe-based assays enable multiple targets within one run, reducing the time and costs of quantification compared with running consecutive singleplex assays. A recent example from Canada compared performance of the multiplex assay to four singleplex assays (Hayes et al., 2023), illustrating comparable results. Development of the TaqMan array card (TAC) was initiated in gene expression studies and has been applied to WW data to detect many pathogens. A head-to-head comparison with singleplex qPCR testing illustrated only a moderate reduction in sensitivity that was dependent on the sample matrix (Lappan et al., 2021). The availability of multiplex assays for the simultaneous detection of 3-7 targets can enable "multi-pathogen" surveillance, further improving the efficiency of wastewater surveillance for infectious diseases. To support interpretation of data, studies should ideally report the percentage recovery, limit of detection and limit of quantification of the selected method. The sample concentration methods are known to influence these parameters, where the methods chosen will likely be a balance of optimising sensitivity versus scalability, resource, and lab capacity required to implement.

Digital PCR (dPCR) is an emerging tool that works on a similar basis as qPCR, but implements a multitude of small volume reactions in parallel. Viral concentration can be directly quantified by counting the fraction of partitions reaching a positive endpoint, relinquishing the need for empirical standards to be established. Studies showed that dPCR may be more sensitive and less affected by inhibitors than qPCRbased (Ahmed et al., 2022; Ciesielski et al., 2021; Flood et al., 2021; Jahne et al., 2020; Malla et al., 2024; Sanders et al., 2013). Additionally, dPCR can easily incorporate multiple assays, again facilitating multi-pathogen surveillance (Malla et al., 2024). However, dPCR suffers from saturation issues when faced with high concentration (>10⁴ genome copies/ μ l) samples, (Ciesielski et al., 2021) and hence samples may require dilution.

Isothermal amplification, especially loop-mediated isothermal amplification (LAMP), has been successfully applied for the detection of SARS-CoV-2 and norovirus in wastewater (Amoah et al., 2021; Suzuki et al., 2011). LAMP is highly specific and, using three primer sets instead of one, produces more amplicons than PCR rapidly (within 15-60 minutes) at steady temperatures (37-65°C), hence, thermal cyclers or other specialised equipment are not required. However, LAMP-based approaches only provide semi-quantitative or presence/absence data and may be less sensitive than PCR-based approaches (Suzuki et al., 2011; Cao et al., 2022). Other rapid detection methods, e.g. biosensors (Amoah et al., 2021) may also be useful, but have not yet been trialled within WBE applications. Paper-based technologies that utilise RT-LAMP and do not require laboratory testing have a reported limit of detection of 10 RNA copies μL^{-1} and a field comparison with standard lab-based RT-PCR indicating equivalent performance in detecting the presence of RNA (Cao et al., 2022). The development of field-based technologies is an exciting area of innovation. A current challenge, which is also the case for RT-PCR methods, is the need for a sample concentration step that typically requires laboratory analysis and transportation to ensure sufficient sensitivity and this can result in a long lead-time to results. However, many research groups continue to develop innovations in all technology discussed, potentially enabling quantitative results for RT-LAMP technologies, or scalable field laboratories for qPCR and dPCR.

Next Generation Sequencing (NGS) is now widely used for the identification of viruses in environmental studies (Mavrikou et al., 2020). For WBE applications, amplicon-based sequencing is the most common approach (Garner et al., 2021) that enables the sequencing of a full or partial viral genome within days at a high sensitivity. The disadvantage of this method is that it requires an initial PCR amplification of the target region and, hence, is not suitable for discovering previously unidentified viruses. Metaviromic approaches enable the sequencing of all viral RNA or DNA within a sample without the need for previous knowledge of the genome. However, this assay is less sensitive and time-consuming and can be biased toward/against certain sequences and, hence, may not be suitable for routine wastewater monitoring (Tamáš et al., 2022; Adriaenssens et al., 2021; Rothman et al., 2021; John et al., 2024a).

