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Correlative analysis of wastewater trends with clinical cases and hospitalizations through five dominant variant waves of COVID-19

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Supporting Information

The Supporting Information is available free of charge at: https://pubs.acs.org/doi/XXXX. The supporting information includes tables of SARS-CoV-2 summary statistics and tables of Pearson and Spearman correlation coefficients between wastewater and clinical data.

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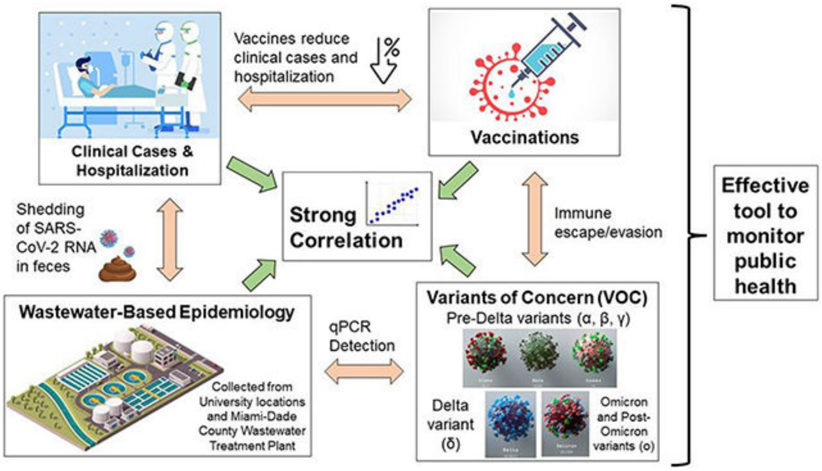
Abstract

Wastewater-based epidemiology (WBE) has been utilized to track community infections of Coronavirus Disease 2019 (COVID-19) by detecting RNA of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), within samples collected from wastewater. The correlations between community infections and wastewater measurements of the RNA can potentially change as SARS-CoV-2 evolves into new variations by mutating. This study analyzed SARS-CoV-2 RNA, and indicators of human waste in wastewater from two sewersheds of different scales (University of Miami (UM) campus and Miami-Dade County Central District wastewater treatment plant (CDWWTP)) during five internally defined COVID-19 variant dominant periods (Initial, Pre-Delta, Delta, Omicron and Post-Omicron wave). SARS-CoV-2 RNA quantities were compared against COVID-19 clinical cases and hospitalizations to evaluate correlations with wastewater SARS-CoV-2 RNA. Although correlations between documented clinical cases and hospitalizations were high, prevalence for a given wastewater SARS-CoV-2 level varied depending upon the variant analyzed. The correlative relationship was significantly steeper (more cases per level found in wastewater) for the Omicron-dominated period. For hospitalization, the relationships were steepest for the Initial wave, followed by the Delta wave with flatter slopes during all other waves. Overall results were interpreted in the context of SARS-CoV-2 virulence and vaccination rates among the community.

Graphical Abstract



Keywords

SARS-CoV-2; Variants; Wastewater; Wastewater-Based Surveillance; Clinical Cases; Hospitalization

1.0 Introduction

SARS-CoV-2, the severe acute respiratory syndrome coronavirus-2, is the causative virus of the Coronavirus Disease 2019 (COVID-19) illness, which first emerged in late 2019 in Wuhan, China.[1, 2] The World Health Organization (WHO) declared that COVID-19 was a global pandemic in early 2020 and the virus has since impacted populations all over the globe, with 764 million reported global infections as of late-April 2023.[1-3] The evolution of the SARS-CoV-2 virus from the original Wuhan wild-type strain has been defined by the WHO and discussed within scientific literature coined as variants of concern (VOC).[4-9] The classification for a SARS-CoV-2 variant to become a VOC relate directly to its threat towards human and public health, specifically towards transmissibility between individuals, the severity of disease, evasion of the immune system, and its ability to reduce the effectiveness of currently available vaccinations and treatments.[5, 7, 9-12] The five most-common VOC's, and their specific genetic mutations that have been described within scientific literature since the onslaught of the COVID-19 pandemic are: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529).[4-8, 10, 11, 13]

