Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Building-level wastewater surveillance of SARS-CoV-2 is associated with transmission and variant trends in a university setting

Sarah C. Sellers, Emily Gosnell, Dillon Bryant, Stefano Belmonte, Stella Self, Maggie S.J. McCarter, Kirsten Kennedy, R. Sean Norman*

* Corresponding author.
E-mail address: rsnorman@sc.edu (R.S. Norman).

ARTICLE INFO

Keywords:
Wastewater-based epidemiology
SARS-CoV-2
Building-level surveillance
Variants of concern
COVID-19
University

ABSTRACT

The University of South Carolina (UoSC) was among the first universities to include building-level wastewater surveillance of SARS-CoV-2 to complement clinical testing during its reopening in the Fall 2020 semester. In the Spring 2021 semester, 24h composite wastewater samples were collected twice per week from 10 residence halls and the on-campus student isolation and quarantine building. The isolation and quarantine building served as a positive control site. The wastewater was analyzed using RT-ddPCR for the quantification of nucleocapsid genes (N1 and N2) to identify viral transmission trends within residence halls. Log$_{10}$ SARS-CoV-2 RNA concentrations were compared to both new clinical cases identified in the days following wastewater collection and recovered cases returning to sites during the days preceding sample collection to test temporal and spatial associations. There was a statistically significant positive relationship between the number of cases reported from the sites during the seven-day period following wastewater sampling and the log$_{10}$ viral RNA copies/L (overall IRR 1.08 (1.02, 1.16) p-value 0.0126). Additionally, a statistically significant positive relationship was identified between the number of cases returning to the residence halls after completing isolation during the seven-day period preceding wastewater sampling and the log$_{10}$ viral RNA copies/L (overall 1.09 (1.01, 1.17) p-value 0.0222). The statistical significance of both identified cases and recovered return cases on log$_{10}$ viral RNA copies/L in wastewater indicates the importance of including both types of clinical data in wastewater-based epidemiology (WBE) research. Genetic mutations associated with variants of concern (VOCs) were also monitored. The emergence of the Alpha variant on campus was identified, which contributed to the second wave of COVID-19 cases at UoSC. The study was able to identify sub-community transmission hotspots for targeted intervention in real-time, making WBE cost-effective and creating less of a burden on the general public compared to repeated individual testing methods.

1. Introduction

A major challenge of the ongoing COVID-19 pandemic has been containing the spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). There are many limitations to clinical testing-based surveillance: asymptotically infected individuals often do not get tested; many clinical tests are less sensitive during the early stages of infection; clinical tests are costly, burdensome on the individual, and were in short supply during the initial months of the pandemic (Buitrago-Garcia et al., 2020; Daughton, 2020; Ferretti et al., 2020; Johansson et al., 2021). Wastewater-based epidemiology (WBE) approaches for SARS-CoV-2 surveillance have several advantages over an approach based on clinical testing. Because WBE methods measure the amount of SARS-CoV-2 viral RNA present in wastewater, they can detect infections missed by clinical testing. Furthermore, WBE methods are a passive form of surveillance that places no economic or time burdens on the general public.

WBE is a public health tool that was previously established as a method to monitor licit and illicit drug use, antibiotic resistance, poliovirus, and other viruses (e.g., adenoviruses, astroviruses, enteroviruses, hepatitis A and E viruses, noroviruses, and rotaviruses) within communities (Burgard et al., 2013; de Oliveira Pereira et al., 2016; Du
environmental research 215 (2022) 114277

