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Cross-continental comparative experiences of wastewater surveillance and a vision for the 21st Century

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Declaration of competing interest

There are no competing interests to declare.

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Abstract

The COVID-19 pandemic has brought the epidemiological value of monitoring wastewater into sharp focus. The challenges of implementing and optimising wastewater monitoring vary significantly from one region to another, often due to the array of different wastewater systems around the globe, as well as the availability of resources to undertake the required analyses (e.g. laboratory infrastructure and expertise). Here we reflect on the local and shared challenges of implementing a SARS-CoV-2 monitoring programme in two geographically and socio-economically distinct regions, São Paulo state (Brazil) and Wales (UK), focusing on design, laboratory methods and data analysis, and identifying potential guiding principles for wastewater surveillance fit for the 21st century. Our results highlight the historical nature of region-specific challenges to the implementation of wastewater surveillance, including previous experience of using wastewater surveillance, stakeholders involved, and nature of wastewater infrastructure. Building on those challenges, we then highlight what an ideal programme would look like if restrictions such as resource were not a constraint. Finally, we demonstrate the value of bringing multidisciplinary skills and international networks together for effective wastewater surveillance.

Key words: COVID-19, SARS-CoV-2, São Paulo, Brazil, Wales, One Health, Wastewater Based Epidemiology

1. Introduction

A vital tool in the global response to the COVID-19 pandemic has been wastewater surveillance (table 1). Monitoring viral (SARS-CoV-2) load in the sewers provides estimates of the infection rate in a community and trends in virus circulation in the population. Unlike traditional public health surveys based on individual testing, viral load in wastewater has been shown to provide a relatively low-cost estimate of disease prevalence that are not biased by testing capacity or behaviours, and that often precede public health data by a few days (Kumar et al., 2021). Efforts to implement wastewater surveillance to track SARS-CoV-2 across the globe have been met with different types of challenges. In some regions, such as São Paulo state, Brazil, there has been a long tradition of utilising wastewater surveillance to track the prevalence or the outbursts of serious diseases, in particular, poliovirus, as a supplementary approach to the Global Polio Eradication Initiative (De Melo Cassemiro et al., 2016; Martins et al., 1983; WHO, 2014, 2022). However, in many other countries, such as the Wales, wastewater monitoring for public health surveillance had never been routinely implemented other than for environmental monitoring. This is surprising considering the demonstration as an effective monitoring tool for infection outbreaks in the UK over 75 years ago (Moore, 1948, 1950).

Table 1. Examples of SARS-CoV-2 monitoring programmes from around the globe. Details of these programmes are provided, such as the number of sample sites, the population coverage (where available) as well as the frequency of sampling per week. Data was extracted from various sources for each of the programmes, as outlined in the reference column. Some of the estimates are not current, and instead reflect peak coverage. End date is characterised by the national programme finishing, being drastically reduced, or no longer providing data to their public dashboard.

Country or region	Reference	Number of sites	Population coverage (%)	Frequency of sampling per week	End date
England	(UK Health Security Agency, 2022)	302	74	3	March 2022
Scotland	(Fang et al., 2022)	120	80	3 to 4	Ongoing
Northern Ireland	(S. Bell et al., 2022)	31	62	2	March 2023
Netherlands	(van Boven et al., 2023)	300	99.6	1 to 4	Ongoing
Switzerland	(SWI swissinfo.ch, 2022)	117	~70	3	Ongoing
Austria	(BMSGPK, 2022)	48	58	2	Ongoing
Germany	(Robert Koch Institute, 2023)	175		2	Ongoing
Turkey	(Turkish Ministry of Agriculture and Forestry, 2023)	189	67	1 to 2	Ongoing
Israel	(Bar-Or, 2022; Bar-Or et al., 2022)	135	>55%	2	July 2023
Pune, India	(The Pune Knowledge Cluster, 2024)	32		2	Ongoing
Hong Kong, China	(Chui, 2023)	154	80	3	Ongoing
South Africa	(SAMRC, 2023)	76		1	April 2023
Ottawa, Canada	(Delatolla et al., 2024)	2	92	7	Ongoing

Besides previous experience, local or regional factors are likely to have also played significant roles in the way this type of surveillance system is implemented or optimised. Examples of such factors include: the nature and extent of existing wastewater infrastructure, the governance infrastructure and data sharing, the nature of the relationship between different actors that need to interact (e.g., water utilities, researchers, public health organisations), or the availability of financial resources, testing facilities and trained personnel. The success of wastewater surveillance programmes depends on their capacity to innovate and overcome challenges to adapt to a changing world. A 21st century wastewater surveillance system will be faced with significant global scale challenges. Some are linked intrinsically to water and wastewater management systems, including climate change, rising sea levels, emerging pollutants, rising demographics and concerns over public health, biodiversity loss and ecosystem services. Other challenges will be prompted by epidemiological concerns, such as new SARS-CoV-2 variants, the shift from pandemic to endemic status of COVID-19, the emergence of future pandemics as well as the routine monitoring of other diseases and biomarkers of human health. Beyond challenges, water surveillance programmes also hold great opportunities, namely the potential for meeting the United Nations Sustainable Development Goals, contributing to sustainable cities and communities; industry, innovation, and infrastructure; clean water and sanitation; as well as good health and wellbeing.

Here we reflect on the local and shared implementation challenges of the SARS-CoV-2 monitoring programmes of two geographically and socio-economically distinct regions: São Paulo state (Brazil's most populous state) and Wales (one of the four countries making up the United Kingdom), to identify potential guiding principles of a wastewater surveillance programme fit for 21st century scenarios.

The benefit of using these two examples is that they have both approached wastewaters surveillance in systems with differing structural constraints. These structural constrains include historical context that have led to the present-day wastewater infrastructure, as well as social, governance and economic landscapes. Therefore, understanding the evolution of these two wastewaters surveillance programmes, created under different selection pressures, can provide valuable insights into overcoming common challenges while also providing a roadmap to achieve the characteristics of an ideal international wastewater surveillance programme fit for the 21st century.

To structure this reflection, here we:

1. Provide a brief overview of the historical development, current infrastructure and governance that shape the current wastewater surveillance programmes of São Paulo state and Wales.
2. Compare SARS-CoV-2 wastewater surveillance in terms of design, laboratory methods, data analysis and utilisation.
3. Imagine what an ideal SARS-CoV-2 wastewater surveillance system would look like if resources and logistics were not a constraint, reflecting on opportunities for future wastewater monitoring.

2. History of wastewater and wastewater surveillance in São Paulo state and Wales

In response to the SARS-CoV-2 pandemic, wastewater surveillance programmes have been put in place across the globe, each example having been shaped by wastewater infrastructure, socio-economic context and governance (Arora et al., 2022; Carcereny et al., 2021; Izquierdo-Lara et al., 2021; Tlhagale et al., 2022). São Paulo state (Brazil) and Wales (United Kingdom) provide models to assess how these structural constraints may shape wastewater surveillance programmes and thus Wastewater Based Epidemiology (WBE).

2.1 Evolution of two contrasting wastewater systems – historical context for surveillance

By the turn of the 20th Century, both São Paulo state and Wales had emerged from the Sanitary Enlightenment with the foundations of a modern wastewater system, forged by shifts in organisation, infrastructure, policy, society, and monitoring (Fig. 1). Due to high levels of rainfall in Brazil, especially in summer, the direct or indirect introduction of rainwater into domestic sanitary sewer branches is expressly prohibited (Decree No. 5916/75 in the State of São Paulo). Although, in many areas a fully separated system is not always achieved (FUNASA, 2019) due to illicit discharges of rainwater into the wastewater system and water entering old wastewater pipes in times of high rainfall (Fig. 2b,e,h). In São Paulo, whenever there is a public sewer system in service conditions, industrial effluents must be discharged into it, in which case it is necessary to comply with specific legislation (*Decreto n.8.468*, 1976) (Fig. 2b). If it is not feasible to connect to the public system, the effluent may be released into the water body if it does not alter its conditions and meets specific emission standards (*Decreto n.8.468*, 1976; CONAMA, 2011)(Fig. 2j). Alternatively, Wales has a largely combined wastewater system, where wastewater (e.g. blackwater, greywater, industrial (Fig. 2b), clinical (Fig. 2a)) are actively combined with rainwater. This creates a large volume of wastewater with a cocktail of contaminants, whose removal can be highly variable (Comber et al., 2019) due to dilution capacity, outdated facilities (Gardner et al., 2012) and the expense of effective methods of contaminant removal (Rout et al., 2021).