Previous literature illustrates the presence of Alpha first emerging in September of 2020 in the UK and late December of 2020 within the United States. [4, 7, 13, 14] Beta emerged in South Africa in December of 2020, and Gamma emerged in Brazil in December of 2020, yet both Beta and Gamma emerged within the United States as of January 2021. [4, 7, 13, 14] Delta first emerged within India in December of 2020, although Delta was not seen in the United States until March of 2021.[4, 7, 13, 14] The United States saw a surge in positive cases during the Delta variant, which dominated one phase of the pandemic occurring from roughly June through November of 2021.[15] Omicron first emerged in South Africa during November of 2021, and by the end of that month was found in the United States with widespread distribution by January of 2022.[16] Within this study we describe five "waves" that are associated with SARS-CoV-2 detection within wastewater and correspond to the date ranges of Table 1. These waves are defined based upon timeframes with evident increases in SARS-CoV-2 levels within the study sewersheds, and the timing of the variants in U.S. patient populations. A surge was defined to start upon a general trend of increasing SARS-CoV-2 signal being detected by qPCR and ends when the levels return to baseline levels. The wave which we describe as the Initial wave includes the period when the wild-type virus was present in the United States as well as possible variants of interest that are not discussed here. The Pre-Delta wave corresponds to when Alpha, Beta, and Gamma emerged within the United States, and the Delta wave describes specifically around the time when Delta emerged and clinical cases drastically increased.[15] The Omicron wave corresponds to when Omicron emerged and case numbers also continued to climb from previous months, and the Post-Omicron wave describes what has been measured throughout 2022.[15, 17]

The reproductive number, or number of new people infected per infected individual, is a measure of transmissivity. For the Alpha variant, described by Lin et al. 2021[7], the reproductive number was 75-78% higher than the wild-type virus, the Beta variant was roughly 50% more transmissible than previous variants, and Gamma was 1.4-2.2 times more transmissible than the wild-type; contrarily, the Delta variant was described to be 97% more transmissible than previous variants, including the wild type.[7, 18, 19] Further research applied the concept of the higher viral load, seen with the Delta strain, supported by higher direct qPCR measurements of patient samples,[20-22] and found that the Delta strain may cause higher hospitalization instances with more severe disease and intensive care unit (ICU) admissions due to its morphology.[18] Breakthrough infections, due to variant turnover within a community, increase speculation on vaccine failure. Yet what has been seen with the Delta variant, compared against Alpha[12, 14], is that the Delta is sixfold less sensitive to neutralizing antibodies, eightfold less sensitive to vaccine-elicited antibodies, and has varying results of vaccine-evasion dependent upon the vaccine.[23-26]

Examples of changes in case numbers, or severity of illness of infected patients due to variant turnover, can be found in the state of Florida. This state was, historically, an epicenter during the emergence of the Zika virus in 2016, so the dynamics of epidemiological spread from this earlier virus were subsequently applied to evaluate the potential spread of the Delta strain .[27] Florida saw few new cases of Delta between February 2021 and June 2021, which coincided with the widespread distribution of the COVID-19 vaccines.[15] But a new wave of epidemics hit the state after that, resulting in a significant increase in incidence.[9, 15, 27] This wave in the state was marked by an increase in the proportion of the genome of the Delta lineage, from 28.7% to 54.3%.[27] Within 3 months of its emergence, the Delta variant had completely replaced the previously prevalent Alpha variant as the most dominant.[27-29] While the Delta variant found in Florida showed fewer common mutations, it was 3 to 21 times more prevalent in communities in Florida as compared to global data.[8, 27]

The Delta variant was quickly replaced by Omicron worldwide. Omicron, the most heavily mutated variant thus far spread quickly throughout the globe.[16, 17, 30, 31] Within weeks, the spread of this new VOC coincided with large increases in the prevalence of COVID-19 cases likely due to enhanced immune evasion and enhanced transmissibility from previous VOCs.[16, 30, 31, 30, 32-35] The emergence of Omicron and its subvariants continue to be evaluated as SARS-CoV-2 continues to evolve.[16, 30, 36-38]

