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Sewage surveillance of SARS-CoV-2 at student campus residences in the Western Cape, South Africa

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HIGHLIGHTS

• Spatiotemporal targeted surveillance of SARS-CoV-2 viral RNA linked to student residences.
• A steep rise in SARS-CoV-2 viral RNA was observed at the onset of the 4th wave.
• The surveillance acted as an additional source of information to reduce student activities on campus.
• Campus activity ceased, which coincided with reduced viral load.
• Targeted surveillance proved effective for risk minimization and student activity decision-making.

ABSTRACT

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostic capacity is limited in defined communities, posing a challenge in tracking and tracing new infections. Monitoring student residences, which are considered infection hotspots, with targeted wastewater surveillance is crucial. This study evaluated the efficacy of SARS-CoV-2 targeted wastewater surveillance for outbreak mitigation at Stellenbosch University's student residences in South Africa. Using torpedo-style passive sampling devices, wastewater samples were collected biweekly from manholes at twelve Stellenbosch University Tygerberg (SUT) campus and Stellenbosch University-Main (SUM) campus student residences. The surveillance led to an early warning detection of SARS-CoV-2 presence on campus, followed by an informed management strategy leading to restriction of student activities on campus and a delay in the onset of the third wave that was experienced throughout the country. Moreover, the study highlighted the extent...
1. Introduction

Wastewater-based epidemiology (WBE) has been widely adopted as a complementary mechanism to clinical surveillance for risk prediction and monitoring of infectious diseases such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Ahmed et al., 2020; Johnson et al., 2021; Street et al., 2021). The method is based on the principle that genetic material (genomic RNA) from SARS-CoV-2 can be detected and quantified using wastewater, and this information can be used to monitor community outbreaks in real time (Colosi et al., 2020). More recently, the approach for tracing SARS-CoV-2 infection has been extended to more defined communities such as hospital sewer networks (Acosta et al., 2021), aged care facilities, and student campus residences (Betancourt et al., 2021; Brooks et al., 2021; Gibas et al., 2021; Scott et al., 2021). For example, monitoring of campus wastewater has shown value in containing the spread of infection amongst university residences, especially asymptomatic and undiagnosed infections, exposure to which can be the initiator of superspreading events (Brooks et al., 2021; Gibas et al., 2021; Lemieux et al., 2021). A WBE approach to monitoring coronavirus disease 2019 (COVID-19) in student residences has been performed at 279 universities globally as of early July 2022 (COVIDPoops19 Dashboard, 2022).

Studies using WBE at student campus residences used various sampling methodologies such as grab (Brooks et al., 2021; Scott et al., 2021) and composite (Gibas et al., 2021). Either approach presents limitations due to unique challenges that impact the consistency of routine surveillance at the institution level compared to conventional sampling at wastewater treatment facilities. Such challenges include high variability in wastewater flow and solid particulate matter (SPM) which can lead to a high rate of missed shedding events. A recent study suggests that the sampling methodology does affect the viral load discoverable in wastewater, especially in small defined communities (Liu et al., 2022). For this reason, it is necessary to explore the most suitable method of sampling for viral RNA in sewage lines from defined communities where conventional sampling methodologies may prove troublesome. The use of passive sampling devices has its advantages by serving as a hybrid between grab and composite sampling through its permanent contact with the water body for a defined time period, thus reducing sampling error and missing shedding events, along with its ease of use and deployment (Schang et al., 2021).

In addition to quantifying RNA viral load and alerting communities to infection surges, WBE can be used to detect variants of concern (VOC) at the community level using quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) genotyping as a cheaper alternative to sequencing (Caza et al., 2021). During the time of the study, there were two VOCs circulating in South Africa at a high frequency, first the Beta and then the Delta VOC. The Beta (B.1.351) VOC was discovered in South Africa, with the earliest reported case on October 8, 2020 (Tegally et al., 2021; Cov-lineages.org, 2022a, 2022b, 2022c). It is characterized by eight lineage-defining mutations, with key mutations in the receptor-binding domain (RBD) being K417N, N501Y, and E484K (Tegally et al., 2021). The Delta variant was discovered in India on March 1st, 2021, and is characterized by 12 mutations, with two key mutations being L452R and P661R mutations (Umair et al., 2021). The Delta (B.1.617.2) variant had a higher infection and hospitalization rate compared to the Beta and Alpha variants (Hussey et al., 2021).

