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Wastewater-based epidemiology for preventing outbreaks and epidemics in Latin America – Lessons from the past and a look to the future

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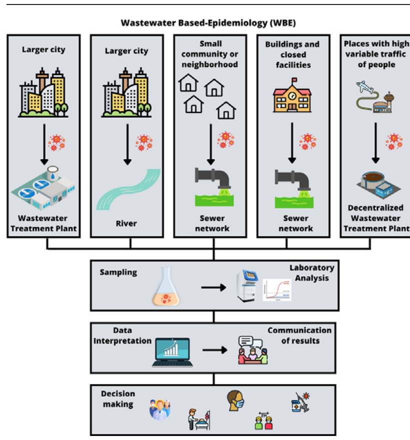
HIGHLIGHTS

- Main concepts of WBE in public health are addressed.
- Critical review of WBE in Latin America described.
- Approach to use WBE in socially vulnerable areas proposed.
- WBE framework for Latin America suggested.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater-based epidemiology (WBE) is an approach with the potential to complement clinical surveillance systems. Using WBE, it is possible to carry out an early warning of a possible outbreak, monitor spatial and temporal trends of infectious diseases, produce real-time results and generate representative epidemiological information in a territory, especially in areas of social vulnerability. Despite the historical uses of this approach, particularly in the Global Polio Eradication Initiative, and for other pathogens, it was during the COVID-19 pandemic that occurred an exponential increase in environmental surveillance programs for SARS-CoV-2 in wastewater, with many experiences and developments in the field of public health using data for decision making and prioritizing actions to control the pandemic. In Latin America, WBE was applied in heterogeneous contexts and with emphasis on populations that present many socio-environmental inequalities, a condition shared by all Latin American countries. This manuscript addresses the concepts and applications of WBE in public health actions, as well as different experiences in Latin American countries,

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and discusses a model to implement this surveillance system at the local or national level. We emphasize the need to implement this sentinel surveillance system in countries that want to detect the early entry and spread of new pathogens and monitor outbreaks or epidemics of infectious agents in their territories as a complement of public health surveillance systems.

Contents

1. Introduction

The ability to rapidly monitor the emergence and spread of infectious agents is essential for disease prevention, intervention, and control. However, there are limitations of the current epidemiological surveillance systems to deal with rapid population growth, the appearance of new pathogens, and the resurgence of previously controlled infections. In this context, wastewater-based epidemiology (WBE) emerged as an important tool in support of epidemiology. The approach is based on the assumption that the concentration of a substance excreted in feces or urine by the population over a defined period, can be estimated from the concentration in wastewater that reaches a wastewater treatment plant (WWTP) or the sewer system, in the corresponding interval (Daughton, 2020). This same concept can be applied to human virus surveillance (Polo et al., 2020; Mousazadeh et al., 2021).

Wastewater-based epidemiology for evidence of pathogens has a long history of use in public health, but the best-known example was the use of this approach in the Global Polio Eradication Initiative (Cassemiro et al., 2016; Deshpande et al., 2003; Manor et al., 1999; Brouwer et al., 2018; WHO - Word Health Organization, 2015; https://polioeradication.org/who-we-are/strategic-plan-2013-2018/surveillance/). These studies/reports highlighted that sustained genomic surveillance in wastewater could be used as an early warning to assess the introduction of a new infectious agent into the community or the re-emergence of known pathogens.

In 2013 and 2014, a polio outbreak in Israel was avoided due to the country's robust environmental surveillance program, which identified the presence of wild poliovirus type I (WPV1) in the sewage system, allowing the rapid mobilization of a population vaccination campaign (Brouwer et al., 2018). Recently, vaccine-derived poliovirus (VDPV) has been detected in several wastewater samples in North and East London, and the authorities have warned that the virus may be circulating in the community, creating an alert. The authorities have urged people to make sure that their vaccines against poliomyelitis are up to date (Grassly, 2022; Wise, 2022).

While the region of the Americas has maintained its polio-free status for almost 30 years, a polio case was recently confirmed on 13 September 2022 in the United States of America (PAHO - Pan American Health Organization/World Health Organization, 2022a; PAHO - Pan American Health Organization/World Health Organization, 2022b). The expansion of the environmental surveillance in USA, as part of the public health response to the confirmation of a polio case (MMWR, 2022. https://www.cdc.gov/mmwr/volumes/71/wr/pdfs/mm7133e2-H.pdf) highlighted the use of this surveillance as a tool to monitor the circulation of poliovirus. The case of paralytic polio in an unvaccinated adult in Rockland County and in several wastewater samples from communities near the patient's residence, met the World Health Organization (WHO)'s criteria for circulating vaccine-derived poliovirus (cVDPV). Given this situation, the Pan American Health Organization / World Health Organization (PAHO/WHO) reiterated to Member States the need to join efforts to maintain and strengthen epidemiological surveillance of acute flaccid paralysis (AFP) for the rapid detection of cases, to achieve polio vaccination coverage of >95 %, and to have an up-to-date response plan for polio outbreaks or events (PAHO - Pan American Health Organization/World Health Organization, 2022a; PAHO - Pan American Health Organization/World Health Organization, 2022b).

Therefore, poliomyelitis seemed to be a disease of the past, but these recent findings are a warning to the public and governments that it is necessary to improve the surveillance systems, capable of anticipating health actions before an outbreak occur or epidemic settles in a territory.

At the beginning of the coronavirus disease 2019 (COVID-19) pandemic, scientific studies showed that the genetic material of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), an enveloped virus, could be frequently detected in the feces or urine of infected people (Chen et al., 2020; Foladori et al., 2020; Wang et al., 2020). This provided a strong indication that genetic material could potentially be detected in the wastewater. Some countries moved quickly to monitor the circulation of the virus in the population using the viral RNA (ribonucleic acid) detection approach in wastewater (Ahmed et al., 2020; Medema et al., 2020; PAHO - Pan American Health Organization, 2020; Randazzo et al., 2020; Thompson et al., 2020; Wurtzer et al., 2020; Shah et al., 2022) and also, although to a lesser extent, in surface waters impacted by wastewater (Haramoto et al., 2020; Kumar et al., 2020; La Rosa et al., 2020; Rimoldi et al., 2020; Mahlknecht et al., 2021; Maidana-Kulesza et al., 2022). The data generated by this surveillance tool served as an additional source of information that could be used to support public health decision-making (WHO, 2020a). Evidence of SARS-CoV-2 circulation in a community through wastewater has been important in reinforcing public health measures to reduce transmission (for example, health system preparedness, better sanitation and hygiene, use of masks, physical distancing and vaccination campaigns). Fig. 1 presents a surveillance model based on wastewater and public health actions that can be developed from the data obtained.

Some important considerations for the use of WBE in support of health actions include: (i) the use of environmental surveillance to estimate the increase or decrease in the transmission (circulation) of a pathogen in a community and to detect outbreaks early; (ii) monitor changes over time in response to public health interventions; (iii) detect the reintroduction of pathogens or the emergence of new agents; (iv) help identify high-risk groups; (v) to monitor antimicrobial resistance (AMR) in communities.

2. Applications of WBE in the health actions

2.1. Early warning

The term "early warning" can be interpreted in two ways in the context of surveillance for COVID-19 or other pathogens: (i) signaling an early stage of an outbreak or the introduction of new or re-emerging pathogens into a territory or (ii) heralding an imminent surge in infected people. The basic assumption is that since viral replication precedes the onset of symptoms, people infected with SARS-CoV-2 may excrete the virus in their feces before clinical symptoms appear and cases are reported (Zhu et al., 2021). Therefore, if proper sampling strategies and quantification methods are adopted, an increase in viral concentration in wastewater can be observed and reported before newly infected individuals develop symptoms and seek medical attention (Sodré et al., 2020; Zhu et al., 2021). This type of surveillance can be an important decision-making tool for health systems to control outbreaks and epidemics in a community (Fig. 2).