Surveillance strategies such as wastewater-based epidemiology (WBE), which is also known as wastewater surveillance (WS) as well as wastewater-based surveillance (WBS), have been used throughout the COVID-19 pandemic to track and trace the prevalence of SARS-CoV-2 within communities.[6, 15, 22, 28, 39-48] Resulting evidence of wastewater-based studies has provided that wastewater surveillance can prove useful in predicting hospitalizations, clinical cases, and ICU admissions.[15, 19, 42, 49, 50] Although relationships have been established between SARS-CoV-2 wastewater levels and prevalence of COVID-19, few have evaluated the change of these relationships by variant wave. This study addresses this data gap by evaluating the association between wastewater SARS-CoV-2 levels and the number of community cases under the influence of different variants, as well as the number of hospitalizations.[6, 28, 40, 42, 43, 49, 51] Starting in September 2020, the University of Miami (UM) established a WBE surveillance program as a tool for measuring the COVID-19 spread within its campuses.[44] For two years since then, targeted sampling and research[15, 41, 44, 45, 52] has been utilized to inform policies for the safety of students, faculty, and staff. The Central District Wastewater Treatment Plant (CDWWTP) of Miami-Dade County was an early collaborative partner with UM facilitating routine weekly collection of wastewater servicing a large population allowing for comparisons between campus level and community level relationships between human case data and wastewater.[15, 41, 52] The objective of this study was to describe relationships between clinical case data, and WBE surveillance data for the five distinct internally defined "variant waves" of the COVID-19 pandemic. We hypothesize that the clinical case data correlates strongly with wastewater data but shifts in relationships occur during times dominated by differing variant strains. This study therefore focused on evaluating prior to and following the mass distribution of vaccinations against COVID-19, correlations between SARS-CoV-2 and clinical cases, and hospitalizations within communities.

2.0. Materials and Methods

2.1. Clinical Data Collection

Aggregated campus level clinical case data was published throughout the pandemic on UM's public COVID-19 dashboard. This data from August 16, 2020, through September 30, 2022, which reported the number of positive COVID-19 cases among students and employees (faculty plus staff) daily throughout the course of the pandemic, was made available for correlation analysis with wastewater (and approved by Internal Review Board, IRB 20210164).

COVID-19 clinical case and hospitalization data in Miami-Dade County was available through the Centers for Disease Control and Prevention (CDC) public data base.[1, 53] Data collected from the CDC database included a 7-day moving average of positive cases and hospitalization data divided-up by regions with differing zip codes. Clinical data, provided by the CDC, was collected from March 2020 through September 2022, while the hospitalization information was available from July 2020 through September 2022.

Vaccination data reporting the number of students and employees receiving the primary and booster vaccines was provided through the UM administration which had developed databases for tracking vaccinations for both populations. Similarly, vaccination for the Miami-Dade County population was available through the public databases of the CDC.

2.1.1. Wastewater Sample Collection—Wastewater was collected from the UM Gables campus and from the community wastewater treatment plants in Miami-Dade County. Basic water quality parameters, per sample, were collected in the field using a Xylem YSI ProDSS sonde. Water quality measurements included temperature, pH, turbidity, dissolved oxygen, and specific conductivity.[15, 41] Summary statistics for water quality are provided in the supporting information within Table S-1. All persons collecting, or handling wastewater followed the University's policies of Health and Safety including the use of PPE, and disinfection of equipment.

The UM Gables campus samples were collected weekly from September 30, 2020, until September 21, 2022, and were gathered from the long-standing WBE surveillance program at UM.[15, 41, 44] The UM Gables campus wastewater enters the municipal sewer system at three locations, at sites referred to as WG01, WG02, and WG0U. All three sites are lift stations fitted with wet wells which allow for mixing of wastewater. At WG02 (n=102) composite samples were collected (24 equal volume hourly samples during the prior 24 hours) using an autosampler (ISCO 6712, time paced). Samples at sites WG01 (n=102) and WG0U (n=96) were collected as grab samples from the wet wells. Grab samples were collected at these two sampling sites as only one autosampler was available, which was installed at WG02 given that it received the largest proportion of wastewater from the residential dorms on campus. All weekly campus wastewater collection was collected in the morning at about the same time and on the same day of the week to maximize the consistency in the population-wastewater-use patterns between sampling intervals. Upon collection, samples were immediately transported to the University's Biospecimen Shared Resource laboratory in coolers on ice packs.

Two different sampling efforts were conducted at the community wastewater treatment plants. One effort was conducted by UM and the other effort by Miami-Dade Water and Sewer Department (MD-WASD). Miami-Dade County has three major community wastewater treatment plants: the North District (ND, mean flow 93 million gallons per day (mgd)), Central District (CD, mean flow 111 mgd), and South District (SD, mean flow 92 mgd) Plants servicing populations of 780,000, 830,000, and 920,000, respectively.

