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Delineating the Spread and Prevalence of SARS-CoV-2 Omicron Sublineages (BA.1–BA.5) and Deltacron Using Wastewater in the Western Cape, South Africa

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This study was one of the first to detect Omicron sublineages BA.4 and BA.5 in wastewater from South Africa. Spearman rank correlation analysis confirmed a strong positive correlation between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral RNA in wastewater samples and clinical cases ($r = 0.7749$, $P < .0001$). SARS-CoV-2 viral load detected in wastewater, resulting from the Delta-driven third wave, was significantly higher than during the Omicron-driven fourth wave . Whole-genome sequencing confirmed presence of Omicron lineage defining mutations in wastewater with the first occurrence reported 23 November 2021 (BA.1 predominant). The variant spread rapidly, with prevalence of Omicron-positive wastewater samples rising to >80% by 10 January 2022 with BA.2 as the predominant sublineage by 10 March 2022, whilst on 18 April 2022 BA.4 and BA.5 were detected in selected wastewater sites. These findings demonstrate the value of wastewater-based epidemiology to monitor the spatiotemporal spread and potential origin of new Omicron sublineages.

Keywords. genotyping; SARS-CoV-2; B.1.5.9 (Omicron); B.1.617.2 (Delta) lineages; wastewater-based epidemiology; BA.1; BA.2; BA.3; and BA.5.

South Africa announced the emergence, of a new severe acute respiratory coronavirus 2 (SARS-CoV-2) variant on 24 November 2021, which resulted in a steep surge in coronavirus disease 2019 (COVID-19) cases and marked the advent of the Omicron-driven fourth wave [1]. Following this announcement, on 26 November 2021, the World Health Organization classified the new variant, B.1.1.529 (Omicron) as a variant of concern (VOC) [2]. Since its introduction, Omicron has driven the fourth wave of infections in South Africa and has been detected globally in more than 150 countries [3].

As of its first introduction, Omicron became known as a highly transmissible strain with reduced virulence [4]. At the same time, COVID-19 disease severity during the fourth wave appeared to be lower in most countries than in the Delta-driven third wave [5–7]. Furthermore, outcomes of laboratory-confirmed SARS-CoV-2 cases suggested that higher vaccination coverage and or natural immunity due to previous infection(s) could have reduced disease severity and virulence of the Omicron-driven fourth wave [6, 8, 9]. Notably, the continuous emergence of new VOCs marked by several nucleotide variations, which allow for escalated immune escape and increased disease severity with high transmissibility, contribute to the persistence of the current COVID-19 pandemic. Thus, tracking genetic variations from positive SARS-CoV-2 cases may yield crucial information regarding Omicron's immune evasion properties that threaten global efforts to control the pandemic.

The highly transmissible Omicron variant exhibits multiple mutations (>30) in the immunogenic regions, receptor-binding domain/motif (RBD/RBM) and the N-terminal domain (NTD) that are associated with enhanced cell entry and transmissibility

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[10]. In total, the Omicron VOC has approximately 24 overlapping mutations and 13 new mutations that contribute to its transmissibility [10]. Phylogeny of Omicron shows the presence of various sublineages with different mutational profiles: BA.1, BA.1.1 (BA.1 and R346K); BA.2 (lacks Δ69/70); BA.3 (remaining in the parent lineage B.1.1.529), with BA.1 and BA.2 identified as the predominant circulating sublineages during the Omicron fourth wave. According to Chassalevris et al, as of 15 December 2021, BA.1 accounted for 99% of the sequences submitted to the Global Initiative on Sharing All Influenza Data (GISAID), with >95% harboring the Δ69/70 [11] linked to S-gene target failure (SGTF). However, in late January 2022, the Network for Genomic Surveillance in South Africa (NGS-SA) reported that while BA.1 was the predominant sublineage in December (84%) the proportion of BA.2 increased to 86% in February 2022, with BA.3 continuing to be detected at low levels (https://www.nicd.ac.za/wp-content/uploads/2022/04/Update-of-SA-sequencing-data-from-GISAID-1-Apr-2022.pdf). In early April 2022, 2 new Omicron sublineages were identified. These have been designated as lineages BA.4 and BA.5. Nonetheless, prominent mutations linked to BA.4 include all BA.2 mutations with the additions of the Δ69/70, L452R, F486V, NSP4, L438F with Q49, D61, L11F, and P151S reverted to wild type, whilst BA.5 shares the same mutations/deletions including D3N, L11, P151, A27038G, and C27889T (NGS-SA).

Wastewater-based epidemiology (WBE), through targeted surveillance, can rapidly detect and track the geographical distribution of these variants as well as differentiate changes in disease burden caused by different VOCs in communities [12]. Needless to say, wastewater testing has emerged as a valuable tool for ongoing monitoring and a predictive system to detect, track, and trace SARS-CoV-2 and VOC in sewage [13, 14]. Whole-genome sequencing (WGS) constitutes a reference methodology for the detection of known and novel SARS-CoV-2 mutations. Accordingly, Thermofisher's Ion AmpliSeq WGS targeted technology allows for a rapid 72-hour turn-around time, an important surveillance component to identify different circulating variants following a positive polymerase chain reaction (PCR) test result. This study aimed to track circulating VOC using WBE and provide credible evidence that wastewater can be used as a proxy to detect Omicron sublineages, BA.1 and BA.2, and identify newly emerging BA.4 and BA.5 sublineages for the first time in wastewater.