A review that included studies conducted by several countries that used wastewater surveillance as an early warning to assess the circulation of SARS-CoV-2 in communities showed that viruses could be detected in wastewater samples up to 63 days before the start of clinical cases in the community (Shah et al., 2022). Several other studies have also shown this application (Randazzo et al., 2020; Hata et al., 2021; Zhu et al., 2021). In addition, efforts have been made to analyze historical samples of wastewater for evidence of SARS-CoV-2 circulation on dates before the declaration of the pandemic (Fongaro et al., 2021a; Zhu et al., 2021). These studies demonstrated the presence of viral RNA fragments obtained through RT-qPCR, without, however, sequencing the complete virus genome for confirmation (Fongaro et al., 2021a; Zhu et al., 2021). However, these studies if combined with genomic surveillance suggest that processed and stored sewage samples can be used in future research to track the past occurrence of an emerging virus in the community and/or the circulation of a virus around a community.

2.2. Spatial and temporal trends

Another application widely used by WBE programs is the ability to monitor spatial and temporal trends of an infectious disease during the course of

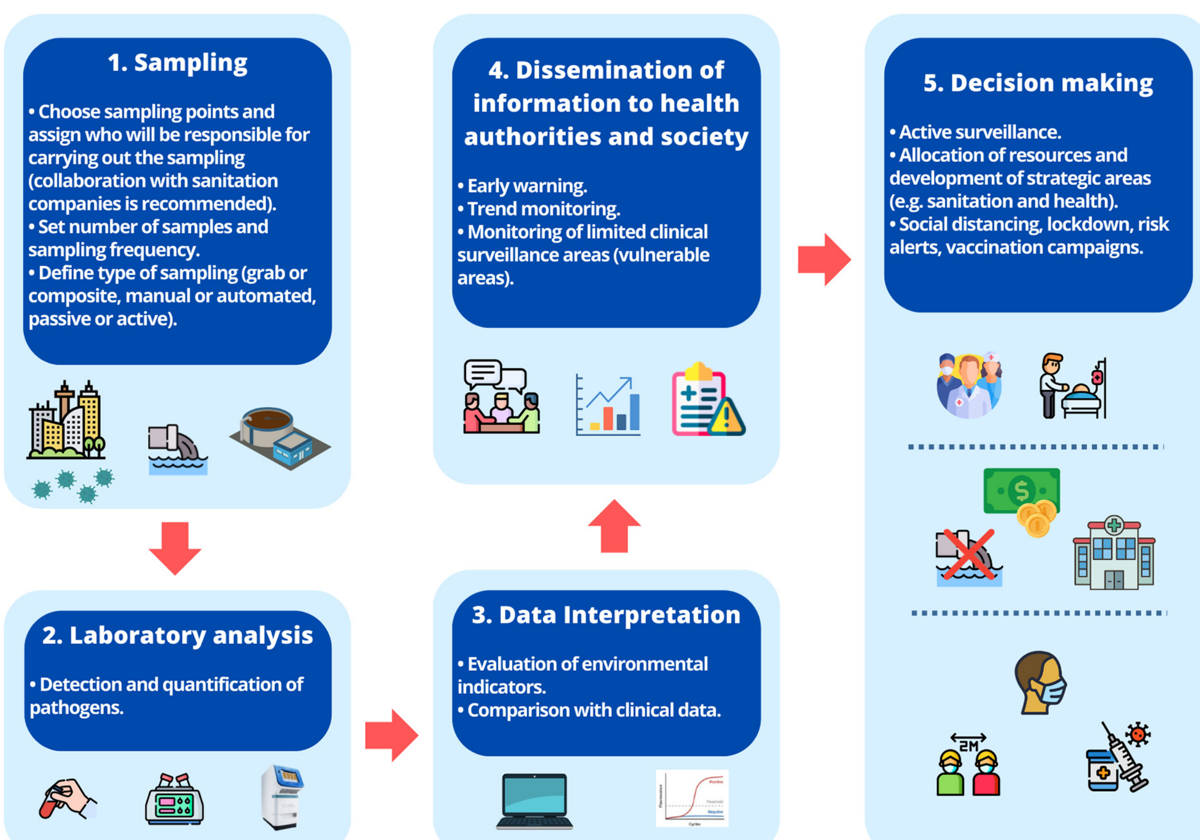


Fig. 1. Wastewater based-epidemiology (WBE) and public health actions for disease prevention or control.

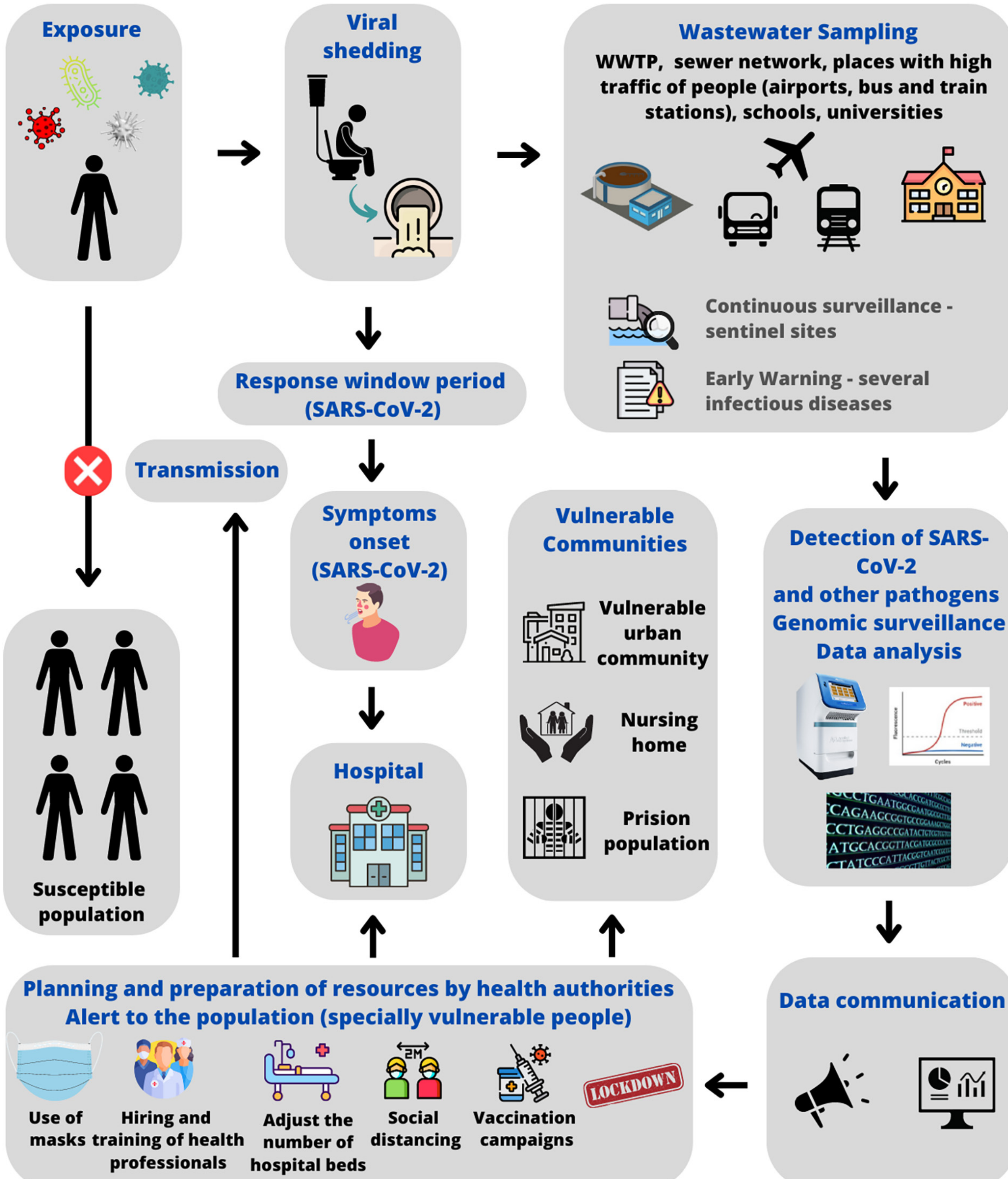


Fig. 2. Early warning of COVID-19 and other diseases through wastewater-based epidemiology and health system preparedness.

an epidemic in a territory (Sims and Kasprzyk-Hordern, 2020; Thompson et al., 2020; Wurtzer et al., 2020; McClary-Gutierrez et al., 2021) (Fig. 3).