UM collected two sets of samples weekly at the CDWWTP, one set consisted of composite (WC0Dc) samples (collected from January 2021 to September 2022, n=90), and the second set consisted of grab samples (WC0Dg) (collected from January 2021 to May 2022, n=71). The purpose of the grab sample collection was for comparative purposes to determine whether grab and composite collection methods provided equivalent results. Grab samples (1 L) were collected by UM staff from the influent to the wastewater plant at the head of the grit chambers. The composite samples consisted of 1 L volumes provided as the staff members of the CDWWTP create daily wastewater composites, in much larger volumes, for in-house water quality analysis.

The second sampling effort by MD-WASD was conducted at all three community WWTPs. Samples were collected either weekly or biweekly at each plant and these samples were then shipped to a commercial company, Biobot Analytics. All composite samples at the wastewater treatment plants (inclusive of those provided to UM) were collected using a refrigerated autosampler (HACH AS950 fitted with an IO9000 for flow proportional sampling over a 24-hour period corresponding to midnight to midnight the prior day). Samples were analyzed from March 2020 through September 30, 2022, by the MD-WASD group (n=170 for CDWWTP, n=155 for NDWWTP, and n=158 for the SDWWTP).

2.1.2. Wastewater Concentration & Analysis—Following the methodology described by Sharkey et al. 2021[44] and Babler et al. 2022[41], the steps utilized for laboratory analysis were sample pretreatment, concentration, nucleic acid extraction, and detection of molecular targets by qPCR. The primary concentration method utilized in UM's laboratories was electronegative (EN) filtration.[39, 45] Pretreatment of wastewater included the addition of human coronavirus-OC43 (OC43) as a recovery control for the entire process. The amount of OC43 added corresponded to 10^6 copies per Liter of wastewater. OC43 was chosen as it was a positive-sense single-stranded RNA coronavirus, like that of SARS-CoV-2. To prepare the sample for EN filtration, additional pretreatment included the adding of magnesium chloride ($MgCl_2$) and the reduction of pH between 3.5-4.5 with 10% hydrochloric acid (HCl). The intention of this pretreatment was to improve the binding affinity of SARS-CoV-2 by adjusting the viral particles' chemical charge to positive. By doing so, we prepared the ambient viral particles for more effective trapping on the negatively charged membrane. After pretreating the wastewater, it was then filtered by vacuum filtration through HAWP filters (hydrophilic, mixed-cellulose ester, 0.45 μm pore size, EMD-Millipore: #HAWP04700). This method allowed for capturing SARS-CoV-2 particles through physical straining for those adhered to suspended solid particles and through surface charge interactions. Samples were filtered through HAWP filters until clogging wherein volumes varied from 15 to 150 mL. Wastewater samples from the campus were generally less turbid requiring larger filtration volumes to ensure clogging. Samples from the WWTP were generally more turbid and were characterized by smaller filtration volumes (from 15 to 30 mL), due to the high suspended solids which quickly saturated the membranes with particles. Sample volumes were likely impacted by wastewater age. The average travel time of wastewater through the sewer system is estimated at 1 day allowing for the formation of iron precipitates which imparted a dark color to the samples collected at the plant. The increased solids at the WWTP site resulted in decreased sample volumes and thus increased detection limits. The levels of SARS-CoV-2 at the wastewater plant, however, were less variable and no samples measured below detection limits. Upstream sample volumes were influenced by larger variations in turbidity (and thus more variable SARS-CoV-2 levels) as the wastewater had not yet undergone as much mixing as downstream samples collected at the WWTP. These variations in sample volumes could have influenced the detection limits of the measurements especially when COVID-19 levels in the upstream community was low.

Once samples were filtered, filter membranes were then folded in on themselves four times and placed within 1.5 mL of 1X DNA/RNA Shield. These lysates were used for RNA extractions within 24 hours of preparation using a Zymo Research Quick RNA-Viral Kit, with a modified in-house protocol to reduce qPCR inhibition at the University's Center for AIDS Research (CFAR), the method of reducing qPCR inhibition in combination with this commercial kit is described in detail within Babler et al. 2023.[52] Concentrate samples were transported to the CFAR laboratory immediately on ice and stored in 4 °C until they were extracted (within 24 hours).[41, 45, 52] The optimized methodology described in Babler et al. 2023[52] underwent initial extraction efficiency evaluation and quality assessments by measuring nucleic acid concentrations (ng/μL) of extracted samples. To augment these initial assessments, we utilized B2M RNA present within wastewater as a qualitative measure to confirm that RNA was extracted from freshly collected samples. For the B2M molecular target, 99% of all collected wastewater samples were above detection limits.