New methods often enable analysis of more complex material, or improves sensitivity or the scalability of the approaches, but carried out in isolation makes epidemiological inference a challenge. A recent example from South Africa summarised data collected from 87 wastewater treatment plants by eight independent laboratories (Iwu-Jaja et al., 2023). Owing to the different methods utilised (and other challenges) comparisons between sites were not possible, limiting the utility of the collected data. However, the joint analysis enabled evaluation of methodologies and protocols were further refined and could be used across laboratories. A recent PATH report (PATH, 2025) summarises stakeholder opinion on standards required for WW tools that enable continued innovation while ensuring quality control and standardisation. As well as data metrics and reporting standards, development of reference material was highlighted as a useful tool to compare methods across laboratories and time, and supporting epidemiological inference. Comparisons between settings are particularly important when using wastewater data to inform public health stakeholders. A challenge to accomplishing these ambitions is that they are not equally important across settings and wastewater surveillance goals. Diversity in wastewater collection, conveyance and treatment systems, as well as resource availability, dictate some heterogeneity in technologies (e.g., automate samplers versus simple grab sampling; centralised water resource recovery facilities versus septic tanks, open sewers or pit latrines). This may pose a significant challenge if data generated from separate settings or studies is to be analysed jointly, for example to understand relative burden of pathogen loads across a region, or to assess transmission dynamics between neighbouring communities. Standardisation of methods provides one solution when there are no resource limitations, but where there are physical or economic barriers to standardisation, assessing and accounting for sources of uncertainty and variability between methods may be required by, for example, using data normalisation approaches or by conducting inter-laboratory trials (Endo et al., 2024; Pellett et al., 2024; Wade et al., 2022). A further challenge is establishing consistent methods for estimating faecal contamination of a sample, which is important to translate data back to the population and estimate sensitivity. In sewered systems wastewater flow is often available and measures such as ammonia and dissolved oxygen can also be used to inform calculations of pathogen load in the population. For non-sewered or complex sewer systems, there is currently no consensus within the community on the appropriate methods to estimate fecal contamination. However, this is a topic being tackled by several research groups and a consensus should emerge in due course. Since the production of the PATH report (October 2023) there has been limited consensus on the way forward, despite the recognised need for standards within the community, possibly because it is

unclear who might be responsible for holding and distributing any developed reference material or having responsibility for developing best practice (Paracchini et al., 2024).

3. A framework for identifying the utility of WBE for specific pathogens

Based on experience in applying WBE to several examples we propose a decision framework to support its future use for additional pathogens and settings (Fig. 1). Central to the requirement are four components; evidence or plausibility of detection of genetic fragments (i.e., DNA/RNA) in stool and/or other excreta, consideration of the likely infected hosts within a setting, the available mechanisms for sewage disposal that can be sampled, the need for presence/absence or other quantitative data to inform the public health action, and challenges inherent with current surveillance data.

WW surveillance for SARS-CoV-2 was effective because early clinical studies illustrated that despite the virus largely replicating in respiratory tissues, stool samples of hospitalised patients contained RNA fragments of sufficient quantity that could result in detection in sewage. Transmission of SARS-CoV-2 has been predominantly human to human, and with limited clinical testing at the start of the pandemic and increasingly after 2022, data and analysis from WW data has filled a gap in understanding the extent of infection in many populations. The application of WW surveillance to the zoonotic Influenza A H5N1 strain, recently observed in the United States' cattle and some humans, is more complex. Within the United States the national WW surveillance programme has been rolled out to include Influenza A since October 2023 (CDC Influenza A Virus Wastewater Data, 2024). The primers in use are specific to the influenza A virus genotype but not the H5N1 subtype, which means that WW positivity could correspond to infection beyond the H5N1 subtype (i.e., seasonal influenza A infections). Case reports for the current H5N1 pandemic have detailed outbreaks within several animal species, including both dairy (cattle) and poultry (chicken) farms in the United States (as of 25th July there are 171 dairy herds affected within 13 states (CDC Influenza A Virus Wastewater Data, 2024)). Therefore, if positivity occurs for Influenza A in WW, the provenance for the infected species would remain uncertain. In the United States sewage systems are 'combined' meaning that run-off from farms are frequently included alongside household waste in the in-flow to sewage treatment works, and there is anecdotal reporting that unsold milk (that could be incidentally contaminated) is disposed of via combined sewers (CNN, 2025).

