


Contribution of wastewater-based epidemiology to SARS-CoV-2 screening in Brazil and the United States

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ABSTRACT

Wastewater-based epidemiology (WBE) is a valuable tool for investigating the existence, prevalence, and spread of pathogens, such as SARS-CoV-2, in a given population. WBE, proposed as part of the SARS-CoV-2 surveillance strategy for monitoring virus circulation, may complement clinical data and contribute to reducing the spread of the disease through early detection. In developing countries such as Brazil, where clinical data are scarce, information obtained from wastewater monitoring can be crucial for designing public health interventions. In the United States, the country with the largest number of confirmed SARS-CoV-2 cases worldwide, WBE programs have begun to be carried out to investigate correlations with coronavirus disease 2019 (COVID-19) clinical data and support health agencies in decision-making to prevent the spread of the disease. This systematic review aimed to assess the contribution of WBE to SARS-CoV-2 screening in Brazil and the United States and compare studies conducted in a developed and developing country. Studies in Brazil and the United States showed WBE to be an important epidemiological surveillance strategy in the context of the COVID-19 pandemic. WBE approaches are useful for early detection of COVID-19 outbreaks, estimation of clinical cases, and assessment of the effectiveness of vaccination program.

Key words: COVID-19, epidemiology, SARS-CoV-2, wastewater, WBE

HIGHLIGHTS

- Early alert of SARS-CoV-2 through the use of wastewater-epidemiology strategies.
- Wastewater surveillance can complement clinical data to provide a tool of surveillance SARS-CoV-2 circulation.
- Wastewater monitoring could help identify the presence of asymptomatic individuals in the community.
- Contribution of WBE to decision-making in public policies.
- Contribution of WBE to the health system.

INTRODUCTION

The first outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China, in December 2019. The disease soon progressed to a global pandemic, causing great apprehension and constituting a public emergency worldwide (Cucinotta & Vanelli 2020). According to the World Health Organization (WHO), as of September 1, 2022, there have been more than 600 million confirmed cases globally and more than 6 million deaths. The United States, the country with the highest number of cases, has had 94 million confirmed cases and 1 million deaths, whereas, in Brazil, there have been 34 million confirmed cases and 683 thousand deaths (WHO, accessed on May 12, 2022).

Coronavirus is a group of positive-sense, single-stranded RNA viruses that cause respiratory tract infections. The new coronavirus strain, SARS-CoV-2, is genetically related to SARS-CoV (Gorbalenya *et al.* 2020). Clinical presentation of the disease may be asymptomatic or include symptoms such as cough, fever, fatigue, myalgia, and temporary loss of smell and taste (Gao *et al.* 2020; Holshue *et al.* 2020). SARS-CoV-2 is transmitted by disease carriers mainly through small respiratory droplets produced by sneezing or coughing (Wu *et al.* 2020). However, evidence has shown the presence of SARS-CoV-2 RNA in human excreta, including feces and urine, discharged as wastewater (Collivignarelli *et al.* 2020).

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A person infected with SARS-CoV-2 can quickly spread the virus to many others, especially through routine international travel, mass gatherings in public places, and direct physical contact (Ding & Liang 2020). Studies indicate that SARS-CoV-2 is found in wastewater in different regions of the world, underscoring the need for effective environmental surveillance systems (Medema *et al.* 2020; Peccia *et al.* 2020). Wastewater-based epidemiology (WBE) has been used as a tool for investigating the existence, prevalence, and spread of pathogens, such as SARS-CoV-2, in a population, through wastewater monitoring. In addition to surveillance, WBE has been used to assess correlations between viral detection in wastewater samples and confirmed cases from clinical samples, even before the emergence of SARS-CoV-2 (Carducci *et al.* 2006; Bisseux *et al.* 2018). WBE has served as an effective tool for surveillance of other viruses through wastewater monitoring, such as poliovirus and hepatitis A (Hellmer *et al.* 2014; Wigginton *et al.* 2015), and may be just as important for surveillance of SARS-CoV-2, predicting outbreaks and the resurgence of COVID-19, and controlling the pandemic.