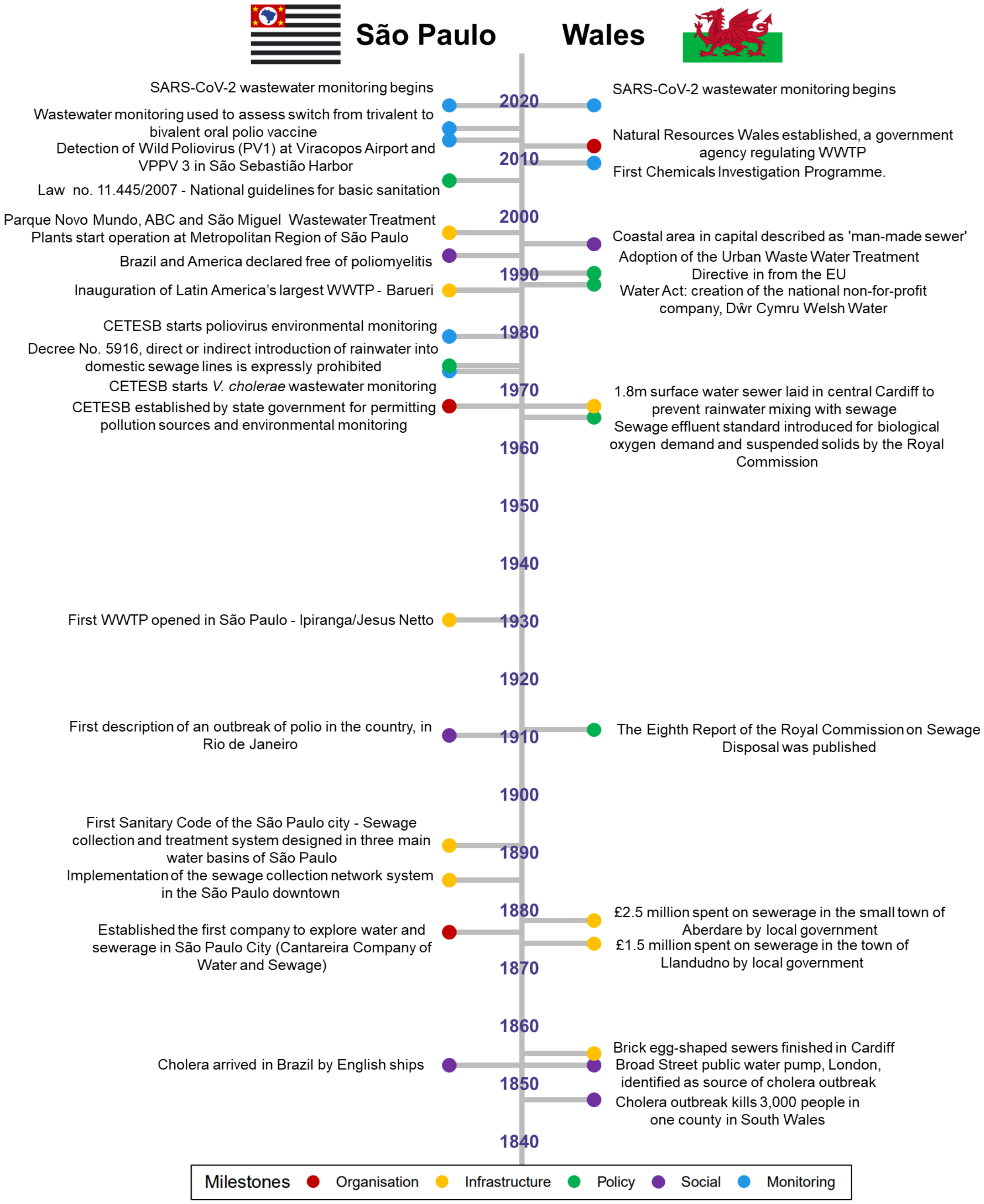


Figure 1 Timeline of milestones (coloured by the nature of the event) in the establishment of the wastewater systems in São Paulo state (left) and Wales (right).

In both São Paulo state and Wales, not all wastewater reaches a wastewater treatment plant (WWTP) (Fig. 2g). The main indicators for São Paulo state shows that 64.5% of the sewage is collected and treated (Fig. 2f,g), 22.6% is collected but not treated, 9.2% is not collected nor treated (Fig. 2e,h), while the remaining 3.7% correspond to individual local solutions (Agência Nacional de Águas, 2021) (e.g. septic tanks, rudimentary pits, open sewers, the launching of wastewater into watercourses and rainwater galleries (Stepping, 2016)). Therefore, a significant portion of the population is 'off-grid', and left without adequate sanitation services, especially in the many of the irregular/unplanned settlements and favelas, which have high population densities and levels of deprivation.

Like São Paulo, Wales is home to many 'off-grid' settlements, but these settlements are rarely found in urban zones, but located in low density rural areas like the uplands. In addition to these rural settlements, there are also many irregular settlements which are primarily used for tourism. These include large areas of static caravans and camp sites, which have a highly seasonal population, often found on the coast, which also rely on holding infrastructure such as septic tanks. In total, there are 7,116 registered septic tanks in Wales, however, little is known about the number of unregistered tanks, or how much waste is released from leaky tanks. Based on household mapping in rural regions it is estimated that ca. 10% of the population in Wales are not connected to mains sewerage. Finally, in Wales, outdated brick-lined Victorian networks still support a large portion of the wastewater network (Heathcote et al., 2003), which due to increasing wastewater volumes, have exceeded capacity. When capacity is exceeded combined sewer overflows (CSOs) are used to remove excess wastewater, allowing the wastewater to flow, untreated, into the environment (Perry et al., 2024), a process which is likely to worsen with climate change (Abdellatif et al., 2015; Petrie, 2021; Zan et al., 2023).

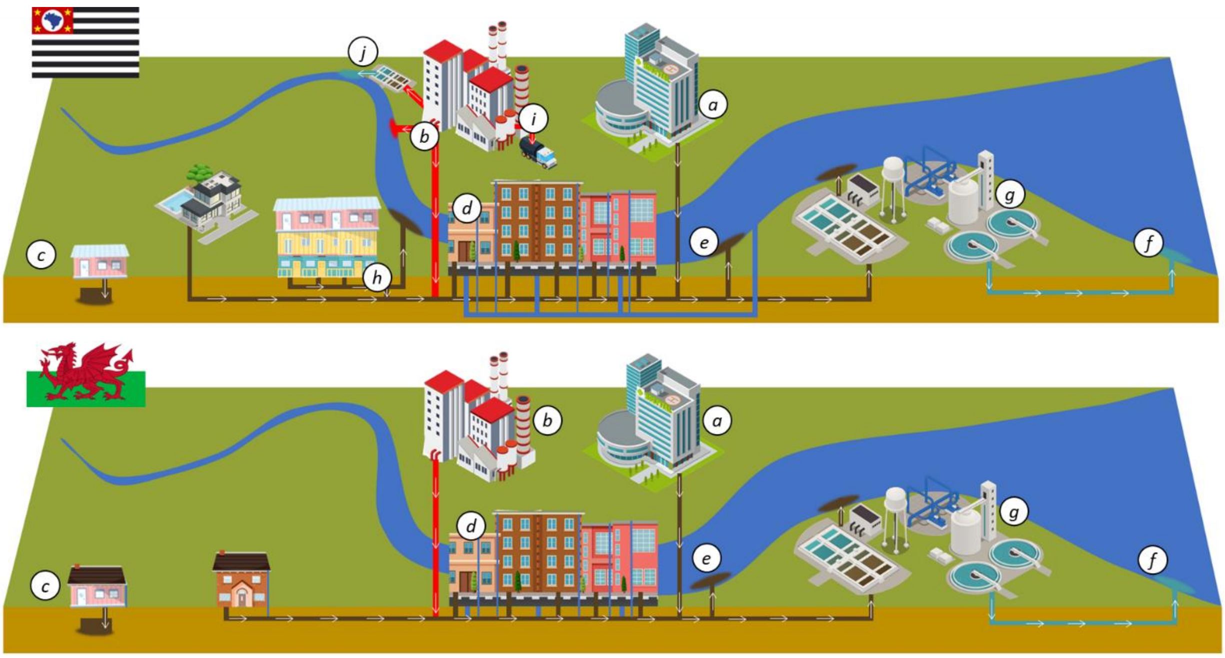


Figure 2 Diagram of wastewater systems in Wales (UK) (top) and São Paulo state (Brazil) (bottom), highlighting wastewater sources (e.g., (a) hospitals, (b) industry, (c) off-grid domestic, (d) high density domestic with surface runoff, (h) informal high density domestic with wastewater misconnections) and outputs (e.g., (c) individual local solutions, (e) untreated release, (f,g) WWTP treated release, (i) transport to specialty treatment and (j) on-site WWTP).

2.2 Social, governance and economic contexts for wastewater surveillance

Geographic size, demographics and history have shaped different contexts for wastewater surveillance in São Paulo state and Wales. Consequently, the governance around wastewater and public health is very different between them. In São Paulo state, multiple branches of government are involved in policy related to wastewater which is not the case in Wales. São Paulo state also has a multitude of governmental regulators, each of which will have different priorities, including sanitation services, monitoring of waterborne infectious diseases or wastewater discharges into the environment. Wales only has two government sponsored bodies to regulate wastewaters, one mainly focused on the environmental impact of wastewater discharge and the other on consumer prices. Economically, wastewater service providers are also diverse in São Paulo state with 60% of municipalities serviced by a government owned company, and the other 40% of municipalities serviced by local public and private services. In contrast, in Wales, most of the population is served by one not-for-profit company (Dŵr Cymru Welsh Water), with a smaller second private company (Hafren Dyfrdwy) providing service to a population approximately 7% of the population served by Dŵr Cymru Welsh Water (Hafren Dyfrdwy, 2019).

3. Comparison of SARS-CoV-2 wastewater surveillance programmes

Building on the body of knowledge on WBE accrued from countries and regions where surveillance programmes have been in place for a long time, such as São Paulo, SARS-CoV-2 wastewater surveillance programmes have required a step-change to meet the needs for near real-time and country-wide assessments. The development of a wastewater surveillance programme, be that São Paulo state or Wales, can be split into three broad categories, design, laboratory methods and data analysis (Fig. 3a-c). These fundamental components will be used to structure the comparison here, acknowledging the fact that they are all interconnected and influenced by structural constraints of the system (e.g. funding, resources, logistical constraints, desired outcomes and type of wastewater system) (Fig. 3e). Structural constraints cannot be easily changed, and will not be the focus here.

Each component of the wastewater surveillance programme requires multidisciplinary expertise (Fig. 3a,b,c), for example, molecular biologists are required to develop the methodology for detecting and quantifying pathogens of interest in wastewater; their knowledge will influence the design of the programme, such as where samples can be taken and how long they can be stored and best transported. Another example is statisticians and bioinformaticians, who can extract and interpret relevant trends through data analysis, but those insights should also feedback into the design (Fig. 3d) of a programme, as they can influence how variance is better accounted for. In this section, we compare the outcome of these multidisciplinary interactions in the programmes in São Paulo state and Wales.