METHODS

Wastewater Sample Concentration and RNA Extraction

Raw sewage samples were collected on a Monday between 8:00 and 11:00 AM weekly from 24 wastewater treatment plants (WWTPs) in Cape Town and 4 WWTPs in the Breede Valley Municipality, located in Cape Town, South Africa, between 15 November 2021 and 28 March 2022. For RNA extraction, 100 mL of an influent wastewater grab sample was mixed and centrifuged at 2500*g* for 20 minutes, whereafter 2.5 mL of the resultant pellet was used for total RNA extraction using a previously described protocol by Johnson et al [12]. Following extractions, the consequent RNA (70 µL) was aliquoted and stored at −80°C until required for molecular analysis.

Quantitative Real-Time Polymerase Chain Reaction Analysis

SARS-CoV-2 RNA from both sewage and spiked control samples were reverse transcribed, amplified and quantified using the Bio-Rad iTaq Universal Probes One-Step Kit (Bio-Rad) according to the manufacturer's instructions. Extracted RNA was standardized at a concentration of 0.2 µg/µL, and quantitative real-time PCR (RT-qPCR) was performed as previously described by Johnson et al [15]. Briefly, 1 µL of RNA was added to a 2.5-µL master mix in a final volume of 10 µL containing 0.25 µL of the 2019-nCoV Centers for Disease Control and Prevention-approved nucleocapsid (N1 and N2) primer/probe set purchased from Whitehead Scientific. For quality assurance and control, the 2019-nCoV N Positive Control (catalogue No. 10006625; Whitehead Scientific) was used to construct a standard curve (200 000–20 genome copies [gc]/µL), the setting cycle threshold (Ct) of 0.02 and ensure that all samples amplified above the threshold with a correlation coefficient >0.99 and a PCR efficiency between 90% and 100% (slope −3.3 to −3.6). PCR was conducted on an Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (Life Technologies) as previously described by Johnson et al [12]. All reactions were done in duplicate, and a plasmid SARS-CoV-2 positive control at a viral titer of 200 gc/µL was included as a qRT-PCR–positive control. Subsequently, selected samples positive for SARS-CoV-2 were submitted for Ion Torrent sequencing.

Whole-Genome Sequencing Using Thermo Fisher Ion Torrent Research Panel

For sequencing and mutation detection using Variant Caller version 5.16.0.5 with default parameters, RNA was sent to the Central Analytical Sequencing facility (Stellenbosch, South Africa) where library preparation was performed using the Ion AmpliSeq SARS-CoV-2 research panel (Life Technologies) according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 8.0.1. The SARS-CoV-2 viral load of wastewater were reported using summary statistics. The Student *t* test was used to investigate the difference between SARS-CoV-2 RNA viral load during the Omicron fourth wave compared to the Delta third wave. A Spearman correlation analysis was performed to assess the association between SARS-CoV-2 viral load in wastewater and Ct values as well as the correlation between

SARS-CoV-2 wastewater viral load and positive clinical cases. Where appropriate, descriptive statistics were used to assess wastewater's SARS-CoV-2 RNA copies per mL.

RESULTS

Wastewater Epidemiology Predicts Onset of the Fourth Wave

Routine surveillance was conducted on 620 wastewater samples from 27 WWTPs in the Cape Town and Breede Valley Municipalities for 23 weeks. The samples were collected between 15 November 2021 and 25 April 2022. More than 70% of the wastewater samples tested positive for SARS-CoV-2, with 871 gc/mL (high Ct values, data not shown) detected on the 15 November 2021 (Figure 1A). The latter was exactly 1 week before the Omicron-driven fourth wave emerged, whilst the lowest Ct value of 31.7 (data not shown), equivalent to 8892.3 gc/mL, was detected on 13 December 2021 (Figure 1A), which was during the fourth wave. As it is expected that high Ct values are indicative of reduced SARS-CoV-2 viral load, a Spearman rank correlation was computed that showed a negative but strong correlation and statistically significant relationship between Ct value and viral load ($r = -0.8446$, $P = .0010$; data not shown).

Wastewater as a Proxy for Clinical SARS-CoV-2 Detection

A comparative analysis between SARS-CoV-2 viral load detected in wastewater and clinical data was performed (Figure 1B and Figure 2A and 2B). Temporal analysis of SARS-CoV-2 RNA detected in the influent of municipal wastewater sites displayed a steady increase in viral load on 15 November 2021 and reached its highest value on 13 December 2021 (Figure 2A and 2B). The rapid increase in viral loads coincided with an upsurge in COVID-19 clinical case data (23 November–13 December 2021), which also marked the onset of the Omicron-driven fourth wave. However, the period between 27 December 2021 and 24 January 2022 was characterized by a decrease in viral load that paralleled the decline in SARS-CoV-2 clinical case data (Figure 2). Spearman rank correlation analysis confirmed a strong positive correlation between SARS-CoV-2 viral RNA and positive case data over the same time ($r = 0.7732$, $P = .0001$; Figure 1B). Furthermore, Western Cape wastewater data depicted that the Omicron-driven fourth wave had significantly lower SARS-CoV-2 RNA viral loads with less-severe clinical cases as compared to the Delta-driven third wave ($P = .006$; Figure 3A).

