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RESEARCH ARTICLE

SARS-CoV-2 surveillance-based on municipal solid waste leachate in Brazil

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Abstract

Municipal solid waste leachate-based epidemiology is an alternative viral tracking tool that applies fresh truck leachate as an early warning of public health emergencies. This study aimed to investigate the potential of SARS-CoV-2 surveillance based on solid waste fresh truck leachate. Twenty truck leachate samples were ultracentrifugated, nucleic acid extracted, and real-time RT-qPCR SARS-CoV-2 N1/N2 applied. Viral isolation, variant of concern (N1/N2) inference, and whole genome sequencing were also performed. SARS-CoV-2 was detected on 40% (8/20) of samples, with a concentration from 2.89 to 6.96 RNA Log_{10} 100 mL^{-1}. The attempt to isolate SARS-CoV-2 and recover the whole genome was not successful; however, positive samples were characterized as possible pre-variant of concern (pre-VOC), VOC Alpha (B.1.1.7) and variant of interest Zeta (P.2). This approach revealed an alternative tool to infer SARS-CoV-2 in the environment and may help the management of local surveillance, health, and social policies.

Keywords SARS-CoV-2 · Variant of concern · Fresh truck leachate · Municipal solid waste leachate-based epidemiology · Public health · Epidemiological surveillance

Abbreviations

COVID-19	Coronavirus disease-19
MSW	Municipal solid waste
MSWL-BE	Municipal solid waste leachate-based epidemiology
NGS	Next-generation sequencing
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
VOC	Variant of concern
VOI	Variant of interest
WBE	Wastewater-based epidemiology
WTS	Waste transfer station
WWW-BE	Wastewater, water, and waste-based epidemiology

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Introduction

Coronavirus disease-19 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic on March 11th, 2020 (WHO 2020). Globally, 630,387,858 confirmed cases of COVID-19 have been reported, including 6,583,163 deaths, according to the last update on November 9th, 2022 (WHO 2022b). According to SARS-CoV-2 genomic surveillance, different lineages, variants of interest (VOI), and variants of concern (VOC) have been described (WHO 2022a). Different factors can influence the dynamics of COVID-19 transmission by aerosol or droplets, such as biological characteristics of the

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virus, incubation period, virus interaction with air pollution, meteorological conditions, viral infectivity, interpersonal contact rate, population density, and host characteristics such as socioeconomic status, occupational exposure, habits, immune system, age, and sex (Coccia 2020).

Non-pharmacological measures to decrease SARS-CoV-2 human-to-human transmission included quarantine, lockdown, social and workplace distancing, school and store closures, and the use of masks (Coccia 2020), important to control the virus spread and consequently increased household wastewater and waste generation (Filho et al. 2021; Iyer et al. 2021; Jia et al. 2022). Since individuals who are presymptomatic, asymptomatic, or presenting mild symptoms in home isolation can also eliminate SARS-CoV-2 in feces, the presence of viral RNA in wastewater and solid waste can be applied for virus surveillance (Chen et al. 2020; Kumblathan et al. 2023).

Different approaches have been proposed for SARS-CoV-2 pandemic preparedness and control (Coccia 2021, 2022a, b). Wastewater-based epidemiology (WBE) is a useful tool to track SARS-CoV-2 and support public health, especially in developing countries (Barrios et al. 2021; Fongaro et al. 2021; Prado et al. 2021; Rosiles-González et al. 2021). This approach is based on the hypothesis that the concentration of SARS-CoV-2 eliminated by the feces of a population can be estimated on the virus quantification in the wastewater after normalization (Langeveld et al. 2023). Data generated by wastewater surveillance can serve as an additional tool to support decision-making in public health (Prado et al. 2023; Zhao et al. 2023).

Recently, a new integrative approach including epidemiology based on wastewater, raw untreated water, and solid waste, named WWW-BE, has been proposed for low-income settings (Gwenzi 2022). Previously, solid-waste-based epidemiology using swabs from saliva residues present in disposable waste was described as an alternative method for SARS-CoV-2 environmental monitoring (Di Maria et al. 2021), and the application detection of SARS-CoV-2 in solid waste leachate was also described (Mondelli et al. 2022).

Different types of solid waste, such as toilet paper, disposable diapers, and human excreta, can carry microorganisms to the solid waste leachate (Gerba et al. 2011). Fresh truck leachate is the liquid collected from the basin of the waste collection truck (Saadoun et al. 2021), which depends on the moisture content of the waste, the amount of precipitation, and the compaction process (Abouri et al. 2016, Shatkin et al. 2005). This study aimed to investigate the potential of SARS-CoV-2 surveillance based on solid waste fresh truck leachate. For this purpose, we analyzed the occurrence, concentration, infectivity, and molecular characterization of SARS-CoV-2 in this matrix.