2

Due to the versatile nature of WBE, it can be applied broadly at wastewater treatment plants and more granularly at point-source locations (e.g., effluent for a single building). After SARS-CoV-2 RNA was found in the feces of both symptomatic and asymptomatic COVID-19 patients, WBE was adapted to monitor SARS-CoV-2 transmission within the population of a given geographical area (Ahmed et al., 2020; Chen et al., 2020; Daughton, 2020; Xagoraraki and O’Brien, 2019). It has been observed that viral shedding can be detected in wastewater 4–10 days before the onset of symptoms and up to several months after the first symptoms arise (Miura et al., 2021; Nararajan et al., 2022; Wong et al., 2021; F. Wu et al., 2022; Y. Wu et al., 2020). As the COVID-19 pandemic reaches a point of endemicity and less resources are dedicated to routine clinical testing, wastewater surveillance can provide the broad-scale surveillance needed to monitor the potential emergence and spread of novel SARS-CoV-2 variants. The success of this surveillance approach has been demonstrated in the monitoring of the initial Omicron variant and the more recent Omicron sub-lineages, BA.4, and BA.5 (Johnson et al., 2022; La Rosa et al., 2022). Early detection of novel SARS-CoV-2 variants in community wastewater can be followed with a more targeted public awareness and vaccination campaign to minimize viral transmission within the impacted communities, similar to the public health response following the detection of poliovirus in routine wastewater monitoring programs. Additionally, the establishment of sustainable wastewater surveillance programs provide public health laboratories with the capability to monitor a range of potential pathogens other than SARS-CoV-2, such as monkeypox, poliovirus, and antibiotic resistance bacteria in order to prevent future pandemics (Link-Gelles, 2022; Wolfe et al., 2022).

Since its conceptualization, several universities have integrated WBE into their reopening strategies, including the University of South Carolina (UofSC) (Betancourt et al., 2021; Gibas et al., 2021; Harris-Lovett et al., 2021; Karthikeyan et al., 2022; Lu et al., 2022; McMahan et al., 2021; Scott et al., 2021). During the Spring 2021 semester, UofSC-Columbia, located in Columbia, South Carolina, had 25,391 total undergraduate students and 24,973 full-time undergraduate enrollments (University of South Carolina, 2021). During this semester, 5955 students lived on-campus in 28 campus-affiliated housing locations (University of South Carolina, 2021). As a component of the UofSC SARS-CoV-2 mitigation strategy, a WBE approach was implemented for 11 sites, including 10 residence halls and the designated on-campus student isolation/quarantine building. A critical criterion for selecting testing sites was ensuring that the point source location exclusively received effluent from one tractible residence hall. The goal of this study was to use WBE in a campus setting to (i) assess the effectiveness of wastewater surveillance of SARS-CoV-2 as a public health mitigation tool in high-density facilities, (ii) predict potential trends in clinical cases based on the viral RNA concentration in the wastewater, and (iii) monitor the potential emergence of SARS-CoV-2 variants and their effects on transmission trends. The use of WBE methods at a fine temporal and spatial scale provided an early warning system that supported and guided the implementation of residence hall targeted testing protocols, thus enhancing campus-wide viral mitigation.

2. Materials and methods

2.1. Clinical case data

Prior to the spring semester move-in day on January 10, 2021, students were required to provide proof of either a negative COVID-19 test in the last 10 days or a prior COVID-19 infection within the last 90 days. During the semester, all students, faculty, and staff were assigned to groups labeled 1–4 based alphabetically by last name or by residence hall for students residing on campus. Each group was required to participate in mandatory COVID-19 testing, either on-campus or off-campus, once per month based on assigned group testing weeks. On-campus clinical testing was performed using RT-qPCR analysis of saliva samples, processed by the UofSC College of Pharmacy, and reported daily to UofSC Health Services and weekly to the publicly available UofSC COVID-19 dashboard. Data sharing between UofSC Health Services and University Student Housing identified residential locations of positive cases through a real-time occupancy management database. The clinical case data used in this study was obtained from the UofSC dashboard and from de-identified data provided from University Student Housing. Students who tested positive for COVID-19 or were directly exposed to SARS-CoV-2 resided in either the isolation/quarantine site or an off-campus isolation location of their choosing. After 10 days of isolation/quarantine, students could return to their residence halls and classes, assuming they were asymptomatic within 24 h of their return date.