Following the extraction of RNA, 30 μL of HIV RNA, provided by the CFAR laboratory, was added to the 10 μL eluate of wastewater RNA to assess inhibition with following qPCR analyses. An additional "HIV RNA control" sample was generated weekly alongside the wastewater sample set by combining 10 μL nuclease-free water with 30 μL of HIV RNA and were included as an additional sample alongside the wastewater RNA within qPCR.

Samples were quantified by Volcano 2nd Generation (V2G)-qPCR (with the same cycling parameters as those mentioned in our previous studies[15, 41, 44, 45, 52, 54]). Quantification focused on five targets: the SARS-CoV-2 nucleocapsid gene (N3) described by Babler et al. 2022[41], two target parameters for normalization purposes, Pepper Mild Mottle Virus (PMMoV) and Beta-2 Microglobulin (B2M)[15, 41, 52], and two control parameters OC43, and HIV.

As mentioned above, OC43 was spiked into the samples and measured by V2G to determine the recovery of the virus from concentration through qPCR detection (inclusive of extraction). On average, percent recoveries were 27%, see Table S-2 in the supporting information for calculated recovery per sampling site (between 22 to 30%). Background levels of OC43 were checked periodically and were consistently below detection limits. However, it is recognized that humans may shed OC43, especially during surges of seasonal illness. A study focused on wastewater treatment plant in California found seasonal human coronavirus in wastewater.[55] The levels observed in this study were estimated to represent about 1% of the level of the OC43 spike suggesting a relatively small contribution of background OC43 to the spike recovery measurement.

HIV was also measured wherein the water control sample's Ct values were compared against the wastewater Ct values of the same V2G assay; if the wastewater RNA Ct values were within ±2 cycles of the water control, then results of the wastewater RNA were considered uninhibited. Each qPCR assay that was performed for this study had seven NTC's included per plate, alongside target-specific standards ranging serially from 10^1-10^5 to develop the standard curve for quantification ($R^2>0.95$). All data quantified by qPCR were back calculated and reported as genomic copies per Liter (gc/L) of raw wastewater. No corrections were made to the SARS-CoV-2 gc/L values including the percent recovery of OC43. The detection limit for SARS-CoV-2 by V2G-qPCR was estimated at 100 gc/L[44].

Wastewater collected by MD-WASD, and analyzed by Biobot Analytics, followed methodology as described by Duvallet et al. 2022[56]. In brief the method employed by Biobot Analytics involved pasteurization of the sample (60 °C for 1 hour) and filtration of 45 mL of sample through a 0.2 μm pore size filter. The subsequent laboratory methods varied over the course of the measurement period. From March to June 2020, 40 mL wastewater filtrates were concentrated using polyethylene glycol precipitation (PEG) (Wu et al.)[57]. The pellets were resuspended in TRIzol and RNA was purified using phenol/chloroform extraction and ethanol precipitation. Samples were then analyzed using two-step RT-qPCR targeting the N1 and N2 regions of the SARS-CoV-2 nucleocapsid gene. In June 2020, Biobot changed the concentration method to centrifugal ultrafiltration (Amicon Ultra-15) which processed 15 mL of wastewater. The viral particles concentrated were lysed by adding AVL buffer. Ethanol was added to the lysates and the lysate ethanol mixture was applied to RNeasy Mini columns or RNeasy 96-well cassettes and eluted into 75 μL of nuclease-free water. RNA extracts (3 μL) were then subjected to one-step RT-qPCR targeting the N1 and N2 regions as described above. Biobot ran positive synthetic SARS-CoV-2 RNA controls and two no-template controls on every qPCR plate. Matrix inhibition was assessed by reviewing the slopes of the qPCR curves.