To understand the use cases for specific pathogens, validation studies are essential and can vary from reliable reports of the shedding profile of pathogens in the stool of infected individuals to ES studies within discrete populations. Even for validation studies it is important to clearly define the intended goals of surveillance as this will inform sites selected for sampling and the diagnostic methods. ES within convergent sewer networks is most advanced, using different scales of investigation such as sampling only from sewage treatment works and within the network. Assuming pathogen material is sufficiently well preserved in the network, populations with wide usage of such systems are the simplest and easiest to sample in a representative way. ES data from non-sewered networks, including river systems and latrines, have revealed the potential to identify hot-spots from quantitative data and the ability to consistently detect variants, (Barnes et al., 2023) but there remain challenges to identify reliable measures of faecal contamination. For populations where waste is collected via latrine systems, scalability of sewage surveillance is challenging but could be met by low-cost detection systems, positioned correctly for representativeness. For each pathogen, it is important to evaluate the effectiveness of current clinical surveillance to detect the presence of the pathogen, and (where necessary) provide reliable evidence of incidence. We suggest that pathogens with evidence of faecal shedding, a relatively high proportion of asymptomatic infection and a high probability of severe outcome would benefit from ES in at-risk settings. Challenges with case reporting due to



Fig. 1. Flow chart to guide the utility of WBE for a new pathogen in humans. The flow chart guides the user through the impact of biological detection, influence of pathogen natural history, the effect of setting, and the metrics of the pathogen required to inform on suitable use cases.

low availability of resources, incidence in vulnerable populations, or with clinical diagnostics may provide circumstances where ES can supplement information where clinical surveillance data is limited or unavailable. For this reason, specific use cases for ES may well depend on the level of clinical surveillance available in a specific setting, region or country. While low availability of resources for surveillance is encountered in most countries, this is especially problematic in low-income countries, and this is exactly where infectious diseases have the highest disease burden. Low-income settings also coincide with non-convergent sewerage systems, further highlighting the need to develop and validate ES within non-sewered settings (Anon, 2025). Consequently, use of ES in low-income settings, alongside suitably designed intervention programmes have the potential to make a considerable impact. To support the assertion that ES would be beneficial, economic evaluation studies are keenly required for specific use cases

The complexity of the framework points towards WBE being an interdisciplinary science. Input from laboratory and clinical scientists is essential to establish the reliability of pathogen shedding and detection. Environmental engineers are needed to map the connectivity of potential sampling points and understand how drainage (or lack of drainage) will impact the representativeness of collected samples. Data scientists and bioinformaticians are an essential component of translating the data into information. Epidemiologists, pathogen specialists and clinicians are required to provide input into how information from ES can be incorporated into situational awareness of a pathogen, and utilise the information generated to make public health decisions. This should form part of a dedicated public health program to determine best use for each

pathogen area, for example as signal intelligence or directed for outbreak response to monitor effectiveness of interventions such as vaccination. For WBE to be useful for pandemic preparedness, interdisciplinary teams need to be established prior to pandemic emergence to have the capability to pivot sampling and analyses towards a pathogen of concern. For this reason we welcome recent governmental and stakeholder investments in capacity building (HERA, 2023).

4. Application of analytics to WBE: the stages of an emerging infectious disease

As an emerging infectious disease progresses through a population, variation in the underlying infection incidence (Fig. 2) is determined by pathogen-specific factors, infectious contact rates in the host population and the host immune response. Initially, the host is susceptible to infection as no pathogen is present and there has been no history of this pathogen in the community (and for simplicity we assume no cross immunity from other pathogens). This circumstance will be the case for a new infectious disease, such as SARS-CoV-2 in early 2020, or the emergent MPox epidemic.

Should importation occur and with a sufficient growth rate (R > 0), the incidence of new infections will increase, but due to randomness and variation in transmission incidence can be "patchy" or spatially heterogeneous. Detection of the pathogen during the early stages of introduction has the potential to provide early warning of disease incursion, and indeed illustration of the opposite (absence of infection) may be just as useful since interventions can be avoided or may cease. Statistical methods to estimate 'freedom from infection' have been applied to



Fig. 2. Stages of an epidemic where WW surveillance has provided insight on the epidemiology of an emerging pathogen. The lower panels illustrate the spatial aspects of WBE, where a proportion of the target population is included in wastewater sampling (green circles). The upper panel illustrates the time course of an epidemic, and we highlight different stages of an epidemic where data and analysis of wastewater data can inform the epidemiology.