Materials and methods

Sample, data, and research settings

Leachates from municipal solid waste (MSW) were collected from a waste transfer station (WTS) located in the city of Rio de Janeiro, State of Rio de Janeiro State, Brazil. The WTS service area is represented by Jacarepaguá, Cidade de Deus, and Barra da Tijuca, with approximately 900,000 inhabitants and 19 different neighborhoods, represented by the colored area according to the number of inhabitants, varying from yellow to red intensity on the geographic map (Fig. 1). Twenty samples with 200 mL of fresh truck leachate were collected from November 2020 to January 2021, after manual household collection, in order of arrival in the WTS directly from the basin of different MSW trucks and representing different neighborhoods. All samples were collected in sterile polyethylene bottles, transported to the laboratory at 4 °C, and processed in 24 h.

Measures of variables

A total of 42 mL of fresh truck leachate samples were ultracentrifuged in a Sorvall® WX Ultra Centrifuge Series (Thermo Scientific, Waltham, MA, USA) according to a previous study (Lanzarini et al. 2020) and stored at − 80 °C until nucleic acid extraction.

Nucleic acids were extracted by the QIAamp Fast DNA Stool Mini Kit® (Qiagen, Valencia, CA, USA), which has an InhibitEX Buffer for PCR inhibitor removal and can be used for both DNA and RNA extraction (Monteiro et al. 2022). SARS-CoV-2 RNA was detected and quantified by real-time RT-qPCR using primers and probes targeting the nucleocapsid gene targets N1 and N2, according to CDC protocols (Lu et al. 2020). Viral loads were estimated using standard curves with tenfold serial dilutions from 10^5 to 10^1 genomic copies (GC) per reaction for N1 ($y = -3.672x + 37.996$) and N2 ($y = -3.533x + 41.627$) containing the SARS-CoV-2 amplification region sequence. The samples showing cycle threshold (Ct) values below 40 cycles were considered positive, therefore the limit of detection was 2.065 RNA Log_{10} 100 mL^{-1} (N1) and 3.071 RNA Log_{10} 100 mL^{-1} (N2). Reactions were applied with the SuperScript™ III Platinum™ One-Step RT-qPCR Kit (Invitrogen, Carlsbad, CA, EUA) on TaqMan® System (2019-nCoV RUO Kit, Integrated DNA Technologies, Coralville, IA, USA) and ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Amplifications were tested in duplicate (undiluted and diluted 10^{-1}) samples totalizing four wells, and positivity was assumed at least two wells tested for each sample. SARS-CoV-2 viral concentration was expressed as GC per 100 mL. Quality controls