2.2. Data Analysis

Wastewater and human health data were consolidated in Excel and analyzed with the statistical software package SPSS (version 28.0.0). Shapiro-Wilks Tests of Normality performed on each dataset indicated that the data were not normally distributed. Thus, differences on the mean values between populations was assessed using the Mann-Whitney U test. Correlations were evaluated using Spearman coefficients. To augment the statistical results, Pearson coefficients were also evaluated for the purpose of assessing linear correlations between wastewater and clinical cases. Best fit lines were used to establish the desired quantitative relationships between SARS-CoV-2 RNA levels in wastewater and human case data. Campus wastewater data was evaluated by averaging the SARS-CoV-2 data from the three sites that represent wastewater from the Gables campus (WG01, WG02, and WG0U). The WWTP data corresponded to only the Central District Plant (as composite or grab samples) for the UM dataset. For the MD-WASD, the WWTP data corresponded to the mean from all three plants (North, Central, and South). Correlations were made between the three-week moving average of the $\log_{10}$ of the wastewater genomic copies per liter versus the 7-day moving average of clinical data. The correlations were considered statistically significant for p-values less than 0.05. We normalized the slope value of the Pearson correlations with the population of each area, and its unit became clinical cases/((copies/L) × person). The population contributing within the campus sewershed was the sum of the employee and student population. Given that the population on campus was variable (especially between academic year and summer periods), the denominator of the prevalence (cases per 10,000 people) was adjusted for the changes in population contributing to the sewershed.

The vast majority of the community-level WWTP data (>99%) was above detection limits (Table 2). Between 71% and 84% of the campus samples were above detection limits. When evaluating the data, the values below detection limit were set to the detection limit values of 100 gc/L[44].

3.0. Results and Discussion

3.1. Variant Waves on Campus

Wastewater levels of SARS-CoV-2 RNA, measured by V2G-qPCR at UM, and campus clinical case comparisons showed significant correlations in most variant-dominant periods; the distinct peaks of cases corresponded to different periods of dominant variants. Five waves of sharp rises in COVID-19 cases (Figure 1a) were observed on the UM campus from the end of September 2020 until the end of September 2022, and wastewater SARS-CoV-2 levels were shown to coincide with human cases by showing increases and decreases to reflect these defined waves. In an academic campus setting, the trend of these cases was noticed to be influenced by the school system's calendar for holiday breaks and return-to-campus with the start of each semester. As the summer break period of 2021 began, the number of cases on campus was at very low levels from May to July compared to the main academic year. This was likely due to the combined effects of low campus densities during the summer and the increase in vaccination rates among employees and students (Figure 1a). The employee population remained relatively constant throughout the calendar year

(4,000 to 4,400) whereas the student population varied significantly between the academic year (15,300 to 16,700) and the summer semesters (4,200 to 4,400). Towards the end of the summer of 2021, the impacts of the Delta variant were apparent although the number of people impacted was relatively low compared to other wave periods. These low numbers were likely driven by the lower student population on campus at the time, and the high vaccination rates of employees (91%) and students (78%) at the time the students returned at the end of August for the start of the Fall 2021 semester.

Vaccination rates and the number of vaccines administered seemed to influence wastewater data and the trend of cases, especially after the administration of the first vaccine dose.[23, 25, 31, 34, 35] As shown in Figure 1a, when the completed full vaccine ratio of employees and students started to increase rapidly in March 2021 during the Pre-Delta wave, the number of cases on campus and the SARS-CoV-2 RNA concentrations in the monitored wastewater decreased rapidly. During the end of the Delta wave period, students and employees were predominantly vaccinated (students at 79% and employees at 93% by August 30, 2021). In addition, employees began to receive boosters, which likely contributed to the drop in the number of cases. However, presumably due to the declining vaccine effectiveness in the face of new variants,[32, 34, 35] high vaccination rates did not appear to have much of an impact on the number of infections during the Omicron variant wave. Although, the campus community was highly vaccinated, with 95% of the employee population vaccinated (and 27% with boosters) and 84% of the student population vaccinated (and 9% with boosters) by November 30, 2021.[23, 34]