surveillance data such as that measured from wastewater samples to estimate the effect of location and frequency of sampling on the sensitivity of detection (O'Reilly et al., 2020). These methods can also be used to determine the probability that a population is free from infection. The methods were applied in England to determine the sampling locations and provide assurance of a polio-free status; by sampling the 20 highest risk localities approximately 80 % of the estimated risk was accounted for. ES based information may also contribute to uncovering characteristics of a new disease at this point - in particular, it may contribute to understanding and evidence if emergence is in locations where clinical or survey data is more unreliable or difficult to collect, leading to improved early detection. ES collected from transportation hubs (eg. airports) has seen considerable recent expansion, with the aim to support pandemic preparedness and early pathogen detection (Jones et al., 2023). The data and analytics are in initial stages but we look forward to developments in this area. In these examples, the goals of collecting ES data have been clearly defined and informed how sampling should occur; defining goals is a key element of study design to ensure that the questions will be appropriately addressed and helps avoid unnecessary sampling.

Looking prospectively, for WBE to inform early detection of pathogen incursion, further research is required to establish the sample size and locality of risk-based sampling for a sufficiently accurate and timely early warning. While specific examples have been reported for many pathogens, this doesn't always translate into a consistent pattern of early detection and the reasons for this are not well understood. To fill this research gap, systematic analysis of surveillance systems are needed across settings, and mathematical models applied to specific pathogens would inform understanding of factors and be used to explore surveillance scenarios. For early detection of SARS-CoV-2 in WW (within Scotland) the sensitivity was higher in smaller catchment sizes, (Fitzgerald et al., 2021) which creates a tension between higher sensitivity of smaller sites and population sensitivity of larger sites. The feasibility of early detection will be affected by the resources available to carry out surveillance. Consequently, analytics should include economic analyses and consider multiple stakeholder perspectives. Although not strictly within an analytics perspective, analysis should be communicated to public health stakeholders in a timely manner to inform actions and decisions. Collaborative research is therefore very important to understand proportionate decisions based on analytics alongside acknowledgement that these decisions may evolve as the epidemiology evolves.

Through time, if the exponential growth rate remains positive, disease incidence will continue to increase until the depletion of susceptibles in the population limits further infection or interventions reduce transmission sufficiently. In this epidemic stage estimating the growth rate has been useful to support planning (eg. hospital bed capacity) in healthcare settings. Some time later incidence will peak and a decline in infection will occur. These dynamics can repeat themselves in "epidemic boom and bust" cycles according to the rate of infection, which can be mediated by inherent seasonal dependency and public health interventions, and changing susceptibility of the population.

For WBE to inform quantification of the early stages of the epidemic, reliable estimates of prevalence and incidence are important, or as a minimum, an indicator of epidemic direction is required. These interpretable metrics are useful for public health stakeholders, and their rationalisation is akin to the estimation processes used in WW surveillance for illicit drug use, where WW data are converted to "doses per day" for each drug investigated (Metcalfe et al., 2010). The development of analytical frameworks to support these outcomes has rapidly progressed during the COVID-19 pandemic. Initially, several groups illustrated the correlation between RNA concentrations in ES data and reported cases, and more advanced methods validated the predictive ability of RNA in ES data against cross-sectional data on SARS-CoV-2 positivity (Morvan et al., 2022). Estimation of the epidemic growth rate from case data is typically performed by estimating the effective reproduction number ('Rt'), which translates as the expected number of secondary infections from a primary case in time. Values greater than one indicate an increase in incidence (Nash et al., 2023). Translation of these analytical methods to quantitative ES data requires smoothing methods to account for increased uncertainty and variability in this data (Lewis-Borrell et al., 2023; Huisman et al., 2022).

