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Environmental genomic surveillance of SARS-CoV-2 in wastewater in Rio de Janeiro, Brazil

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ABSTRACT

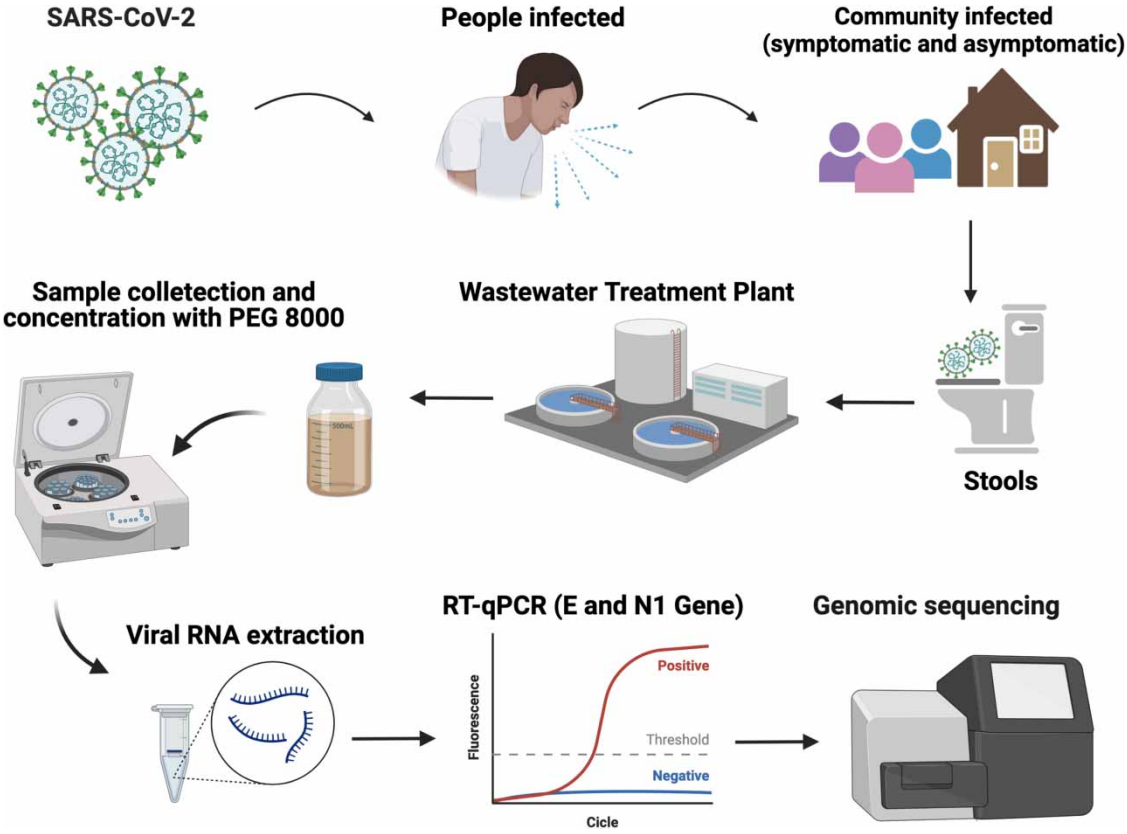
Wastewater-based epidemiology can be a complementary approach for monitoring SARS-CoV-2 prevalence, diversity, and geographic distribution. It is a complementary approach regarding its prevalence and diversity, and geographic distribution. The study aimed to evaluate the genetic diversity of SARS-CoV-2 in two wastewater treatment plants (WWTPs) in Rio de Janeiro, Brazil. Samples were collected over a period of January to December 2021 and were concentrated with PEG8000 and the presence of SARS-CoV-2 was detected using *E* and *N1* genes. Partial sequencing of the SARS-CoV-2 genomes resulted in the identification of variants of concern and variants of interest throughout the collection period. It was possible to identify the Mu, Delta, Gamma and Omicron variants in WWTP1; on the contrary, no variants were observed in WWTP2. To the best of our knowledge, we detected the variant Mu (B.1.621) containing characteristic mutations (S:E484K, S:N501Y) from WWTP, for the first time, in Brazil. Another Mu variant detected from clinical surveillance was announced one month after our finding. The detection of SARS-CoV-2 in wastewater can serve as a tool to monitor the prevalence and epidemiology in each community, helping to understand the spread of the virus among the population.