Spatiotemporal Spread of Omicron

A rapid increase in COVID-19 cases was observed towards mid-November 2021 in the Western Cape of South Africa, which marked the onset of the Omicron-driven fourth wave. This was concomitant to a decline in the Delta variant (Figure 3B). The frequency of mutations that characterize the Delta variant decreased from 15 November 2021 onwards (Figure 3B). At this time, mutations that define the Omicron VOC were not detected within any of the wastewater sites as classified by GISAID (https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/ accessed 16 April 2022), except for the Airport sample (Supplementary Material 1). However, 1 week later, the appearance of mutations that characterize the Omicron VOC was detected within various WWTPs (Figure 3B and Supplementary Material 2). On 23 November 2021, the Omicron VOC viral loads intersected with that of Delta VOC (Figure 3B). Subsequently, except for the Rawsonville WWTP, the frequency of mutations that characterize the Delta variant decreased in most sites between 23 November and 6 December 2021, while the frequency of mutations that characterize the Omicron variant increased in all other WWTPs (Figure 3, Figure 4, Figure 5, and Supplementary Material 2). It was of interest to observe how the Rawsonville WWTP continued to harbor both Delta and Omicron mutati ons, where previously the Delta variant was predominant duri ng early November 2021 and March 2022 and where subsequently the Omicron variant reverted to dominance on the 28 March 2022. In April 2022, the Delta variant was dominant in Rawsonville. The predominance of Omicron was concomitant with a high viral load within the Rawsonville WWTP (Supplementary Material 2 and Figure 4).

Mutational Profile and Temporal Analysis of Omicron Sublineages BA.1, BA.2, BA.3, and BA.5

Next, the pattern of mutations in the spike protein, including mutation in NTD, SD1/SD2, and RBD/RBM regions that define the BA1, BA.2, and BA.3 sublineages, were assessed. On 4 April 2022, a new Omicron sublineage was announced in South Africa, and the Nextstrain database defined it as Omicron (BA.4 and BA.5) harboring the following spike protein amino acid substitutions and insertion: BA.4 and BA.5 (T19I, L24S, ins25PPA, A27, 69/70 DEL, G142D, V143/145DEL, V213I, G339D, S371L, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K) (https://www.nicd.ac.za/wp-content/uploads/2022/04/Update-of-SA-sequencing-data-from-GISAID-8-Apr-2022.pdf). Compared to BA.4, the BA.5 sublineage lacks Δ69–70 and is not detected by SGTF, as defined by GISAID and the Pangolin COVID-19 Lineage Assigner (version 4.0.5, lineages version 9 April 2022) (Figure 4, Figure 5, and Supplementary Material 1 and 2). The mutational detection was performed on WWTP using Ion Torrent sequencing (Supplementary Material 1 and 2) and sublineage classified as per GISAID (accessed 16 April 2022).

BA.1, BA.2, BA.3, and BA.5 share multiple common spike protein mutations, but each also has unique mutations that define the different sublineages (Figure 4, Figure 5, and Supplementary Data 1 and 2). On 10–17 January 2022, most

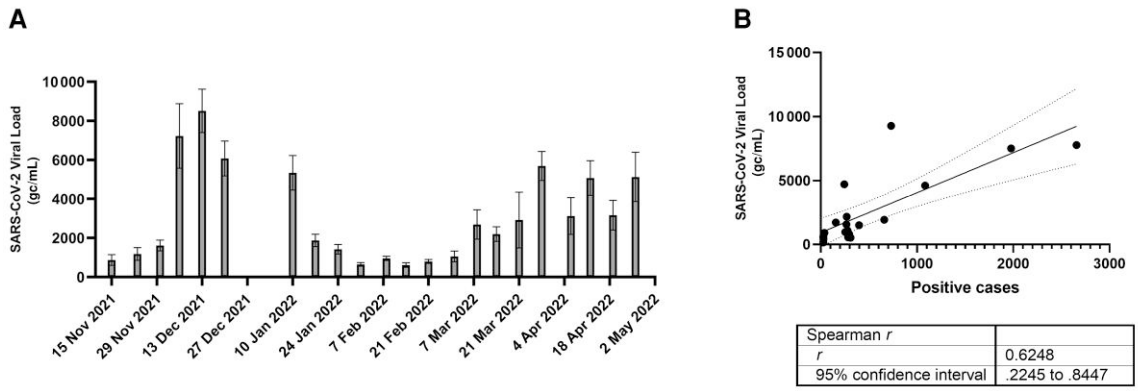

Figure 1. Correlation analysis and quantification of SARS-CoV-2 viral RNA in wastewater. *A* and *B*, Positive correlation between SARS-CoV-2 viral RNA per mL of wastewater at 27 Western Cape wastewater treatment plants and positive clinical case data. Results were expressed as standard deviation (SD). Clinical data was sourced from the publicly available South African Western Cape Government's Department of Health COVID-19 Response dashboard (https://coronavirus.westerncape.gov.za/COVID-19-dashboard). Abbreviations: gc, genome copies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

sites (87%), except Melkbosstrand and Westfleur Domestic WWTP, harbored unique BA.1-defining mutations: Δ69/70, S371L, G446S, G496S, T547K, N856K, and L981F. In early January 2022, the presence was confirmed of BA.2.9 in the Melkbosstrand WWTP, whilst Westfleur Domestic harbored the BA.1 as well as the BA.5 sublineages at 0.3 of the read frequency, as classified by Pangolin, indicative of the appearance of nucleotide variation (https://pangolin.cog-uk.io/), and GISAID (Supplementary Material 1 and 2).

By 10 March 2022, the BA.2 sublineage became dominant (81%) with key defining mutations in most sites: T191, Δ24–26, A275, V213G, S371F, T376A, D405N, and R408S. Conversely, Rawsonville WWTP was the only site that reverted to Delta (sublineage AY.32), with key BA.1 mutations present at a low read frequency as compared to the Delta-defining mutations (Supplementary Material 2). Interestingly, the Athlone WWTP harbored a BA.2.9 Omicron lineage, Touws River a mixture of BA.2 and BA.5 at 0.5 frequency, whilst Gordons Bay harbored the BA.2 and BA.5 at a read frequency of 0.33 (Figures 4–6 and Supplementary Material 1 and 2).