A limited number of studies to date have investigated the use of wastewater to routinely screen circulating VOCs. As a result, this study aimed to use passive sampling devices for spatiotemporal surveillance of COVID-19 infection on student campuses, along with routine screening for circulating VOCs. As such, two major student campuses at Stellenbosch University (SU), Western Cape, South Africa, were selected to develop a WBE platform for early detection of COVID-19 infection and routinely screen for VOCs in student residences for informed decision-making.

2. Methodology

2.1. Sample location

Samples were collected in two geographical locations of SU campuses (Fig. 1), namely the SU Main Campus (SUM; Stellenbosch, South Africa) and the SU Tygerberg Medical Campus (SUT; Cape Town, South Africa), which are approximately 40 km apart, over 28 weeks (19 May 2021 to 27 November 2021). A total of twelve defined sewage manhole sites (seven SUM and five SUT) were selected, with seven receiving sewage from single residence buildings and five receiving sewage from up to three different residences on campus. The sewage network did not allow for these devices to be placed further upstream at defined residence locations due to logistics, low sewage flow rates, or the safety of the personnel deploying the devices.

The sewer lines on the SUM campus residences were covered by 44 % torpedo-styled passive sampling devices, with a total student population residing on campus being 6500, which was a fraction of the total Cape Winelands district population in 2021 (n = 958,400). Whereas, SUT campus residence sewer lines were covered by 63 % torpedo-styled passive sampling devices with a total student population residing on campus being 1000, which was a fraction of Tygerberg's sub-district total population (n = 743,139). The respective districts' population estimate data for 2021 were accessed through the Western Cape government department of health portal (https://www.westerncape.gov.za/assets/departments/health/h_102_2020_covid-19_population_data.pdf).

2.2. Passive sampling and processing

The study made use of a 3D printed torpedo-style passive sampling device (Schang et al., 2021), of which the blueprints were made freely available by the research group from Monash University, Australia (http://www.bosl.com.au/wiki/passive_sampler). The device was attached to a nylon rope for recovery after deployment, which allowed wastewater to enter through multiple entry points incorporated in the housing, allowing wastewater solid particle matter (SPM) to be trapped within the sampling material that fills the device's inner section. Five pieces of standard medical gauze (75 mm × 75 mm) were chosen as the sampling material because they allowed the aqueous component to flow through the sampling device while capturing sufficient SPM content over the deployment period (Habtwold et al., 2022). To prevent ragging and the introduction of other large materials into the sample device, the gauze-filled sampling device was wrapped in shade cloth and secured with cable ties.

Twelve passive sampling devices were deployed at the two SU campus settings for a period of 21 h (1 pm to 10 am the next morning), as this period...
allows for the capture of most major flushing events from the residents, 24-h sampling was not feasible due to logistical issues. All sampling events were done twice a week (Sundays-Mondays and Tuesdays-Wednesdays). Upon completion of the 21-h passive sampling period, the devices were retrieved and pre-processed, which included the removal of the shade cloth covering and the SPM-filled gauze packing into labelled sealed bags using tweezers that were cleaned with 70 % ethanol and wiped off between sampling locations.

All waste materials that were used for the on-site pre-processing (paper towels, shade cloth, cable ties, and gloves) were disinfected with 70 % ethanol and placed in an autoclavable biohazardous bag that was taken to the laboratory for further disinfection and disposal in biohazardous waste containers. The pre-processed samples were transported to the laboratory and further processed on the same day of collection. Upon arrival at the laboratory, the gauze packing from each sampling location was immediately eluted within the same sealed bag that was used to transport them from the field, thus limiting the unnecessary handling of samples. Elution was done with 20 mL of a 1× double-autoclaved phosphate buffer solution (PBS) mixed with 0.05 % Tween 80 (Fisher Scientific, Leicestershire, United Kingdom). The PBS solution containing the SPM-filled gauze material was then massaged gently for 2 min, after which the gauze was moved to the top of the elution buffer in the sealed bag and squeezed to collect all the liquid from the material. The suspended SPM eluate from the wastewater sample was then poured into 50 mL Falcon tubes, the lids sealed with parafilm, and taken for RNA extraction and RT-qPCR analysis within 24 h.

2.3. Sample concentration and viral RNA extraction

A 25 mL aliquot of PBS-eluted passive sample was centrifuged at 2500 g for 20 min to yield a pellet used for subsequent RNA extraction. Thereafter, RNA was extracted following a method described by Johnson et al. (2021). For primary concentration recovery rate in the spiked matrix, a 25 mL previously SARS-CoV-2 negative passive wastewater sample was spiked with a known concentration of inactivated SARS-CoV-2 wild type (E.62) or variant (501Y.V2) (supplied by Medical Virology labs Tygerberg). The pellet used was not weighed after centrifugation. Therefore the resultant viral load was calculated based on the volume used to resuspend the SPM and the surface area of the gauze used. The viral recovery was calculated as (copies recovered/copies spiked) × 100.