Studies point to limitations in clinical data collection resulting from a high number of asymptomatic individuals and a lack of clinical testing in underserved populations, among other factors, affecting the capacity of authorities to deal with the COVID-19 pandemic (Mota *et al.* 2021). The seriousness of the situation emphasizes the need for alternative monitoring techniques. A growing number of studies report the use of WBE, mainly in developed countries such as the United States (Bar-On *et al.* 2020; Haramoto *et al.* 2020; Kumar *et al.* 2020; La Rosa *et al.* 2020; Medema *et al.* 2020; Nemudryi *et al.* 2020; Randazzo *et al.* 2020; Rimoldi *et al.* 2020; Wurtzer *et al.* 2021).

This review aimed to assess the contribution of WBE to SARS-CoV-2 screening and compare studies conducted in Brazil, a developing country with the third highest prevalence of confirmed COVID-19 cases, with those conducted in the United States, a developed country with the highest number of confirmed cases worldwide.

METHODS

A systematic review was performed using the Bibliometrix tool of RStudio software (R version 4.1.2) to remove duplicates and select articles found in the databases. The PRISMA guidelines were followed, and the searches for indexed articles were performed in Scopus and Web of Science. The purpose of this review was to determine if the WBE is contributing or can contribute to rapid tracking of SARS-CoV-2 in Brazil and the United States from the beginning of the first outbreak in 2020 to the present date according to peer-reviewed literature. The search strategy included the following keywords: 'SARS-CoV-2' AND 'virus concentration' AND 'wastewater-based epidemiology' AND 'COVID-19' AND 'SARS-CoV-2 wastewater monitoring.' The results were limited to the countries of interest, namely, Brazil and the United States.

Subsequently, article titles and abstracts were screened for inclusion criteria, which were as follows: (i) epidemiological data on SARS-CoV-2 in wastewater, (ii) clinical data on confirmed COVID-19 cases, (iii) wastewater surveillance, and (iv) articles indexed in Scopus or Web of Science. We excluded other reviews, manuals, resolutions, editorials, letters, comments, books, proceedings, and duplicate publications. Subsequently, articles were read in full, and those that met all inclusion criteria were used as a theoretical basis in this review (Figure 1). A total of 16 articles were selected (Table 1).

No meta-analysis was carried out in the present review because the objective of this review was to show whether studies in the countries of choice showed an association between the positive results found in sewage and the number of confirmed cases for the study location, in addition to showing whether in Brazil and in the United States, WBE can contribute to the screening of SARS-CoV-2 in new outbreaks of the disease.

Table 1 shows the 16 articles selected for this review, presented by the name of the authors and the country where the study was carried out.

RESULTS AND DISCUSSION

Wastewater-based surveillance

WBE is a valuable epidemiological tool to detect the movement of viruses in a community and monitor viral loads in a given catchment area, constituting a practical and reliable approach to assess the viral prevalence and predict the onset of outbreaks, which allows preventing the viral spread and identifying critical points in a population (Kitajima *et al.* 2020; Venugopal *et al.* 2020). WBE is defined as the normalization of influent analyte concentration to per capita mass loads using daily flow and wastewater treatment plant (WWTP) population, and provides population-scale information on human activity within basin boundaries hydrographic (Choi *et al.* 2018). This tool comprises the extraction, analysis, processing, and interpretation of biological markers detected in wastewater as health information. First developed as a strategy to