2.2. Wastewater collection

During the Spring 2021 semester, wastewater samples were collected twice per week, beginning on January 6, 2021, and continuing until April 26, 2021. Composite wastewater samples were collected over 24 h using ice-packed ISCO GLS Compact Composite Samplers (Teledyne ISCO, Lincoln, Nebraska, USA) with a collection rate of 30 mL of wastewater every 15 min (Fig. S1). Samplers were deployed between 8:30–10:00 a.m. on Mondays and Thursdays and recovered on Tuesdays and Fridays between 8:30–10:00 a.m. Each sampler was suspended using a Teledyne ISCO ProHanger Suspension Bracket within a sewage maintenance hole designated as a direct access point for wastewater exiting the specific monitored locations. In total, 13 locations were surveyed across the UofSC-Columbia campus, but Sites 2 and 12 were not single access points and therefore not included in this study. Of the 11 sites included, 10 were residence halls, and one was the on-campus student isolation/quarantine building (Fig. S2, Table S1). At the end of each sampling period, the samplers were collected, and the samples were placed on ice for transport to the laboratory. Samples were processed within 2 h of collection. To monitor the sample temperature within the samplers during the 24 h composite sampling, HOBO Pendant® Temperature/Light 64 K Data Loggers - UA-002-64 (Onset, Cape Cod, MA, USA) were placed directly in the collection bottles at multiple sampling locations, and the temperature was recorded every 5 min (Fig. S3). The similarities in temperature profiles at each site suggest that sample storage conditions during the 24 h collection period remained consistent and were not a source of variability between sites.

2.3. Wastewater processing and RNA extraction

Immediately following sample collection, each composite sample was homogenized for 10 min at medium speed using Waring LB10S variable speed laboratory blenders (Waring Laboratory, Torrington, CT). A preliminary study was performed and showed that this homogenization step was critical to disrupting solid aggregates and increasing SARS-CoV-2 RNA recovery in the liquid fraction before sample concentration (Fig. S4). Fifty milliliters of homogenized samples were decanted into conical bottom centrifuge tubes and centrifuged using a Beckman Coulter Allegra X-30 R refrigerated centrifuge at 4 °C and 4577 × g for 20 min without braking to pellet solids. Sample supernatants were collected and concentrated using Millipore Amicon centrifugal ultrafiltration units with a molecular weight cutoff of 30 kDa. Samples were centrifuged at 4 °C and 4000 × g for 10 min repeatedly until the supernatant was concentrated to 400 μL. From the concentrate, 200 μL was stored at −80 °C, and nucleic acids were extracted from the remaining 200 μL. Total RNA was extracted using the AllPrep PowerViral DNA/RNA kit as specified by the manufacturer’s instructions (Qiagen, Valencia, CA, USA). The final nucleic acids were eluted into 51 μL RNase-free water.
2.4. SARS-CoV-2 quantification

Reverse transcription droplet digital PCR (RT-ddPCR) was used to detect and quantify SARS-CoV-2 RNA copies/L in wastewater within 12–24 h following sample collection. Multiplexed probe-based RT-ddPCR assays were performed using the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad, Hercules, CA, USA) and manufacturer instructions for the quantification of SARS-CoV-2 and SARS-CoV-2 variants. The N1/N2 assay targeted the SARS-CoV-2 viral nucleocapsid (N) gene for viral RNA quantitation in both wild-type and variant strains. Variants of concern (VOCs) were routinely monitored in the isolation/quarantine building and residence halls, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2). The N501Y assay targeted the spike protein gene of the Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1) variants. N501Y positive samples were further tested for specific variant determination using the 69/70del (B.1.1.7), E487K (B.1.351 and P.1), K417N (B.1.351), and T487K (B.1.617.2) assays. In the isolation/quarantine site, the Pepper Mild Mottle Virus (PMMoV) assay was used to quantify the concentration of PMMoV, one of the most abundant RNA viruses in human feces (Kitajima et al., 2018; Scott et al., 2021). As the amount of PMMoV is correlated to the amount of fecal material present in the wastewater, dividing the amount of SARS-CoV-2 in the wastewater by the amount of PMMoV allowed normalization of SARS-CoV-2 RNA relative to the amount of fecal material present. A binomial regression model was used to assess the association between the number of positive individuals in the isolation/quarantine building and the SARS-CoV-2 RNA concentration normalized to PMMoV RNA concentration. It was observed that the positive association between viral RNA copies in wastewater and COVID-19 cases was more significant in the non-normalized data (Fig. 4b) compared to the normalized data (Fig. S5). Therefore, all analyses throughout this study used non-normalized log_{10} SARS-CoV-2 RNA copies/L for the wastewater data.