On 28 March 2022, BA.2 remained the predominant sublineage (87%) in all WWTP except for the WWTP in Rawsonville. At this time Deltacron, which has both Omicron and AY.32 Delta mutations was predominant, whereas Gordons Bay continued to harbor both BA.2 and BA.5 mutations at a read frequency of 0.3 (Supplementary Material 1 and 2). Hence, it is noted that from 10 March 2022, the newly arisen sublineage BA.4 and BA.5 as well as the Deltacron mutations could be detected in the Rawsonville, Gordons Bay, Westfleur Domestic, Camps Bay, Fisantekraal, Green Point, and Melkbosstrand WWTPs.

DISCUSSION

Globally, it has been suggested that SARS-CoV-2 wastewater monitoring can be used as a proxy for clinical testing to detect and study the emergence and spread of SARS-CoV-2 variants on a community level. This key priority and cost-effective strategy allow for community-wide screening and fills a critical surveillance gap left by limited testing on a global scale. This type of surveillance is high priority within South Africa, especially with the lifting of lockdown restrictions and the implementation of prioritized COVID-19 testing strategies. Hence, we suggest that the national government should embark on a new wastewater-based testing strategy to accurately track and trace SARS-CoV-2 viral loads and detect VOC within communities [16, 17].

To date, South Africa has experienced 4 SARS-CoV-2 waves caused by distinct variants. With each wave, a rise in clinical case numbers was observed, which marked the start of that wave. Concurrently, a resurgence of viral load coincided with a steady rise in SARS-CoV-2 viral RNA in wastewater. The current study confirmed that a positive correlation exists between a rise in the clinical case number and increased SARS-CoV-2 viral RNA in wastewater. Similar findings have been observed by other researchers [10, 18, 19], corroborating that SARS-CoV-2 viral load detected in wastewater correlated with clinically established cases [1, 9, 20–22]. Furthermore, over the past 2 years, multiple studies have corroborated that the detection of SARS-CoV-2 in wastewater could be a leading indicator of COVID-19 prevalence. These studies showed that WBE could detect an increase in viral signal 4–7 days before the onset of any clinical cases [20, 23–25]. Similarly, during the Omicron wave the current study showed an increase in SARS-CoV-2 viral load a few days (1 week) before any rise in Omicron clinical case data was reported. The reliability of the model and lead time could have been impacted by various factors, including the sampling strategy used.

Next, the spatiotemporal spread of SARS-CoV-2 VOC was tracked over time and WBE samples were compared to clinical