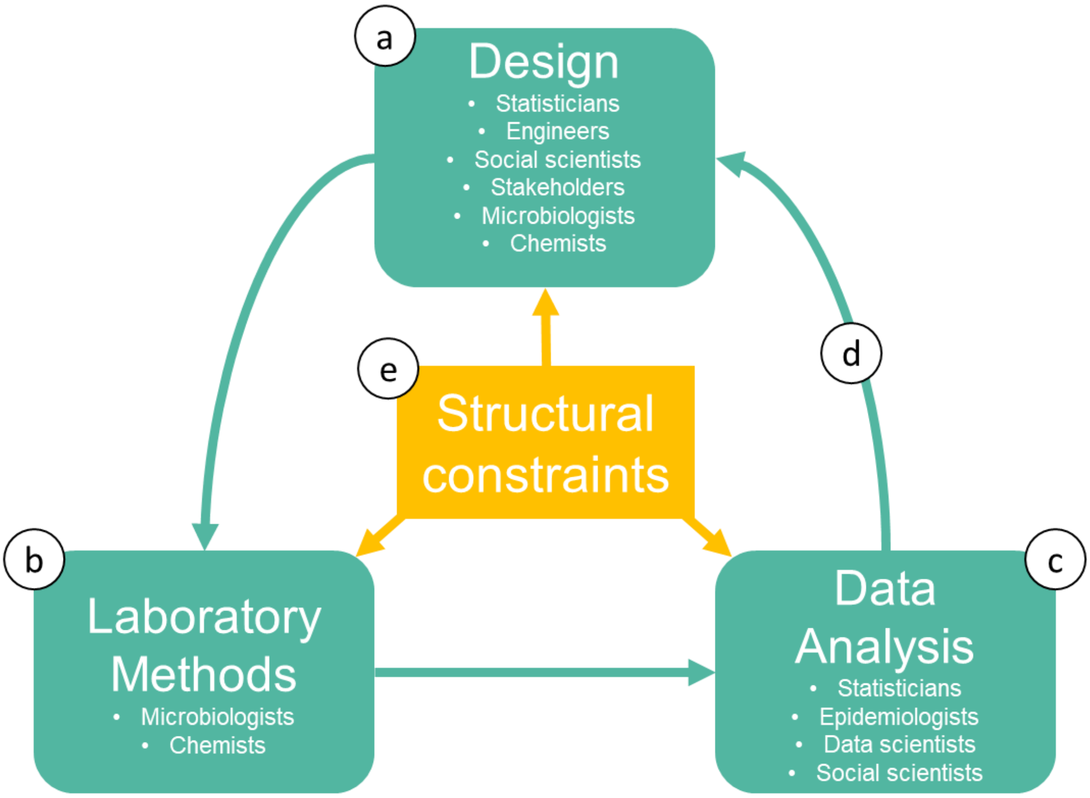


Figure 3 Schematic representation of the main interacting components needed to build an operational wastewater surveillance programme: (a) design, (b) laboratory methods, (c) data analysis, as well as the (e) feedback loop finalised between data analysis and design. Also included is the influence of (e) structural constrains on each of those components.

3.1 Design

3.1.1 Stakeholders

Decisions on choosing sample sites are made by a combination of stakeholders in both São Paulo state and Wales. In São Paulo state, sampling design was conceived between the wastewater utility, São Paulo State Sanitation Company (SABESP), who provided logistics and wastewater flow data, the Epidemiological Surveillance Centre, who brought their knowledge of transmission routes, and the Environmental Company of São Paulo State (CETESB), who provided environmental surveillance expertise. University of São Paulo and UNICAMP participate in the process in the support of data analysis. Wales followed a similar multi-stakeholder development, which included Dŵr Cymru Welsh

Water and Hafren Dyfrdwy, who provided access to WWTPs and wastewater flow data, Public Health Wales, who provided clinical expertise, academics at Cardiff University and Bangor University, who provided environmental surveillance. Finally, the end users, the Technical Advisory Cell of Welsh Government, who used insights gathered to advise on public health policy, with insights from the wastewater surveillance ultimately preventing two lockdowns in 2021 and 2022 during the Christmas period.

Criteria driving the decision-making regarding sampling locations were similar in Wales and São Paulo: size of the population represented by the sample, and logistics such as site accessibility. Previous monitoring programmes in São Paulo state had focused on faeco-orally spread enteric pathogens, with transmission through contact and contamination of water and food (e.g. enteroviruses). Therefore, previous wastewater sampling designs reflected the previous transmission types, focusing on areas with poor sanitation and low immunisation rates, as well as reflecting the endemic, rather than pandemic, nature of the pathogens, focusing on entrance sites (e.g. airports, harbours, bus stations). SARS-CoV-2 required a different sampling approach, as it is primarily a respiratory disease and its presence was far more ubiquitous, which meant sampling efforts were largely concentrated on WWTPs, although not entirely. In Wales, there had been no previous wastewater monitoring for health, but like São Paulo, Wales focused on WWTPs, even more so than São Paulo.

3.1.2 Sampling locations

In São Paulo, out of a total of 24 sites, 14 were located within the conurbation of the MRSP as half of the population of the São Paulo state live in the MRSP and since the beginning of the pandemic the largest number of cases of COVID cases was concentrated in this region. Seven sites were located on the Tietê river system that crosses the city of São Paulo which are directly influenced by the releases from the MRSP sanitary drainage basins, and three at the WWTPs of three large cities in the interior of the State. However, due to the organic growth of the RMSP and the complexity of its watersheds, the selection of sampling points was a challenge, and high priority areas included high-density urban areas and regions with vulnerable populations, such as slums, where people are concentrated in a small indoor space (Barbosa et al., 2022). Effluents from hospitals and WWTPs from cities that received large immunisation campaigns (Serrana and Botucatu) were also monitored for a year. More recently, human entry sites such as international ports, airports and bus stations were included in the wastewater surveillance program. A focus on transmission routes is also what prompted the São Paulo state programme to choose a sampling site on the coast of São Paulo city, where the largest port in Latin America is located, and where the base population of around 800,000 doubles in the summer season. When lockdown measures are suspended, sites that see high levels of population influx such as tourism hotspots are key to follow epidemiological sources and transmission routes.

The programme in Wales initially started with 19 WWTP sites, and in January 2022, the number of sites increased to 47, representing approximately 66% of its population (2,070,883 people). Due to resource constraints, the number of sites was however decreased again in November 2023 to 11 representative WWTPs, after almost four months of no sampling. The largest of these sites, and the capital, Cardiff, serves an estimated 602,990 people, and the smallest site, Betws-y-Coed in north Wales servicing an estimated 375 people. Wales also regularly monitored four prisons, due to the congregation of people in a small indoor space, as well as it being an isolated community of vulnerable people. In addition to these sites, near to source sampling has previously conducted on an ad hoc basis, including sea/airports due to their role in the entry of new SARS-CoV-2 strains. Further, the programme was expanded in 2022 to include the routine monitoring of wastewater from 10 hospitals covering ca. 40% of the patient population in Wales, although this was cut completely in July 2023 due to resource constraints. Periodic sampling of other near-source environments has also been undertaken on a limited basis (e.g. meat processing plants, mass quarantining hotels, university halls of residence). Unlike England, a decision was not made to monitor other near-source environments of perceived high transmission risk (e.g. schools, care homes) due to a lack of perceived benefit relative to the cost.

The trade-off between site representativity and site accessibility were important considerations for both programmes. In the São Paulo state programme, 8 sites out of 24 were located within WWTP basins: the influent of the five WWTP that served the RMSP, and the WWTPs of the three inland cities, unlike Wales, where all sites were located at the influent of WWTPs, other than some sampling of ports and prisons. This is linked to the difference in scale between São Paulo state and Wales, both in terms of population size (Wales = 3.14 million people, São Paulo state = 46.65 million people) and geographic size (Wales = 20,779 km^2, São Paulo state= 248,220 km^2), and so even though Wales focused almost entirely on WWTPs, it could cover a greater proportion of the population, including sparsely populated areas. The Wales programme is different from many other WBE programmes that solely focused on capturing large urban areas, as it actively sought to understand how the disease evolved and moved between urban and rural communities. Wales has a high density of WWTPs (total = 833) over a small geographic area and population. On average, there is a WWTP for every 3,765 people (Dŵr Cymru Welsh Water, 2023), a granularity which facilitates the accessibility and surveillance of smaller populations, unlike São Paulo state, where relatively fewer WWTPs (total = 897) service a larger population, equating to a WWTP for every 49,097 people on average (Agência Nacional de Águas, 2019). Sampling within the network of a large WWTP catchment to capture smaller sections of the population can be more difficult due to accessibility. In addition, in Wales, there is, on average, one WWTP for every 25km^2, whereas in São Paulo state there is one WWTP for every 276km^2.

To ensure population representativity, the São Paulo state programme samples sewage pumping stations, accessible sites in the sewage network through manholes, accessible sewage sites from hospitals, and accessible sewage or open river flows where infrastructure is scarce or absent such vulnerable favela communities. For example, SARS-CoV-2 wastewater surveillance was carried out in São Remo (May to November 2020) and Paraisópolis (May 2020 to June 2021) communities in São Paulo city, which have a population of approximately 8,000 and 51,000 inhabitants, respectively. These communities are supplied with treated potable water, but not connected to a sewer system. Wastewater is therefore discharged into streams which run to Rio Pinheiros. Samples collected from these streams revealed a correlation between cases of COVID-19 cases in the communities and SARS-CoV-2 concentrations in the streams (Barbosa et al., 2022; Pepe Razzolini et al., 2021). A similar study in Wales has also shown the presence of SARS-CoV-2 RNA in WWTP effluent with hydrodynamic modelling predicting that it can travel many kilometres downstream from the WWTP eventually reaching the coastal zone (Hillary et al., 2021; Robins et al., 2022).

Many rural households in Wales are 'off-grid' and rely on septic tanks or rudimentary pits for wastewater collection (Fig. 2c), however, the percentage this makes up of overall wastewater volume is unknown. A definitive publicly available estimated percentage of wastewater that is released directly into the environment is also unknown (Fig. 2e). The Welsh programme does not sample 'off-grid' communities, which would involve sampling cesspits, or untreated wastewater released into rivers. This has not been considered as apriority because 'off-grid' communities, while often the most deprived, are also living in the most sparsely populated areas of Wales where transmission rates are lowest. In São Paulo, on the other hand, 'off-grid' communities live in some of the most densely populated areas of the region where transmission rates are likely to be the highest.