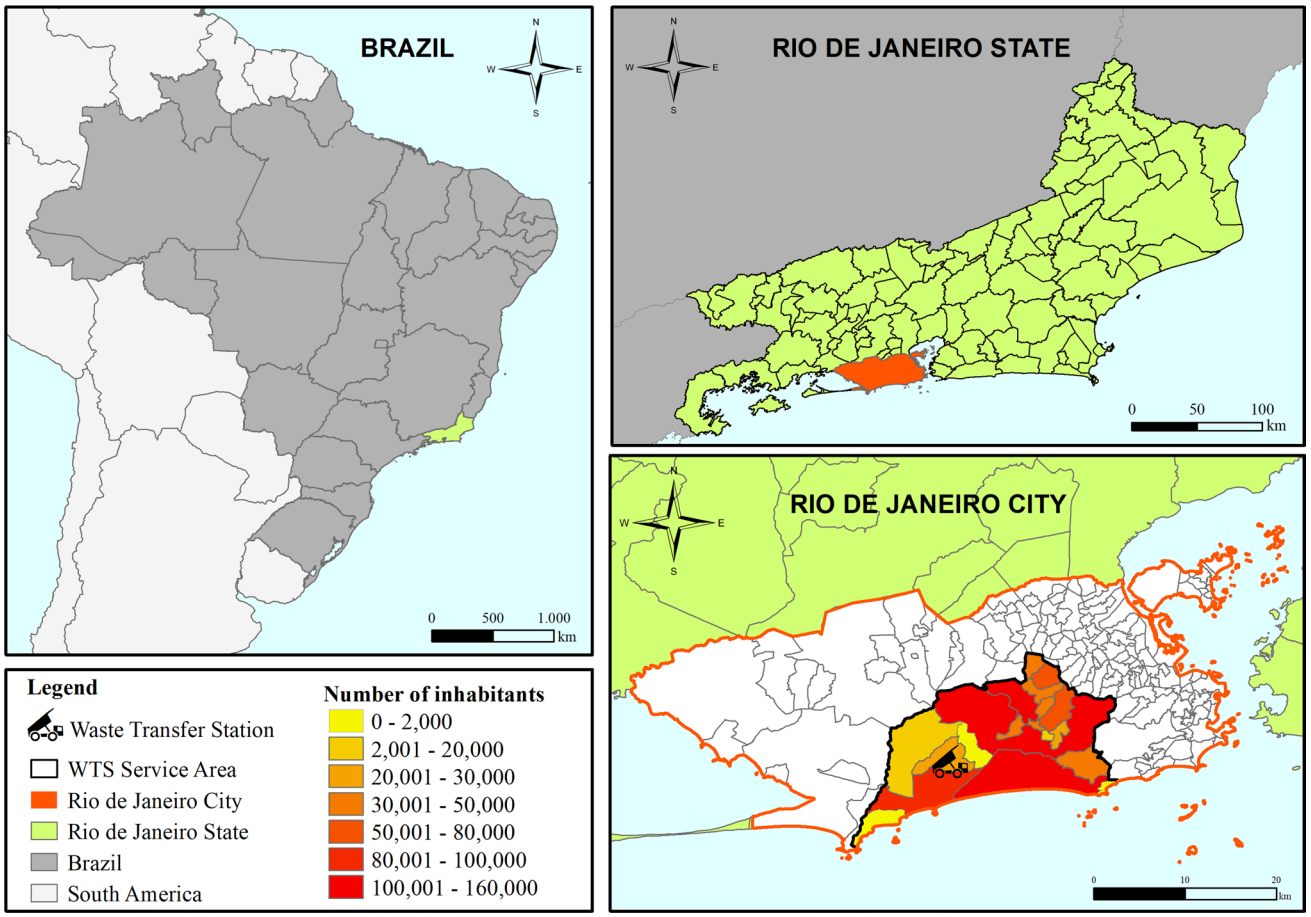

Fig. 1 Geographic map of the city of Rio de Janeiro (orange line), state of Rio de Janeiro (green filled), and Brazil (gray filled). The study area is a waste transfer station (WTS) represented by the truck symbol and respective colored WTS service area, with the number of inhabitants varying in intensity from yellow (less) to red (more)

included non-template control (NTC), positive (2019-nCoV RUO Kit, Integrated DNA Technologies), and negative controls (DNA/RNAse-free water), and the use of different rooms for master mix preparation, nucleic acid extraction, qPCR, and product analysis to prevent cross-contamination.

For detection of SARS-CoV-2/VOC, positive samples were analyzed by multiplex real-time RT-qPCR 4Plex SC2/VOC (Bio-Manguinhos, Fiocruz), Ministry of Health registration number 80142170057. The kit consists of RT-PCR detection of specific targets for detection at the SARS-CoV-2 nucleocapsid (N) gene and human constitutive gene RNAseP (RP), and for the VOCs inference at the ORF1a gene NSP6 target S106, G107 and F108 region, which suffer the deletions (106-108Del NSP6) and spike gene target failure (SGTF) the H69 and V70 regions, which suffer the deletions (69–70 Del), the combination of this absence or presence of this deletion allows infer the VOCs: Alpha and Omicron BA.1/BA.1.*, BA.4 and BA.5/BA.5.* (106-108Del NSP6 and 69-70Del Spike present), Beta, Gamma and Omicron BA.2/BA.2.* (106–108Del NSP6 present and 69–70Del Spike absent), Pre-VOC lineages and Delta (106-108Del NSP6 and 69–7 0 Del spike absent). The regions were amplified using specific primers and TaqMan probes with reporter fluorophores described below: gene N-FAM, gene RP-CY5, gene NSP6-ROX, and gene Spike-HEX/VIC. The RT-PCR reaction was performed in the 7500 Real-Time PCR System equipment. The conditions consist of 1 cycle of 15 min at 50 °C, 2 min at 95 °C, followed by 40 cycles of 20 s at 95 °C, and 30 s at 61 °C. Briefly, 10 µL of extracted RNA was added to 4.4 µL of PCR Mix and 5.6 µL SC2/VOC Mix. The result is valid with a threshold > 50,000 and Ct values ≤ 40.0. The interpretation of the result consists of verifying the presence or absence of the deletion together with the epidemiological data at the time of sample collection.