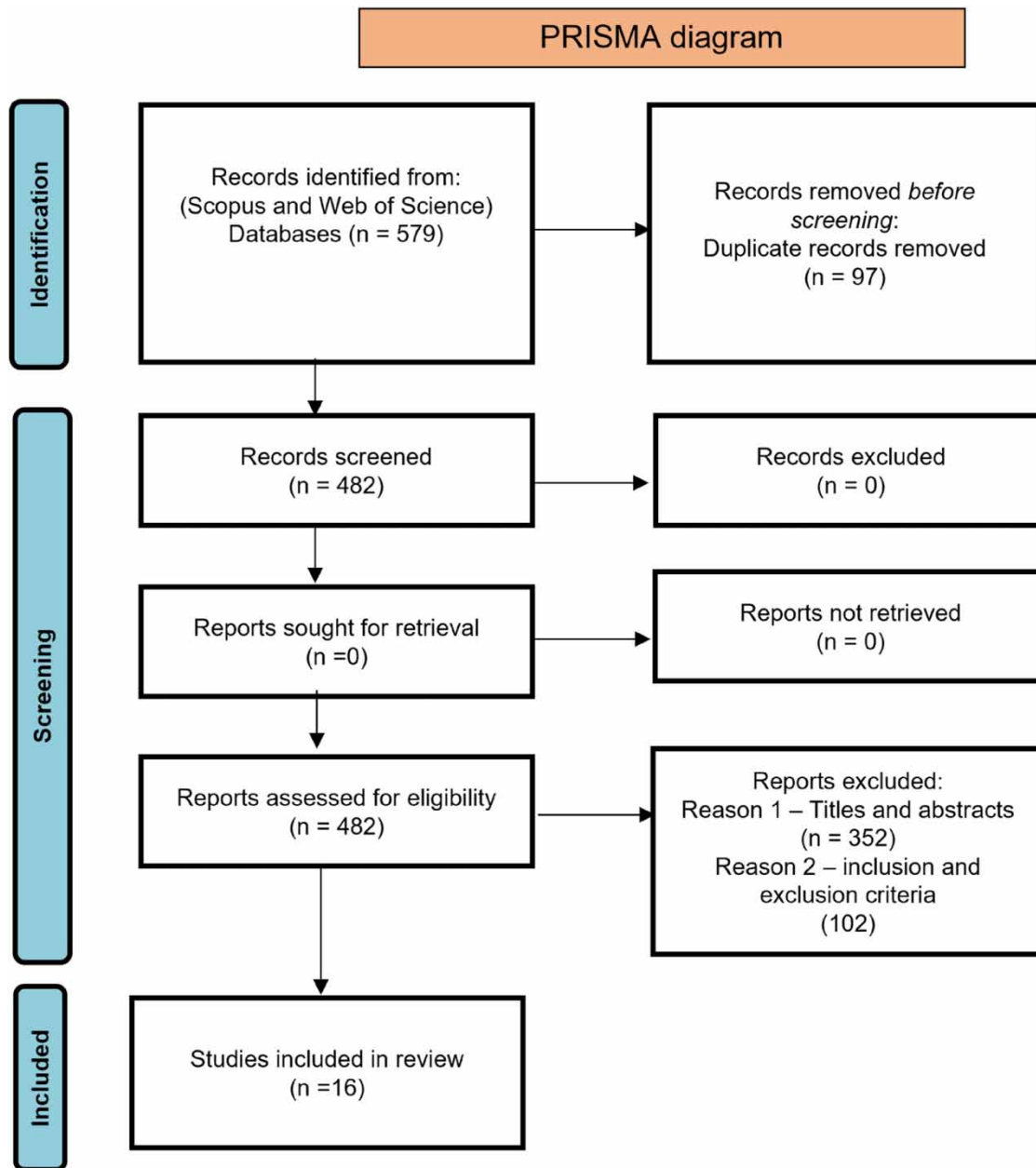


Figure 1 | PRISMA flow diagram of the database search and article selection process (from Page *et al.* 2021).

systematically track chemical residues in wastewater to monitor illicit drug consumption, WBE is currently applied for the analysis of several different chemical compounds and microorganisms, serving as a complementary source of information for outbreak and disease surveillance systems (Banta-Green *et al.* 2009; Feng *et al.* 2018). Briefly, representative samples of wastewater are collected from the influent of a WWTP. Sample preparation usually includes filtering or centrifuging the sample and a pre-concentration step. Recent studies have shown different concentration methods, reverse transcription polymerase chain reaction targets, process controls, and criteria for calculating incidence and interpreting results (Lorenzo & Picó 2019).