3.1.3 Sampling timings

Given previous expertise, initial monitoring of SARS-CoV-2 started 39 days after the first case of COVID-19 was recorded for the São Paulo state programme. In Wales, the first wastewater samples were taken for analysis a few days after citizens were evacuated from Wuhan, China and arrived back in the UK (March 2020), however, this was confined to 3 locations in Wales (Cardiff, Wrexham, Bangor (Hillary et al., 2021)). At this stage the use of WBE for tracking SARS-CoV-2 was unproven and as such this was the validation phase that led to the development of the national programme in both England and Wales (Tlhagale et al., 2022). It took a further 6 months for the programme to be expanded more widely in Wales, and there was reliance on grab sampling. Two WWTPs in the São Paulo state programme (ABC and São Miguel) were also already equipped to provide 24-hour composite samples, which meant that they could be sampled immediately. However, in Wales, composite samplers were not being installed until November 2021, approximately 1 year and 8 months after the first case of COVID-19 in Wales, which meant the programme was reliant on grab samples for a large portion of the pandemic, which are a snapshot in time, and less representative than the longer time periods captured by composite samples. Currently, both programmes utilise a mix of grab and composite sampling, but due to constraints at some of the São Paulo state sites, they are unsuitable for permanent composite samplers. The Welsh programme, however, is predominantly based within WWTPs, and has now installed refrigerated composite sampling across all its sites. In terms of sampling frequency, the São

Paulo state programme samples bi-weekly whereas the programme in Wales did samples five times a week and was reduced to three times a week in November 2023, but this choice was primarily resource driven.

3.2 Laboratory methods

3.2.1 Concentration

The initial step in processing wastewater for molecular methods is concentration and removal of large debris. Originally, the São Paulo state programme used an ultracentrifugation and glycine elution method (Pina et al., 1998), unlike in Wales where an overnight polyethylene glycol (PEG) precipitation is used (Farkas et al., 2021). The latter method was chosen in Wales based on previous studies isolating a range of viruses from wastewater and the ability to process large volumes of wastewater (100-250 mL (Farkas et al., 2018)). Both ultracentrifugation and PEG precipitation methods offer similar viral recovery, but each have advantages and disadvantages (Ahmed et al., 2020; Crocetti et al., 2021). An advantage of overnight PEG concentration is that the only laboratory equipment required is a centrifuge capable of reaching 10,000 *g*, which is a standard piece of equipment in most molecular laboratories, unlike ultracentrifugation which can require speeds of upwards of tenfold greater. Further, ultracentrifuge filters are expensive and their supply chain was erratic at the start of the pandemic. Due to the high speeds required and expensive equipment, it is also more difficult to process larger volumes (> 50 mL) using ultracentrifugation (Lu et al., 2020). However, the ultracentrifugation method is less time consuming, and concentration can be achieved in 3 hours, rather than the 24-hour process required for overnight PEG precipitation, which can be an obstacle for the rapid turnaround time required to make wastewater monitoring useful. In addition to this, PEG precipitation can also suffer from co-concentration of inhibitors, which can impact on SARS-CoV-2 detection (Scott et al., 2023), which is not such an issue for ultracentrifugation (Warish Ahmed et al., 2020). Recently, however, due to the decrease in the number of COVID-19 cases in São Paulo, the ultracentrifugation method was replaced by the electronegative membrane filtration method (W. Ahmed et al., 2015) which allows the concentration of high volumes of wastewater (100-200 mL) when compared with ultracentrifugation method (40-50 mL). Importantly, methods used in São Paulo state and Wales can concentrate viruses from both solid and liquid fractions of the wastewater, which can be important for maximising SARS-CoV-2 recovery (Ahmed et al., 2020; Kaya et al., 2022). Both methods also have a low cost per sample once the laboratory equipment has been acquired, which makes the scaling of operations more economic, vital for regular and widespread monitoring.

3.2.2 Extraction

Following concentration, viral RNA is extracted. In São Paulo state, a spin column extraction kit is used, whereas in Wales magnetic silica beads are used. Much like the differing concentration steps, both methods offer similar viral recovery of SARS-CoV-2, but each have advantages and disadvantages. There is evidence to suggest that SARS-CoV-2 recovery is marginally lower using magnetic silica beads when compared to spin columns, however, magnetic silica beads have also been shown to provide greater sensitivity and the process can be automated (Pérez-Cataluña et al., 2021). Another important factor in selecting which extraction methodology to adopt includes availability of equipment, and because the production of silica magnetic beads is relatively easy, it means that they are more readily available, unlike specialised commercial plasticware involved in the spin column extractions (Klein et al., 2020). Plasticware supply chain issues, like those seen during pandemics, brought on by factors such as high demand, mean that reliance on specialised commercial plasticware can hinder the progress of smaller wastewater programmes that are unable to stockpile. Finally, protocols involving magnetic silica beads can be easily automated using liquid handling robots, unlike those involving spin columns, due to the requirement of centrifugation. The latter allows the simultaneous processing and preparation of 96 samples within a few hours.

3.2.3 SARS-CoV-2 measurement

The focus for both São Paulo state and Wales has been SARS-CoV-2 prevalence in wastewater, which is measured using reverse transcription quantitative real-time PCR (RT-qPCR), involving the amplification of target regions from the fragmented SARS-CoV-2 genome found in wastewater (Farkas et al., 2021). The first step in method development is choosing a region of the SARS-CoV-2 RNA genome to target. In São Paulo, both the N1 and N2 regions are amplified, whereas in Wales the N1 gene is used. The multiple marker approach allows for multiple regions of the fragmented SARS-CoV-2 RNA genome to be characterised. To assess the presence of PCR inhibitors, in São Paulo, the N1 assay is performed in a multiplex reaction with a synthetic oligonucleotide RNA positive control. In Wales, a pseudomonas virus phi6 positive control is measured. The final element of the RT-qPCR is the addition of standards which are used to produce standard quantification curves, which, in São Paulo, consist of serial dilutions of a SARS-CoV-2 plasmid control. In Wales, however, a synthetic positive-strand SARS-CoV-2 RNA is used.

In addition to monitoring persistence, molecular methods are also used to monitor SARS-CoV-2 variants present in wastewater. In Wales, sequencing is used to detected variants using the EasySeq™ RC-PCR SARS-CoV-2 Whole Genome Sequencing kit on the Illumina NextSeq platform, which is conducted at all WWTPs once a week. In the São Paulo state, no regular sequencing is conducted for variant detection, although some samples have been sent to project partners in the Karolinska Institute, Sweden.

3.3 Data analysis

3.3.1 Data processing and normalisation

In São Paulo, data streams include field measurements (temperature, pH and rainfall), wastewater flow (instantaneous and daily average) provided by Sanitation Companies, and laboratory determinations for SARS-CoV-2, total suspended solids, CrAssphage and ammoniacal nitrogen. SARS-CoV-2 data are originally measured in gene copies (gc) per litre but for WWTPs and other sampling sites where wastewater flow is available, this measure is converted to daily viral load of gc per day. The data are normalised by population, considering the population of the sewer catchment. Studies are being conducted to assess the need for data normalisation for sewage dilution, based on CrAssphage and ammoniacal nitrogen. CETESB laboratory data have shown that total suspended solids (TSS) concentration interferes with the recovery of enveloped viruses; samples with higher TSS values had lower bovine coronavirus recovery rates (Barbosa et al., 2022). Recovery rates are not used to correct the concentration of SARS-CoV-2 detected in wastewaters.

For the programme in Wales, there are multiple data streams, all of which are used to produce a normalised signal. The first type of data stream received by the programme is that produced by the laboratories, which includes SARS-CoV-2 and chemical markers (ammoniacal-N, electrical conductivity, orthophosphate, and turbidity). The second type of data stream is flow data, which is provided weekly by Dŵr Cymru Welsh Water, and is recorded in minute intervals. The final data stream is static and is an estimate of population size within the WWTP catchment. Due to issues with the accuracy and reliability of flow measurements taken at the WWTPs, chemical markers are measured and are used to produce an estimated flow, which in combination with the population estimates are used to calculate viral load per capita. Using this methodology is important as often flow is lost, or capped, before it is measured at the WWTP through outlets in the system such as diversion channels, storm tanks and CSOs, but using the dilution of chemical markers, total flow can still be estimated despite capping. The details flow estimation and SARS-CoV-2 normalisation can be found in Wilde et al. (2022). 10 day rolling averages are used to smooth the signal over time. Unlike São Paulo, there is no outlier removal or missing data imputation.

3.3.2 Reporting

In Wales, reports are sent weekly to Welsh Government, with both national and regional overviews of SARS-CoV-2 levels in the wastewater, and were later made publicly available (Welsh Government, 2022), although reports were no longer made public after the budget cuts in September 2023. Regions of Wales were split into the management units of the main water utility company, Dŵr Cymru Welsh Water, with the country being divided into 14 regions, but this later changed to four larger geographic regions based on health boards, due to the cut in the number of sites sampled after budget cuts. Data is primarily visualised and communicated in line graphs of rolling mean SARS-CoV-2 gc/day per 100 people over time, a national heatmap and before budget cuts, four bullet points of descriptive text. The text provided nationally, and for each region, contains the following information: four-week trend, trend compared to the previous week, indicators triggered and sampling issues or inconsistencies. One of the most informative aspects of the text are the indicators, of which there are three, which indicate high signal level (viral loads exceed half of the highest weekly average recorded in the previous 6 months), rapid increase (weekly average of the viral load has increased by at least 100% since the previous week) and increasing signal level (weekly average of the viral load has increased since the previous week for at least 3 weeks in a row).

In São Paulo, reports with N1 and N2 concentrations (gc/L) for all sites monitored are sent fortnightly to State Epidemiological (CVE) and Sanitary Surveillance (CVS) Centers (Health Secretary) and São Paulo Municipal Health Surveillance Coordination (COVISA) with a graphic showing the temporal evolution for each site. This information is also available at CETESB website where it is possible to follow up the spatial and temporal variation of SARS-CoV-2 concentration at WWTPs, vulnerable areas and surface water (https://cetesb.sp.gov.br/sars-cov-2/). Other than the evolution of time trends, no indicators are routinely established in the technical report of São Paulo state, but such a tool can easily be developed and incorporated into the website.