For SARS-CoV-2 sequencing, the genomes were amplified, and the library was constructed by the Illumina COVIDSeq Test next-generation sequencing (NGS) approach using Illumina set of primers ARTIC v4 and additionally the adaptations performed by Fiocruz surveillance genomic network (Naveca et al. 2021). High-throughput sequencing

of RNA-positive SARS-CoV-2 samples was performed on the Illumina MiSeq platform with the MiSeq Reagent Kit v2-Micro (150 × 2 bp, paired-end) and on the Illumina NextSeq 1000/2000 platform with NextSeq 1000/2000 P2 Reagents 200 Cycles-v3 (100 × 2 bp, paired-end) (Illumina, San Diego, CA, USA).

Genome assembly was carried out through Viral Flow version 0.0.6 (https://github.com/dezordi/ViralFlow), an automated workflow for SARS-CoV-2 genome assembly (Dezordi et al. 2022). Molecular characterization was performed using the PANGO Lineage version 4.0.6 for lineage assignment (https://pangolin.cog-uk.io/) (O'Toole et al. 2021) and Nextclade version v.0.14.2 (https://clades.nextstrain.org/) (Aksamentov et al. 2021).

RT-qPCR SARS-CoV-2 positive leachate samples were processed for viral isolation in cell culture in a biosafety level 3 facility (BSL3, Pavilhão Hélio Peggy Pereira, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil). Vero cells E6 were cultured at 37 °C in a humidified incubator with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, USA), 0.25% sodium bicarbonate (Sigma-Aldrich, USA) and 40 mg/mL gentamicin (Gibco, Thermo Fisher Scientific, USA), for viral passage. For isolating infectious virus from leachate sample, 200 µL aliquots were inoculated on Vero E6 cells seeded at cells 40,000 cells/cm2 in T-25 flasks 24 h before the inoculation. After 1 h of incubation with periodic shaking, 5 mL of DMEM medium was added to culture cells and checked daily for both cytopathic effects (CPE) under a microscope and viral load by RT-qPCR using the molecular kit 4Plex SC2/VOC (Bio-Manguinhos, Fiocruz). For each sample, isolation was attempted in a maximum of three consecutive blind passages.

Data analysis procedure

Maps data preparation, conversion, and analyses were performed using ArcGIS commercial software, supported by Environmental System Research Institute (ESRI) (version 10.5). All data in raster and vector format were worked on Sirgas 2000 UTM Zone 23 S projection system. All information used in this research is made available by public, federal, and state agencies.

Graphs and figures were developed using the software GraphPad Prism 8.0. SARS-CoV-2 were log-transformed, and concentrations were described as RNA Log_{10} 100 mL^{-1} GC per mL. For SARS-CoV-2 N1 and N2 comparison, a contingency table was generated on GraphPad Prism 8.0, and a Fisher exact test was applied. Data were considered statistically significant when ρ-value < 0.05.

Results and discussion

SARS-CoV-2 has been detected in different environmental matrices (Anand et al. 2022; Núñez-Delgado et al. 2021). In this work, we investigated the potential of SARS-CoV-2 municipal solid waste leachate-based epidemiology (MSWL-BE) as an early warning tool. Comparing the detection of SARS-CoV-2 RNA nucleocapsid genes N1 and N2, targets were detected by real-time RT-qPCR in 40% ($n = 8/20$) and 15% ($n = 3/20$), respectively. The mean concentration values ranged from 2.89 to 6.96 RNA Log_{10} 100 mL^{-1} for N1 and from 4.07 to 4.93 Log_{10} 100 mL^{-1} for N2 (Fig. 2). In the comparison between the performance of N1 and N2 targets for SARS-CoV-2 detection, although the difference was not statistically significant using Fisher's exact test (p-value = 0.1552), N1 has demonstrated more sensitivity for analyzing fresh truck leachate than N2, with higher detection and quantification. Differences in detection between the targets N1 and N2 were also obtained in leachate samples collected from August to September 2021 at a WTS in the city of São Paulo, Brazil (Mondelli et al. 2022). This is in accordance with previous studies on sewage samples (Fahrenfeld et al. 2021; Pérez-Cataluña et al. 2021). Raw wastewater samples in vulnerable urban communities in São Paulo, Brazil, showed similar concentrations of N1/N2, ranging from 1×10^3 to 1×10^6 GC/L in the same period (Barbosa et al. 2022). N1 quantification range (2.89 to 6.96 RNA Log_{10} 100 mL^{-1}) on fresh truck leachate presented higher concentrations compared with untreated wastewater from Buenos Aires, Argentina (1.00 to 5.00 RNA Log_{10} 100 mL^{-1}), Brisbane, Australia (2.13 to 4.07 RNA Log_{10} 100 mL^{-1}), and Quintana Roo, México (2.25 to 2.87 RNA Log_{10} 100 mL^{-1}) (Ahmed et al. 2021; Barrios et al. 2021; Rosiles-González et al. 2021).