Several countries (mainly in Europe, the United States, Australia, Japan, India, China, countries in Latin America and also in Africa) are using this approach to evaluate temporal and spatial trends in a territory with significant correlations between the variations obtained between viral concentrations in wastewater and the trend of variations in the number of cases in the communities (Ali et al., 2021; Agrawal et al., 2021; Ahmed et al., 2020; Barrios et al., 2021; Chakraborty et al., 2021; Claro et al., 2021; Fuschi et al., 2021; Giraud-Billoud et al., 2021; Hata et al., 2021; Hillary et al., 2021; Li et al., 2021; Medema et al., 2020; Pillay et al., 2021; Prado et al., 2021; Razzolini et al., 2021; Thompson et al., 2020; Wu et al., 2021; Wurtzer et al., 2020).

A recent review of the experiences acquired by Latin American countries (including Argentina, Brazil, Chile, Costa Rica, Ecuador, Mexico, Peru, Paraguay and Uruguay) showed that the vast majority used the wastewater-based surveillance approach to monitor the course of epidemic evolution along time (Barrios et al., 2021; Claro et al., 2021; Gallardo-Escárate et al., 2021; Giraud-Billoud et al., 2021; Mota et al., 2021; Prado et al., 2021). Most of the experiences began when there were already reported cases in the respective monitoring regions. However, monitoring has also been developed in rural areas, and closed places for the early

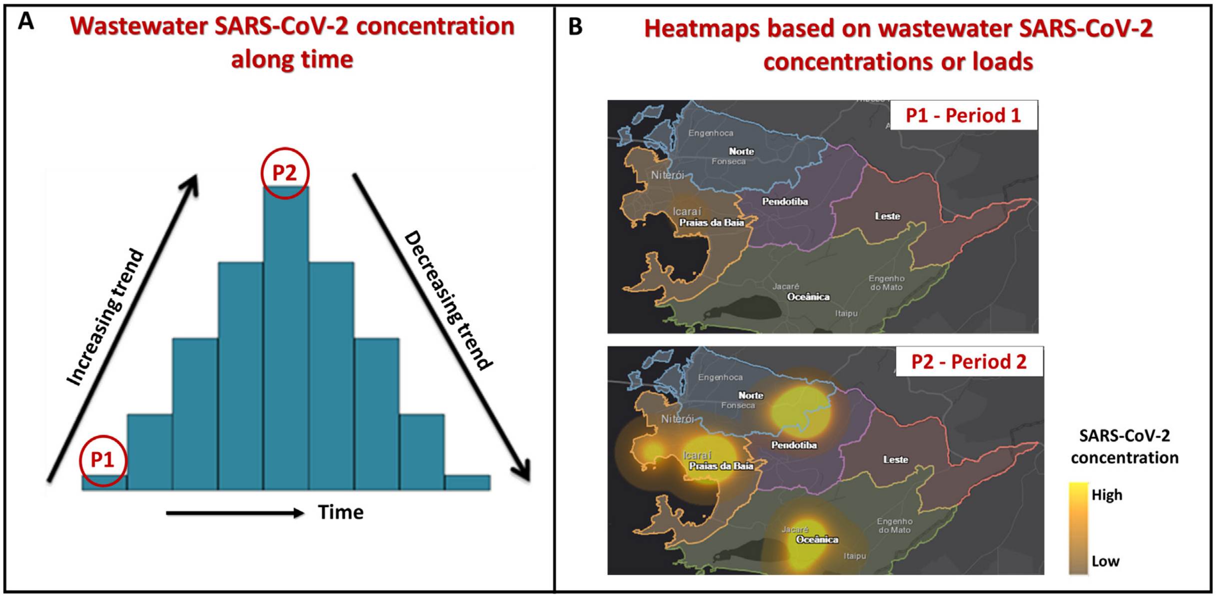


Fig. 3. Examples of temporal and spatial trends based on data generated from wastewater analysis. (A) Evolution of SARS-CoV-2 concentration in wastewater; and (B) Heatmap based on SARS-CoV-2 concentrations in wastewater.

evaluation of the virus circulation in the population (Fongaro et al., 2021b; Carrillo-Reyes et al., 2021). Evaluation of surface waters or groundwater was also observed in many countries (Maidana-Kulesza et al., 2022; Coronado et al., 2021; Cruz-Cruz et al., 2021; Iglesias et al., 2021; Guerrero-Latorre et al., 2020; Mahlknecht et al., 2021; Razzolini et al., 2021; Rosiles-González et al., 2021, Barbosa et al., 2022). In practically all the cases evaluated, significant correlations were observed between the concentration of SARS-CoV-2 in the wastewater and the reported clinical cases, reinforcing the usefulness of this approach to monitoring the epidemiological status in regions.

2.3. Emergence/re-emergence of pathogens – post-elimination or post-vaccination era

When the pandemic moves into a new phase – the post-elimination era or post-vaccination period, occur a decrease in the rates of infections and severe cases and deaths, with decrease in the clinical testing. In this case, surveillance of wastewater can be useful for detecting the introduction or re-establishment of a specific pathogen, identifying risk areas.

In Latin America, a successful case to assess the reintroduction of a wild type 1 Poliovirus (WPV1) was reported in 2014 by Companhia Ambiental do Estado de São Paulo, Brazil, which carried out continuous surveillance in wastewater from airports and other sentinel sites (https://cetesb.sp.gov.br/blog/2014/07/01/identificado-poliovirus-selvagem-no-esgoto-do-aeroporto-de-viracopos/). After complete sequencing of the VP1 gene, it was found that the virus belongs to a West African WPV1 genotype, characteristic of Nigeria (WEAF-B). These data were sent to the WHO global reference laboratory for poliomyelitis, which characterized that the poliovirus circulated in some regions of Central Africa, originating in Equatorial Guinea. On May 5, 2014, the WHO issued the Declaration of Public Health Emergency of International Concern (ESPII) due to situation of polio in the world, listing 10 countries with the potential to export the virus: Afghanistan, Cameroon, Ethiopia, Equatorial Guinea, Iraq, Israel (wild poliovirus environmental circulation only), Nigeria, Pakistan, Syria and Somalia (https://cetesb.sp.gov.br/blog/2014/07/01/identificado-poliovirus-selvagem-no-esgoto -from-viracopos-airport/).

In American countries, preliminary data indicate that regional immunization coverage of the third dose of polio vaccine (Pol3) was 79 % in 2021, the lowest coverage since 1994 (PAHO - Pan American Health Organization/World Health Organization, 2022b). Currently, due to the low vaccination rate in the region, four countries (Brazil, Dominican Republic, Haiti, and Peru) were classified as very high risk for the event of a poliovirus importation or the VDPV emergence, and eight countries (Argentina, Bahamas, Bolivia, Ecuador, Guatemala, Panama, Suriname, and Venezuela) as high risk (PAHO - Pan American Health Organization/World Health Organization, 2022b).