3.4 Funding and cost

Funded through CETESB, the São Paulo state programme received approximately US$292,000 (R$1,435,150) over a three-year period, equating to US$8111 a month. 45% of the spend was on human resources and 55% was on supplies and materials. At its peak, the Welsh programme received US$5,442,000 (£4,270,000) of funding from Welsh Government for a 12-month period, equating to US$453500 a month, which was then reduced to US$791,400 (£621,000) between September to March 2023, equating to US$113,057 a month. The longest continuous contract awarded at any one time was 12 months.

Due to differences in the price of goods and services between São Paulo state and Wales, an exact comparison of cost is difficult, however, Purchasing Power Parities (PPP), offer a methodology to compare costs through a "basket of goods" approach (table 2). We accept that there are variations in standards of living and values of indicators such as gross domestic product per capita within nation states. However, Brazil and UK national figures, in terms of PPP, provide an estimated comparison of purchasing power between São Paulo state and Wales. These comparisons highlight the disparity in funding between the two programmes and begins to explain differences in frequency and geographic spread of sampling, as well as the extent of monitoring conducted on other biological markers other than SARS-CoV-2.

Table 2. Comparison of wastewater programmes costs in São Paulo state and Wales. Included are the actual monthly costs of the programmes in their home countries and local currencies, as well as an estimated comparable cost, using Purchasing Power Parity from 2022 (OECD, 2024). Figures are given to the nearest thousand.

Wastewater programme	Actual monthly cost in source country and currency	Purchasing power parities, Total, National currency units/US dollar	Estimated monthly equivocal cost in US dollars
São Paulo state, Brazil	R$40,000	2.583	US$15,000
Wales, UK (scaled down)	£80,000	0.651	US$136,000
Wales, UK (peak)	£356,00	0.651	US$547,000

4. Common challenges

4.1 Design

By comparing the Wales and São Paulo, the main challenges in designing an efficient wastewater surveillance program include: lack of existing experience and infrastructure, the level of collaboration between stakeholders, the extent to which the sample can provide a representative measurement of SARS-CoV-2 levels in wastewater as well as the extent to which these levels can be related to known populations and infectivity. The design of both programmes has involved the participation of a range of stakeholders including researchers with an understanding of epidemiology and water systems, water utilities that can provide information and access to sewerage systems and data, governmental institutions that can provide funding and public health data to support the programme. In São Paulo, many of these collaborations were already in place because of existing poliovirus and cholera programmes. However, given the historical nature of these previous programmes, collaborations and initiatives for environmental monitoring have developed at municipal, state or regional levels. The SARS-CoV-2 programme has reflected this legacy and a National Wastewater Monitoring Plan for SARS-CoV-2 has not yet been developed. On the other hand, the urgent need for national decision-making tools as well as the lack of existing wastewater surveillance systems has required investment at a scale that could only be directly led and resourced by Welsh Government. Lack of experience in large-scale routine wastewater surveillance in Wales, as well as the United Kingdom more broadly, has brought the additional challenge of rapidly developing operational collaborations between environment agencies, academia, public health and water utilities. While academic institutions often develop collaborative links, these often operate around research and innovation departments and over short timescales, thus they rarely involve long term operational demands required for wastewater surveillance.

Factors affecting the quality or quantity of genetic material in the sample have also been a challenge to design, with one of the main factors being dilution. Extreme weather events such as floods and storms are common in tropical regions, including Brazil, and high rainfall associated with storm events are also common in Wales, particularly when excess water enters the wastewater system, they cause dilution of the SARS-CoV-2 signal. Indeed, this problem is only set to get worse, as climate change has been identified as one of the main challenges faced by urban wastewater systems globally, with increased frequency of high-intensity storm events (Hughes et al., 2021; Langeveld et al., 2013). Ideally, sewage would be collected separately from surface-runoff, reducing the impact of dilution. Even where sewage is separated from wastewater, such as São Paulo, surface-runoff can still enter the sewage network through misconnections. One way of accounting for the changes in SARS-CoV-2 signal induced by dilution is by normalising by wastewater flow. However, access to reliable flow data is often not possible, due to logistical, technical, or financial constraints. Even if a flow meter is present at the WWTP, flow data can become more unreliable during high flow events due to issues caused by debris, and because wastewater can be diverted before the flow meter into storage tanks or overflow into rivers or the sea. Not only this, but where the flow meter is placed in the WWTP can influence the flow measurements, making comparability between plants difficult. When flow is not available, or when flow data is unreliable, there are methods that can account for dilution based on chemical or biological constituents of the wastewater (Wilde et al., 2022). However, these methods require further laboratory capacity, at least some flow data (e.g. historic flow) and may not be as effective in near-source applications. The measurement of flow within the sewer network (e.g. at manholes) is particularly problematic, but not impossible, as within Welsh programme flow gauges have recently been installed for near-source monitoring of wastewater at hospital sites.

If dilution can be adequately accounted for, there still remains the issue of dilution and its impact on viral detection rates (Aguiar-Oliveira et al., 2020), with the possibility that significant dilution events could reduce the SARS-CoV-2 signal below detection limits, rendering dilution normalisation techniques redundant in these scenarios. In addition to dilution, decay is another challenge when designing a wastewater monitoring programme. Sewage infrastructure and sampling design can impact the length of time it takes for particles to get to a sampling point, and then to reach the laboratory. Given the nature of SARS-CoV-2 and other pathogens, transit times can affect the decay level of the material and thus the quality of the sample (Burnet et al., 2023). Other wastewater contributions to the sewage system can also affect the quality of the sample. For example, industrial contributions can, in addition to diluting sewage, affect the viability of pathogens in the environment (Bayati et al., 2022) as well as cause PCR inhibition (Scott et al., 2023).

Finally, variation in the population numbers being serviced by a wastewater system is a challenge to wastewater monitoring and relating a wastewater sample to its shedding population. Populations serviced by a wastewater treatment plant can be estimated by looking at population census. These can be at best annual, but always represent a population residing in the area at a static point in time.

Movement of individuals during the day to commute, but also for tourism, can significantly affect viral loads. This is true for example for tourism hotpots like the Welsh coastal towns, or the main port of São Paulo. Populations not serviced by a wastewater treatment plant are more difficult to estimate.

4.2 Laboratory methods

SARS-CoV-2 concentrations measured in wastewater can be strongly influenced by sampling method, sample preservation, storage time, concentration method, RNA extraction method, RT-PCR assay selection and overall performance of each step in the molecular pipeline (Beattie et al., 2022; McClary-Gutierrez et al., 2021). Yet decision making processes in response to operational constraints have led to a great diversity of molecular pipelines, with no standardized protocols for the determination of SARS-CoV-2 in wastewater.

Previous poliovirus monitoring has influenced the processing of wastewater samples for SARS-CoV-2 detection in São Paulo. The government agency, CETESB, had existing environmental virology laboratory infrastructure and well-established collaborative networks from environmental poliovirus surveillance which began in 1980. In Wales, however, academic environmental virology laboratories were, and continue to be, the foundation of the national programme. SARS-CoV-2 is an enveloped virus, unlike poliovirus and other enterovirus, which had been previously monitored by government laboratories in São Paulo state. Therefore, although there was valuable expertise and equipment available, new methodologies for SARS-CoV-2 had to be validated, making them more specific, more sensitive and reducing degradation of the non-enveloped virus, all while working within the parameters of existing laboratory infrastructure. The academic, and fundamentally experimental, beginnings of the SARS-CoV-2 monitoring in Wales meant that it was not constrained by the infrastructure of previous monitoring programmes, which offers benefits, but also came with its own constraints. Laboratory infrastructure had to be created during a time where equipment was scarce, and molecular methods and analytical pipelines are constantly evolving, which has consequences for data consistency and long-term comparisons. The consistency of long-term comparisons is likely to change over time, even without methodological approaches, as new strains arise, and populations become more widely vaccinated. However, flexibility in the molecular approaches used in Wales has also contributed to a lack of standardised molecular protocols used in the two laboratories in the Welsh programme, which at times made comparability between samples difficult. Ultimately this led to the primary processing of samples in one laboratory with subsequent molecular analysis carried out in two laboratories.

In addition to variations in the molecular pipelines, SARS-CoV-2 can be influenced by the properties of wastewater itself, including matrix composition, physicochemical characteristics of wastewater and viral form (Kantor et al., 2021; LaTurner et al., 2021; Li et al., 2021). The combined sewage systems found in Wales has the potential for producing highly complex wastewater due to varied inputs, including industry and surface runoff. Each of the inputs could contain a different cocktail of PCR inhibitors, such as multi-ringed polysaccharides (e.g. humic and fulvic acids), salts, fats, proteins, surfactants, metal ions (e.g. iron and aluminium) and RNases (Warish Ahmed, Simpson, et al., 2022), all of which can cause false reduced SARS-CoV-2 signals when using RT-qPCR. Not only this, but inhibitors can vary in both time and space in ways that are often hard to predict, such as human behaviour (Pons et al., 2020). The properties of the wastewater may also affect downstream sequencing with some sites routinely failing despite having strong RT-qPCR signals. The same issue is also seen in the São Paulo state programme, however, the greater separation of industrial waste and surface runoff means that, in theory, the wastewater matrix should be more consistent in composition.

Finally, trying to detect small concentrations of SARS-CoV-2, and establishing an adequate limit of detection/quantification can also be a challenge, especially when wastewater samples are highly dilute, because of high rainfall events, or if SARS-CoV-2 cases are low in the population. Increasing sensitivity in RT-qPCR is one of the primary drivers for selection of molecular methods (Warish Ahmed, Bivins, et al., 2022), which is also the case in São Paulo state and Wales.