In brief, RNA was extracted using the Qiagen RNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. To stabilize viral RNA, the pellet was transferred to a 15 mL PowerBead Tube containing lysis buffer. The sample was then homogenized and phase-separated with an equal volume of phenol/chloroform, with the upper aqueous phase transferred to a new 15 mL tube and combined with the RNA extraction kit’s buffers. After that, the aqueous phase was transferred to the RNeasy JetStar Mini Column to elute the bound RNA before centrifugation at 13,000 g for 15 min. The resulting pellet was dried and dissolved in 70 μL of ribonuclease-free water. Total RNA was quantified using spectrophotometry on a NanoDrop® ND-1000 (Nanodrop Technologies, Wilmington, NC, USA) instrument.

2.4. Quantitative reverse transcriptase-polymerase chain reaction analysis

RT-qPCR was carried out as previously described by Johnson et al. (2021). The primer and probe sets were selected in accordance with the Centers for Disease Control and Prevention, targeting the SARS-CoV-2 nucleocapsid gene (N1 and N2) (2019-nCoV CDC EUA Kit, Integrated DNA Technologies, USA) (U.S. Centers for Disease Control and Prevention, n.d.). One-step RT-qPCR reaction utilizing iTaq Universal Probes One-Step
reaction mixture (Bio-Rad Laboratories, USA) was carried out in 10 μL reaction volume. The assay reaction included 1 μL of 0.2 μg/μL total RNA, 5 μL of iTaq universal probes reaction mix (Bio-Rad Laboratories, USA), 0.25 μL iScript reverse transcriptase (Bio-Rad laboratories, Hercules, CA), and 0.5 μL primers and probes (Integrated DNA Technologies, USA). The RT-qPCR was performed on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (ABI instrument, Life Technologies, USA) using thermal cycling conditions where reverse transcription was performed at 50 °C for 10 min; enzyme activation at 95 °C for 1 min, followed by amplification at 95 °C for 15 s and 60 °C for 1 min for 40 cycles. To quantify SARS-CoV-2 viral load for N1/N2 genome copies (GC/cm2/21 h) Eq. (1) was used (Hayes et al., 2022). A standard curve method was used with a 10-fold serial dilution (200,000–20 GC/μL) of 2019-nCoV-N-positive plasmid control (Quantabio, Beverly, MA, USA). For each plate reaction, a reagent blank, a positive, and negative control were conducted in duplicates. The reaction threshold was set at 0.02, while the correlation coefficient was >0.99 and the PCR efficiency was between 90 and 100 %, with a slope ranging from −3.3 to −3.6.

\[
\text{RNA concentration} = \frac{\text{viral load (GC)} \times \text{eluted volume (25ml)}}{\text{gauze surface per sampler (cm}^2) \times 21 \text{ hours}}
\]

(1)

2.5. Variant genotyping

The Beta (N501Y, E484K, and K417N) and Delta (L452R and P681R) variants were genotyped using a technique described by Johnson et al. (2022). Briefly, 2.5 μL of RNA with a viral load >133.3GC/cm² was added to 2.5 μL of TaqPath one-step RT-qPCR master mix and 0.25 μL of TaqMan™ SARS-CoV-2 Mutation Panel to make a final volume of 10 μL. The QuantStudio™ 7 Flex Real-Time PCR System instrument (ThermoFisher Scientific, Massachusetts, United States) was used. All RT-qPCR experiments were performed in duplicates, including a non-template control (NTC) and wild-type AccroMetrix COVID-19 RNA control (RUO) (ThermoFisher Scientific) was supplied with the assay with each plate reaction. RNA sequencing was performed at the Central Analytical Facility of Stellenbosch University using the Ion Torrent next-generation sequencing (NGS) technology (ThermoFisher Scientific), with the Ion AmpliSeq SARS-CoV-2 research panel (Thermo Fisher Scientific) according to the manufacturer’s instructions.

2.6. COVID-19 clinical case data

The COVID-19 clinical case data was sourced from the publicly available South African Western Cape Government’s Department of Health COVID-19 Response dashboard. The clinical case data was represented as a 7-day moving average of clinically diagnosed cases from the respective districts where SU campuses are situated (https://coronavirus.westerncape.gov.za/ covid-19-dashboard). The VOC genomic data was sourced from the publicly available NICD's VOC prevalence weekly reports, focusing on the clinical case data from the Western Cape, South Africa (https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/sars-cov-2-genomic-surveillance-update).