Key words: SARS-CoV-2, environmental genomic surveillance, wastewater-based epidemiology

HIGHLIGHTS

- Sequencing analysis showed the circulation of the Mu variant from the wastewater one month before being detected in the clinical surveillance in Rio de Janeiro city.
- Wastewater-based epidemiology (WBE) revealed Delta variant AY.32, Mu, B.1618, B.1469, XD presenting characteristic mutations by the WHO.
- The potential for WBE to track the emergence and spread of variants of concern in wastewater before being detected through clinical surveillance in Rio de Janeiro city.

GRAPHICAL ABSTRACT



INTRODUCTION

The coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified for the first time in Wuhan, China at the end of 2019 and on 11 March 2020, the World Health Organization declared COVID-19 a pandemic (la Rosa *et al.* 2020). SARS-CoV-2 is an enveloped, positive-stranded RNA virus, with a spiky crown-shaped surface and belongs to the Coronaviridae family; other members of the family such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1) emerged in China in 2002, and Middle East Respiratory Syndrome (MERS-CoV) emerged in Saudi Arabia in 2012 (Algaissi *et al.* 2020; Magro *et al.* 2020; Mordecai & Hewson 2020; Girón-Navarro *et al.* 2021).

While primarily infecting lung cells, SARS-CoV-2 can infect the gastrointestinal tract as well (Kitajima *et al.* 2020). Alongside the usual symptoms of COVID-19, including fever, dry cough, dyspnea and loss of smell, some studies indicate that patients may also display symptoms associated with the gastrointestinal tract (Li *et al.* 2020). Detection of viral RNA in the stool of both symptomatic and asymptomatic individuals implies that SARS-CoV-2 can be excreted in stool and other bodily secretions, and consequently transported to wastewater treatment plants (WWTPs) or indirectly into water resources (Kitajima *et al.* 2020; Xiao *et al.* 2020). The SARS-CoV-2 RNA has been detected in wastewater samples in several countries, including Italy, Japan, Netherlands, Australia, Spain, United States and Brazil (Ahmed *et al.* 2020; la Rosa *et al.* 2020; Medema *et al.* 2020; Randazzo *et al.* 2020; Sherchan *et al.* 2020; Fongaro *et al.* 2021). Increased circulation of the virus in the population enhances the viral load in sewage systems, as well as the risk of transmission of pathogens from humans to the aquatic environment (Achak *et al.* 2021; Black *et al.* 2021).

In the context of the COVID-19 pandemic, wastewater-based epidemiology (WBE) is being applied for the detection of SARS-CoV-2 discharged into sewage via feces (Kitajima *et al.* 2020). The WBE has also been employed in previous cases to screen for infectious diseases, monitor illicit drug use and address other health concerns (Zuccato *et al.* 2008; Farkas *et al.* 2020). WBE provides a non-invasive and cost-effective way to track the spread of disease and has become increasingly

important in the context of the ongoing COVID-19 pandemic (Cervantes-Avilés *et al.* 2021). It allows detection of SARS-CoV-2 in wastewater samples before they are clinically screened and can be used to estimate the dissemination virus in each region (Wu *et al.* 2020). SARS-CoV-2 was detected as early as 27 November 2019, 56 days in advance of the first confirmed COVID-19 case in the Americas (in the USA) (Fongaro *et al.* 2021). Medema *et al.* (2020) observed the presence of SARS-Coronavirus-2 RNA in sewage in the early stage of the epidemic in the Netherlands. In the same way, SARS-CoV-2 has been repeatedly detected in human sewage since January 2020 in Barcelona, Spain (Chavarria-Miró *et al.* 2021).