For a public health response, the alternatives used are monitoring of mortality rates and clinically based surveillance. In these approaches, confirmation of cases is based on clinical, epidemiological, or laboratory tests. With these approaches, asymptomatic individuals may not be identified. In this context, WBE can be an alternative to complement these data,

Table 1 | References included in the systematic review

Reference	Country
Chernicharo <i>et al.</i> (2021)	Brazil
Mota <i>et al.</i> (2021)	Brazil
Claro <i>et al.</i> (2021)	Brazil
Prado <i>et al.</i> (2021)	Brazil
Fongaro <i>et al.</i> (2021)	Brazil
Razzolini <i>et al.</i> (2021)	Brazil
Augusto <i>et al.</i> (2022)	Brazil
de Sousa <i>et al.</i> (2022)	Brazil
Wu <i>et al.</i> (2020)	The United States
Wu <i>et al.</i> (2022)	The United States
Sharkey <i>et al.</i> (2021)	The United States
Barrios <i>et al.</i> (2021)	The United States
Weidhaas <i>et al.</i> (2021)	The United States
Wu <i>et al.</i> (2021)	The United States
Ai <i>et al.</i> (2021)	The United States
Barua <i>et al.</i> (2022)	The United States

since its detection through monitoring of SARS-CoV-2 in wastewater can be performed in the general population (Bivins *et al.* 2020; Medema *et al.* 2020).

The highlight of the WBE is its early warning capability, that is, signaling an early stage of an outbreak and the imminent increase in infected individuals. Thus, sampling and quantification methods are adopted, and the increase in viral load in wastewater can be observed and reported even before individuals show symptoms and seek medical attention. However, there are still significant gaps in this early warning system, and further research is needed (La Rosa *et al.* 2020; Medema *et al.* 2020; Peccia *et al.* 2020; Randazzo *et al.* 2020).

WBE in Brazil and the United States

Brazil is a developing country with limited healthcare, a precarious health infrastructure, and scarce clinical data. By contrast, in developed countries such as the United States, the potential of wastewater surveillance studies is increasingly recognized. The Centers for Disease Control and Prevention and several state health agencies implemented WBE programs to investigate correlations with COVID-19 clinical data and support decision-making in different localities (CDC 2020; Wu *et al.* 2021).

The studies included in this review, conducted in different regions of Brazil and the United States, show that WBE is a useful tool for the surveillance of SARS-CoV-2 and combating COVID-19 outbreaks (Table 2).

A research group in Belo Horizonte, Brazil, created a decentralized wastewater surveillance program to assess the local prevalence of COVID-19 from May 2020 onward. In this study, data were analyzed based on the relative prevalence index (RPI) and SARS-CoV-2 RNA concentration in wastewater. This strategy was applied to circumvent limitations in the accuracy of case numbers estimated from wastewater data, given the great intrinsic variability of parameters used to convert SARS-CoV-2 RNA concentration (Mota *et al.* 2021). WBE-derived RPI analysis proved to be relevant as an early warning tool, helping to identify regions with high COVID-19 prevalence before the worsening of the pandemic throughout the city as a whole. Critical points of the disease had a prevalence almost three times higher than the average and were characterized as vulnerable regions, that is, areas with poor access to sanitation, healthcare, and other basic services. The use of RPI can be advantageous in WBE as it normalizes parameters such as wastewater flow, which can vary greatly and may be difficult to measure accurately. More studies like this can provide information for cities to cope better with the pandemic, particularly in countries where clinical data are scarce, such as Brazil.

Critical points were also evaluated in Lincoln, in Nebraska, the United States, revealing a generalized increase in COVID-19 cases via the detection of SARS-CoV-2 in wastewater (Barrios *et al.* 2021). The collected data allowed for monitoring

Table 2 | Epidemiological data from studies conducted in Brazil and the United States