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism v8.0.1. The SARS-CoV-2 viral loads of wastewater were reported using summary statistics. Normality was tested using the Shapiro-Wilk test. Spearman’s rank correlation was used to determine the correlation between the N1 and N2 targets, as well as the correlation of wastewater SARS-CoV-2 viral load and SARS-CoV-2 positive cases 7-day moving average of the respective districts.

3. Results

3.1. Spatiotemporal quantification

The first samples were collected at the SUT and SUM campuses on May 19th and May 25th, 2021, respectively. In matrix spike analyses, the primary concentration recovery rate of attenuated SARS-CoV-2 ranged from 10.59 % to 17.41 %, which was comparable to other reported studies (Betancourt et al., 2021; Street et al., 2021; Randazzo et al., 2020). The limit of detection for RT-qPCR assay was reported from the group’s previous study reported by Johnson et al., 2021 to be 700GC/mL using field samples. There was a strong correlation between N1 and N2 viral loads for SUT (r = 0.9797, p-value <0.0001) (Fig. S1) and for SUM (r = 0.9561, p-value <0.0001) (Fig. S2). Positive results for the N-gene targets were observed from the start of the study period with SUT and SUM at an average of 73.4 GC/cm²/21 h and 40.9 GC/cm²/21 h respectively, although at low viral RNA loads, corresponding with the low numbers of clinical cases recorded.

On June 9, 2021, all sampling locations at the SUT and SUM campuses saw sharp increases in RNA viral loads with an average of 1610.3 GC/cm²/21 h and 578.3 GC/cm²/21 h at SUT and SUM, respectively (Fig. 2A.I and B.I). On June 10, 2021, the institution limited campus activity, transitioning to online-based learning and teaching. With limited campus activities, wastewater surveillance data had a reduction in SARS-CoV-2 viral loads in the monitored campus residence. Moreover, this was also the transition phase toward the long winter recess, which was followed by both online and in-person examinations. Students returned to their residences on August 9, 2021, with campus activities resumed, sporadic viral RNA loads increased in almost all campus residence settings, with SUT increasing from 29.9 GC/cm²/21 h to 159.7 GC/cm²/21 h (Fig. 2A. II) while SUM increased from 15.6 GC/cm²/21 h to 1373.5 GC/cm²/21 h (Fig. 2B.II). These increases corresponded to the number of daily cases reported in the respective areas, as seen in the clinical case data overlaid in Fig. 2A. and B. The SUT and SUM campuses’ wastewater viral load data were correlated to the 7-day moving average positive cases reported in the districts. The SUT campus had a positive correlation with the Tygerberg sub-district reported cases (r = 0.5008, p-value = 0.0048). Furthermore, the SUM wastewater data was correlated to the Cape Winelands district having a stronger positive correlation (r = 0.6612, p-value<0.0001) (Fig. 3).

At the beginning of September 2021, reported positive cases decreased in the respective districts, corresponding with a decline in viral loads detected in wastewater samples across all campus residential settings. The viral loads on the SUT campus surged during the last week of sampling (November 27, 2021), which marked the start of the fourth wave in South Africa (Fig. 2A. III), whereas the viral loads on the SUM campus remained low (Fig. 2B.III). The first case of Omicron was reported on November 8, 2021 (Cov-lineages.org, 2022a, 2022b, 2022c), and in the final three weeks of November 2021, 74 % of the sequenced samples in the outbreak’s epicentre, Gauteng, South Africa, were Omicron cases (Dyer, 2021). These findings corroborate with what was observed at the SUT campus on November 24, 2021, with increased viral copy numbers in wastewater (Fig. 2A.III), validated by Ion Torrent NGS of the selected residence samples (Table 1).