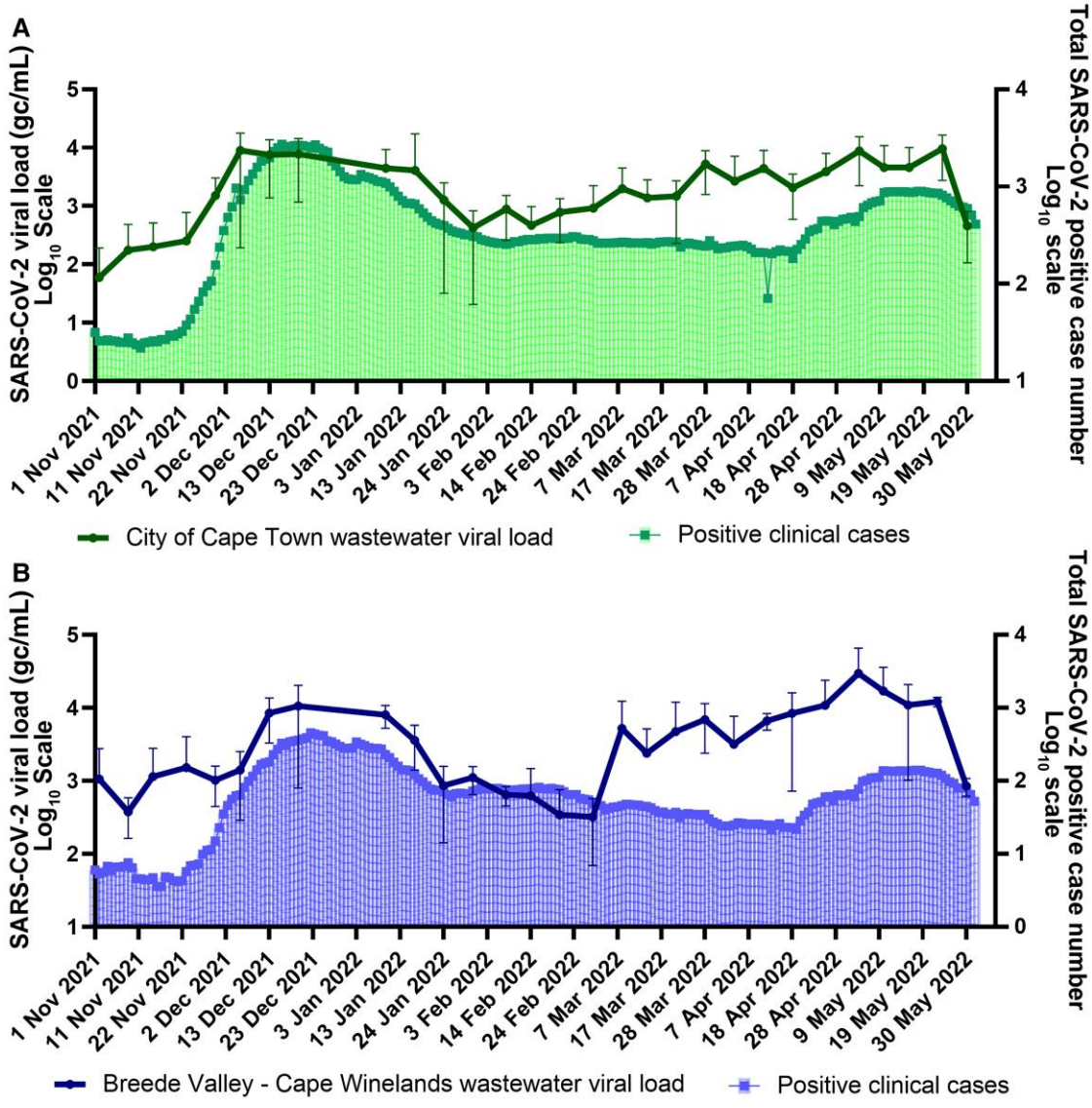

Figure 2. Temporal dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in municipal wastewater superimposed on the epidemiological clinical data. Trends in SARS-CoV-2 viral RNA from (*A*) City of Cape Town and (*B*) Breede Valley superimposed on clinical case data that tested positive for SARS-CoV-2 (area graphs). Results were expressed as standard deviation (SD). Line graphs represent SARS-CoV-2 gc/mL of RNA concentration in municipal wastewater measured with q-RT-PCR using the N1 and N2 primer pairs. Clinical data was sourced from the publicly available South African Western Cape Government's Department of Health COVID-19 Response dashboard (https://coronavirus.westerncape.gov.za/COVID-19-dashboard). Abbreviations: gc, genome copies; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

case data. Following the first detection of Omicron in mid-November 2021, it swiftly replaced Delta as the dominant variant in the WWTPs of the Western Cape, South Africa [26–30]. Furthermore, the current study supported clinical findings that the spatiotemporal spread of Omicron accelerated from late November 2021 with a concomitant decline in the Delta-driven third wave [29]. Accumulated scientific evidence confirmed that Omicron is a highly mutated VOC that is highly transmissible with increased infectivity compared to Delta [10, 26, 29]. This was confirmed by Mallapaty et al [26] who further reported that Omicron has a short incubation period (3 days) and lower viral load with infected individuals having a 48% increased risk of infectivity when compared to Delta [26]. Likewise, other studies have also reported that disease severity and hospitalization rate were significantly lower in the Omicron-driven fourth wave [31, 32]. Conversely, although this study observed a lower viral load during the Omicron wave as compared to the Delta wave, this has not been universally observed. For example, Oloye et al reported that the total viral load detected by RT-qPCR for 3 Canadian cities showed the Omicron VOC to be 2-fold greater than that observed for the Delta wave [33].

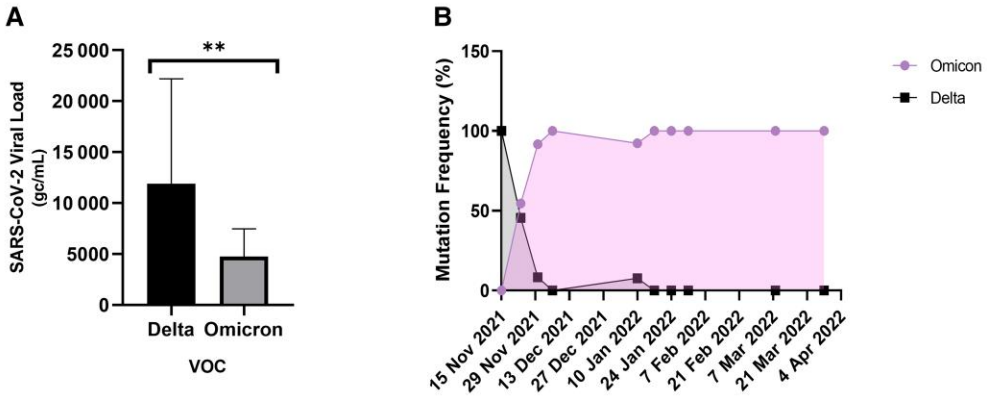

Figure 3. Temporal analysis of SARS-CoV-2 VOCs in wastewater treatment plants in the Western Cape. *A*, Viral load in Delta-driven third wave versus Omicron-driven fourth wave. *B*, Data obtained from SNP genotyping and genomic sequencing data as classified by Pangolin (version 3.1.20, lineage version 28 February 2022; https://pangolin.cog-uk.io/) and mutation detection as classified by most likely lineage to a given SARS-CoV-2 genome sequence ** $P = .006$. Results were expressed as standard deviation (SD). Abbreviations: gc, genome copies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNP, single-nucleotide polymorphism; VOC, variant of concern.