The N1/N2 duplex reaction mix for the SARS-CoV-2 RT-ddPCR assays (22 μL) consisted of 1X Supermix, 20U/μL of RT Enzyme, 15 nM of DTT, 900 nM of N1/FAM probe, 250 nM of N2/HEX probe, 6 μL of nuclease-free water, and 5 μL of the sample. The reaction mixes for the RT-ddPCR variant assays (22 μL) consisted of 1X Supermix, 20U/μL of RT Enzyme, 15 nM of DTT, 900 nM/250 nM (FAM/HEX) of the ddPCR mutation detection assay, 7.1 μL of nuclease-free water, and 5 μL of the sample. The Exact Diagnostics SARS-CoV-2 control and nuclease-free water were used as positive and negative controls, respectively, in all N1/N2 assays. Positive controls for the variant assays consisted of unique gBlock Gene Fragments obtained from Integrated DNA Technologies, Inc. (IDT, Coralville, Iowa, USA). For ddPCR, droplets were generated using a Bio-Rad QX200 Automated Droplet Generator. Droplets were thermal cycled using a Bio-Rad C1000 Touch thermal cycler and quantified using a Bio-Rad QX200 as described previously (Isanovic et al., 2022). Detailed ddPCR procedures and SARS-CoV-2 quantification are described in the supplemental methods. Information on primers, probes, and gBlocks used in this study are listed in Table S2 and according to the updated dMIEQ guidelines, other information is located in Table S3.

2.5. Statistical analysis

2.5.1. Association of residence Hall occupancy and positive wastewater samples

Each statistical analysis was performed with either RStudio 2022.02.1 or 1.4.1106. Associations were assessed between sample positivity rates (defined as the proportion of wastewater samples from a site in which SARS-CoV-2 RNA was detected) and the number of occupants at the site. A binomial regression model was fit with the number of positive samples from each site as the response, the total number of samples over the course of the semester as the number of trials, and the number of occupants at the beginning of the semester as the predictor. Detailed methods used for statistical analyses are located in the supplemental methods document.

2.5.2. Assessment of temporal trends in identified cases and wastewater viral RNA concentration

Individuals who tested positive were removed from their university residence to an isolation location and therefore contributed to their residence wastewater only prior to testing positive. Consequently, we expected to see a positive relationship between the viral RNA concentrations in wastewater collected from residence halls and the number of individuals who tested positive in the following days. Site-specific associations were assessed between the number of residents who tested positive for COVID-19 and the concentration of viral RNA in wastewater. Since the RNA copies/L was noticeably right-skewed, log_{10}-transformation was performed before analysis. A negative binomial mixed regression model was fit with the number of cases detected at each site during the combined seven-day period following sampling as the response and the log_{10} RNA copies/L from the corresponding site as the predictor. Site-specific random intercepts and random slopes were included, along with an offset term for the residence hall occupancy at the beginning of the semester.

2.5.3. Assessment of temporal trends in recovered cases and wastewater viral RNA abundance

Many individuals continue to shed SARS-CoV-2 into wastewater even after they are no longer contagious (Xiao et al., 2020). As a result, we expected to see a positive relationship between the amount of viral RNA present in the wastewater of a residence hall and the number of priorly infected individuals who recently returned to the residence hall after completing isolation. Site-specific associations were assessed between the number of priorly infected individuals who returned to their residence hall after completing isolation and the log_{10} viral RNA copies/L of wastewater. A negative binomial mixed regression model with the number of priorly infected individuals who returned to each residence hall over the seven-day period prior to wastewater sampling as the response and the log_{10} viral RNA copies/L of the corresponding residence hall as the predictor was used to assess this association. The model included site-specific random intercepts and random slopes and an offset term for the residence hall occupancy at the beginning of the semester.

2.5.4. Assessment of trends in the isolation/quarantine building

Unlike the residence halls, positive individuals contribute to the wastewater in the isolation/quarantine building after testing positive, necessitating a separate analysis for this site (Site 13). As the total occupancy of the isolation/quarantine building was at times too small for a Poisson or negative binomial model to be appropriate (e.g., one occupant), a binomial regression model with the logistic link function was fit with the number of positive individuals in the building as the response and the log_{10} viral RNA copies/L of wastewater as the predictor. The number of occupants of the building (including both confirmed cases and close contacts without a positive test) was used as the number of trials for the binomial model.