In relation to COVID-19, the absolute shortage of vaccines that marked the initial phase of the pandemic gave way, then, to an extremely unequal vaccination process in the region (Drexler and Hoffman, 2021). Currently, in South America, the number of people who received at least two doses or the recommended single-dose schedule of vaccine was >70 %, while for the region of Caribbean this coverage reaches about 50 % of the population, according to the last PAHO report (https://ais.paho.org/imm/IM_DosisAdmin-Vacunacion.asp - last accessed 11/28/2022). In many places, vaccine availability is still a bottleneck. Another issue is hesitancy to get vaccinated. In Brazil, for example, public health institutions have a long-standing reputation for social progress, with vaccinations having gained wide acceptance over the decades (Drexler and Hoffman, 2021). Fortunately, the attitude of getting vaccinated prevailed over the disinformation of social networks and the denialist attitude of the federal government during the pandemic. However, in other countries, including many Caribbean countries, health authorities are struggling with skepticism about the vaccines (Drexler and Hoffman, 2021).

Due to reductions in vaccination coverage rates, particularly observed for the polio vaccine, as well as the need to continue the surveillance of recent emergent pathogens like SARS-CoV-2, it would be important to improve WBE in Latin American countries, especially in the current global, regional and national epidemiological scenario. This complementary surveillance system could help to identify areas of greatest risk or circulation of pathogens, allowing to health authorities allocate resources and prepare the health system to promote actions to prevent or control outbreaks or epidemics. This approach can also be useful for evaluating the impact of vaccination, helping health authorities target resources and vaccine doses to specific or higher-risk areas and reduce the number of hospitalizations or deaths in a population.

2.4. Monitoring in areas with a silent or incomplete epidemiological profile (vulnerable populations)

Environmental surveillance also has the potential to be used to complement clinical surveillance or to trigger more comprehensive surveillance in areas with a silent or incomplete epidemiological profile. Areas with this profile can be identified, for example, in places with excessive population density, very few resources, such as informal settlements or, more generally, in marginalized populations or living in conditions of socio-environmental vulnerability. In these contexts, access to health facilities may be limited, care-seeking behavior may be low, testing capacity may be low, and clinical surveillance capabilities may be overwhelmed (PAHO – Pan American Health Organization, 2020).

It is important to observe whether these vulnerable populations have adequate sanitation services, such as sewage collection networks, to implement this type of surveillance. Many cities in Latin and Central America countries do not have adequate coverage of sanitation services (Madrigal et al., 2020; Filgueira et al., 2020; Fuchs et al., 2022). In these settings, researchers obtained data on rivers or streams contaminated with sewage in areas of vulnerable communities or sites with limited or absent clinical surveillance data, where monitoring of these sources was important to obtain information on the virus circulation in the population (Maidana-Kulesza et al., 2022; Barbosa et al., 2022; Fongaro et al., 2021b; Guerrero-Latorre et al., 2020; Iglesias et al., 2021; Razzolini et al., 2021; Prado et al., 2021).

The sewage monitoring in collectors of well-defined coverage areas (decentralized sewer pipe) was also important to trigger faster and focused health actions in vulnerable areas (Prado et al., 2021; Mota et al., 2021). In the cities of Salta and Salvador Mazza (Salta Province, Argentina), wastewater was also analyzed in a decentralized manner from samples obtained from main collectors in well-defined coverage areas (unpublished data; Rajal, V.B., personal communication) from July 2020 to the end 2021. Through the results it was possible to observe that the prevalence and spatial distribution of COVID-19 based on sewerage data indicated that the regions with the highest rates of social vulnerability in the city were the most affected for the pandemic.

2.5. Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is a complex issue and increasing threat to global health (Aarestrup and Woolhouse, 2020; Sims and Kasprzyk-Hordern, 2020). After the COVID-19 pandemic, the problem associated to AMR became more evident because the population consumed more drugs, including antimicrobials, resulting in the selection and spread of bacteria and other microorganisms resistant to antibiotics in the environment (Harrington et al., 2022). In this way, WBE has emerged as a promising tool for AMR surveillance in the population, especially in communities with low clinical testing capacity (Aarestrup and Woolhouse, 2020). This type of surveillance that could be coupled with next generation sequencing technologies can accurately describe and characterize the global occurrence and distribution of AMR over time, setting national and global priorities, assessing the impacts of interventions, identifying new genes of resistance, and supporting investigation of outbreaks of resistant pathogens (Aarestrup and Woolhouse, 2020; Sims and Kasprzyk-Hordern, 2020; Harrington et al., 2022).

3. WBE program for SARS-COV-2 and other pathogens of interest for Latin America - barriers and challenges

With the emergence of new pathogens, especially after the COVID-19 pandemic, the WBE has emerged as an important complementary epidemiological surveillance system, currently being adopted in >70 countries and 3807 locations around the world. (COVIDPoops19 Summary of Global SARS-CoV-2 Wastewater Monitoring Efforts by UC Merced Researchers - https://ucmerced.maps.arcgis.com/apps/dashboards/c778145ea5bb4daeb58d31afee389082 - updated 11/23/2022).

Examples of some countries that have developed national surveillance programs for SARS-CoV-2 in wastewater are shown in Table 1.

During the last decades, the pathogens surveillance in wastewaters in Latin America was carried out sporadically as scientific research in Universities, environmental agencies and Research Institutes and has not yet been incorporated into routine monitoring systems, with the exception of some countries that have recently structured a surveillance system at national level (Table 1) due to the current relevance of the topic for public health.

The health sector as the end-user of wastewater signals supporting the public health response should have a leading and coordinating role in the design of the surveillance programme, convening relevant environment departments, wastewater system operators, testing laboratories, and other relevant partners and stakeholders (WHO - World Health Organization, 2022b). Every surveillance system must be backed by a legal framework of the State that guarantees the efficient functioning of the system (PAHO, 2010).

In Brazil, the Ministry of Health, in partnership with National Agency for Water and Sanitation (ANA) and National Institute of Science and Tecnology on Sustainable Sewage Treatment Plants (INCT ETEs Sustentáveis) is currently leading efforts to build the Brazilian National WBE Programme, which is intended to include infectious diseases beyond COVID-19 and antimicrobial resistance.

For some Latin American countries, research and sanitation sectors have been taking a leading role in organizing projects or surveillance systems for pathogens in sewage, with the participation or not of the health sector. However, the environmental surveillance of pathogens of interest in public health is practically new in most countries in the region. During decades, the allocation of financial resources to implement wastewater surveillance has not been guaranteed, mainly to integrated national or multinational programs in region involving health, environmental and sanitation sectors (Korc and Hauchman, 2021). Health prevention policies and environmental surveillance have never been a priority in Latin American countries, so this is an excellent opportunity to leverage this sector.

The construction and implementation of a WBE Program in Latin American countries may face other series of challenges that need to be addressed.

First, it is necessary to determine which pathogens will be part of the scope of monitoring Program. Neglected and notifiable diseases in Latin American countries can be prioritized and considered for environmental monitoring, beyond emerging infectious diseases.

A second challenge needs to be overcome, the methodology that should be used for pathogen detection. The methodologies already described and currently in use in several countries can be complex and laborious and therefore, may require considerable laboratory resources and highly trained professionals in sufficient numbers to carry out the analyses. Depending on the pathogen to be monitored, there are requirements regarding the biosafety laboratory level and there are also concerns about containing infectious agents in the laboratory, in order to minimize the risk of human infection and its dissemination into the environment. This can become a significant barrier to the implementation of country wide environmental surveillance. It is important to mention the importance of funding research aimed to developing methods for the detection of pathogens of interest for environmental surveillance in places with limited resources.