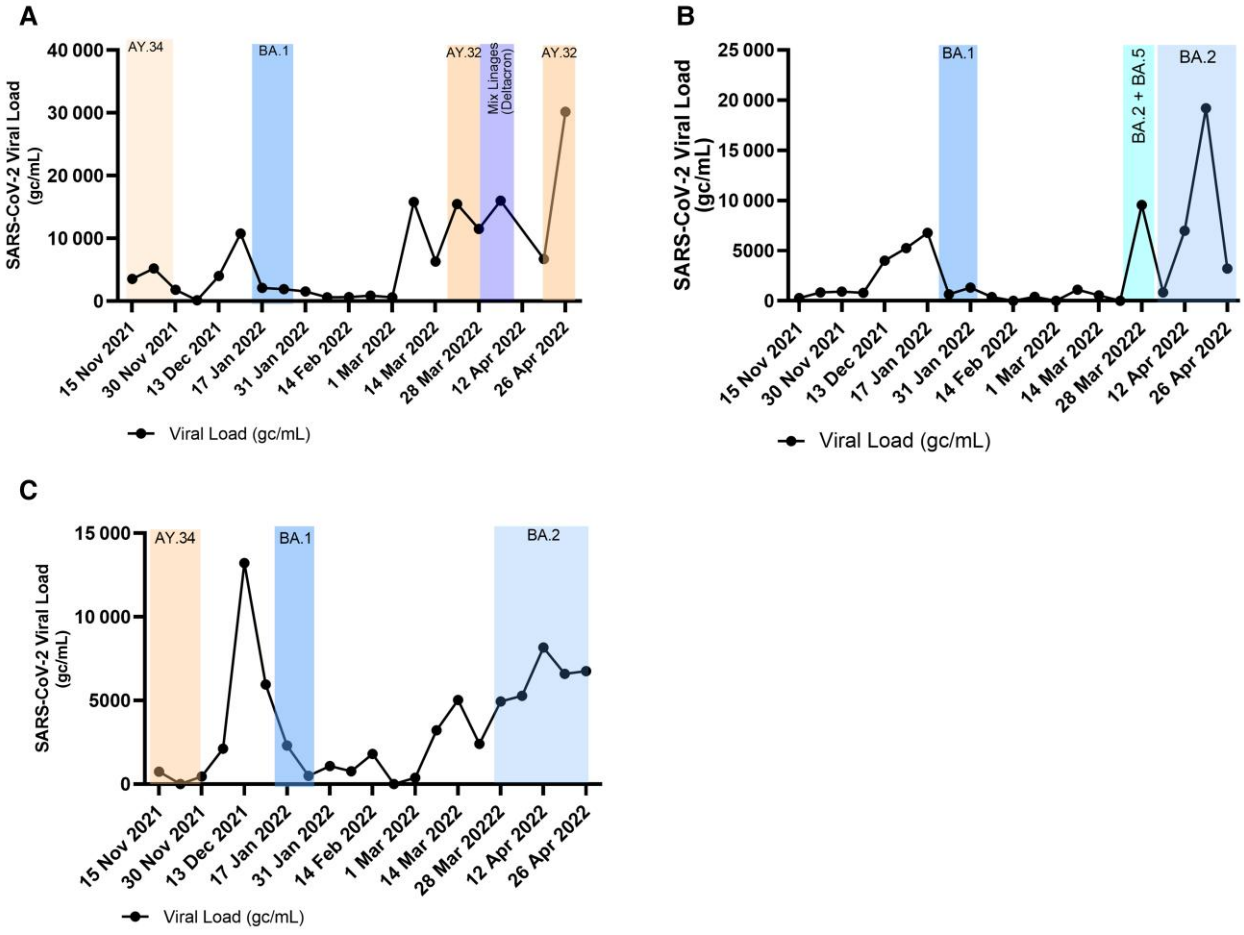

Figure 4. Spatiotemporal analysis of variants of concern in Breede Valley area Omicron and its sublineages. Pangolin classified sublineages as classified by key amino acid mutations of the Omicron variant in (*A*) Rawsonville, (*B*) Touws River, and (*C*) Worcester wastewater treatment plants, classified by Pangolin https://pangolin.cog-uk.io/ version 4.0.5, lineages version 9 March 2022. Abbreviations: gc, genome copies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