3. Results

3.1. Wastewater collected from individual residence Halls reveal spatial and temporal trends of sars-cov-2 transmission on campus

There were spatial and temporal differences in the number of positive samples and the log_{10} concentration of SARS-CoV-2 RNA in the wastewater collected at each site (Fig. 1; Table S4). In the 10 residence halls, SARS-CoV-2 RNA was detected in 189 of 285 samples (66.3%) from January 6, 2021, to April 26, 2021, where the percentage of positive samples at each site ranged from 44.8% to 92.9% (Table S4). There was no significant relationship between the odds of a wastewater sample testing positive and the number of occupants in a residence hall (p-value
the semester, the average \( \log \) may be driving the spatial differences in viral abundance. Throughout the semester on January 24, contributing to decreased viral RNA concentration in the wastewater sample from this site on January 25. This pattern of case identification contributing to reduced wastewater SARS-CoV-2 RNA concentration in Site 1 occurred throughout the semester on February 2–4, March 20–22, April 13–15, and April 15–18. There was a statistically significant positive relationship between the number of cases reported in Site 1 during the seven-day period following wastewater sampling and the \( \log_{10} \) viral RNA copies/L (IRR 1.07), meaning that for every unit increase in \( \log_{10} \) viral RNA copies/L of wastewater, the number of new cases per occupant per day for Site 1 increased by a factor of 1.07 (or 7%) (Fig. 2B). Similar trends were observed in the remaining residence halls at different time points throughout the semester (overall IRR 1.08 (1.02, 1.16) p-value 0.0126) (Fig. S6).

3.2. Recovered COVID-19 cases affect SARS-cov-2 RNA concentration in campus wastewater

The number of returning recovered COVID-19 cases was compared to wastewater viral RNA concentration at each of the 10 residence halls (Fig. 3; Fig. S7). For each residence hall, increases in viral RNA concentration were observed following the return of previously positive cases. For example, Site 1 had eight case returns between January 14–18, 2021, which contributed to increased viral RNA concentration in the wastewater sample on January 19 (Fig. 3A). Similarly, two returning cases were again observed for this site on January 26 and January 27, contributing to increased wastewater viral RNA concentration on January 28. Site 1 had additional return events on February 3–4, February 12–15, March 8–11, March 23–29, and April 3–5 that contributed to increased SARS-CoV-2 RNA concentration at this site. In some instances, Site 1 had a return case that did not contribute to increased viral RNA concentration, such as on January 22 and March 30. The opposite effect for those two events is likely due to a low viral titer in the stool at the point of return or the removal of a new COVID-19 case at the same time.

There was a statistically significant positive relationship between the number of cases that returned to Site 1 after completing isolation during the seven-day period preceding wastewater sampling and the viral RNA concentration in the wastewater (IRR 1.11), meaning that for every unit increase in the number of cases that returned to Site 1 as the response and the wastewater RNA copies/L as the predictor. Site-specific random intercepts and random slopes were included, along with an offset term for the residence hall occupancy at the beginning of the semester (Overall IRR 1.08 (1.03, 1.16), p-value 0.0126). Site 1 is shown as an example; see Fig. S6 for trends observed in Sites 3–11.
increase in $\log_{10}$ viral RNA copies/L of wastewater, the number of returning cases per occupant per day for at Site 1 increased by a factor of 1.11 (or 11%) (Fig. 3B). Similar trends were observed at the other monitored sites throughout the semester (overall IRR 1.09 (1.01, 1.17) p-value 0.0222) (Fig. S7).

### 3.3. SARS-CoV-2 RNA concentration in isolation building wastewater correlates with COVID-19 cases

During the Spring 2021 semester, from February 8, 2021, to April 26, 2021, samples were collected at the isolation/quarantine building (Site 13) twice per week, resulting in 20 wastewater samples. Of the 20 wastewater samples, 100% were positive for SARS-CoV-2 RNA (Table S4). The mean $\log_{10}$ viral RNA copies/L for Site 13 over the course of the semester was 6.41, with a maximum of 7.35 on March 15 and a minimum of 3.78 on March 4 (Fig. 4A; Table S4).