Latin American countries have large deficits in sewage collection and treatment infrastrucuture (Table 2). This is a factor that brings challenges to the implementation of WBE in the region. When a city has its sewage collection and treatment services universalized, good coverage of the population can be achieved by monitoring the influent of Wastewater Treatment Plants (WWTP). However, this is not the reality of several Latin American countries. In addition, even in cities with high sewage services coverage, it is important to expand the sampling points beyond the WWTPs, in order to monitor different areas with distinct levels of social and health vulnerability. In this sense, the monitoring of pathogens can be expanded to sewer network level, or to pumping stations, rainwater channels and water bodies that receive sewage contribution.

The participation of sanitation companies in environmental surveillance programs is also important. Sanitation companies play a fundamental role, both in defining the sampling points and support sewage sampling in addition to providing data. The engagement of this sector is very important for the success of any WBE program, including countries or cities where the sewerage services are privatized.

Table 1
Countries with national surveillance systems for SARS-CoV-2 and other pathogens in wastewater.

Country	Reference
United States	https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater surveillance.html?CDC_AA_refVal = https%3A%2F%2Fwww.cdc.gov%2Fcoronaviru %2F2019-ncov%2Fcases-updates%2Fwastewater-surveillance.html
United Kingdom	https://www.gov.uk/government/publications/monitoring-of-sars-cov-2-rna-in-england-wastewater-monthly-statistics-1-june-2021-to-7-march-2022/emhp-wastewater-monitoring-of-sars-cov-2-in-england-1-june-2021-to-7-march-2022
Finland	Finnish Institute for Health and Welfare, 2021 - https://thl.fi/en/web/thlfi-en/-/coronavirus-found-in-wastewater-in-helsinki-and-turku-but-not-at-other-sites-monitored-weekly
Australia	Water Research Australia, 2021 - https://www.waterra.com.au/projectdetails/264
Netherlands	National Institute for Public Health and the Environment - Ministry of Health, Welfare, 2021 - https://www.rivm.nl/en/news/sewage-research-decline-of-novel-coronavirus-in netherlands
South Africa	https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-reports/wastewater-based-epidemiology-for-sars-cov-2-in-south-africa/
Brazil	The pilot project of the Brazilian Network for Covid Monitoring in Sewage can be accessed at the following links: https://www.gov.br/ana/pt-br/assuntos/acontece-na-ana/monitoramento-covid-esgotos and https://etes--sustentaveis.org/
Argentina	Federal Network for the Detection in the Environment, organized and coordinated by the National Ministry of Science, Technology and Innovation (MINCyT), working together with the National Research Council (CONICET) and the National Agency for the Promotion of Research, Technological Development and Innovation (Agencia I + D + i). https://www.argentina.gob.ar/noticias/una-red-federal-estudia-el-coronavirus-en-aguas-residuales

Table 2
Coverage of sewage services in urban areas of Latin America.

Country	Connection to the sewer network (%)	Sewage Treatment (%)
Bolivia	69,0	44,9
Chile	98,5	80,7
Mexico	91,3	56,2
Peru	88,3	59,7
Brazil	78,2	42,5

Source: Fuchs et al., 2022.

Finally, it is worth mentioning the challenges related to communicating the results generated from WBE programs. Health authorities may not be familiar with the data interpretation. Thus, the data should be translated and transformed into useful information to health authorities. In addition, there must be agility between the stages of data generation and data communication, so the information can reach decision-makers as soon as possible to ensure prevention and control actions for the monitored diseases. In cases that it is important to communicate the information to the general population, it is essential to think how to translate the data in accessible language to population. So, the information can be easily understood, and its dissemination can bring positive impacts to public health.

In the face of these scenarios, we have identified key aspects for a surveillance system using wastewaters and its application in public health actions, considering the particularities of the Latin American context.

3.1. How to implement a WBE program?

3.1.1. Representativeness of the population

For environmental monitoring to be of maximum utility, it would be important for it to be as representative as possible of the target population (Polo et al., 2020; Keshaviah et al., 2021). Choosing an area to monitor, as well as the size of the population and its sociodemographic, epidemiological, and environmental characteristics, is the first step in employing a WBE program (Table 3).

Wastewater monitoring data is intended to complement other health and environmental monitoring indicators. No public health intervention or action should be based solely on wastewater data. A sampling strategy must balance available resources and testing capacity with the needs of public health data and may need to be updated over time, with changes in scientific knowledge and public health needs.

3.1.2. Sampling strategy: where, how and what to collect? Sampling strategy overview

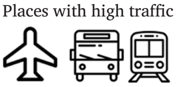
Sampling is one of the most important steps in research related to WBE. This is due to variations in the contribution of biomarkers of interest to the sewage system, as well as variations in the flow of wastewater leading to the WWTP or any other sampling point of interest (Keshaviah et al., 2021).

Samples should be collected at sites preceding the addition of chemicals or waste stream mixtures at the WWTP. There are three types of wastewater monitoring samples:

- ✓ Untreated wastewater (affluents from WWTPs and sewers (sewer pipes),
- ✓ Primary sludge,
- ✓ Surface water contaminated with wastewater (rivers or streams). These types of samples are especially important for countries or cities that do not have adequate wastewater treatment systems and wastewater is discharged into receiving water bodies.

The monitoring of WWTP and pumping stations is generally less challenging, due to the greater infrastructure that these sampling points have for sewage collection. Sampling in sewer pipes, rainwater channels or water bodies can be more complex. In some places, for example, communities dominated by drug trafficking, the challenges could be related to lack of security to sampling personnel and equipment, which can make sample

Table 3
Monitoring areas, populations served and objectives.

Monitoring areas	Population	Objectives	Note
Larger cities 	50,000 to >1000,000	Early warning, evaluation of temporal and spatial trends, genomic surveillance	If local sanitation systems are not available, surveillance can be carried out in water bodies contaminated with sewage.
Community sites 	1000 to 50,000	Temporary test sites (hotspots areas)	Testing can be performed on WWTPs, but, the monitoring in the sewer pipes (in collectors or interceptors in well defined areas) helps to reach the most difficult access areas and in the identification of outbreaks, with focused health action and faster control.
Buildings, and closed facilities (prisons, university residences, homes for the elderly) 	Up to 2000	Identification of new groups and outbreaks before individual cases are diagnosed	The alert of the virus in the sewer can trigger preventive measures, such as clinical testing and the isolation and treatment of individuals who have tested positive. The transmission can be interrupted.
Places with high traffic of people 	Variable	Early warning	Ports, airports, railroads can act as sentinel sites. Also allows identifying reappearance or introduction of new or known pathogens

collection difficult or unfeasible. In rainwater channels (combined sewer system), it is important to consider the dilution effect of rainwater in the samples, especially in regions and/or periods with high rainfall.

In addition to sampling sites and sample types, it is also important to establish the sample volumes collected, the sampling method and the sampling frequency so that surveillance is most useful, especially in pandemic situations. Table 4 presents a design for monitoring wastewater at different stages of a pandemic.

Sampling points vary according to the pandemic phase, because when there is outbreak or pandemic situations, monitoring should be extended for any area (with or withouth coverage sewer systems). In other situations, monitoring may be focused on areas with greater circulation of people, sentinel sites (border regions) or at greater risk. This could reduce program costs, particularly relevant in developing countries.

Establishing a validated and reliable sampling strategy, laboratory testing and data analysis and interpretation is necessary for wastewater surveillance to inform public health action. Therefore, instructions on how to safely collect, store, and ship samples, test methods, including quality controls (matrix recovery control, human fecal normalization, quantitative measurement controls, inhibition evaluation, negative controls, and biosafety criteria) can be consulted in *The guide for the analysis and quantification of SARS-CoV-2 in wastewater* (PAHO - Pan American Health Organization, 2021. https://iris.paho.org/bitstream/handle/10665.2/54698/CDECECOVID-19210014_spa.pdf?sequence=1&isAllowed=yproduct), which was prepared with the contribution of several specialists on the subject in Latin America.