We evaluated N1 viral load in different neighborhoods of the city of Rio de Janeiro, Brazil, and quantification is geographically represented in Fig. 3. The intensity varied

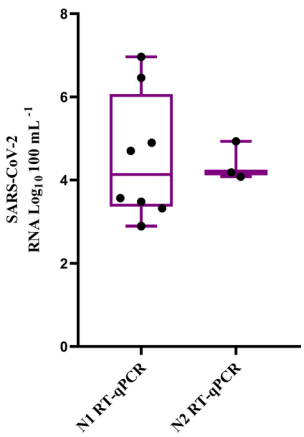

Fig. 2 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA Log_{10} per 100 mL quantification of nucleocapsid genes N1 and N2 by real-time reverse transcription–quantitative polymerase chain reaction (RT-qPCR) in fresh truck leachate at a waste transfer station (WTS) located in the city of Rio de Janeiro, Brazil

according to WTS service area, and in this preliminary analysis, neighborhoods closer to the WTS had a higher N1 RNA concentration than those distant from this location. SARS-CoV-2 RNA monitoring for a larger period is necessary to verify its occurrence. This variation could be influenced by the collection time until arrival at the WTS, population density, meteorological conditions, and physicochemical characteristics of the fresh truck leachate. More studies are needed to confirm this hypothesis. The physicochemical characterization of the fresh truck leachate from this WTS was previously analyzed in 2019, showing an acidic pH, high apparent color, turbidity, total solids (dissolved, suspended, fixed, and volatile), total organic carbon, and chemical oxygen demand (Lanzarini et al. 2022). The influence of household collection time on MSWL-BE could be related to the characteristics of SARS-CoV-2, as enveloped viruses are less persistent in the environment when compared with nonenveloped viruses (Wurtzer et al. 2021). In wastewater, SARS-CoV-2 RNA varied according to fecal shedding, population size, in-sewer factors, including solid particles, organic load, travel time, flow rate, pH, temperature, and sampling strategy (Bertels et al. 2022; Saingam et al. 2023).

4Plex SC2/VOC kit was applied for VOC variants inference and characterized three types of VOC among the eight positive fresh truck samples (Table 1). In our study, we could not recover the SARS-CoV-2 whole genome sequencing; however, using the 4Plex SC2/VOC (Bio-Manguinhos, Fiocruz) inference test, we could detect the possible pre-VOC in November 2020, possible VOC Alpha (B.1.1.7), and possible VOI Zeta (P.2) in January 2021, according to the molecular clinical epidemiology scenario from human nasopharyngeal swab presented on Fig. 4 (data from EpiCoV at GISAID).

In the same period, variant Alpha (B.1.1.7) of RNA SARS-CoV-2 was detected in wastewater treatment plants from different regions of Israel (Bar-Or et al. 2021), England (Wilton et al. 2021), and Spain (Carcereny et al. 2021). To our knowledge, this is the first report about the VOI Zeta