4.3 Data analysis

To assess the validity of wastewater surveillance, one of the most straightforward assessments that can be carried out is a correlation between COVID-19 cases and SARS-CoV-2 signal in the wastewater. This does come with two assumptions, the first being that case data is complete, reliable and representative. This was not the case at the start of the pandemic when testing was often sporadic and targeted to outbreaks causing a mismatch between wastewater and clinical surveillance data (Hillary et al., 2021). The São Paulo State Health Department provides daily data for COVID-19 cases per municipality which is compared with SARS-CoV-2 levels, however, due to the lack of intensive testing in Brazil, most reported cases correspond to symptomatic patients and health seeking behaviours, invalidating any comparison with wastewater SARS-CoV-2 levels. In Wales, although during the height of the pandemic there was intensive routine testing, however, national policy has now moved away from providing free testing services, to a reliance on self-reporting and finally no need to report a positive case. Consequently, comparison of this data with wastewater SARS-CoV-2 levels became more and more futile.

Subsequently, an estimated national percentage of the population with COVID-19, which was modelled by the Office for National Statistics as part of their COVID-19 Infection Survey (Office for National Statistics, 2022), was used for comparisons with the SARS-CoV-2 wastewater signal. The COVID-19 Infection Survey estimates had their limitations, however, such as the decreasing number of SARS-CoV-2 tests they were based on, as well as the lack of regional data. However, even this programme was terminated in March 2023, and so wastewater provides the only reliable way to estimate national levels of SARS-CoV-2 infections.

The second assumption of comparing SARS-CoV-2 levels in wastewater and case data is whether the two have a meaningful relationship. The levels of SARS-CoV-2 in wastewater corresponds to the number of people who are infected with SARS-CoV-2 and their shedding rates. A host of factors can impact shedding, such as age (Bertels et al., 2022; Jones et al., 2020; Omori et al., 2021; Prasek et al., 2022), and shedding can last a week after detectable respiratory SARS-CoV-2 (Zhang et al., 2021).

Another challenge when utilising levels of SARS-CoV-2 in wastewater is understanding the source of variation and trying to limit that variation so that a representative signal can be achieved. This starts with the sample design, for example, grab samples will be inherently less representative of the wastewater over a 24-hour period than composite samplers, yet in the São Paulo state and Welsh programme, results from both grab and composite samples are compared together. We have also already outlined how the programmes in both São Paulo state and Wales try and limit variation through improved laboratory methods, however, data collected in the laboratory on precipitation volumes, extraction efficiency and qPCR efficiency are not used in downstream data analysis, and so that explanatory variable for variation is therefore lost.

Neither São Paulo state nor the Welsh programme routinely implement an outlier removal method. Removal of large values in signal could be erasing a genuine rapid increase in SARS-CoV-2 in a community, which would be important for policy makers. Finding and implementing a robust statistical approach for understanding outliers in this context has not yet been achieved. If an exceedingly high value is detected in the Welsh programme, data sources contributing to that signal are investigated (e.g., physiochemistry, flow data, qPCR result), and if there is doubt on the quality of that sample, it is withdrawn and re-analysed. Not having a robust outlier removal method risks the inclusion of anomalous data.

One of the greatest sources of variation on levels of SARS-CoV-2 in wastewater is from the sewage infrastructure, primarily through dilution and population sizes. The Welsh programme uses a robust methodology to account for dilution and population size, whilst also overcoming the challenges imposed by sewage infrastructure (mainly capped flow measurements through CSOs). Yet, despite this, the Welsh programme only have access to static population estimates, when in some cases, large proportions of the population are not static, due to phenomenon such as commuting and tourism, which will lead to unaccounted variation in levels of SARS-CoV-2 in wastewater. In São Paulo, even getting static population estimates for a wastewater catchment can pose a challenge. The Demographic Census is the best database, and the information is collected by households, but it is held every 10 years and the last one, in 2020, has not yet been finalised. The SNIS is a national database on Water and Sewage services, Urban Solid Waste Management and Drainage and Urban Stormwater Management for all Brazilian municipalities, and it too has detailed annual information, but the data is self-reported by the sanitation companies and is not audited or validated. It therefore contains inconsistencies introduced by errors in filling out official forms, difficulties verifying data by local teams, or even intentional manipulation of information by service providers. For example, population resident in regularized areas, or areas under their responsibility, as defined in the service concession contract, and not the total population of the municipality. Therefore, populations that reside in watershed protection areas are intentionally made invisible. Indeed, households used by the tourist population or commercial activities, but which maintain the residential register, also pay lower costs and contribute to overestimating the proportion of the population with access to the water network and sewage.

Further progress also needs to be made on characterising the impacts different inputs have on signals detected at a WWTP, for example, the age of a population, the number of hospitals and population density. Similarly, better linking results obtained from wastewater with healthcare interventions could provide a valuable tool in validating results from wastewater programmes, but also validating the impact of different types of intervention (e.g. transport restrictions, targeted vaccination). Yet these real-world examples are lacking in both the São Paulo state and Welsh programmes. One barrier in progressing crossover between wastewater surveillance and public health is that often the areas monitored for wastewater overlap multiple health boards or administrative boundaries, in addition to the difficulties of working with public health stakeholders who can have different aims and objectives.

On a practical level, working with multiple stakeholders, not only in public health, but also within a wastewater programme (e.g., water utilities, government, laboratories) can also pose its own challenges. For example, in Wales, due to the constraints of data sharing between institutions imposed by firewalls, prior to the implementation of a new data system in 2023, two separate databases existed in the North and South Wales laboratories, both of which had different data formats and required frequent manual updates. In addition, accessing regional data on the number of clinical COVID-19 infections and SARS-CoV-2 variants from individual patients proved almost impossible to obtain due to ethical and data compliance issues. Fundamental databasing and data sharing issues such as these can cost time and impact cross collaboration.

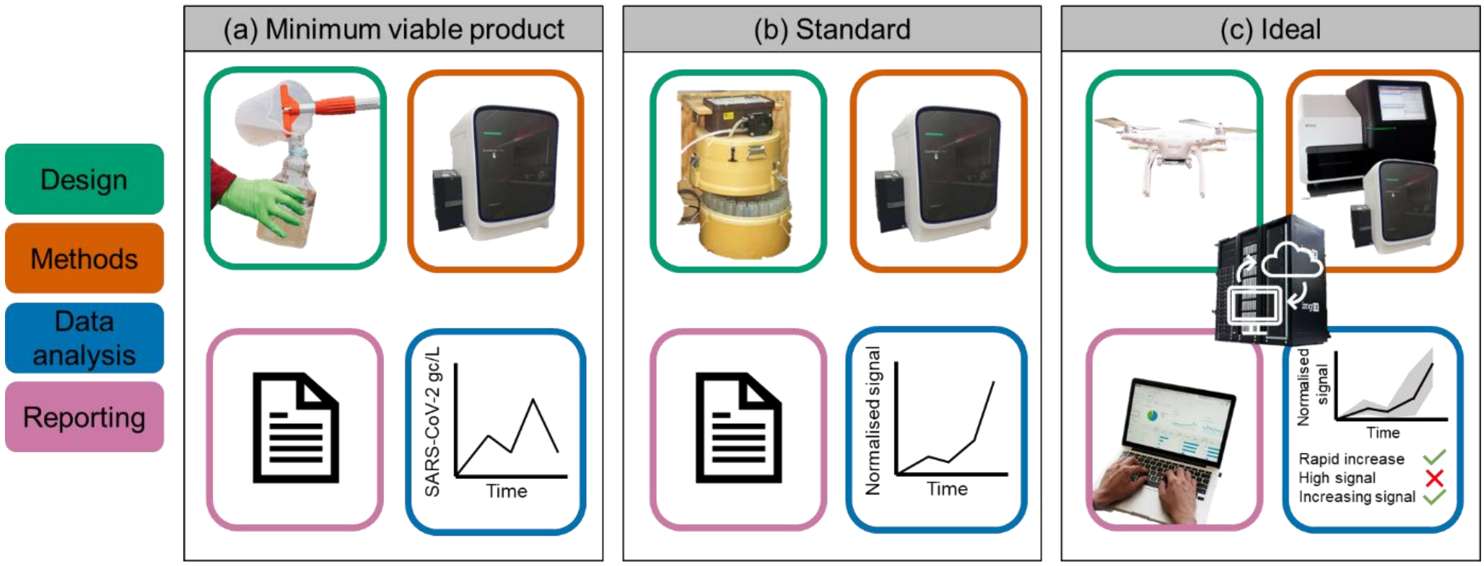
In the State of São Paulo, data sharing must comply with the Brazilian General Data Protection Regulation (Law 13.709/2019), however, although data on mortality and hospital admission is available on an open access platform, there is a delay of about two months for data on hospitalizations and two years for mortality, with the smallest scale of data being entire municipalities, except for the municipality of São Paulo state where the data are available on the census tract scale. Additional difficulties with health data involve underreporting; lack of an integrated outpatient database in basic health units; the use of different administrative divisions for the areas of health, sanitation, environment, among others. Besides, these databases usually have high dimensionality, missing data and can suffer from user inputting errors, thus requiring specialist teams with computing, data handling and statistical skills.

4.4 Novel insights

While other reviews have examined challenges of wastewater surveillance design (e.g. number of samples taken, geographic spread, degradation and the value of composite samples) (Medema et al., 2020), laboratory methods (e.g. extraction yields, concentration and purification) (Kumblathan et al., 2021) and, to a lesser extent, data analysis (e.g. wastewater-clinical comparisons and population normalisation) (Polo et al., 2020), our cross-continental comparison and use of case studies has allowed us to identify and go beyond discussing technical challenges. Novel insights into more holistic challenges are presented, such as the collaboration between stakeholders required to bring an effective wastewater surveillance system together, how choosing stakeholders can impact long term laboratory processes and how data sharing issues between stakeholders can seriously limit novel insights and analyses.