Several studies have shown that SARS-CoV-2 RNA can persist in wastewater for several days, even after patients have recovered from COVID-19 (Wu *et al.* 2020; Robinson *et al.* 2022). This means that WWTPs may serve as a source of ongoing exposure to the virus, especially in communities with high rates of COVID-19 transmission (Gholipour *et al.* 2021; Girón-Navarro *et al.* 2021). With the onset of the pandemic and the emergence of SARS-CoV-2 variants, the scientific community began to track and monitor genomic alterations present in the virus through Genomic Surveillance (Cyranoski 2021). Whole-genome sequencing followed by bioinformatic analysis are essential to understand the factors affecting SARS-CoV-2 occurrence, including genetic mutations and new variants description (Lauring & Hodcroft 2021).

This study applied wastewater surveillance to investigate the presence of SARS-CoV-2 from hospital and mixed wastewater effluents between January and December 2021. For this purpose, we evaluate the genetic diversity of SARS-CoV-2 in raw and treated sewage from two WWTPs. Sewage samples concentrated by PEG8000 were analyzed by RT-qPCR using primers toward genes *E* and *N1*. Although we were not able to assemble whole genomes in our samples, interpretation in data analysis software resulted in possible variants of concern (VOCs) such as Delta, Gamma, Omicron and Mu, according to characteristic mutations. The data obtained in the present study suggest that genomic surveillance could be considered an innovative approach capable of revealing SARS-CoV-2 variants in wastewater samples.

MATERIALS AND METHODS

Wastewater sampling and concentration

Weekly samples were obtained from two WWTPs, between January and December 2021. The WWTP1, localized in a hospital in Rio de Janeiro city, exclusively receives effluents from hospitals and has a tertiary treatment system, with biological treatment using the Moving Bed Biofilm Reactors (MBBR) process followed by disinfection with sodium hypochlorite. Meanwhile, the WWTP2, localized in a research institution in Rio de Janeiro city, receives effluents composed of a mixture of laboratory and domestic wastewater. This plant has a biological treatment system at the secondary level with the activated sludge process, extended aeration variant and has the following units: grating, raw sewage lift, desander, aeration tank, secondary decanter, sludge recirculation lift, digester tank and drying beds. Through sampling consisting of 50 mL aliquots every 1 h, 500 mL was collected from each point (affluent and effluent). The samples were transported under refrigeration to the laboratory and processed within 24 h.

Viral concentration

The samples were filtered using a 0.22-μm syringe filter to remove probable bacterial cells and other debris (Wu *et al.* 2020; Nour *et al.* 2021). Then, 4 g of PEG 8000 (10% w/v, Sigma) and 0.9 g of sodium chloride (NaCl) were added in an aliquot of 40 mL of the filtrate. The samples were homogenized to dissolve the solutes and then subjected to centrifugation at 15,000*g* for 2 h at 4 °C. After centrifugation, the supernatant was discarded and the pellet was resuspended with 1 mL of saline-phosphate buffer solution (0.1M PBS, pH 7.2) (Lu *et al.* 2020; Wu *et al.* 2020).

SARS-CoV-2 detection

The extraction of RNA viral was performed using the Bio-Gene DNA/RNA extraction of Viral Kit (Bioclin) according to the protocol of the manufacturer. The RNA concentration was measured using Nanodrop (Thermo Fisher Scientific) to assess the quality of the genetic material extraction process. For the detection of SARS-CoV-2 through real-time reverse transcription polymerase chain reaction (RT-qPCR), the molecular kit SARS-CoV-2 (Biomanguinhos) was used for detection of the *E* gene using the primers E_Sarbeco_F (5′-ACAGGTACGTTAATAGTTAATAGCGT-3′), E_Sarbeco_R (5′-ATATTGCAGCAGTACGCACACA-3′) and probe E_Sarbeco_P1 (5′-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ-3′) (Corman *et al.* 2020). Thus, the *E* gene was the initial marker used to detect SARS-CoV-2 and the *N* gene was used to confirm that the sample would not be a false negative. Negative samples for the *E* gene were subjected for amplification of the *N1*

gene using primers 2019-nCoV_N1 (5′-GACCCCAAAATCAGCGAAAT-3′), 2019-nCoV_N1-R (5′-TCTGGTTACTGCCAGTT-GAATCTG-3′) and probe 2019-nCov_N1-P (FAM-ACCCCGCAT/ZEN/TACGTTTGGTGGACC-3IABkFQ) (CDC 2020).