Composite sampling is more representative for assessing the concentrations of pathogens excreted by a population in sewage surveillance programs. However, in the absence of instruments to perform composite sampling, grab sampling can be performed when the sample is taken on the same day of the week at the same time, preferably at the time of the highest peak inflow of feces into the sewer (PAHO - Pan American Health Organization, 2021).

Ideally, a monitoring frequency of at least two to three times per week would be suitable at any stage of the pandemic phase to achieve better results of epidemiological data and evaluation of trend curves. However, the sampling frequency may vary according to the local capacity and infrastructure to carry out this type of surveillance, since, in the Latin American context, limitations related to the costs were observed to establish this programme.

Table 4
Design for surveillance of wastewater at different stages of a pandemic based on the Pandemic Interval Framework of the CDC - Centers for Disease Control and Prevention, United States.

	Pandemic phase			
	Initial	Acceleration	Deceleration	Preparedness for future pandemics
Hypothetical number of cases	Low	Increasing	Decreasing	Low
Objective	Early warning	Evaluate trends	Evaluate trends	Evaluate the appearance or reappearance of a pathogen
Target population	Sentinel sites	Locations with reported cases	Locations with reported cases	Sentinel sites
Sampling sites	Ports, airports, border regions, closed places, suspicious or higher risk areas, larger cities	Any area with reported cases	Any area with reported cases	Ports, airports, border regions, suspicious or higher risk areas, larger cities
Where to collect	WWTPs, sewer pipes	WWTPs, rivers* or streams*	WWTPs, rivers* or streams*	WWTPs, sewer pipes
Sampling volume	100 mL – 1 L	100 mL – 1 L	100 mL – 1 L	100 mL – 1 L
Sampling method	Composite samples	Composite samples	Composite samples	Composite samples
Sampling frequency	Weekly (at least once a week)	Weekly (at least once a week)	Weekly (at least once a week)	Weekly (at least once a week)

Source: Keshaviah et al., 2021. Adapted. * Areas without basic sanitation coverage.

Viral concentration methods were based on the most used methodologies around the world, such as membrane filtration, ultrafiltration, ultracentrifugation and polyethylene glycol (PEG) precipitation, in addition to lower cost alternative methodologies, such as skimmed milk flocculation (PAHO - Pan American Health Organization, 2021). Several methodologies and kits for the extraction and purification of nucleic acids were included in the guide, as well as the quantitative polymerase chain reaction with reverse transcription (RT-qPCR) for SARS-CoV-2 detection, using primers to amplify fragments of the nucleocapsid gene (N1 and N2) (PAHO - Pan American Health Organization, 2021). Due to high rates of mutation of RNA viruses, particularly SARS-CoV-2, and constant technological advances, the laboratory protocols should be timely updated.

Additional useful technical information that is desirable to public health agencies includes (WHO - World Health Organization, 2022a, 2022b): gene target, assay detection limits, quality assurance and quality control process, and performance on method sensitivity and specificity.

These minimum quality criteria required for SARS-CoV-2 detection have been recommended in the recently published guide (PAHO - Pan American Health Organization, 2021). For other viruses, some modifications in the molecular detection methods (primers for gene target amplification, PCR cycles, among others) are required, but the quality control criteria could be used as models in future documents.

3.2. Genomic surveillance

Genomic surveillance in wastewater has been developed in recent years as an important strategy within the scope of public health surveillance actions (WHO - World Health Organization, 2022a, 2022b). Genomic surveillance, based on SARS-CoV-2 genome sequencing to monitor the circulation of variants of interest (VOI – Variants of Interest) (Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda and Mu) or concern (VOC – Variants of Concern) (Alpha, Beta, Gamma, Delta and Omicron) is an important step in epidemiological surveillance, especially for the impact they can have on the efficacy and effectiveness of currently available vaccines (https://www.who.int/activities/tracking-SARS-CoV-2-variants).

Currently, genomic surveillance is used for many pathogens found in wastewaters. One example is the recently discovered potential for wastewater-based environmental monitoring of arboviruses, as the genetic material of viruses that cause dengue, chikungunya, and Zika virus have been found in wastewater (Lee et al., 2022; Chandra et al., 2021; Muirhead et al., 2020). The potential for sewage surveillance of influenza virus types and subtypes has also been investigated (Heijen and Medema, 2011; Wolfe et al., 2022). Monkeypox virus DNA was also recently detected at nine WWTPs in the Greater San Francisco Bay Area, California, United States, highlighting the potential of WBE for surveillance of this pathogen that has been of concern worldwide (Wolfe et al., 2022).

In addition to microorganisms, it is worth mentioning the surveillance of antibiotic resistance genes from wastewater, which has shown promise given current surveillance programs, which are based on monitoring of hospitalized patients and research of antimicrobial agents used as a last resource. Wastewater-based antibiotic resistance gene surveillance uses techniques such as metagenomics and has the potential to identify all known resistance genes circulating in the population. A single sample collected has the potential to generate data from large populations (not limited to data obtained only from hospitalized patients). In addition, the identified genes come from various taxa of microorganisms, and it is not necessary to culture these microorganisms to obtaining information on antimicrobial resistance. Currently, a global effort has been carried out to monitor antimicrobial resistance genes to generate a database using metagenomics of wastewater samples. These data can be very useful for public health (Aarestrup and Woolhouse, 2020; Hendriksen et al., 2019).

In Latin American countries, we can highlight the difficulty of establishing continuous monitoring or surveillance based on genomic sequencing of SARS-CoV-2 or other pathogens in wastewater, involving the rapid or real-time release of these results. The methodological problems due to the complexity of the samples (pool of samples of the population), the lack of laboratory structure that implies the acquisition or maintenance of expensive equipment, the costs and the difficulty in the acquisition of materials and reagents (more accentuated in a pandemic situation due to the great demand from developed countries), investments for the hiring and training of human resources and financial resources, in general, are pointed out as the greatest obstacles for the establishment of environmental surveillance programs and monitoring actions by public laboratories.

For these reasons, protocols for the rapid detection and triage of VOIs and VOCs based on the amplification of specific genomic segments used in quantitative or real-time reverse transcription polymerase chain reaction (RT-qPCR) assays have been developed to provide greater agility in obtaining results and reducing the costs involved in the analyzes (Oh et al., 2022; Peterson et al., 2022; https://www.bio.fiocruz.br/index.php/br/produtos/reativos/testes-moleculares/novo-coronavirus-sars-cov2/kit-molecular-4plex-sc2-voc-bio-manguinhos).

These protocols can provide a quick screening and inference of the VOIs and VOCs circulating in a locality, however, for the confirmation of these variants or the evaluation of new strains or pathogens that may emerge in the epidemic or post-pandemic context, genomic sequencing is still required. Therefore, the countries must allocate more resources for this type of surveillance, in addition to training human resources and investing in laboratory infrastructure. International aid in some cases also promotes the development of the region through the training of human resources, financial aid or analysis and genomic sequencing of strains or pathogens identified in some countries. We have identified a Pan-American network for Environmental Epidemiology (PANACEA) (https://www.ncl.ac.uk/press/articles/latest/2021/11/panacea/) led by Newcastle University in collaboration with Karolinska Institute, the University of Santiago de Compostela, and MGI-tech and with the participation of 14 Latin American countries to build a resilient and sustainable monitoring network capable of obtaining real-time data to help protect public health against chemical and microbiological exposures in the region. The scientists will develop and implement new molecular tools in environmental epidemiology and training new professionals capable of producing, analysing, and comprehensively interpreting data, strengthening technical capabilities in the countries.