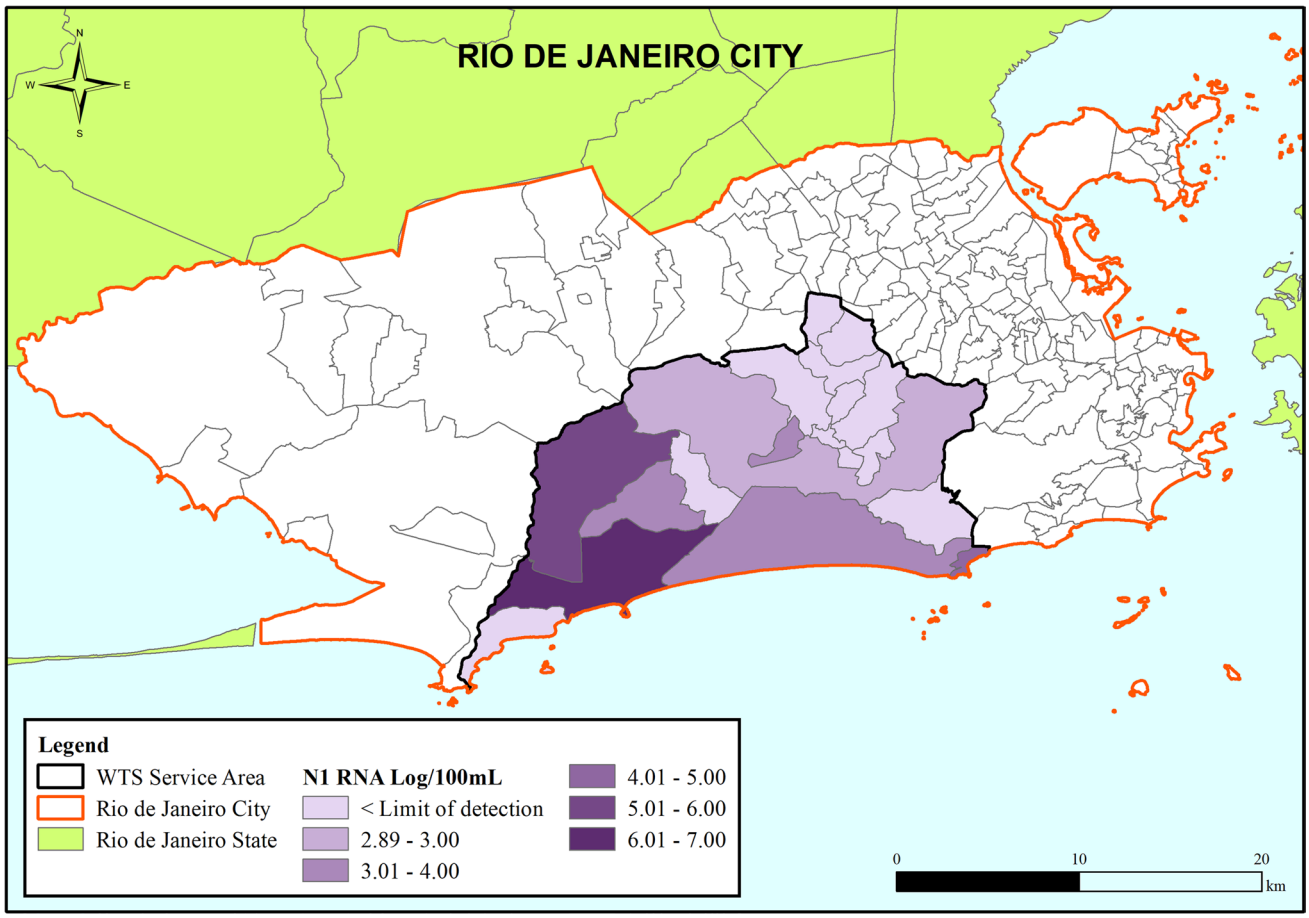

Fig. 3 Geographic representation of real-time reverse transcription–quantitative polymerase chain reaction (RT-qPCR) nucleocapsid gene N1 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA Log_{10} per 100 mL of fresh truck leachate from the waste transfer station (WTS) service area (black outline) located in the city of Rio de Janeiro (orange outline), state of Rio de Janeiro (green filled), and Brazil. The N1 RNA Log/100 mL concentration varied in intensity from light (< limit of detection) to dark purple (6.01–7.00)

Table 1 Possible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages and variants screened by real-time reverse transcription–quantitative polymerase chain reaction (RT-qPCR) SC2/VOC inference from fresh truck leachate samples according to date of collection and neighborhood origin of Rio de Janeiro, Brazil

Date of collection	Rio de Janeiro neighborhood	ORF1a 106-108Del	Spike 69-70Del	Possible SARS-CoV-2 lineage or variant
27th November 2020	Vargem Grande	Detected	Detected	Possible lineages pre-VOC
30th November 2020	Vargem Pequena	Detected	Detected	Possible lineages pre-VOC
18th January 2021	Vargem Grande	Undetected	Undetected	Possible Alpha (B.1.1.7)
18th January 2021	Joá	Detected	Detected	Possible Zeta (P.2)
19th January 2021	Recreio	Undetected	Undetected	Possible Alpha (B.1.1.7)
19th January 2021	Riocentro	Undetected	Undetected	Possible Alpha (B.1.1.7)
19th January 2021	Barra da Tijuca	Undetected	Undetected	Possible Alpha (B.1.1.7)
19th January 2021	Jacarepaguá (Merck)	Detected	Detected	Possible Zeta (P.2)

(P.2) variant detection in environmental matrices. VOI Zeta (P.2), a descendant of the B.1.1.28 strain, was first described in December 2020 in the state of Rio de Janeiro, Brazil, and rapidly spread across the country (Voloch et al. 2021). Different SARS-CoV-2 variants have been detected in wastewater, and since then, different methods have been compared for virus sequencing (Cha et al. 2023; Girón-Guzmán et al. 2023). Recent studies suggest a combination of centralized and decentralized WBE implementation in high-, middle-, and low-income countries for genomic surveillance (Amin et al. 2023; Gonçalves et al. 2022; Jarvie et al. 2023; Prado et al. 2023).