Viral genome sequencing and bioinformatics analysis

The positive samples for SARS-CoV-2 were sequenced on the MiSeq platform (Illumina) at the National Institute for Quality Control in Health (INCQS) using the CovidSeq kit (Illumina) as described by the manufacturer's instructions. The libraries were sequenced on a V2-type cartridge for 300 cycles according to kit protocols. The kit was used as described by the manufacturer's instructions. The libraries were sequenced on a V2-type cartridge for 300 cycles according to kit protocols. The Fastp was used to trim reads with a cutoff >30 quality Phred Score (Chen *et al.* 2018). The trimmed reads were assembled using SPAdes through pipeline coronaSPAdes, a special mode of rnaviralSPAdes specifically aimed for SARS-CoV-2 *de novo* assembly (Meleshko *et al.* 2022). For lineage classification, the output FASTA file from coronaSPAdes was submitted to Next-Clade (https://clades.nextstrain.org) and Pangolin (Phylogenetic Assignment of Lineages of Named Global Outbreaks) (https://github.com/cov-lineages/pangolin) (Cleemput *et al.* 2020; Rambaut *et al.* 2020). The presence of characteristic mutations in the VOCs and variants of interest (VOIs) obtained in our study were confirmed on the website (https://outbreak.info/) in accordance with the WHO recommendation (WHO 2022).

Availability of sequence data

The sequence sets were deposited in GenBank under BioProject PRJNA950421.

RESULTS

Viral concentration and detection

Out of 152 wastewater samples, 76 were from WWTP1 (38 from the affluent and 38 from the effluent) and 76 from WWTP2 (38 from the affluent and 38 from the effluent). In WWTP1, 47.4% (36/76) of the samples presented the *E* gene (22 in the affluent and 14 in the effluent). The negative samples for the *E* gene ($n = 40$) were evaluated for the presence of the *N1* gene, where 11.6% (13/40) of the WWTP1 samples presented the *N1* gene (5 in the affluent and 8 in the effluent). In WWTP2, only 5.3% (4/76) of the samples presented the SARS-CoV-2 *E* gene. In contrast, none of the samples presented the *N1* gene. Weekly samples from WWTP1 were grouped according to the month of collection to carry out genomic sequencing (Table 1).

Table 1 | Detection of SARS-CoV-2 throughout the year 2021 at WWTP1

Month	Localization	Detected gene	Monthly average CT
January	Affluent	*E* and *N1*	33.19
	Effluent	*E* and *N1*	35.99
February	Affluent	*N1*	34.06
	Effluent	*N1*	34.22
March	Affluent	*E* and *N1*	32.98
	Effluent	*E* and *N1*	35.31
May	Affluent	*E*	32.31
	Effluent	*E* and *N1*	35.32
June	Affluent	*E*	35.26
	Effluent	*E*	35.84
July	Affluent	*E*	32.33
	Effluent	*E*	34.41
August	Affluent	*E*	34.91
	Effluent	*E* and *N1*	36.54
September	Affluent	*E*	34.14
	Effluent	*E* and *N1*	36.05
December	Affluent	*E*	32.73
	Effluent	*E*	36.11

SARS-CoV-2 genome sequencing

Partial sequencing of the SARS-CoV-2 genomes resulted in the identification of VOCs and VOIs throughout the collection period. It was possible to identify the Mu, Delta, Gamma and Omicron variants in the affluents and effluents of WWTP1 (Table 2); on the contrary, no variants were observed in WWTP2. The table with all suggested variants is available in Supplementary Table S2.