3.2. Variant genotyping

With increasing viral loads of SARS-CoV-2 detected in wastewater, the emergence of SARS-CoV-2 VOCs circulating in the student residences needed to be investigated using an affordable screening method, the RT-qPCR genotyping assay, when compared to the sequencing methods currently used (Itarte et al., 2021). This is to better understand the dynamics of the highly virulent circulating variants for informed decision-making. As part of the two campuses’ routine surveillance, SUM and SUT’s positive samples with viral loads of >133.3GC/cm²/21 h were genotyped using the TaqMan® SARS-CoV-2 genotyping technique, with a focus on signature mutations linked with the VOCs. The Beta (K417N, N501Y, and E484K and (Delta) L452R and P681R mutations were monitored (Figs. 4 and 5). Fig. 6(A and B) depicts the average mutational frequency for the Beta and Delta variants for SUT and SUM campuses, respectively overlayed with VOC genomic surveillance of the Western Cape, South Africa during the same period. The mutational frequency was calculated by dividing all positive sites by the total number of sites tested for each campus setting. On June 9th, the frequency of mutations defining the Beta variant
Fig. 2. Temporal change in viral RNA genome copies per milliliter of the N-gene SARS-CoV-2 targets at the Stellenbosch University campuses superimposed with 7-day moving average clinical cases for the respective districts during the period of the current study. The 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). A) Stellenbosch University Tygerberg campus wastewater data (line graph) with 7-day moving average of Tygerberg subdistrict moving average clinical cases of SARS-CoV-2. B) Stellenbosch University Main campus wastewater data (line graph) with a 7-day moving average of Cape Winelands district moving average clinical cases of SARS-CoV-2. I) Yellow shade represents the beginning of the surveillance and the introduction of the Delta variant (reduction of student campus activity) II) Red shade represents the period after recess when students had moved back to their residences III) Green shade represents a period where wastewater viral load and reported cases were low.

Fig. 3. Correlation of Stellenbosch University’s residence wastewater viral load to 7-day moving average clinical case data in the respective districts. A) Correlation of SUT campus wastewater data to Tygerberg sub-district B) Correlation of SUM campus wastewater data to Cape Winelands district.
the SUM campus, except for one residence (Met-residence), in which the Delta variant was introduced earlier at rated by Ion Torrent sequencing (Table 1) results, where UBU a residences underlining strain (Fig. 6A). The results observed in Fig. 6A were corroborated on June 30th and July 13th, 2021, while the Beta variant became the observe how the Beta variant reverted to dominance on the SUT campus delaying the emergence of Delta variants on the SUT campus. This data indicates how the Beta variant was supplanted by the Delta variant well with its dominance over time in the Western Cape. The appearance of the Delta and Beta variants, with the Delta variant dominating. The increase decreased marginally at the SUT campus (Fig. 4). It was interesting to observe how the Beta variant reverted to dominance on the SUT campus after June 10th. However, the Delta variant became dominant between June 30th and July 13th, 2021, while the Beta variant became the underlying strain (Fig. 6A). The results observed in Fig. 6A were corroborated by Ion Torrent sequencing (Table 1) results, where UBU a residences at SUT campus was characterized to have Delta VOC on June 9, 2021.

The frequency of mutations that characterize the Beta variant decreased at SUM campus between June 9th and June 16th, 2021 (Fig. 5), while the frequency of mutations that characterize the Delta variant increased. The genotyped data indicated that the Delta variant was introduced earlier at the SUM campus, except for one residence (Met-residence), in which the Beta variant was still circulating (Fig. 6B). This data was corroborated by the Ion torrent NGS data (Table 1), confirming the presence of both the Delta and Beta variants, with the Delta variant dominating. The increase in the Delta variant dominance at the respective campuses corroborated with its dominance over time in the Western Cape. The appearance of the Delta variant was responsible for the spikes noticed on June 9th, 2021, at both campus settings. The decision to move to online learning aids in delaying the emergence of Delta variants on the SUT campus. This data indicates how the Beta variant was supplanted by the Delta variant well after its introduction.

Ion Torrent NGS data (Table 1) from the beginning of the third and four waves confirmed the introduction of the Delta and Omicron variants, respectively. Therefore, confirming the surge noticed in Fig. 2(A.III) at the end of November 2021 as a spike caused by the introduction of the Omicron
variant on the SUT campus. Our data confirmed the use of specific TaqMan® genotype mutation panels to be accurate and can be used as a real-time and affordable method to screen for known VOCs.

4. Discussion

The rapid increase in viral loads on the SUT campus on June 9, 2021, caught the interest of campus health officials, as the WBE results preceded the marked increase in positive clinical cases in the residence, which prompted the decision to limit, if not suspend, various campus activities and transition to an online-based teaching regime beginning June 10, 2021. According to various studies, viral loads in stool samples exhibit a more irregular pattern than those in the upper respiratory tract (Walsh et al., 2020; Zheng et al., 2020; Betancourt et al., 2021). This could explain the "erratic" pattern of viral signals from the student campus residences seen in the current spatiotemporal investigation where reported cases in the areas were deemed low (Figs. 2A and B). However, there are a number of factors influencing the erratic patterns of the viral signals as flow rate and population normalization factors were not accounted for.