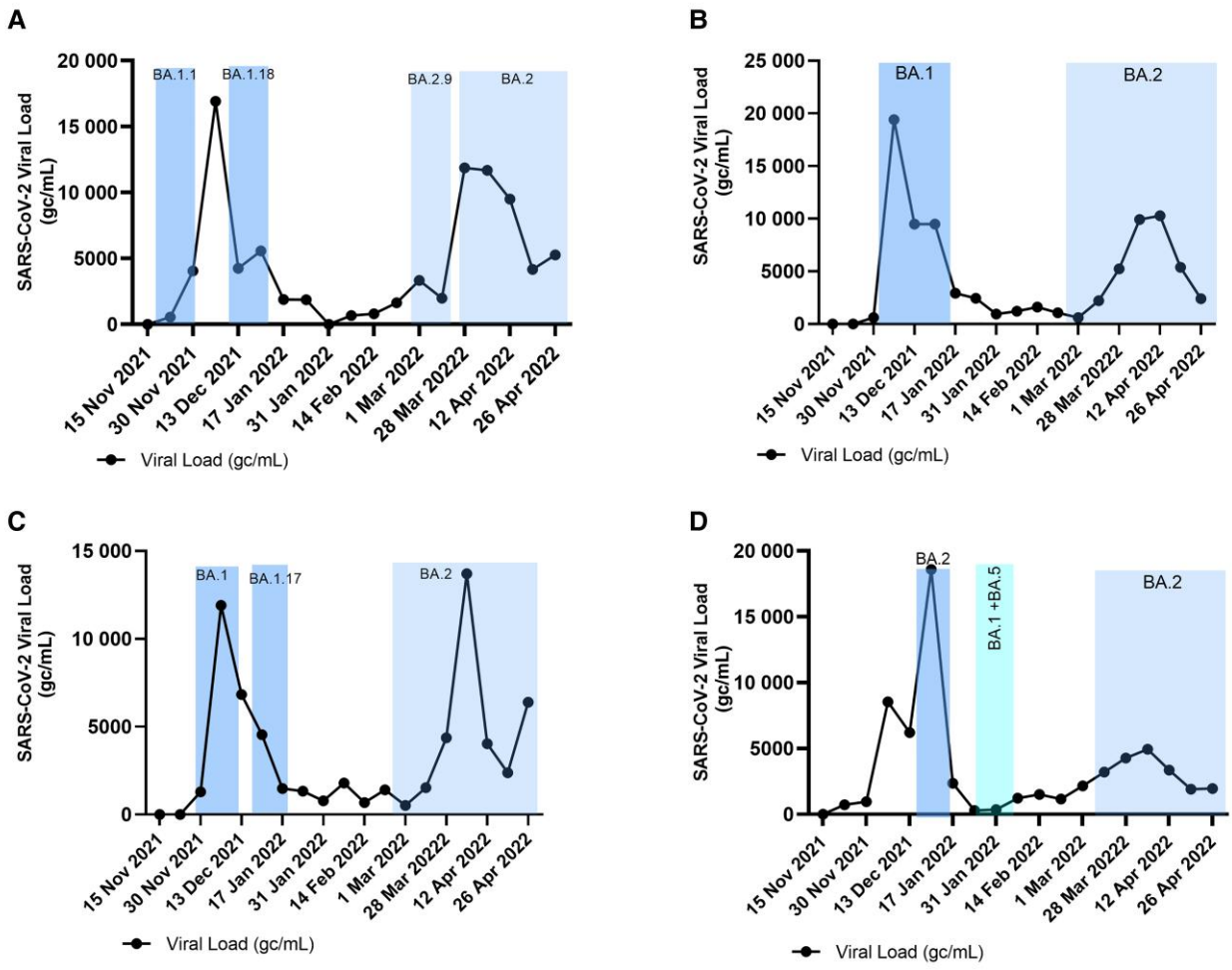

Figure 5. City of Cape Town wastewater temporal trends: (*A*) Athlone, (*B*) Mitchells Plein, (*C*) Macassar, and (*D*) Wesfleur WWTPs. Pangolin classified sublineages by key amino acid mutations of the Omicron variant in WWTP in the City of Cape Town, classified as per Pangolin https://pangolin.cog-uk.io/ version 4.0.5, lineages version 9 March 2022. Abbreviations: gc, genome copy; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WWTP, wastewater treatment plant.

According to Gupta et al, a constant change in the frequencies of dominant SARS-Co-V-2 viral lineages, which arise from nucleotide variation through favorable selection, is the major determinant of disease severity and immune escape [34]. Hence, globally, efforts have been made to identify the different virus sublineages as the variants change dynamics. This study analyzed genetic variations in SARS-CoV-2 detected in wastewater over several months. The current study, through WBE, confirmed the presence of 4 Omicron sublineages BA.1, BA.2, BA.3, and BA.5 (classified by Pangolin, accessed 16 April 2022). This was also confirmed by Kumar et al [36], who reported that BA.1, BA.2, and BA.3 had spread globally [36]. Kumar et al [36] and numerous other researchers [3, 35] also reported that BA.2 has a selective advantage over BA.1, thus rationalizing the swift replacement of BA.1 by BA.2. In our study, we noted an increase in viral load throughout December 2021 to January 2022, which was driven by the Omicron BA.1 sublinage. Most WWTPs in Cape Town spiked on 13 December 2021. However, by 10 March 2022 BA.1 (distinct mutations S371L, G446S, and G496S) was completely replaced by BA.2 (distinct mutations S371F, T376A, D405N, and R408S). Of interest was the mix Deltacron mutation observed in the Rawsonville WWTP, which presented mixed lineages (Omicron-like genetic signature) with both Delta and Omicron being present. Whether the latter was a recombinant event remains unclear, especially in wastewater that contains a combination of genomes. However, between November 2021 and January 2022, SARS-CoV-2 Delta and Omicron variants were cocirculated, allowing for coinfections and possible recombinant events [36]. Even so, our data continuously confirmed the predominance of the Delta variant in the Rawsonville WWTP, even during the period when Omicron was the primary circulating strain. Likewise, in a study by Yaniv et al, the authors reported the presence of cryptic circulating Delta variants during increased levels of the Omicron variant [37]. Of interest was the study of Rockett et al [38] and Menni et al [32], who