DISCUSSION

WBE could become an extremely crucial tool in the management of public health during and after the COVID-19 pandemic, capable of obtaining epidemiological information and signaling risk factors for the environment and public health (Cervantes-Avilés *et al.* 2021). This requires the application of effective methods in the concentration and detection of SARS-CoV-2 in wastewater (Ahmed *et al.* 2020). The advancement of molecular approaches, such as RT-qPCR, allowed obtaining information on the presence of viruses in different environments, including wastewater (Haramoto *et al.* 2018). It is important to note that many assays were aimed at clinical samples and, therefore, may have limitations when performed in environments containing inhibitors, in addition to the presence of other viruses (Ahmed *et al.* 2022).

Although there is no consensus on the best targets, the detection of SARS-CoV-2 has been carried out using the *N*, *E*, *ORF*, *M* and *S* genes (CDC 2020; Corman *et al.* 2020; Vogels *et al.* 2020). In the present study, the SARS-CoV-2 was detected by *E* and *N1* genes in wastewater samples. Meanwhile, in Spain's sewage, the assay suggested by CDC/USA using the detection of the *N1* gene outperformed the assay toward *M* gene by Charité Institute (Kim *et al.* 2020; Pérez-Cataluña *et al.* 2021). On the other hand, another study by Corman *et al.* (2020) reported that analyses using the *E* gene were superior to analyses using the *N* gene.

Table 2 | Suggested variants of concern after analysis of sequencing of SARS-CoV-2 of WWTP1

Collect month	Collect point	Variant suggested by databases	Gene:Mutation
January	Affluent	XK	ORF1b:A854R, ORF1b:Y954A
March	Affluent	P.1 (Gama)	ORF1a:G3846P, ORF1a:G3848V
		B.1.618	N:A119S, ORF9b:Y42N
		XBJ (BA.2.3.20 + BA.5.2)	ORF1a:A1708S, ORF1a:N1709P, ORF1a:F1710A, ORF1a:C1711L
		AY.34.1.1 (Delta)	ORF1a:T609V, ORF1a:Q611P, ORF1a:W612S, ORF1a:L613K, ORF1a:T614K
		AY.22 (Delta)	ORF3a:G224C, ORF3a:S253P
		XA (B.1.1.7 + B.1.177)	ORF1b:D249N, ORF1b:P314L, ORF1b:L379S, ORF1b:L380N
May	Affluent	BF.24 (Omicron)	ORF1a:D629L, ORF1a:W630G, ORF1a:E632L, ORF1a:K636A, ORF1a:E637*, ORF1a:G638V, ORF1a:V639G, ORF1a:E640Q
		XD (Delta + BA.1)	ORF1b:M592K, ORF1b:L593S, ORF1b:K594E, ORF1b:T595S, ORF1b:Y597*, ORF1b:D599S, ORF1b:E601V
		B.1.621 (Mu)	S:E484K, S:N501Y
June	Affluent	B.1.469	ORF1b:K2557R
		P.1 (Gama)	ORF3a:S253P
August	Affluent	XBA (BA.2 + AY.45)	ORF7a:A106L, ORF7a:I107G, ORF7a:V108N, ORF7a:F109A, ORF7a:I110P, ORF7a:T111S, ORF7a:C113A, ORF7a:F114P, ORF7a:T115R, ORF7b:L6R, ORF7b:T40I
		AY.99 (Delta)	ORF1b:G662S
		AY.32 (Delta)	ORF1a:V2930L
		AY.99.2 (Delta)	ORF1a:F1642*, ORF1a:L1643M, ORF1a:N1662S, ORF1a:G1663P, ORF1a:L1664V
September	Affluent	XD (Delta + BA.1)	N:D63G, ORF9b:T60A
December	Affluent	XD (Delta + BA.1)	N:D63G, ORF9b:T60A, ORF8:S69L
		XAA (BA.1+BA.2)	ORF1a:K856R, ORF1a:A903V
		BF.24 (Omicron)	ORF1a:D629L, ORF1a:W630G, ORF1a:E632L, ORF1a:K636A, ORF1a:E637*, ORF1a:G638V, ORF1a:V639G, ORF1a:E640Q, ORF1a:V774S, ORF1a:E775T, ORF1a:P777S
		XAW (BA.2 + AY.122)	ORF1a:A1306S
		A.28	ORF3a:S171L, ORF3a:T170K
		XBA (BA.2 + AY.45)	M:I82T
		BA.5.2.41 (Omicron)	ORF1a:S2661H, ORF1a:D2662L
		AY.33.1 (Delta)	ORF1b:H1807R, ORF1b:F1764L

Genes:Mutations marked in red represent the characteristic mutations of variants deposited in databases (https://outbreak.info/).