Reference	Sampling location	Sampling period	Concentration method	SARS-CoV-2 detection rate in wastewater	Correlations between SARS-CoV-2 rate in wastewater, clinical data, and case estimates
Chernicharo <i>et al.</i> (2021)	Belo Horizonte, Brazil	March to August 2021	Negatively charged membrane filtration	100%	Detection estimated to be 3% higher than the number of confirmed cases
Mota <i>et al.</i> (2021)	Belo Horizonte, Brazil	May to August 2021	Negatively charged membrane filtration	100%	Detection estimated to be 3% higher than the number of confirmed cases
Claro <i>et al.</i> (2021)	São Paulo, Brazil	June 2020 to March 2021	PEG precipitation	100%	Detection estimated to be 4.3% higher than the number of confirmed cases
Prado <i>et al.</i> (2021)	Rio de Janeiro, Brazil	April to August 2021	Ultracentrifugation	100%	Detection rate correlated with the number of confirmed cases
Fongaro <i>et al.</i> (2021)	Santa Catarina State, Brazil	October 2019 to March 2020	PEG precipitation	100% from November onward	Detection before the presentation of confirmed cases
Razzolini <i>et al.</i> (2021)	São Paulo, Brazil	May to November 2020	Ultracentrifugation	100%	Detection rate correlated with the number of confirmed cases
Augusto <i>et al.</i> (2022)	São Paulo, Brazil	April to August 2021	PEG precipitation	Up to 88% in composite samples and 75% in individual samples	Estimated cases were up to 4.7% higher than the number of confirmed cases
de Sousa <i>et al.</i> (2022)	Goiânia, Brazil	January to August 2021	PEG precipitation	43.63%	Estimated cases were up to 4.2% higher than the number of confirmed cases
Wu <i>et al.</i> (2020, 2022)	Massachusetts, USA	January to May 2020	PEG precipitation	100% from March onward	Estimated cases were up to 5% higher than the number of confirmed cases
Sharkey <i>et al.</i> (2021)	Miami, USA	September to December 2020	Negatively charged membrane filtration	100%	Detection rate higher than the number of cases per sampling day
Barrios <i>et al.</i> (2021)	Lincoln, Nebraska, USA	July to September 2020	Negatively charged membrane filtration	41.6%	Detection rate correlated with the number of confirmed cases
Weidhaas <i>et al.</i> (2021)	Utah, USA	April to Maio de 2020	Negatively charged membrane filtration	61%	Detection rate below the number of confirmed cases
Wu <i>et al.</i> (2021)	159 USA municipalities	February to June 2020	PEG precipitation and Amicon Ultra Centrifugal Filter	49.1%	62% of samples correlated with confirmed cases
Ai <i>et al.</i> (2021)	Ohio, USA	July 2020 to January 2021	Adsorption – precipitation and hollow fiber polysulfone concentration pipette	100%	Detection rate correlated with the number of confirmed cases
Barua <i>et al.</i> (2022)	Charlotte, North Carolina, USA	June to December 2020	HA type filtration	100%	Detection rate correlated with the number of confirmed cases

outbreaks of the disease among marginalized populations (Barrios *et al.* 2021). In Ohio, the United States, a research group developed a polynomial model for the wastewater data analysis. The model showed a correlation between confirmed cases and detection of the virus in wastewater (Ai *et al.* 2021). The referred study was the first to apply the model to track COVID-19 cases from WBE data.

Estimation of COVID-19 cases using WBE data

Studies have estimated the number of COVID-19 cases from SARS-CoV-2 detection rates in wastewater, helping public bodies control the spread of the disease through preventive measures. In Belo Horizonte, Brazil, SARS-CoV-2 spread was made evident by the presence of the virus in sewage sub-basins (Chernicharo *et al.* 2021). Wastewater-based estimates of infected individuals were significantly higher (about 3% more) than confirmed cases reported in epidemiological bulletins of the city (Table 2). From March to May 2021, 1,766 cases were confirmed; however, according to wastewater surveillance data for the same period, about 20,000 individuals were infected. Such a marked difference might be due to the low number of clinical tests performed and due to the fact that it is possible to detect asymptomatic carriers through wastewater surveillance, in addition to symptomatic ones (Bai *et al.* 2020; La Rosa *et al.* 2020). Wastewater surveillance indicated a significant increase in COVID-19-infected individuals in the city coincident with the easing of social distancing measures, followed by an exponential growth trend in both clinical and wastewater data (Chernicharo *et al.* 2021). In São Paulo, Rio de Janeiro, and Goiânia, Brazil, the number of cases estimated on the basis of wastewater surveillance was about 4% higher than the number of reported cases (Prado *et al.* 2021; Augusto *et al.* 2022; de Sousa *et al.* 2022).