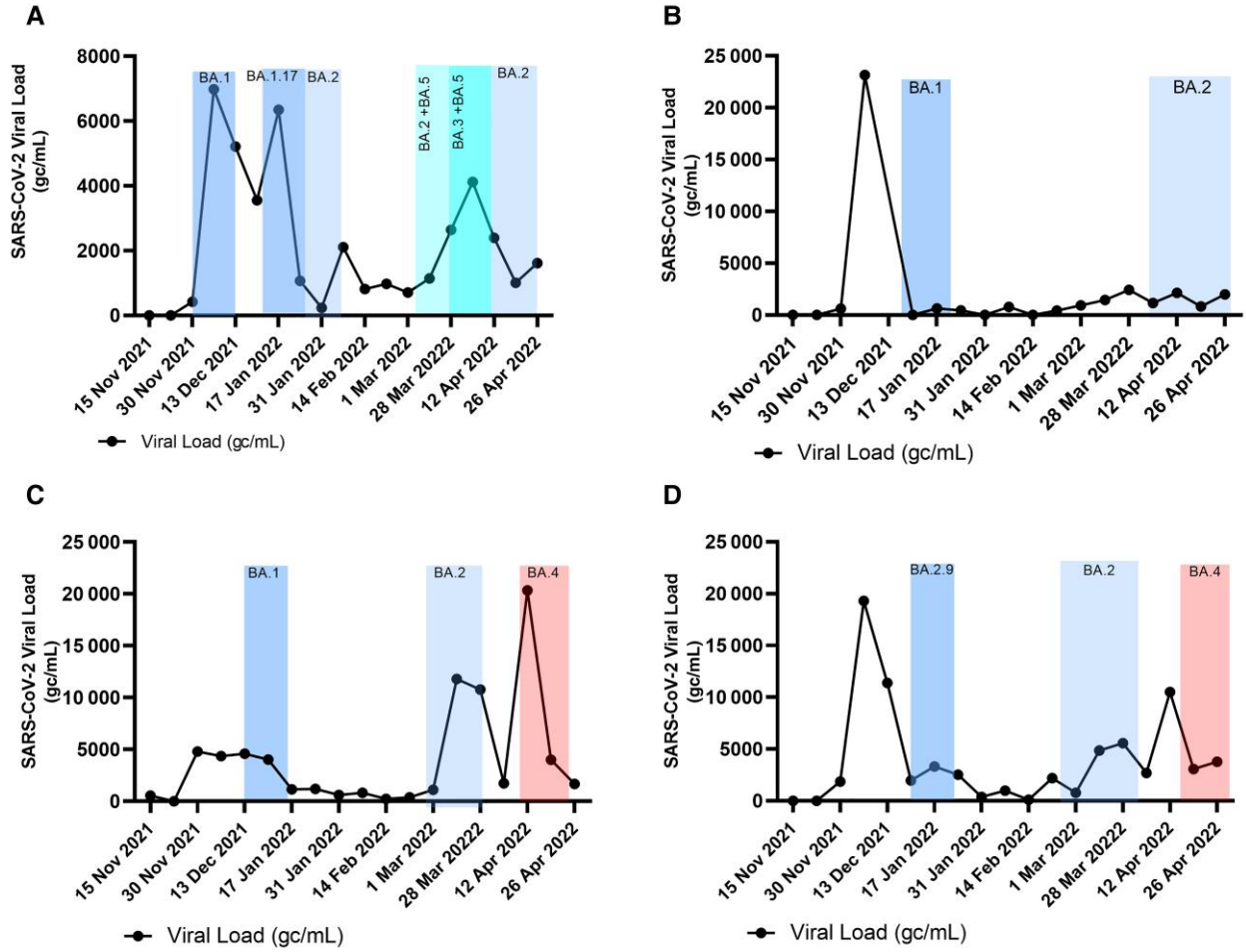

Figure 6. City of Cape Town wastewater temporal trends: (*A*) Gordons Bay, (*B*) Wildvoelvlei, (*C*) Camps Bay, and (*D*) Melkbosstrand WWTPs. Pangolin classified sublineages by key amino acid mutations of the Omicron variant in WWTP in the City of Cape Town, classified as per Pangolin https://pangolin.cog-uk.io/ version 4.0.5, lineages version 9 March 2022. Abbreviations: gc, genome copy; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WWTP, wastewater treatment plant.

reported on a dual infection, with Omicron and Delta, in immunocompromised individuals that were unrelated, thus confirming that the same host can be infected with 2 phylogenetically distinct SARS-CoV-2 variants.

GISAID analyses demonstrate that BA.3 shares most of its mutations with BA.1 and BA.2, with BA.3 causing the lowest number of cases out of the 3 Omicron lineages [36, 39]. Our study confirmed that Gordons Bay harbored BA.2 mutations from 10 March 2022 onwards; however, 3 weeks later, the emergence of BA.3 could be identified. Of interest was the newly identified BA.5, which was detected at a low read frequency in early March 2022 at the Gordon's Bay and Touws River WWTPs. Nonetheless, on 18 April 2022 BA.4 and BA.5 were detected in Camps Bay, Fisantekraal, Green Point, and Melkbosstrand.

WBE has emerged as an alternative approach to monitor the spread of asymptomatic and symptomatic SARS-CoV-2 viral loads within a catchment area. However, even though a positive correlation between wastewater viral load and clinical data exists, a substantial amount of optimization is still required to make WBE scalable and to ensure that wastewater data are accurate for public health action. For example, the current study reported on the presence of a mixed Deltacron mutation within the small rural district of Rawsonville. It is noteworthy that the Rawsonville treatment plant receives waste from a holiday resort and both formal and informal settlements within the surrounding area. Hence, the dominance of the circulating variant between the different collection points might have differed if sampling was done from the sewershed (that is the community area served by a wastewater collection system) instead of the WWTP. Sampling from a sewershed would have been a more accurate measure to pinpoint infection trends/hotspots and to link the circulating variant in the event of an outbreak to a specific community before waste flows into the treatment plant. This would also have allowed for a more accurate public health response. Therefore, a follow-up study is currently

underway, which will investigate the presence of circulating VOC by screening sampling points upstream of the Rawsonville WWTPs.

CONCLUSION

WGS is an important component of SARS-CoV-2 surveillance to identify new or circulating lineages within communities. Accordingly, this study made use of WBE to track the Omicron variants and their sublineages in Cape Town South Africa, and by doing so, this study was the first to identify BA.4 and BA.5, as classified by GISAID, within the wastewater. More importantly, this study provides plausible evidence that WBE can complement clinical studies, allowing public policymakers to have a comprehensive and sustainable tool to improve public health responses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the authors.

Notes

Author contributions. R. J., N. M., J. S., K. M., F. M., P. R., C. K., S. D., A.V., and B. G. performed experiments, analyzed data, and wrote the manuscript. A. V. performed whole-genome sequencing. R.J., S. S.-N., J. L., S. N., C.W., M. M., G. G., A. M., R. S., W. P., and C. M. applied for funding, provided scientific input, and edited the manuscript.

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