In the present study, it was possible to detect SARS-CoV-2 in WWTP1 during all analyzed months of 2021. This plant exclusively receives effluents from hospitals and performs tertiary treatment with MBBR followed by disinfection with sodium hypochlorite. Although MBBR treatment is considered an efficient procedure in reducing SARS-CoV-2 RNA, it does not guarantee the complete removal of viral RNA fragments (Kostrytsia *et al.* 2022). The WWTP2, which presented SARS-CoV-2 only in January 2021, receives mixed sewage and secondary treatment, where there is sludge formation, which can lead to low RNA concentration in the liquid part and high concentration in the solid part. Westhaus *et al.* (2021) revealed that the RNA of SARS-CoV-2 was detected in the sludge, while the aqueous phase was negative, suggesting a possible adhesion of viral particles to the sedimented solids. It is important to emphasize that virus detection is correlated with the prevalence of the number of cases of COVID-19 infection in each location (Medema *et al.* 2020). These aspects associated with the lockdown in 2021 probably contributed to the sharp reduction in SARS-CoV-2 detection in WWTP2 samples. Furthermore, although viruses can remain in the aquatic environment by adhering to suspended solids in the environment, factors such as temperature, pH change and the presence of other microorganisms can also affect viral detection (Rollemberg *et al.* 2020).

Viral genetics and evolution have been the main topics investigated since the first published Sars-CoV-2 genome, which boosted the investigation of the viral genome through sequencing (Zhu *et al.* 2020). The data generated in this study, based on the genomic sequencing of WWTP1 samples, resulted in the recovery of partial genomes, which enabled the identification of characteristic mutations of different SARS-CoV-2 variants, based on database analyses (Table 2). It is necessary to consider that the SARS-CoV-2 present in wastewater may be inserted into a complex set of genomic RNA of circulating variants in each community. This RNA may be present in an intact or fragmented viral capsid in the environment and may make the recovery of genomes unfeasible (Wurtzer *et al.* 2020; Robinson *et al.* 2022). On the other hand, a clinical sample may contain numerous RNA copies of a given SARS-CoV-2 variant.

In the present study, it was possible to detect suggestive mutations of the Delta, Gamma and Omicron VOCs. The Gamma (P.1) variant was detected in genomes of clinical samples from December 2020 to August 2021 (Fiocruz 2022). However, this variant was only detected in WWTP1 in March and June, a period in which there was an increase in the number of clinical genomes deposited in the Fiocruz Genomic Network (FGN) (Fiocruz 2022). The Delta (AY.99.2) variant was revealed in August 2021 (Table 2), where an increase in clinical genomes deposited from the FGN was also observed (Fiocruz 2022). Lamarca *et al.* (2022) revealed that over 98% of clinical genome sequences from the state of Rio de Janeiro originated from a single introductory event of the AY.99 lineage, which diverged into AY.99.1 and AY.99.2 lineages between May and June 2021. Both strains spread across the state from this point onwards, with AY.99.2 being dominant. Another Delta variant, AY.32 detected in wastewater, in August, presented the mutation ORF1a:V2930L, described as a characteristic mutation by the WHO (WHO 2022). In addition to the Delta variant AY.32, other variants Delta, Mu, B.1618, B.1469, XD (recombinant variant Delta + BA.1) and XBA (Table 2) also showed characteristic mutations. Another significant finding was the detection of the Mu (B.1.621) variant containing characteristic mutations described by the WHO (S:E484K, S:N501Y) in the WWTP1 entry, in May 2021. In June 2021, a Mu variant, containing the same mutations, recovered from a clinical sample and had its genome deposited in the GISAID database. It is important to point out that the WWTP1 sample comes from the effluent of the same hospital where the clinical variant B.1.621 was found. These data allow us to suggest that this variant was present in wastewater one month before it was detected in the clinical sample. As in the present study, Karthikeyan *et al.* (2022) detected the B.1.621 variant through genomic surveillance of wastewater approximately 4 weeks before its first detection through clinical genomic surveillance. It was possible to detect mutations suggestive of the Omicron variant in May 2021 in our wastewater samples, the variant in question was only found in clinical samples in Brazil in December 2021 (Fiocruz 2022).