In Utah, the United States, by contrast, the number of estimated cases was lower than that of confirmed cases (Weidhass *et al.* 2021). Such findings might be explained by the facts that (i) not all individuals with COVID-19 eliminate the virus in feces, (ii) the duration of elimination may vary between individuals, (iii) weekly or biweekly sampling of wastewater may underestimate RNA abundance, providing different results from daily case counts, (iv) RNA may degrade in wastewater, or (v) sample processing methods may be inefficient (Weidhass *et al.* 2021). Studies conducted by Wu *et al.* (2020, 2022) in Massachusetts, the United States, showed that the number of cases estimated through WBE was about 5% higher than the number of confirmed cases, similar to that observed in Brazil (Table 2).

Correlation of SARS-CoV-2 detection in wastewater with clinical data

Studies have correlated WBE data with clinical data of individuals infected with the disease to evaluate whether this tool is effective for virus monitoring. In Santa Catarina State, Brazil, SARS-CoV-2 was detected in wastewater even before the first confirmed case (Fongaro *et al.* 2021). In the cited study, the SARS-CoV-2 load remained constant until March 2020 and increased thereafter, coinciding with the onset of COVID-19 cases in Santa Catarina State. The authors sequenced a positive sample and compared it with sequences available in the NCBI database, confirming the presence of SARS-CoV-2 (100% similarity). The positive sample was detected in November 2019, that is, 56, 91, and 97 days before the first confirmed case of COVID-19 in the Americas, Brazil, and Santa Catarina State, respectively, demonstrating that the virus had been introduced to the community months before it was first reported (Fongaro *et al.* 2021). Ai *et al.* (2021) sequenced SARS-CoV-2 genomes in wastewater samples from Ohio, the United States, and observed genetic variations according to sampling point; nevertheless, almost 100% of samples were of the D614G variant, which dominated the global pandemic. In Niterói, Rio de Janeiro State, Brazil, 27 positive wastewater samples were sequenced to construct a library (Prado *et al.* 2021). Three lineages were identified by nucleotide substitutions and classified as the B.1.1.33 variant, the most prevalent lineage in Rio de Janeiro State (Resende *et al.* 2020).

In São Paulo, SARS-CoV-2 wastewater detection was positively correlated with clinical data and even preceded trends in clinical data by up to 2 weeks (Claro *et al.* 2021). WBE was found to be a powerful and complementary tool for monitoring the spread of COVID-19 in Brazil and assisting in the prediction of SARS-CoV-2 outbreaks. Razzolini *et al.* (2021), in São Paulo, and Prado *et al.* (2021), in Rio de Janeiro, also found a positive correlation between clinical data and detection of the virus in wastewater. In São Paulo (Razzolini *et al.* 2021), SARS-CoV-2 viruses detected in wastewater samples were analyzed for cytopathic effects on CCL-81 (Vero) cells, and no cytopathic effects were identified; that is, viruses in wastewater were not infectious (Razzolini *et al.* 2021). Similar results were reported in a recent study in the United States: SARS-CoV-2 isolated from wastewater showed no infectious risk to the population (Robinson *et al.* 2022).

Wu *et al.* (2020), in Massachusetts, the United States, found that wastewater samples tested positive for the virus 4–10 days before clinical reports. The correlation of viral titers with new clinical cases was higher when newly confirmed clinical cases

were compared with wastewater samples collected 4–10 days before confirmation. These findings show that the virus was detected in wastewater during the typical incubation period of 4–5 days, suggesting the importance of this type of surveillance for prediction of new cases (Wu *et al.* 2020b). Sharkey *et al.* (2021) detected the virus in wastewater from the University of Miami before clinical confirmation of infections in students.