Methodological approaches have illustrated that Rt can be estimated from ES data alone, although with increased uncertainty compared to clinical data (Lison, 2023). While increased uncertainty in Rt needs to be acknowledged, on the other hand, perhaps a low-cost assessment of whether infections are rising, falling or levelling off (to paraphrase Professor Hans Rosling (Maxmen, 2016)) is sufficiently informative. An inherent challenge in validating wastewater-based Rt estimates is that no ground truth for Rt is known in practice and that estimates from other data sources such as clinical case counts can be biased in different and unforeseen ways. Validation methods using simulated data is a useful approach for assessment of alternative methods, but a set of model assumptions are needed that adequately describe the bias present in data. Ideally, Rt estimates from ES data should be compared to clinical data to assess the reliability of these key metrics, and by working with stakeholders should identify what accuracy is sufficient for long-term use of ES. The accuracy can then be optimised by adjusting sampling, laboratory methods, and analytical approaches. The statistical methods used to estimate Rt have already been described and applied, with steps made towards publicly available tools to support wider use (Lison, 2023). Many of the analytical approaches developed so far are reliant on assuming the viral load per capita of the population. Implicit in this calculation is knowledge of the catchment area, and translation of concentration (gc/L) to a population-based measure, e.g. using information about daily flow volumes. In high resource settings these 'meta-data' are usually collected and well-quantified, but not everywhere. Consequently, for reliable quantification to be possible additional data are required, and methods to infer if data collection is not be possible (Langeveld et al., 2023).

Comparisons of Rt from ES and clinical data might identify weaknesses in clinical surveillance (e.g., under-served populations), but Rt estimates from ES would require a high level of accuracy for these aberrations to be sufficiently sensitive. If ES data are prone to aberrations (e.g., due to the presence of inhibitors or poor flow adjustment) the inference from the data may have limited value. Estimation may also be complicated by the appearance of pathogen variants that present different levels of shedding or efficiency in RNA recovery. Alternatively, if there are known weaknesses in clinical surveillance, such as substantially low and unreliable testing, then ES data and associated analytics can add value. Looking forward, these two alternative approaches both have merit, and establishing use cases for each is likely an active area of future research.

Rapid growth of endemic infectious diseases are often due to the immune escape of variants; this has been regularly observed during SARS-CoV-2 and in seasonal viruses (e.g., influenza and norovirus). Consequently, estimating pathogen diversity from ES data and associated bioinformatics (Karthikeyan et al., 2022) is likely to remain an essential component of pathogen surveillance in wastewater. Rather than identify consensus sequences from a wastewater sample, a probabilistic distribution of multiple variants is outputted from next generation sequencing platforms and bioinformatics pipelines. The platform technology varies, for example Illumina sequencing uses a clonal expansion process and nanopore sequencing reads the RNA as it moves through microscopic pores within a dongle, with each varying in read sensitivity. The impact of lower read sensitivity is a less reliable distribution of pathogen variants and reduced sensitivity and specificity of detecting specific variants of interest. Illumina-based analyses are regarded as a 'gold standard' because of the highly sensitive methods and there are an increasing number of studies that have evaluated platforms in comparison to Illumina sequencing (John et al., 2024b). Owing to the complex and variable matrix of wastewater it is likely that these comparative studies will require repeating across settings. The pipelines (e.g., Freya, (Baaijens et al., 2021) CovMix and Kalisto (Yousif et al., 2023)) require pre-defined reference sequences for variants, and wastewater samples must have high sequence quality for reliable inference: in low resource settings or applications with small sample sizes this can impact the lead-time of variant detection. Going forward, collection methods or sample size guidance would improve the utility of this data analysis. Pipelines that are less reliant on lineage assignment

(based on prior detection), such as that illustrated in Yousif et al. (Yousif et al., 2023), or those developed to support metagenomics, (Quince et al., 2017) may act as an early indication of novel variants. Within the area of antimicrobial resistance (AMR), ES is increasing in use and its application has as recently reviewed by Tiwari et al., 2022). Several studies have compared the prevalence of AMR genes in E. coli with clinical data from individuals within (or near) catchment areas to indicate similarities in prevalence, and regional analyses have indicated good correlation between ES and clinical data across settings (Huijbers et al., 2020). These data and analyses illustrate the potential of ES as a monitoring tool for AMR, especially in settings with limited clinical data. Further research is required to explore how WBE could inform important questions such as mechanisms that facilitate development and persistence of AMR and linkage of identified AMR genes to host, and may require sampling of specific at-risk populations. Spatial analysis of pathogens has also been considered within an ES context, but are currently limited in number: mathematical modelling has been used to explore ES sampling strategies for typhoid infection, (Wang et al., 2020) and statistical modelling have been used to test if socio-economic indicators correlate with reported ES data (Li et al., 2023). Spatial analysis of ES data is likely to increase in scope, but addressing comparability of ES data across multiple sites (and laboratories) will need to be addressed to improve inference.