A statistically significant positive association (odds ratio 1.26 (1.13, 1.40), p-value <0.0001) was detected between the number of confirmed positive occupants in the building and the $\log_{10}$ SARS-CoV-2 RNA copies per liter of wastewater. The number of occupants of the building (including both confirmed cases and close contacts without a positive test) was used as the number of trials for the binomial model (odds ratio 1.26 (1.13, 1.40), p-value <0.0001).

### 3.4. Alpha emergence detected in temporal and spatial variant analysis

Wastewater samples were also monitored for the presence of the SARS-CoV-2 variants: Alpha (B.1.1.7), Beta (B.1.351), Delta (B1.617.2), and Gamma (P.1). Each variant mutation was detected in at least one site except for the Delta variant, which was not detected above the LOD. Site 13 contained confirmed positive students from each on-campus residence hall, including residence halls not participating in the study. The occupation by students from a wide range of residence halls at this site provided a broader perspective of transmission for the entire campus.

It was estimated that VOCs started circulating on-campus during February after the initial occurrence of the 69–70 del mutation in wastewater collected at Site 1 on February 8 (66.67%) and Site 9 on February 22 (25.0%) (Fig. 5A, Table S4). On March 4, the Alpha variant began to compose the majority (mean 62.49%) of the viral genotype in the wastewater samples from Site 13. In addition to Alpha, the Beta variant emerged in the first week of March, with its presence increasing to 80.76% by April 26.
variant was observed in a single instance well above the LOD on March 11 but was not observed at any other time point in the isolation building.

Spatial analysis of VOC occurrences across campus demonstrated that Sites 1, 3, 9, and 11 were the only residence halls to test positive for a variant of concern at the same time as the isolation building, indicating that these were among the first sites to experience VOC transmission on campus. Site 1 was the only residence hall to test positive for Alpha, Beta, and Gamma variants. However, the Beta and Gamma variants were only detected in a small number of samples, while the Alpha variant was the most frequently detected variant. VOCs were not detected in Sites 7 and 8 throughout the semester.

4. Discussion

Building-level wastewater surveillance of SARS-CoV-2 provides an in-depth analysis of sub-community transmissions, especially in high-density facilities. While community-level wastewater monitoring gives an understanding of temporal trends in viral transmission, it cannot account for the spatial trends that occur within the sewer sheds. Spatial trends could occur in certain neighborhoods, businesses, or schools where the viral transmission is increasing, but the dilution and mixing of wastewater from multiple sources at the WWTP masks the underlying hotspots.

In the analysis of the 10 residence halls at UofSC, spatial and temporal differences were observed in the number of positive samples, the SARS-CoV-2 RNA concentration within the positive samples, and the number of identified cases (Figs. 1 and 2, Table S4). The significant positive associations between the viral RNA concentration detected at individual residence halls, and the upcoming confirmed positive COVID-19 cases further support the importance of sub-community level sampling as a predictive public health mitigation tool and its importance in detecting potential hot spots in a sub-community (Fig. 1; Fig. 2). Identified positive cases typically followed the detection of increased viral RNA in wastewater, and case removal typically resulted in decreased viral RNA in wastewater collected from the respective site. However, in some instances, case removal did not result in decreased wastewater viral RNA concentration. These results are likely due to underlying positive cases that were not identified through clinical testing or post-isolation cases with continued viral fecal shedding returning to the residence halls during the wastewater testing period. Most previous studies have failed to analyze the significance of recovered positive cases returning from isolation locations, even though fecal shedding can continue for several months after the initial infection (Miura et al., 2021; Natarajan et al., 2022; Wong et al., 2021; F. Wu et al., 2022; Y. Wu et al., 2020). However, one study monitored the wastewater of a residential building and observed increased SARS-CoV-2 RNA concentration that gradually declined after two recovered COVID-19 cases returned from hospital isolation (Wong et al., 2021). Similarly, the findings of this current study support that both newly identified and recovered cases contribute to viral RNA concentration in residence hall wastewater, making it necessary to compare both types of cases to trends in the wastewater data.