The continued evolution of SARS-CoV-2 and the emergence of VOCs such as Omicron VOC highlight the importance of maintaining an active vigilance for the emergence of unexpected new variants (Callaway 2021; Martin *et al.* 2021). The fact that the origins and initial spread of VOCs Alpha and Omicron have not been observed justifies efforts to detect and monitor new variants (Herold *et al.* 2021). However, as stated earlier, genome-wide sequencing of SARS-CoV-2 isolated from wastewater often suffers from low sequencing depth of coverage in epidemiologically relevant areas of the genome, such as the Spike Receptor Binding Domain (RBD) (Fontenele *et al.* 2021; Swift *et al.* 2021). Furthermore, as wastewater may contain a mixture of viral lineages and whole-genome sequencing depends on sequencing small genome fragments, computational strategies to identify variants with linked mutations often fail to identify lineages present at low concentrations (Baaijens *et al.* 2022). To address these issues, researchers have developed a 'targeted' sequencing approach that amplifies and sequences the RBD region of the Spike protein of the SARS-CoV-2 genome as a single fragment (Gregory *et al.* 2021;

Smyth *et al.* 2022). The RBD region is relevant sequencing coverage. A recent study described a set of unknown lineages from various locations in the United States. While wastewater has its own lineages, the study provided evidence that some lineages could have shared the same common ancestor (Gregory *et al.* 2021). It is important to emphasize that in the present study, unknown lineages not reported in environmental and/or clinical samples in global databases were also revealed, despite some difficulties and limitations in the annotation and interpretation of SARS-CoV-2 genomes. Another study, also using this same approach, demonstrated that the frequencies of variants in wastewater closely followed VOC frequency estimates from clinical sampling in the same areas (Gregory *et al.* 2021; Smyth *et al.* 2022). However, in some locations, the presence of unknown strains not seen in clinical specimens anywhere in the world has been demonstrated. Several of these strains contained amino acid substitutions rarely reported in global databases such as GISAID (N460K, Q493K, Q498Y and N501S) (Smyth *et al.* 2022). Changes in circulating SARS-CoV-2 VOCs may require changes in public health responses to the COVID-19 pandemic, as they have the potential to evade vaccines and pharmaceutical interventions and may be more transmissive than other SARS-CoV variants. As such, it is essential to track and prevent its spread in susceptible communities (Wurtz *et al.* 2021).

The data obtained in this study reinforce the idea that genomic surveillance of Sars-CoV-2, through WBE, can be considered a promising and innovative approach capable of providing important information about the persistence of the virus in environmental samples, in addition to enabling the investigation of the emergence and circulation of variants even before their revelation in patients infected with COVID-19. The WBE has been applied to the surveillance of SARS-CoV-2 and its variants, including the monitoring of outbreaks and the assessment of the effectiveness of public health interventions (Ahmed *et al.* 2022).

CONCLUSIONS

- The detection of a possible B.1.621 (Mu) variant one month before the same variant was recovered from a clinical sample demonstrates the importance of this tool in investigating the emergence and circulation of variants even before their disclosure in patients infected with COVID-19.
- The sequencing of wastewater for SARS-CoV-2 surveillance resulted in small fragments that did not allow assembly of complete SARS-CoV-2 genomes, considering low viral loads, highly fragmented RNA and PCR inhibitors in complex environmental samples. However, database analyses revealed genetic mutations indicative of possible VOCs and VOIs.
- The data obtained in this study reinforce the idea that genomic surveillance of SARS-CoV-2, through WBE, could become an important tool in public health management during and after the COVID-19 pandemic, capable of obtaining epidemiological information and signaling possible risk factors for the environment and public health.

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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