We could not isolate SARS-CoV-2 from fresh truck leachate in cell culture. To date, although SARS-CoV-2 has been detected in fecal samples (Wurtzer et al. 2022) and in some rare cases even infectious (Dergham et al. 2021; Zhang et al. 2020), there is no evidence that SARS-CoV-2 detected in fecal waste, wastewater, and water are infectious, and illness or death has never been reported for this environmental exposure (Brahim Belhaouari et al. 2021). The World Health Organization, Water Environment Federation, US Centers for Disease Control and Prevention, and others do not consider environmental fecal waste exposure to infectious SARS-CoV-2 causing

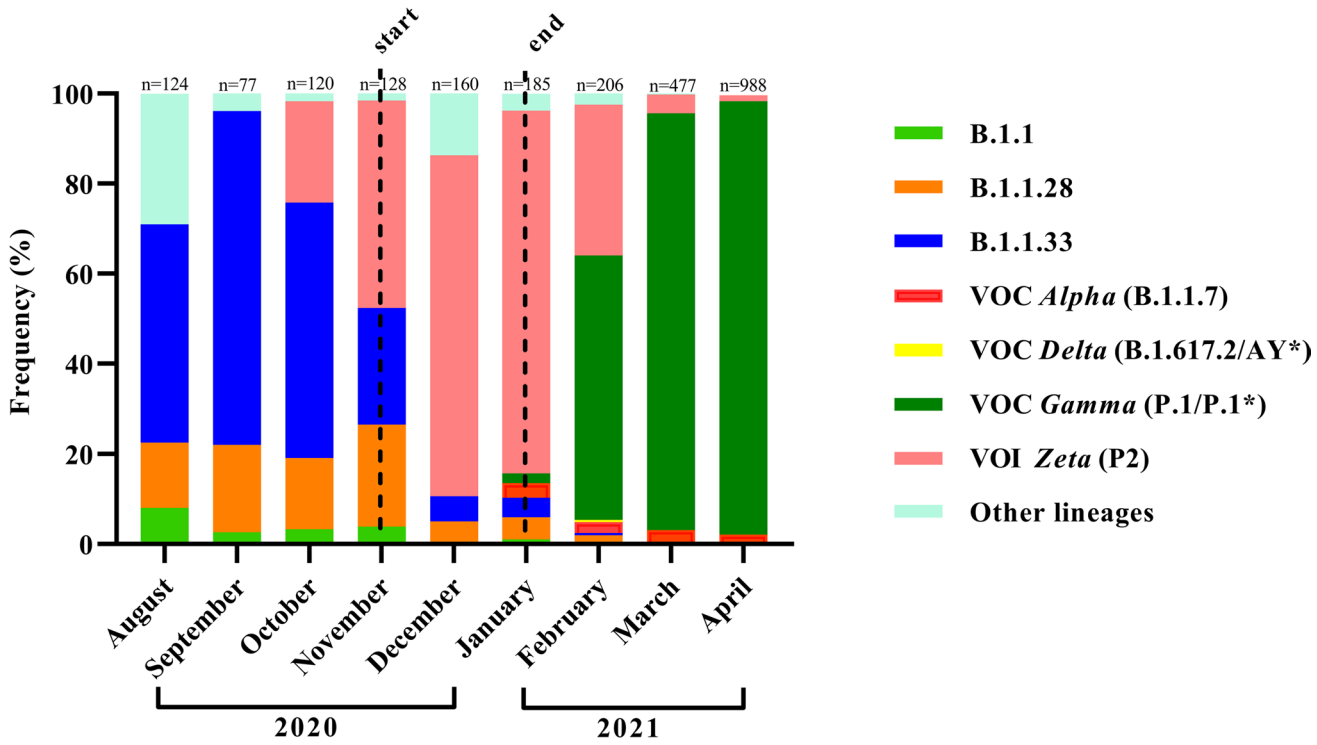

Fig. 4 Molecular epidemiology of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages and variants detected in patients' nasopharyngeal swabs in Rio de Janeiro State from August 2020 to April 2021 (enabled by data from EpiCoV at GISAID). The dashed line indicate the period of beginning and end of fresh truck leachate samples collection

COVID-19 infection and illness (Sobsey 2022), and the most important transmission route of SARS-CoV-2 is airborne spread (McNeill 2022).