Site 13, the university-designated isolation/quarantine building, was used as the positive control site since the number of confirmed positive occupants and quarantined close-contact occupants were known for each sampling period, with the number of isolation cases never exceeding 18. As expected, the temporal relationship between wastewater viral RNA concentration and confirmed positive cases for this site was opposite from that observed in the residence halls. In this building, the viral RNA concentration in wastewater increased only after new confirmed cases were added to its occupancy and decreased when the students’ isolation period ended, and they returned to their respective residence halls. This finding is similar to the trend identified in the isolation building at the University of California, San Diego, where a lag was also observed between isolation case numbers and viral RNA concentration in wastewater (Karthikeyan et al., 2021). While this finding was expected, it further confirmed the significant correlation between COVID-19 case counts and wastewater SARS-CoV-2 RNA concentrations. Site 13 also provided the study with a broader view of viral transmission trends and VOC detection across campus, as it included COVID-19-positive individuals isolated from residence halls not included in the study.

In the Spring 2021 semester, variants of the SARS-CoV-2 virus were first detected at Site 13 during March (Fig. 5). During this time,
increased viral transmission was observed on-campus, contributing to a second case peak in the last half of the semester (Fig. 1). It is likely that the increased transmission was due to the emergence of VOCs, specifically the Alpha variant, which became the most prevalent variant on the UofSC campus on approximately March 4. The temporal and spatial design of this study also provided the opportunity to assess how an emerging VOC may spread in high-density facilities. Some sites tested positive for mutations before others, implicating these sites as possible early hotspots of VOC transmission contributing to the campus-wide VOC emergence. Spatial analysis of VOC transmission throughout the residence halls of a university using RT-ddPCR technology has not yet been published. In general, the use of RT-ddPCR for variant monitoring is limited to a few studies that either focus on the temporal trends of a single broad community or compare a large WWTP to one of its sub-communities (Adusei, 2022; Heijnen et al., 2021; Ho et al., 2022; Kartthikeyan et al., 2022).

With the seemingly constant emergence of new VOCs, transmission rates continue to change, making WBE a powerful tool for monitoring transmission dynamics whose accuracy is immune to changes in the behavior of the public (e.g., testing practices, healthcare-seeking behavior, etc.). WBE can be used to monitor transmission dynamics at the sub-community level that may otherwise be undetectable due to asymptomatic transmission, ever-changing testing practices, and heterogeneity in healthcare-seeking behavior. Identifying sub-community transmission hotspots allows for targeted intervention (i.e., targeted testing, vaccination campaigns, administration of anti-viral medications) in real-time, as seen at multiple universities, including UofSC (Betancourt et al., 2021; Gibas et al., 2021; Harris-Lovett et al., 2021).

Targeted interventions are more cost-effective and create less of a burden on the general public than ‘one size fits all’ interventions, making them highly attractive as COVID-19 transitions from a pandemic to an endemic disease.

5. Conclusion

WBE acts as a passive surveillance system for diseases, such as SARS-CoV-2. Passive surveillance allows for large communities to be monitored without wasting resources or burdening the general public. When used at the building level, WBE provides guidance for targeted interventions (increased testing, masking, social distancing, etc.) as potential transmission hotspots are uncovered. This study observed trends in wastewater SARS-CoV-2 RNA concentration and clinical COVID-19 cases, further showing that WBE methods can predict increased transmission earlier than clinical testing-based surveillance methods. The study also noted the impact of priorly infected individuals returning to residence halls, which has not been discussed in previous wastewater surveillance studies. The University of South Carolina was able to track wild-type SARS-CoV-2 and specific VOCs as they spread and emerged throughout the large campus during the Spring 2021 semester, making building-level wastewater surveillance an effective component of the building-level wastewater surveillance an effective component of the overall COVID-19 mitigation plan. As SARS-CoV-2 becomes less of a threat to the health of our communities, it is anticipated that the newfound national and international WBE surveillance systems will be adapted for the detection of seasonal viruses and future emerging pathogens as a mitigation tool to prevent outbreaks, decrease healthcare costs, and save lives.