5. The ideal: towards a wastewater surveillance fit for the 21st century

There is no doubt that adequate funding and experience of wastewater surveillance provides a solid advantage in implementing an efficient programme for monitoring emerging diseases. Previous knowledge, infrastructure and collaborations between stakeholders in São Paulo state have clearly provided their programme a head-start, and the well-funded Welsh programme is testament to the insights that can be gained if funding is made available. As the pandemic progresses to an endemic state, and as potentially new pandemics may emerge, the swift response of the São Paulo state programme to the COVID-19 pandemic is evidence of the value of experience. Given that many countries, such as Wales, now have experience in wastewater surveillance due to the COVID-19 pandemic, we outline what an ideal wastewater surveillance system may look like and how to progress from minimal viable products (Fig. 4a) and standard wastewater surveillance (Fig. 4b) to an ideal system (Fig. 4c). To do so, we draw on the experiences of both the programmes in Wales and in São Paulo, relevant for any disease with biomarkers in wastewater, not just SARS-CoV-2.



	(a) Minimum viable product	(b) Standard	(c) Ideal
Reliability	• One qPCR machine. • Not central database with data stored on separate machines with no backup.	• Multiple instruments for qPCR, chemistry and sequencing. • Three qPCR replicates. • Central database.	• Multiple laboratories able to analyse qPCR, chemistry and sequencing. • Archived samples. • Ten qPCR replicates. • Bayesian hierarchical models allow for uncertainty to be better modelled for each sample site. • Central database with archive.
Resolution	• Two WWTW sampled which cover large populations. • Sampling occurs once a week. • qPCR only. • No wastewater flow data or alternative measured. • No populations estimates.	• Every WWTW sampled which services more than 100,000 people. • Sampling occurs 3 days a week. • Variants assessed using qPCR. • Wastewater flow measured daily at WWTW.	• Every WWTW sampled in addition to ports, off grid communities, close to source and, where appropriate, natural systems. • Sampling occurs 7 days a week. • Variants assessed using sequencing. • Wastewater flow measured every 15 minutes. • Chemistry data measured every 15 minutes. • Hourly population movement data from mobile phone data.
Accuracy	• Grab samples.	• Composite samples. • Wastewater flow data measured at WWTW but not accounting for the loss of flow in the wastewater system. • Fixed populations estimated based on census data.	• Composite samples. • Wastewater flow data measured at each household from the signatures of water usage measured by household smart meters. • Interlaboratory trials and accreditation. • Population estimates measured from mobile phone data.
Timeliness	• Samples taken from a few sites and collected by car. • Low throughput sampling makes running samples on the qPCR pipeline immediately uneconomical, and so a lag is caused by sample accumulation. • qPCR pipeline requiring overnight steps. • Data requiring manual download, curation, analysis and report generation. • Sample to reporting in 7 days.	• Samples from multiple dispersed sites collected by car, samples start on qPCR pipeline at the end of the collection day. • qPCR pipeline requiring overnight steps. • Data download, curation, analysis and report generation semi-automated. • Sample to reporting in 2-3 days.	• Transportation of samples to a laboratory hub by drone. • qPCR pipeline streamlined and run as soon as sample is received. • Chemical data probes constantly monitoring and relaying data back in the cloud from the WWTWs in real-time. Wastewater flow also measured in real-time from household smart meters. • Data immediately uploaded onto a cloud-based server, where it is automatically processed and displayed on a dashboard. • Sample to reporting in 1 day.

Figure 4 Progress of wastewater surveillance systems, from a (a) minimum viable product, to the (b) standard and then finally, to the (c) ideal system, with developments in design, laboratory methods, data analysis and reporting. In addition to this, there are bullet points containing features of (a) to (c), split into fundamental elements of an effective wastewater monitoring programme, including reliability, resolution, accuracy and timeliness which filter through to all elements of the programme, including design, laboratory methods, data analysis and reporting.

5.1. The ideal: design

One of the most fundamental questions in designing a wastewater surveillance programme is what scale the sampling should be conducted on, both in space and time. Taking multiple samples per week has been highlighted as a key frequency of temporal sampling (Harris-Lovett et al., 2021) and the Centers for Disease Control and Prevention (CDC) recommends a minimum of three samples within a trend period of 15 days (CDC, 2022). The greater the number of samples over a week, the more robust the trends, and therefore, a frequency of 3-7 sample points a week should be aimed for, all of which would need to be taken with composite refrigerated samplers over a 24-hour period (Fig. 4b-c). Preferably the composite sampler should take samples every 10 mins to capture the variability in SARS-CoV-2 concentration. The latter is particularly important when used as an early warning system for pathogen emergence where the levels of SARS-CoV-2 RNA are expected to be highly temporally variable. This sampling frequency is not feasible in the real-world for most countries as it incurs high cost and complex logistics, and often fewer samples can be sufficient in answering the relevant public health questions. Preventing degradation at this stage is also vital, ensuring that samples are kept at 4°C and transported to the laboratory within 24 hours of it being taken. It is likely in the future that preservatives may be added to the samples to prevent the loss of genetic material (S. H. Bell et al., 2023).

For spatial sampling, this will depend on the make-up of the sewersheds and the wastewater infrastructure, but it is beneficial to target large urban populations to that you are able to quickly cover a large percentage of a region's population. However, to get a representative view of a region, rural or off-grid populations must not be neglected. Many of the more nuanced decisions based on geographic sampling may also be politically motivated. Therefore, before designing a surveillance programme, an initial demographic mapping exercise should take place, whereby the surveillance planners are given information on aspects of populations served by sewersheds such as age, population density, deprivation levels, healthcare provision, migration rates (e.g. commuting, national/international tourism). An ideal system would also include the sampling of communities not connected to sewersheds through the sampling of open sewers, the natural environment and septic tanks. This type of sampling would take greater resource and logistics, but it may be important to monitor communities most at risk.

Environmental surveillance should also extend to regions where sewage is discharged without sufficient treatment, to monitor waterborne diseases and other hazardous sewage contaminants that pose a risk to other water users (e.g. recreation and irrigation). There may also be strategic sampling for, example, an early warning system, which would prioritise entry points into a region, such as international borders, airports and seaports, with sampling of the onboard wastewater storage facilities of planes and ferries (Farkas Id et al., 2023).

It is important for wastewater monitoring to be coordinated at national level, implemented at state and municipal level, and have the support of all stakeholders (sanitation companies, laboratories, government) to generate reliable data for decision makers, all the while working with standardised protocols for sample collection, sample processing, data processing and reporting. These initiatives have already been established in other countries such as South Africa, Turkey and the US (Sutton et al., 2022; Tlhagale et al., 2022), as well as within Europe (Izquierdo-Lara et al., 2021). In addition, an International Organization for Standardization standard is currently being developed for SARS-CoV-2 quantification in wastewater. Once established, these wastewater surveillance programmes are not only important for dealing with COVID-19, but can also be applied to other infectious diseases, as well as chemical compounds (e.g. disease biomarkers, pharmaceuticals, microplastics, illicit drugs). Once the sampling regime, infrastructure and stakeholder networks have been put in place, sample processing is the only module which needs to change to tackle a wealth of other problems. For this to be feasible, however, there must be guaranteed funding over the medium and long term to maintain this infrastructure, taking a programme beyond just research. Post pandemic funding is required to stop networks, equipment and expertise from dissipating before the start of the next event which requires wastewater surveillance, be that a critical public health issue (e.g. new pandemics, endemic, antimicrobial resistance (AMR)) or other social issue (e.g. illicit drug taking). A break in funding was seen in the Welsh programme in July 2023, where Welsh Government cuts to healthcare meant that the entire programme was abruptly cancelled. The period of cancellation only lasted a month, after which the need for insights into SARS-CoV-2 levels and variants was so great, there was a reversal to policy made by Welsh Government. Instead, a minimal viable product was put together, cutting sites from 47 to 11 WWTPs and sampling frequency from five to three days a week, along with no hospital sampling, no dashboard and removal of AMR monitoring. However, the disruption resulted in the loss of four months of wastewater surveillance, a loss of trust between stakeholders and a loss of expertise.

Further extension of the current stakeholder networks would also be beneficial, to better integrate wastewater surveillance results into public health decision making. In the São Paulo state programme this would involve cross collaborations between Execution State and Municipal Spheres in collaboration Sanitation Agencies (sampling and laboratory), Universities and Research Institutions, Health Epidemiological and Sanitary Offices, Environmental and Health Laboratories. In Wales, stronger connections could be made with current partners, Public Health Wales and local authorities, to utilise wastewater data on a more localised basis, while also designing surveys to answer public health questions faced by different health boards in Wales. Better connection with international networks would also be beneficial, strengthening those that are already in place, such as with São Paulo state and Wales, but also incorporating other nations, as has been seen with the Pan-American network for Environmental Epidemiology (PANACEA) network, whose work spans 15 countries.

5.2 The ideal: laboratory methods

The trade-off between the choice of different methodologies is largely between cost of the consumables, processing time and the methodology which consistently gives the greatest yield. In an ideal system, the methodology with the highest yield would be preferentially favoured, but for results to be delivered to policy makers in time for them to be useful, processing time is also very important, as is the responsible expenditure of laboratory resources from publicly funded projects. Therefore, an ideal system must be flexible to the constraints in which it is born into and will differ between programmes. In addition to cost, time and yield, accuracy is also important, with good accuracy being dependent on sufficient technical replication. An ideal system would have samples processed in a minimum of triplicate from the concentration phase to qPCR, but preferably more (Fig. 4c). When at the qPCR stage, it would also be beneficial to use a minimum of three probes for the biological marker you are trying to detect. For example, with SARS CoV-2, using a combination of probes that target different regions of the SARS CoV-2 genome. Multiple probes build redundancy and has increased confidence in positive and negative signals in other wastewater surveillance programmes (Huang et al., 2021), in a similar way to increased replication, with the CDC initially recommending the use of three sets of probes for clinical testing (Yaniv et al., 2021), with the N1, N2 and E gene probes showing the greatest sensitivity and correlation with cases (Hong et al., 2021; Huang et al., 2021). This also minimises the potential for false-negative results arising from gene dropouts (Isabel et al., 2022; Wollschläger et al., 2021).