4. Coordination of a WBE program

The coordination of a WBE program, whether at the national or local level, based on the public sphere or with the participation of the private sector, can be variable and will depend on the institutional arrangements that compound the public policies of each government. However, some key institutional actors were observed for the conduction of the process so that this type of program is more effective and oriented to the health needs of the populations.

The wastewater-based program should involve close coordination between public health authorities, research laboratories (public or private), sanitation and environmental companies, and other institutional actors to ensure that sampling and monitoring strategies are based on public health needs and that the results are integrated with other sources of epidemiological surveillance information and linked to actions (Keshaviah et al., 2021). The use of wastewater surveillance for public health actions requires a multidisciplinary approach. Communities interested in conducting wastewater surveillance for COVID-19 should identify the necessary local partners for sampling, testing, and public health actions. Local partners must include:

- ✓ State, local and territorial health departments: epidemiologists and environmental health specialists for COVID-19 and other diseases;
- ✓ Sanitation and environmental companies;
- ✓ Laboratories: public, environmental, academic and/or private health.

The participation of the health sector is very important to identify the regions of public health interest and to coordinate preventive actions and control in specific communities. In addition, the departments of planning, water resources, and sanitation companies can contribute to the definition of collection points in a sewage network and sampling strategies. Sanitation companies can play an important role in: (i) information on the geographic area and population served by a given sewerage system; (ii) distribution of the sewerage system in the urban area; (iii) information about the contribution of sewage from households, businesses, industries; among other information that should be considered. It is also important that sanitation companies act directly in the stages of sample collection and in the provision of auxiliary data, such as flow rates and physical-chemical parameters.

5. Environmental health information systems

Despite technological advances, barriers to the use of wastewater surveillance data to inform public health decisions remain. It should be noted that there is a communication gap between laboratories that quantify SARS-CoV-2 RNA in wastewater and public health authorities tasked with incorporating wastewater data into existing epidemiological information systems. Bridging the gap between research groups generating wastewater surveillance data and the public health sector may help harness the long-term potential of WBE as a tool for the surveillance of public health diseases and decision making (McClary-Gutierrez et al., 2021).

It is necessary to define, quite clearly, what data is required for each indicator, as well as the data source to be identified. Lack of health data at the local level or available data on socio-environmental conditions may be available at different levels of resolution, making it difficult to create links between the data obtained and health conditions, or to identify risk groups. Data may be available for different or inappropriate period of time or intervals and may be insufficient to determine spatial or temporal trends.

Most of the time, the sources of information in Latin America are outside the health sector. This includes routine information collected by different government agencies, universities and research organizations, the private sector, and service providers (Filho et al., 1999). It would be necessary to collect the data at the local level and then insert them into a national information system to group them and facilitate monitoring of the epidemiological situation, helping the different institutional actors in decision-making.

An interesting initiative is the Public Health Environmental Surveillance Centralized Database (PHESD-ODM), developed by CoVRR Net, the Canadian Institute for Health network, which serves as a central repository for open-access wastewater surveillance data (ODM). The ODM helps the wastewater monitoring community collaborate by allowing teams from around the world to share their data in a common structure. This reduces the delay between measurement and analysis and provides more data for better modeling, better collaboration, and better tools for COVID-19 pandemic control.

6. Communication of surveillance data to inform and support public health actions

Health Communication is essential for the development of actions that involve the management of information for decision-making in the field of public health. During the COVID-19 pandemic, we were able to observe in Latin America different experiences of dissemination and communication strategies for SARS-CoV-2 surveillance data in sewerage.

Data were shared in electronic media, where most of the experiences involved reported their results on the pages of city councils or the websites of environmental regulatory agencies, with the presentation of weekly or monthly reports on the spread of SARS-CoV-2 in monitored areas (https://www.covid19wbec.org/).

Many municipalities are publishing the data along with other health indicators on official dashboards, where it is possible for any citizen to access the page and follow the monitoring results by region. A georeferencing system is generally used and the data is translated into heatmaps which represent areas with the highest concentration of virus presence in the sewers of each location. Geographic Information Systems - GIS and heatmaps allow quick identification of risk areas and can be valuable tools in decision-making and, more importantly, in social mobilization and responses of the community (Franch-Pardo et al., 2020). Fig. 4 presents a hypothetical dashboard model that can be used to disseminate SARS-CoV-2 surveillance data in wastewater.

The data presented as heatmaps and published on web pages can also be accessed through mobile phones. This form of communication allows greater dissemination of information not only to decision-makers, but to the population, increasing public awareness and, consequently, promoting preventive health measures.

It is important to note that any form of public diffusion of the information obtained from an environmental monitoring program must occur alongside a

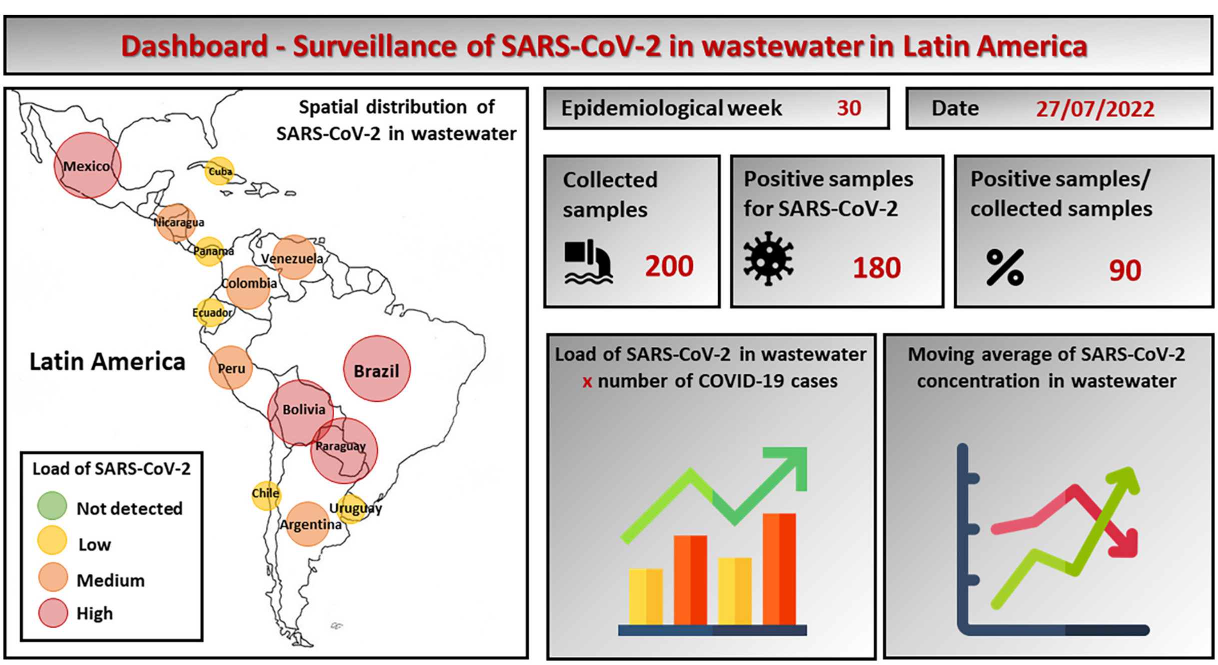


Fig. 4. Hypothetical dashboard model for the divulgation of SARS-CoV-2 surveillance data in sewage through institutional web pages and heatmaps by area. Note: The data presented is not real, but hypothetical to illustrate the model construction example.

strong campaign of dissemination and training for the population. It is crucial that qualified professionals in the field explain to people what wastewater is and that they are population samples that provide abundant information without violating people's privacy. It is also important that they teach people to interpret published information and the implications it has at the individual and population levels. In this way, in addition to providing useful information for the citizen, conflicts between people from different risk areas and social stigmatization of the most vulnerable populations can be avoided.