The technique of sampling appears to be an important element in indicating viral infections in sewage environments. For example, the study by Betancourt et al. (2021) where they used grab as a sampling method, emphasized the issue of wastewater studies failing to produce any positive signal even when a person was known to be infected in the

![Fig. 4. Mutation frequency linked to two VOCs at the Stellenbosch University Tygerberg campus. (A) Mutations characterizing Beta variants, they include N501Y, K414N and E484K. (B) Mutations characterizing the Delta variant which includes L425R and P681R.](image-url)
dormitory. According to Brooks et al. (2021), wastewater surveillance can be used to detect the emergence of outbreaks on college campuses, as their results show that they were able to detect SARS-CoV-2 viral loads, however, at the peak of their infection on campus, the SARS-CoV-2 viral loads did not increase despite the fact that there were more positive students known in the residences (Brooks et al., 2021).

The campuses’ wastewater data were correlated to the district’s SARS-CoV-2 7-day moving average positive case data. The correlation between the SUM and the Cape Winelands district showed a stronger positive correlation compared to the SUT campus and Tygerberg sub-district correlation. This is due to the SUM campus having a larger student representation compared to the SUT campus. Student’s positive case data was not available to be used, however, communication with the student residences management was initiated and it was confirmed that the number of confirmed cases in the residences were not a true measure of actual cases. Students were not forced to get tested regularly, as this was a personal expense for South African citizens. Rapid antigen testing was not available in the country at the time of the study.

The Beta variant was first reported in South Africa on December 18, 2020, accounting for 60% of positive cases in the Western Cape, Eastern Cape, and KwaZulu Natal during the first week of November 2020 (Tegally et al., 2021). The Network for Genomic Surveillance in South Africa (NGS-SA) reported by the NICDs showed that the Beta variant was dominant till May 2021. However, in June 2021, the Delta variant became predominant, supplanting the Beta variant (NICD, 2022). Similar to the data in our study shows the Delta variant became predominant on the

![Fig. 5. Mutation frequency linked to two VOCs at the Stellenbosch University Main campus. (A) Mutations characterizing Beta variants, they include N501Y, K414N and E484K. (B) Mutations characterizing the Delta variant which includes L425R and P681.](image-url)
SUM campus in early June 2021, quickly surpassing the Beta variant. A study by Butt et al. (2021) illustrated how the Delta variant was associated with more severe illness than the Beta variant infecting male patients between 17 and 43 years of age.

It is possible that the introduction of the Delta variant led to the increase in the viral RNA load on June 9, 2021 (Fig. 2A). A small proportion of students might have been infected with the Delta variant before moving out of the residences. There were a number of reported positive cases in some of the student residences after the wastewater data was reported to health officials on campus, confirming the early warning detection.

A review by Harris-Lovett et al. (2021) raised the challenges faced by similar campus surveillance programs globally, including the need for obtaining representative and homogenous samples from sewer locations as well as logistical challenges regarding the deployment of sampling devices in aging sewer systems and at strategic locations that will provide meaningful results. The current study presented the same challenges at the onset of the surveillance program at the two campus locations. These challenges were overcome through constant communication with the campus Facility Management staff and the dedication of students and technical staff members that made routine surveillance possible at the selected sites.

Through the course of the surveillance, it became apparent that the passive sampling approach may introduce a level of uncertainty with respect to the extrapolation of results and comparison between sampling locations. For example, when using a grab/composite sampler, the RNA viral load is influenced by normalizing with the daily flow and population estimates. However, for this current study RNA viral loads were influenced by the surface area of the material used over the exposure time, as flow rate was not measured. However, this may be overcome if sewer flow rates could be measured alongside a passive sampling device in future sampling endeavours. Also, the sewer residence time can vary considerably between sampling locations which results in less homogenization of faecal matter in the aqueous wastewater matrix. However, this limitation also presents itself for discrete sampling and even during composite sampling.

Conversely, a study conducted by Wilson et al. (2022) showed the use of the torpedo-style passive sampling devices presented a cost-effective approach for sampling at defined locations compared to the composite sampling technique. The production and consumables (gauze, rope, etc.) used for a torpedo-styled sampler was 95 % cheaper than the most affordable composite sampler. The current sampling technique provided reproducible results that allowed spatiotemporal monitoring for SARS-CoV-2
viral loads and also prevented unnecessary blockage of the sewer system while the devices were deployed.