Many of the underlying techniques for RNA extraction and concentration have not changed for decades, however, staying at the cutting edge of technological advances regarding measurement of RNA and other nucleic acids is important. For example, the emergence of digital qPCR can allow for improved limits of detection and quantification, which could be important in some contexts, such as early detection, or in highly dilute samples (Ahmed, Bivins, et al., 2022; Tiwari et al., 2022). The characterisation of variants by high throughput sequencing is another area in which emerging sequencing technologies could improve on elements of accuracy, sample processing time and cost. Implementation of nanopore technologies in replacement of sequencing by synthesis technologies, such as Illumina platforms, could eliminate PCR steps which currently makes up part of the EasySeq™ RC-PCR SARS CoV-2 used in Wales, while also reducing sample processing time and cost; although it must also be highlighted that the sequencing accuracy of these technologies is still behind those of the Illumina platform (Barbé et al., 2022; Rios et al., 2021). This highlights another component, additional to cost, processing time and accuracy, and that is the uncertainty of running new technologies, which can pose a significant risk to the stakeholder trust placed in the data being produced by a surveillance programme, especially when it is being used for policy making decisions.

Going forward, design of wastewater surveillance programmes should also incorporate other biological markers for qPCR outside of those relevant to pandemics, as well as regularly sequencing samples (Fig. 4c). Biological markers of multiple pathogens are being regularly monitored in São Paulo state and Wales, such as poliovirus and *Vibrio cholerae* in São Paulo state and as of September 2022, influenza, enterovirus, respiratory syncytial virus, poliovirus, and norovirus in Wales. However, a truly cutting-edge wastewater surveillance programme would go beyond monitoring bacterial and viral pathogens and would use the established infrastructure to investigate other pressing societal issues which lack data. In the case of biological markers, this could include monitoring cancers such as prostate cancer, hormones or, like in the case of the programme in Wales, monitoring of AMR. One tool which is being harnessed by the Welsh programme is metagenomics, which can produce large databases that are not marker specific (Adriaenssens et al., 2021). In Wales, the datasets produced for monitoring of AMR can also be mined for a plethora of prokaryotic and eukaryotic pathogens. Additionally, surveillance programmes do not need to focus solely on biological material and having multiple laboratory process which can process an array of chemical markers (e.g. illicit drugs, pharmaceuticals, emerging contaminants or microplastics) would be value added for relatively little cost, benefiting from the sampling design and analysis already in place. An example of this value added can be seen in the Welsh programme, where after cutting the programme's budget and thus the number of sites (from 47 to 11), the cost per WWTP sampled increased from £7,571 per site per month to £8,065 per site per month, while also losing two days of sampling, a week AMR monitoring, monitoring at hospital sites and a data dashboard.

Irrespective of the type of methodology, making them replicable is key to an ideal system. If the methodologies are run in separate laboratories, detailed standard operating procedures are vital, as even small deviations in the methodology can render results incomparable. Variables such as the supplier of laboratory consumables and equipment, and even down to batch number of the consumables used can significantly impact replication, sometimes due to errors by the manufacturer. Ideally, consumables from the same batch would be used between laboratories to alleviate this problem. Another important step for replicability is the use of ring trials, so that samples and standards are consistently being compared between laboratories to ensure that the methodologies are still aligned. One way in which replication can be assured is to run all samples through the same laboratory, however, this then introduces another problem: having a single source of truth which is not challenged. Not only this, but logistical problems with one laboratory could cripple a national programme, and therefore, a hybrid approach would be beneficial.

Finally, an ideal system would maximise the use of automation and robotics. The greatest potential application of robotics in the processing of wastewater samples is in the liquid handling stages. Not only do robotics allow the high throughput processing of samples, and therefore broader coverage in time and space, but they also eliminate variation introduced by different laboratory members and their approach to processing samples (Hayase et al., 2023). Although, it must be stated that making sure that the robotics between laboratories is identical in terms of manufacturer, programming and mechanics is key, or otherwise these components have the potential of introducing the same, if not more, variation between samples. In addition, the future may also see automated in-sewer detection systems (Ou et al., 2023). One issue of potential major concern for future pandemics is the nature of the organism being tracked. In the case of SARS-CoV-2, no conclusive evidence was presented to suggest that it was infectious when present in wastewater (Jones et al., 2020). This allowed samples to be collected and processed under Biological Safety Containment Level 2 (BSL2) conditions. However, subsequent pandemics might be associated with organisms that remain highly infectious in faeces and urine and thus require sampling handling under BSL3 containment. Based on the COVID-19 pandemic, in this situation it is likely that the BSL3 facilities will be prioritised for clinical surveillance rather than wastewater surveillance, due to the low numbers of BSL3 laboratories outside healthcare settings. A plan of action is therefore needed for this scenario, akin to those that exist for other BSL3 organisms (e.g. Ebola (Jelden et al., 2016)).

5.3 The ideal: data analysis

Much of the data analysis required in wastewater surveillance is accounting for variation introduced by the source (e.g. shedding rates of SARS CoV-2 between variants and age of hosts), sewage network (e.g. dilution, degradation, transport times) and laboratory processes (e.g. qPCR efficiencies, limits of detection). In an ideal system, sources of variation should be reduced or explained and incorporated into the analyses. The goal of design and methods of an ideal surveillance system is therefore accuracy (Fig. 4), which will reduce variation in the measurements being taken, as well as accounting for as much of this variation as possible. For example, population and dilution normalisation of a signal in wastewater would take place in an ideal system but would be further improved by data streams that were more accurate and representative. In the case of population normalisation, instead of static figures like those from a census, dynamic population estimates from anonymized call detail records (mobile phones) would be used (Lai et al., 2019), which could account for daily fluctuations in populations caused by phenomenon such as commuting and tourism. Dilution normalisation would also be improved with the use of chemical markers in addition to the wastewater flow measurements taken at wastewater treatment works (Wilde et al., 2022), as is currently implemented in the Welsh programme, which would reply on the measurement of a suite of chemical markers (e.g. ammoniacal-N, phosphate, electrical conductivity, caffeine).

Another way of better representing variation in the wastewater signal is by providing error margins. Bayesian hierarchical modelling can be a particularly powerful tool in doing so (Medema et al., 2020), as not only could you incorporate existing knowns (priors) such as limits of qPCR quantification, qPCR efficiency and COVID-19 cases, the hierarchical element would allow you to produce sperate estimates of wastewater signal, as well as a margin of error, per sample site.

Invariably, even in an ideal system, due to the nature of wastewater being a complex matrix, and the molecular pipelines for the quantification of viral particles being so complex, there will be outliers which skew the overall trend. Therefore, another element of an ideal data workflow would include an automated outlier detection system for recognising extreme values in a timeseries. Proposed outlier removal methods included Generalized Extreme Studentized Deviate (GESD) test, which have been used in other viral epidemiology contexts (Wiemken et al., 2020). The difficulty with applying such methods is that outbreaks of a disease such as COVID-19, and thus the viral particles in the wastewater, can escalate rapidly, meaning that it can be difficult to sperate outliers from trends if you are sampling on a weekly basis. This reaffirms the need for fine scale temporal sampling, which allows for better discrimination between a real rapid increase of viral particles in the wastewater and outliers introduced through the sampling and laboratory pipelines.

A crucial element to data analysis of wastewater surveillance data is being able to communicate trends in the data to policy makers and public health bodies. Not only does this mean an ideal system must have excellent data visualisation, it also impacts the way in which the trends are delivered to the end user. Many regions around the world are moving away from reporting wastewater surveillance results in a static report, and instead moving to dashboards, whereby live results can be presented in an interactive format that is suited to different audiences, which is part of a wider popularity of displaying data in dashboards. Not only does this allow for a more engaging way to showcase and distribute data insights, it also reduces the timeline between data generation and availability to end users, allowing for quicker dissemination and data-driven action.

Finally, and most fundamentally, for data analysis to be undertaken, no matter how simple, the data must be readily available, which makes effective data sharing and databasing another crucial element of an ideal system. The more complex the analysis, the more data streams are required. For example, multiple molecular laboratories may be providing the SARS-CoV-2, each of which should be able to freely upload results to a central database with ease without the hinderance of firewalls. In the ideal system, those measurements would be easily combined with data sources from the chemistry laboratory providing physiochemical measurements on the sample, the water utility providing wastewater flow at their treatment works, the public health body providing case data, or the weather service providing rainfall data for monitored areas. Collating data from such a broad range of institutions requires extensive data sharing agreements, which in some cases must be purchased, but in many other cases rely on effective partnerships born out of years of collaboration, or the power of government support. An example from the programme in Wales of the prior would be the 2022 strategic partnership between Cardiff University and Dŵr Cymru Welsh Water for future collaboration and research which has facilitated the exchange of expertise and data sharing.

6. Conclusion

Despite the paucity of readily available historical information on wastewater systems in Wales compared to São Paulo, wastewater infrastructure, governance and history were seen to be key drivers in shaping wastewater surveillance programmes. Wastewater is predominantly collected through networked systems in both countries, bringing waste to centralised WWTPs, but significant populations also remain off-grid. While the off-grid populations in São Paulo state are primarily in urban irregular settlements, the Welsh off-grid populations are in mostly in upland rural settings, and in both cases, this usually concerns the most deprived demographics. Ease of access to wastewater samples that capture significant, and representative, portions of the population is clearly a determinant of the cost-benefit of a wastewater surveillance programme. Wales has an advantage when compared to São Paulo state due to the high WWTP density over a small geographic area and population, allowing samples to be taken easily from smaller populations. Besides access challenges, another novel finding is that stakeholder engagement constrains programme design, laboratory methods and data analysis. Despite challenges, achieving a near ideal programme, that delivers reliable, high-resolution, and accurate data in near-real-time, is in the domain of the possible. Costs involved in implementing such a programme will depend on existing infrastructure as well as the level of data and expertise sharing between stakeholders. As the world shifts from pandemic to endemic SARS-CoV-2 levels, surveillance programme funding has come under intense scrutiny. Our work indicates that there are clear benefits in maintaining wastewater surveillance programmes and their network of stakeholders over the long-term for pandemic preparedness, but also as they can broaden their applications to other health indicators.

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