ES data does not result in a reduction in incidence in itself, but actions as a result of interpreting data can. Where the presence of a pathogen, identified through ES, warrants an intervention, the value of ES is clear. Evidence of circulation of poliovirus is one such example, as experienced in London in 2022, where the continued detection of poliovirus (consistent with local circulation) prompted a vaccination response in the wider London area (Polio vaccination campaign letter, 2025). During the acute phase of the COVID-19 pandemic, several examples of interventions as a result of ES detections have been documented. In many university campuses in the United States, ES motivated building-level individual testing. To evaluate interventions, a time series of ES data are required alongside accurate indicators of when specific interventions may be expected to have an effect, and interrupted time-series analysis (or similar) can be applied to the data (Stephens et al., 2022). Additionally, randomised trials of surveillance would also have merit, but few studies exist. During the acute phase of the pandemic, large-scale non-pharmaceutical interventions were implemented and the observable effect in ES data was clear. Looking forward, assessment of the reliability of metrics from ES data on more nuanced interventions will be required to better understand at what spatial scale and strength of intervention it is possible to observe a meaningful effect.

The costs of WBE to detect epidemiologically important changes in incidence is at the heart of establishing the utility of ES. Data collection is comparatively cheaper than the individual clinical data collection required for equivalent prevalence estimation, especially as some elements of an ES programme may be shared between multiple pathogens, or be rapidly pivoted to an emerging data need. Nevertheless, the economic perspective of WW surveillance is an important consideration, and this is an evolving space. The funding of WW surveillance varies considerably; governmental departments fund WW surveillance as 'routine use' but also to support research and development. Nongovernmental organisations (e.g., charities and research funders) and the private sector support and implement WW surveillance across income settings, either as 'routine use' or as research and development. If stakeholders are working towards the vision of routine use, then a framework is needed to evaluate the cost-effectiveness of WW surveillance that incorporates development of suitable cost models, as has been introduced the 'polluter pays' (Trancon and Leflaive, 2025) principle proposed for treatment of pollutants entering wastewater systems. However, this perspective raises questions over whether WW surveillance is a means to monitor health in a human population, or a 'pollution' indicator that considers the potential for onward transmission of viable pathogens and antimicrobial resistance genes, or indeed both.

Stakeholders who choose to financially invest in WW surveillance may be more diverse than those that support clinical surveillance (typically health departments within government), potentially meaning that ES and clinical surveillance may not always be in competition for funds. Additionally, the information gained by investing in clinical or ES differs, and the objectives of surveillance may also differ, and this nuance is required within an economic framework. Application of 'value in information' analyses (De Vries et al., 2021) for infectious disease surveillance would be invaluable to support decision making frameworks and should be prioritised as part of wastewater analytics.

5. Discussion

In this *Perspective* we frame research development around the stages of an emerging epidemic and give an overview on future research directions. We provide a flowchart to guide stakeholders on the utility of WBE for a new pathogen. This guide intends to provide improved clarity on the circumstances under which WBE can be a useful tool and where WBE will have a limited role in understanding epidemiology and informing public health decisions. We anticipate that this flowchart will require revision in the future as the application of WBE evolves; for example, further questions may be included that explicitly consider data collection for routine use or for research.

Across all stages of an epidemic the analytical tools would benefit from increased data collection as this would improve the inference made. In the future WBE analytics will become increasingly available to support analysis and interpretation of wastewater data at all the steps of the epidemic cycle. Many researchers, including the authors of this article and beyond, are developing and validating analytical tools to support analysis. This follows the progress made more broadly in infectious disease epidemiology and bioinformatics to create accessible and open data tools which enables a diversity of applications and in turn fosters innovation and collaboration. We welcome this opportunity to develop new research which has the potential to improve public health.

In conclusion, the increased application of WBE globally has accelerated the development of wastewater and environmental surveillance analytics to maximise the information gained. There are several areas where analytics have the potential to improve, including early detection, development of economic frameworks to support decision-making, and comparing and contrasting measures of epidemic growth to that obtained from clinical data. This research has plenty of opportunities for making innovative leaps, and for ensuring that research is embedded into improving public health.

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Author statement

All authors have made substantial contributions to all of the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
- Drafting the article or revising it critically for important intellectual content.
- Final approval of the version to be submitted.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kathleen O'Reilly reports financial support was provided by Bill and Melinda Gates Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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