Enveloped viruses are less persistent and less resistant to inactivation treatments than naked viruses (WHO 2020). In this study, we also tested real-time RT-qPCR in parallel with the propidium monoazide (PMA) capsid integrity assay (PMA-RT-qPCR) for quantification of intact particles (data not shown). However, we found no significant difference when comparing treated and untreated samples, corroborating the non-infectivity of SARS-CoV-2 fresh truck samples in cell culture. This non-difference is related to the fact that SARS-CoV-2 genomes could be present in different forms: most parts as a ribonucleoprotein complex and as free/unprotected viral RNA, and less than 10% as protected within infectious or non-infectious particles, reinforcing non-infectivity (Wurtzer et al. 2021). Previous studies have already detected infectious HAdV by capsid integrity assay and cell culture infectivity and estimated the quantitative microbial risk assessment for gastrointestinal illness disease (Lanzarini et al. 2022).

Based on the data obtained, it was observed the potential applicability of MSWL-BE, especially in low- and middle-income countries. The association of ultracentrifugation, nucleic acid extraction with QIAamp Fast DNA Stool Mini Kit®, N1 RT-qPCR quantification, and SARS-CoV-2 variant inference with 4Plex SC2/VOC proved to be effective for SARS-CoV-2 surveillance. The fresh truck leachate from solid waste monitoring could be used in future pandemics for local monitoring of pathogens eliminated from feces of infected persons and similar to WBE, applied as a virus surveillance tool.

Conclusions

- MSWL-BE applying the ultracentrifugation method, the QIAamp Fast DNA Stool Mini kit, and the 4PLex SC2/VOC kit proved to be a promising tool for the occurrence, quantification, and SARS-CoV-2 VOC screening in fresh truck leachate from MSW.
- SARS-CoV-2 RNA from fresh truck leachate was detected in 40% (8/20) by real-time RT-qPCR and characterized by SARS-CoV-2 variant screening as possible pre-VOC lineages, VOC Alpha (B.1.1.7) and VOI Zeta (P.2).
- The 4Plex SC2/VOC kit was primarily developed for detection of clinical samples, and to our knowledge, this is the first report applying this commercial kit in environmental matrices.
- We proposed that the MSWL-BE could be an alternative tool for monitoring RNA SARS-CoV-2 in a geographic region at the neighborhood level, especially in developing countries where there is no sewage collection system, and serves as an alert to the importance of solid waste management.
- This surveillance tool could be applied to other microorganisms and opens new perspectives on monitoring public health importance viruses.

This is the first description of a methodology applied to MSWL-BE. We conducted a pilot study, and new monitoring studies in high-, middle-, and low-income countries are needed to test the influence of different variables in detecting SARS-COV-2 in fresh truck leachate. Suggestions about how worldwide nations can prevent the next pandemic include preventive and responsive interventions, focused on different strategies: increase population vaccination, non-pharmaceutical measures of pathogens control, environmental policies focused on environmental protection, wildlife pathogens and biosecurity, prompt-lab diagnostic and news approaches as wastewater and MSWL-BE, investments on health system, and research framework focused on crisis management and social policies for population protection.

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Author contribution Conceptualization: NML, CFM, and MPM. Data curation: NML, CFM, LSV, PCR, and MPM. Formal analysis: NML, CFM, and MPM. Funding acquisition: CFM, MPM, and MMS. Investigation: NML, TP, AVCR, CFM, and MPM. Methodology: NML, TP, AVCR, LSV, and MPM. Project administration: CFM and MPM. Resources: CFM, MPM, and MMS. Software: NML and PCR. Supervision: CFM and MPM. Validation: NML, CFM, PCR, and MPM. Visualization: NML, CFM, PCR, and MPM. Writing—original draft: NML, CFM, PCR, and MPM. Writing—review and editing: NML, CFM, PCR, and MPM.

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Data availability Not applicable.

Declarations

Genetic heritage This article was registered in the "National System for the Management of Genetic Heritage and Associated Traditional Knowledge—SisGen," in compliance with the provisions of Brazilian Law N. 13123/2015 and its regulations, under registration number A1413C9.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interest The authors declare no competing interests.

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