5. Conclusion

Overall, the current study achieved spatiotemporal surveillance for SARS-CoV-2 at two Stellenbosch University campuses in the Western Cape Province, South Africa. A 3-D printed torpedo-style passive sampling device was employed and proved to be a cost-effective and non-intrusive sampling methodology. This study illustrated the effectiveness of the use of torpedo-style passive sampling devices for quantitative surveillance as well as tracking the introduction of VOCs in student campus residences to provide additional information for effective response by the university’s health officials to contain the spread of SARS-CoV-2.

Credit authorship contribution statement

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Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158028.

References

Abdullah, F., et al., 2022. Decreased severity of disease during the first global omicron variant covid-19 outbreak in a large hospital in tshwane, South Africa. Int. J. Infect. Dis. 116, 38–42. https://doi.org/10.1016/j.ijid.2021.12.357.
Acosta, N., et al., 2021. A multicenter study investigating SARS-CoV-2 in tertiary-care hospital wastewater. Viral burden correlates with increasing hospitalized cases as well as hospital-associated transmissions and outbreaks. Water Res. 201, 117369. https://doi.org/10.1016/j.watres.2021.117369.
Ahmed, W., et al., 2020. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers. J. Travel Med. 27 (5), taaa116. https://doi.org/10.1093/jtm/ttaa116. https://doi.org/10.1093/jtm/ttaa116.
Betanzos, W.Q., et al., 2021. COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. Sci. Total Environ. 779, 146048. https://doi.org/10.1016/j.scitotenv.2021.146048.
Brooks, Y.M., et al., 2021. Detection of SARS-CoV-2 in wastewater at residential college, Maine, USA, August-November 2020. Emerging Infectious Diseases 27 (12). https://doi.org/10.3202/crdt27.12.21119.
Butt, A.A., et al., 2021. Severity of illness in persons infected with the SARS-CoV-2/Delta variant vs Beta variant in Qatar. JAMA Internal Medicine https://doi.org/10.1001/jama. internalmed.2021.7949.
Caza, M., et al., 2021. Evaluation of the clinical and analytical performance of the seagene allplexTM SARS-CoV-2 variants 1 assay for the detection of variants of concern (VOC) and variants of interests (VOI). J. Clin. Virol. 144, 104996. https://doi.org/10.1016/j.jcv.2021.104996.
Colosi, L.M., et al., 2020. Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings. preprintFinalesInfectious Diseases (Except HIV/AIDS) https://doi.org/10.1101/2020.10.20.20210484.
COVIDPoops19 Dashboard, 2022. COVIDPoops19 Dashboard.Available at: https://www.covid19wbec.org/covidpoops19 [Accessed 21 January 2022].
Cov-lineages.org, 2022. Cov-Lineages.Available at: https://cov-lineages.org/global_report_B.1.1.7.html [Accessed 21 January 2022].
Cov-lineages.org, 2022. Cov-Lineages.Available at: https://cov-lineages.org/global_report_B.1.351.html [Accessed 9 March 2022].
Cov-lineages.org, 2022. Cov-Lineages.Available at: https://cov-lineages.org/global_report_B.1.3.520.html [Accessed 8 February 2022].
Davies, M.-A., et al., 2022. Outcomes of laboratory-confirmed SARS-CoV-2 infection in the Omicron-driven fourth wave compared with previous waves in the Western Cape Province, South Africa. preprintFinalesInfectious Diseases (Except HIV/AIDS) https://doi.org/10.1101/2022.01.12.220369148.
Dyer, O., 2021. Covid-19: South Africa’s surge in cases deepens alarm over omicron variant. BMJ, n3013 https://doi.org/10.1136/bmj.n3013.
Gibson, C., et al., 2021. Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus. Sci. Total Environ. 782, 116749. https://doi.org/10.1016/j.scitotenv.2021.116749.
Habtewold, J., et al., 2022. Passive sampling, a practical method for wastewater-based surveillance of SARS-CoV-2. Environ. Res. 204, 110258. https://doi.org/10.1016/j.jenvres.2021.110258.
Harris-Lovett, S., et al., 2021. Wastewater surveillance for SARS-CoV-2 on college campuses: initial efforts, lessons learned, and research needs. Int. J. Environment. Res. Public Health 18 (9), 4545. https://doi.org/10.3390/ijerph18094545.
Hayes, Emalie K., Sweeney, Crystal L., Fuller, Megan, Erjavec, Genevieve B., Stoddart, Amina K., Guggen, Graham A., 2022. Operational constraints of detecting SARS-CoV-2 on passive samplers using electronegative filters: a kinetic and equilibrium analysis. ACS ES&T Water https://doi.org/10.1021/acs.estwater.1c00441.
Huyse, H., et al., 2021. Higher mortality associated with the SARS-CoV-2 Delta variant in the Western Cape, South Africa, using RdRp target delay as a proxy. preprintFinalesInfectious Diseases (Except HIV/AIDS).
Itarte, M., et al., 2021. Looking for a needle in a haystack. SARS-CoV-2 variant characterisation in sewage. Curr. Opin. Environ. Sci. Health. 24, 100308. https://doi.org/10.1016/j.joenve.2021.100308.
Jackson, C.B., et al., 2022. Mechanisms of SARS-CoV-2 entry into cells. Nat. Rev. Mol. Cell Biol. 23 (1), 3–20. https://doi.org/10.1038/s41580-021-00418-x.
Johnson, R., et al., 2021. Qualitative and quantitative detection of SARS-CoV-2 RNA in untreated wastewater in Western Cape Province, South Africa. S. Afr. Med. J. 111 (3), 198. https://doi.org/10.7196/SAMJ.2021.v111i13.15154.
Johnson, R., et al., 2020. Tracking the circulating SARS-CoV-2 variant of concern in South Africa using wastewater-based epidemiology. Sci. Rep. 12 (1), 1182. https://doi.org/10.1038/s41598-022-05110-4.
Lemieux, J.E., et al., 2021. Phylogeographic analysis of SARS-CoV-2 in Boston highlights the impact of superspreading events. Science 371 (6529), eabe3261. https://doi.org/10.1126/science.abc3261.