Credit author statement

Sarah C. Sellers: Writing – original draft, Formal analysis, Investigation; Emily Gonnell: Investigation, Writing – review & editing; Dillon Bryant: Investigation, Writing – review & editing; Stefano Belmonte: Investigation, Writing – review & editing; Stella Self: Formal analysis, Writing – review & editing; Maggie S. J. McCarter: Formal analysis, Writing – review & editing; Kirsten Kennedy: Data curation, Writing – review & editing; R. Sean Norman: Conceptualization, Investigation, Writing – review & funding acquisition, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the University of South Carolina internal COVID-19 Mitigation funds. We give special thanks to UofSC Facilities Services Plumbing staff: Tim Crouch, Eddie Durant, and Benjamin Urbates for their assistance in deploying and recovering autosamplers from sewer maintenance sites. Finally, we thank the members of the Norman Molecular Microbial Ecology Laboratory for their continued support from the conception to the completion of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://doi.org/10.1016/j.envr.2022.114277.

References

Adusei, A., 2022. Detection of SARS-CoV-2 variants of concern using wastewater surveillance (Order No. 29210955). Available from ProQuest Dissertations & Theses Global (2681430935). Retrieved from https://www.proquest.com/dissertations-theology-dissertation-detec...-variants-concern-doctoral/2681430935-te-2.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O’Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W., Zaugg, J., Deere, L., Hugenholz, P., Thomas, K.V., Mueller, J.F., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764 https://doi.org/10.1016/j.scitotenv.2020.138764.

Betancourt, W.Q., Schmitz, B.W., Innes, G.K., Preis, S.M., Pogrebna Brown, K.M., Stark, E.R., Foster, A.R., Sprusler, R.S., Harris, D.T., Sherchan, S.P., Gerba, C.P., Pepper, L.L., 2021. COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. Sci. Total Environ. 779, 146408 https://doi.org/10.1016/j.scitotenv.2021.146408.

Butrero-Garcia, D., Egli-Guery, D., Gounette, M.J., Hossmann, S., Imeri, H., Ipkezi, A.M., Salanti, G., Low, N., 2020. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis. PLoS Med. 17 (9), e1003346 https://doi.org/10.1371/journal. pmed.1003346.

Burgard, D.A., Fuller, R., Becker, B., Ferrell, R., Dinglasan-Panililio, M.J., 2013. Potential trends in Attention Deficit Hyperactivity Disorder (ADHD) drug use on a college campus: wastewater analysis of amphetamine and ritalinic acid. Sci. Total Environ. 450-451, 242-249. https://doi.org/10.1016/j.scitotenv.2013.02.020.

Chen, Y., Chen, L., Deng, Q., Zhang, G., Wu, K., Ni, L., Yang, Y., Liu, B., Wang, W., Wei, C., Yang, J., Ye, G., Cheng, Z., 2020. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J. Med. Virol. 92 (7), 833-840. https://doi.org/10.1002/jmv.25825.

Daughton, C.G., 2020. Wastewater surveillance for population-wide Covid-19: the present and future. Sci. Total Environ. 736, 139631 https://doi.org/10.1016/j. scitotenv.2020.139631.

de Oliveira Pereira, J.S., da Silva, L.R., de Meireles Nunes, A., de Souza Oliveira, S., da Costa, E.V., da Silva, E.E., 2016. Environmental surveillance of polioviruses in rio de Janeiro, Brazil, in support to the activities of global polio eradication initiative. Food and environmental virology 8 (1), 27–33. https://doi.org/10.1007/s12560-015-9223-5.

Du, P., Thai, P.K., Bae, Y., Zhou, Z., Xu, Z., Zhang, X., Wang, J., Zhang, C., Hao, F., Li, X., 2019. Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology. Drug Alcohol Depend. 205, 105732 https://doi.org/10.1016/j.drugalcdep.2019.06.034.

Ferretti, L., Wyman, C., Kendall, D., Zhao, L., Nurtay, A., Abeler-Dörner, L., Parker, M., Bonsall, D., Fraser, C., 2020. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. Science (New York, N.Y.) 368, 1033-1036. https://doi.org/10.1126/science.abb6936 eabb6936.

Gibas, C., Lambirth, K., Mittal, N., Roppolo Brazell, L., Hinton, K., Lontai, J., Stark, N., Young, I., Quach, C., Fuss, M., Kaiser, J., Nicolosi, B., Chen, D., Akella, S., Tang, W., Schlueter, J., Munir, M., 2021. Implementing building-level...