7. Costs of a WBE program

In economic and practical terms, WBE is much cheaper compared to clinical screening (Hart and Halden, 2020; Gawlik et al., 2021; Keshaviah et al., 2021; World Bank Group, 2022). Repeated individual testing on a large scale is not feasible. In addition, wastewater testing is less invasive than individual tests and forms part of a routine surveillance system to prevent and/or monitor new outbreaks or epidemics.

The total cost of clinical tests increases considerably during waves, while for wastewater tests the cost generally remains constant (World Bank Group, 2022). This is because in the clinic testing costs are proportional to the number of people tested, while wastewater testing costs are proportional to the number of sites sampled, which does not change much with the number of people covered by the test (World Bank Group, 2022).

The World Bank (World Bank Group, 2022) recently estimated the average proportional costs of testing on clinical and environmental samples for a representative population in Latin America. Both the costs and resources needed for wastewater testing are commonly estimated using simple calculations based on the number of sites, frequency of testing, and equipment and reagent cost estimates. They are estimated per person or per site over periods of time, such as a year; the procedure is illustrated below.

The annual cost for a population (CPA, in US$/year) will depend on the cost per analysis (CA, in US$/analysis) and the number of analyzes performed in a year (NA, in analysis/year), according to:

$$CPA = CA \times NA \tag{1}$$

Consequently, the annual individual cost (CIA, in US$/person/year), considering the number of people (P) that are analyzed, is:

$$CIA = \frac{CPA}{P} \tag{2}$$

Eqs. (1) and (2) are valid both for wastewater analyzes (indicated below with the subscript *ar*) and for clinical analyzes (indicated below with the subscript *c*).

7.1. Estimation of wastewater analysis costs for a population

By way of illustration, the following assumptions were adopted for wastewater analyses:

i. Cost for analysis of SARS-CoV-2 in wastewater (CA_{ar}): US$ 300 (costs based on the number of sites, the frequency of tests and estimates of equipment and reagents costs);
ii. Number of wastewaters analyzes per year (NA_{ar}): 100 (assuming a sample is analyzed twice a week);
iii. Basin size (P): 100,000 people.

In this case, the CPA_{ar} is US$ 30,000/year, while the CIA_{ar} is US$ 0.30/person/year.

7.2. Estimation of the clinical tests cost for a population

In this case, the following data are used (World Bank Group, 2022), corresponding average costs of clinical tests (subindex *c*) by country for Latin America and the Caribbean, between September 2020 and September 2021:

i. Cost per clinical analysis (CA_c): approximately US$ 40 (from US$ 20 to US$ 100);
ii. Number of tests per year: approximately 29 to 94 tests per 100 people, which equates to 2.9 to 9.4 tests per person per year (NA_c).

Considering the lowest values to perform the calculations: CA_c = US$ 20/test and NAc = 2.9 tests/person/year, the annual individual cost (CIA_c) then results in US$ 58/person/year, which is 193 times greater than that corresponding to the CIA_{ar}. On the other hand, for clinical analyses, the annual population cost (CPA_c) (for P = 100,000 people and to be able to compare) is US$ 5,800,000, which is 19,333 times greater than the CPA_{ar}.

The costs of wastewater analysis are much lower than those of clinical analyses, although it is necessary to remember that the type of information it provides is different because it refers to a population. Wastewater surveillance is a complementary monitoring system, and cannot replace clinical surveillance, which should also be promoted by the countries. In that sense, costs of wastewater analyses and of clinical testing should be integrated for cost-effective mass surveillance, as proposed by Wu et al. (2022).

8. Themes under development and perspectives

To make a quantitative correlation based on the viral concentrations present in wastewaters and the estimation of an infected population, it is necessary to determine the amount of genetic material present in the excreta of people and, from the concentration found in the environmental samples, it is possible to extrapolate to the population, using mathematical projections and trend curves (Hart and Halden, 2020). Some studies have aimed to develop calculations and mathematical models that could be more appropriate to estimate the number of infected populations from viral RNA data from wastewater (Ahmed et al., 2020; Hart and Halden, 2020; Chakraborty et al., 2021; Gerrity et al., 2021; Petala et al., 2022).

If the environmental modeling of wastewater is desirable for simulating and estimating the rates of infection in the population, the mathematical models must be used in a complementary way and with great caution (as required by the models), to avoid overestimations and underestimations, beyond the uncertainties (Soller et al., 2022). There are several sources of uncertainty that are generated by unknown relationships between measured variables, for example, between the correlation of the concentration of viral RNA in wastewaters and the estimated infected population. The uncertainty can also be introduced due to the variability in the measurements due to the representative sample, technical precision or error of the measuring instruments (McClary-Gutierrez et al., 2021).

In the case of SARS-CoV-2 in wastewaters, the uncertainties are related to some factors, which include: the viral load excreted, which can vary considerably in each infected individual, the recovery efficiency and the detection limit of the different methods used for detection, the dilution factor and the sampling strategy, the stability of viral particles in the wastewaters, the time involved since the collection of the sample until the detection and data analysis (Ahmed et al., 2022; Saththasivama et al., 2021; Lazuka et al., 2021; Li et al., 2021; McClary-Gutierrez et al., 2021; Zhu et al., 2021; Petala et al., 2022; Wade et al., 2022).

Due to an increase in the number of studies and examples of surveillance monitoring of pathogens in wastewater, many of the uncertainties are becoming clearer. However, it is necessary to incorporate appropriate mathematical models to estimate with greater confidence the number of infected populations within a representative area.

Technological advances involving biosensors and bioanalytical devices have also received significant attention in the WBE (Mahmoudi et al., 2022). These biosensing platforms include mainly lab-on-a-chip (LOC) and microfluidic devices, μpaper-based analytical devices (μPADs), and lateral flow assays (LFAs), which would have desired characteristics, especially in terms of sensitivity and specificity compared to the gold standard method RT-qPCR (Mahmoudi et al., 2022). These technologies combined with digital technologies can lead to smart diagnostics of pandemic infectious diseases, following the concept of "smart cities" that foresees achievement of more efficient and sustainable cities (Bauer et al., 2021; Mahmoudi et al., 2022; Abdeldayem et al., 2022). While the application of biosensors and digital technologies for WBE is not yet a reality in most Latin American cities or countries, the opportunities seem promising. However, their massive application will depend on the availability of resources or how much the costs of these technologies can be reduced in the future. In addition, it is necessary to investments to make these new technologies universal, including 4 or 5G internet access, machine learning, artificial intelligence, computational resources and big data analysis.

Since the use of WBE is still incipient in several Latin American regions, it is recommended that mechanisms for cooperation and exchange of information and experiences within and between countries be strengthened. This would ensure permanent collective learning and the faster incorporation of appropriate techniques and technologies in the countries of the Region.

Countries must be prepared to deal with new socio-environmental challenges and anticipate and prepare for new outbreaks and epidemics that may emerge. Environmental health surveillance should be prioritized and universalized in line with sustainable development goals aimed at improving the quality of life, reduction of inequalities and a look to the future of the next generations.

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Conceptualization, Project administration and Supervision: Agnes Soares da Silva, Gloria Rey-Benito. Data Curation and interpretation: Tatiana Prado. Formal analysis: Tatiana Prado, Marize Pereira Miagostovich, Maria Inês Zanoli Sato, Veronica Beatriz Rajal, Cesar Rossas Mota Filho, Alyne Duarte Pereira, Mikaela Renata Funada Barbosa, Camille Ferreira Mannarino. Writing - original draft: Tatiana Prado. Writing - review & editing: All authors. All authors read and approved the final version of the manuscript.

Disclaimer

Agnes Soares da Silva is a retired employee and Gloria Rey-Benito is a staff member of the Pan American Health Organization. The authors are responsible for the views expressed in this publication, and they do not necessarily represent the decisions or policies of the Pan American Health Organization.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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