A study encompassing 40 states in the United States, carried out from February to June 2020, highlighted that WBE can be implemented to explore viral transmission at different geographical and temporal scales (Wu *et al.* 2021). As in Brazil, SARS-CoV-2 wastewater detection preceded clinical reports. A linear correlation between total viral load in wastewater and new confirmed cases was observed, in agreement with the hypothesis that average spread rates are similar between sewage treatment plants (Wu *et al.* 2021). In North Carolina, the United States, a linear correlation was observed between wastewater viral load and confirmed cases, indicating that wastewater viral load is a better representation of future COVID-19 cases than confirmed cases. Overall, these findings indicate the important potential of WBE for monitoring the increase or decrease in the number of positive cases in a given geographical location. Wu *et al.* (2022) suggested that surveillance data may be more specific for new cases, given the high viral excretion by newly infected individuals (Wu *et al.* 2022). These studies demonstrate that WBE can be used as an early warning of outbreaks for public health and hospital planning in Brazil and the United States.

The use of WBE for pandemic control

In Niterói, Rio de Janeiro State, Brazil, the Oswaldo Cruz Foundation conducted an environmental surveillance project for the detection of SARS-CoV-2 in wastewater to support actions of the Ministry of Health. The study lasted 20 weeks, from April to August 2020. The highest positivity rate was observed between May and June, and the lowest rate in April. Wastewater samples correlated with confirmed cases and deaths in Niterói. WBE supported interventions in communities to search for individuals with COVID-19 symptoms. Infected individuals were identified by testing, and measures were taken against the spread of the disease. Continuous environmental surveillance showed an increase in SARS-CoV-2 loads in wastewater followed by a progressive decrease, reflecting epidemic control measures implemented by the city. The study resulted in the use of SARS-CoV-2 wastewater data to complement conventional indicators, such as mortality and hospitalization rates. Although national and municipal responses to COVID-19 depend on public policies, Niterói pioneered at the national level in the decision to include SARS-CoV-2 wastewater surveillance as a strategy to support pandemic control (Prado *et al.* 2021).

In Goiânia, Brazil, researchers monitored the viral load of SARS-CoV-2 in wastewater and developed a web application to predict the number of infected individuals (de Sousa *et al.* 2022). A Python-based web application (pySewage) was created to help health authorities and the scientific community to handle large amounts of data, such as those produced by WBE. pySewage may also be used to monitor the spatial distribution and prevalence of infections.

In the University of North Carolina, the United States, as reported by Gibas *et al.* (2021), preventive actions such as isolation of dormitories and clinical testing of students were carried out after the detection of SARS-CoV-2. WBE enabled the identification of asymptomatic COVID-19 cases, which would have otherwise gone undetected by on-campus clinical testing. The authors stated that WBE was an important, preventive strategy. Other study groups at different universities and schools in the United States conducted similar studies, in which WBE was used as a tool for detecting SARS-CoV-2, limiting new outbreaks of the disease (Barich & Slonczewski 2021; Betancourt *et al.* 2021; Colosi *et al.* 2021; Crowe *et al.* 2021; Gutierrez *et al.* 2021; Harris-Lovett *et al.* 2021; Scott *et al.* 2021; Wright *et al.* 2022).

WBE has been proposed as part of the SARS-CoV-2 surveillance strategy and has the potential to combat the disease by allowing early detection of the virus. It has been shown that SARS-CoV-2 concentrations in wastewater correlate with the local number of infected individuals (Ahmed *et al.* 2020; Randazzo *et al.* 2020). Wastewater surveillance can complement clinical data to provide a robust tool for surveillance SARS-CoV-2 circulation (Larsen & Wigginton 2020; Medema *et al.* 2020) in both developing countries such as Brazil and developed countries such as the United States, given that these strategies are fast and of low cost. Furthermore, this surveillance system can be used to detect new variants and mutations of SARS-CoV-2 (Medema *et al.* 2020). WBE shows potential for large-scale screening, representing a highly cost-effective approach (Mao *et al.* 2020). This type of study could help identify the presence of infectious asymptomatic individuals in the community.

CONCLUSION

Studies conducted in Brazil and the United States detected SARS-CoV-2 in wastewater, correlating WBE findings with clinical data. WBE-based estimates can contribute to decision-making aimed at preventing the spread of the disease, in addition to

contributing to early warning of new outbreaks. An advantage of wastewater-based testing is that it constitutes a passive monitoring strategy that can indicate the need for individual clinical testing. The results confirm that wastewater monitoring is an important epidemiological tool in the context of the COVID-19 pandemic.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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