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Liu, P., et al., 2022. A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level. Sci. Total Environ. 807, 151047. https://doi.org/10.1016/j.scitotenv.2021.151047.

Meng, B., et al., 2021. Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the alpha variant B.1.1.7. Cell Rep. 35 (13), 109292. https://doi.org/10.1016/j.celrep.2021.109292.

NICD, 2022. SARS-CoV-2 GENOMIC SURVEILLANCE UPDATE - NICD. Available at: https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/sars-cov-2-genomic-surveillance-update/ [Accessed 7 March 2022].

Randazzo, W., et al., 2020. Metropolitan wastewater analysis for COVID-19 epidemiological surveillance. Int. J. Hyg. Environ. Health 230, 113621. https://doi.org/10.1016/j.ijheh.2020.113621.

Schang, C., et al., 2021. Passive sampling of SARS-CoV-2 for wastewater surveillance. Environ. Sci. Technol. 55 (15), 10432–10441. https://doi.org/10.1021/acs.est.1c01530.

Scott, L.C., et al., 2021. Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation. Environ. Res. 200, 111374. https://doi.org/10.1016/j.envres.2021.111374.

Street, R., et al., 2021. Spatial and temporal trends of SARS-CoV-2 RNA from wastewater treatment plants over 6 weeks in Cape Town, South Africa. Int. J. Environ. Res. Public Health 18 (22), 12085. https://doi.org/10.3390/ijerph182212085.

Tegally, H., et al., 2021. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 592 (7854), 438–443. https://doi.org/10.1038/s41586-021-03402-9.

U.S. Centers for Disease Control and Prevention, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel https://www.fda.gov/media/134922/download?fbclid=IwAR1DdEweazD3ixmrpZ2Mc07VXM0.n1.q455eQV76F0AfEaQlQ2Z3ph0Qexpy [Accessed 3 March 2022].

Umair, M., et al., 2021. Detection and whole-genome sequencing of SARS-CoV-2 B.1.617.2 and B.1.351 variants of concern from Pakistan during the COVID-19 third wave. preprint Infectious Diseases (Except HIV/AIDS) https://doi.org/10.1101/2021.07.14.21259909.

Viana, R., et al., 2022. Rapid epidemic expansion of the SARS-CoV-2 omicron variant in southern Africa. Nature https://doi.org/10.1038/s41586-022-04411-y.

Walsh, K.A., et al., 2020. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J. Infect. 81 (3), 357–371. https://doi.org/10.1016/j.jinf.2020.06.067.

Wilson, Melissa, Qiu, Yuanyuan, Yu, Jiaao, Lee, Bonita E., McCarthy, David T., Paeg, XiaoLi, 2022. Comparison of auto sampling and passive sampling methods for SARS-CoV-2 detection in wastewater. Pathogens 11 (3), 359. https://doi.org/10.3390/pathogens11030359.

Zheng, S., et al., 2020. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ, m1443 https://doi.org/10.1136/bmj.m1443.