The relatively modest increase in cases during the Delta wave, illustrated in Figure 1a, coincided with steep increases in wastewater SARS-CoV-2 levels which was observed at levels above 4 $\log_{10}$ gc/L at the end of July 2021. Although the number of cases was generally low at this time, the high wastewater SARS-CoV-2 concentration reflects that the Delta variant likely led to more viral shedding per infected person, supporting claims already stated within the literature.[20] The months of October and November 2021 mark the end of the Delta wave as the SARS-CoV-2 levels were near detection limits in the wastewater and the number of reported cases was also very low. Immediately after the Thanksgiving holiday break of November 2021, when students returned to campus for the end of semester, the levels of SARS-CoV-2 in wastewater started increasing, and the cases followed reflecting the resurgence of COVID-19 associated with the Omicron variant. The Omicron surge continued through winter break. To ameliorate the impacts from the Omicron surge, the University held only remote classes during the first two weeks of the Spring 2022 semester (January 18 to January 30) with in person classes resuming February 1, 2022. The number of documented cases on campus since the February 2022 period remained low in comparison to the numbers documented earlier. This low number of documented cases in the post-Omicron period may be due to under-reporting as described above, especially given that the levels in wastewater remained at elevated levels, at about 4.5 $\log_{10}$ gc/L, through the September 2022 period (Figure 1a).

The correlation between SARS-CoV-2 results, detected from wastewater, and the number of cases on campus was likely affected by human behavior. Such as people commuting to campus, the academic session, full-time employment, and those living on campus year-round. The dominant variant at the time was also an important factor due to the differing characteristics of each variant. Correlations between wastewater SARS-CoV-2 RNA concentrations and campus clinical cases in the four periods (Pre-Delta, Delta, Omicron, and Post-Omicron) resulted in Spearman correlations, r, between 0.49 and 0.83 ($p < 0.03$, except for the Delta wave) (Table S-3).

3.1.1. Normalization for Clinical Cases on Campus—PMMoV or B2M were used to normalize the SARS-CoV-2 measurements. PMMoV is a standard fecal indicator target, utilized with WBE studies and for determining fecal contamination of a water source; it has a dietary origin from humans as it is a plant (i.e., pepper) pathogen, can be found in wastewater in high concentrations, and from various water sources.[58, 59] B2M, a parameter utilized internally at UM, and validated for effective use with SARS-CoV-2 surveillance,[15, 41, 44, 60] is a human-cellular-waste indicator mRNA gene that increases in detectable concentration when an individual is experiencing inflammation or infection, which can readily be detected from wastewater by qPCR. When the SARS-CoV-2 signal was normalized by PMMoV or B2M, it was shown that the trend of normalized SARS-CoV-2 measured from wastewater could better predict the prevalence of clinical cases within the community (Table S-3; Figure 1a). Unnormalized SARS-CoV-2 data in the Pre-Delta wave provided a lower Spearman ($r = 0.55$) correlation between wastewater and clinical case data, but when normalized by PMMoV or B2M the relationship was effectively improved with r values > 0.65 (Table S-3). Similarly, in the other three waves the correlations also improved after normalization (Table S-3). This observation suggests that a standardized approach utilizing different indicators found within human waste can contribute towards a more accurate interpretation of clinical cases in SARS-CoV-2 wastewater surveillance. Among the correlations comparing wastewater data against clinical cases, the Spearman r value of the Omicron wave was the highest, reaching $r = 0.93$ after being normalized by the PMMoV human waste indicator, while the Spearman r value for SARS-CoV-2 normalized by B2M was second highest ($r = 0.87$) (Table S-3). For the Post-Omicron wave, normalization of SARS-CoV-2 also improved the significance of correlations. With normalization by B2M, the Spearman correlation for the Post-Omicron wave increased from $r = 0.49$ to $r = 0.72$ with a unnormalized p-value = 0.024 decreasing to $p <0.001$. Overall normalization of the SARS-CoV-2 signal by indicators of human waste improved the correlations between wastewater SARS-CoV-2 levels and clinical cases at the campus scale (Table S-3).

3.1.2. Correlation Slopes on Campus—SARS-CoV-2 RNA concentration was plotted against clinical cases; the slope of the line provides the relationship between SARS-CoV-2 levels and prevalence of infection within the community (Figure 2a-c). Changes in the slope can be used as a parameter to observe changes in the relationship between wastewater levels and clinical cases. Data show that the employee population was fairly stable throughout the two years at an average population of 4,727 with a maximum per day of 4,505 and minimum per day of 4,007. The student population at UM was much more variable and changed significantly between the regular academic semester and the summer sessions. The student population varied from 15,319 during academic year 2020 to 2021, to 4,234 during the summer of 2021, to 16,730 during academic year 2021 to 2022, and back down to 4,423 during the summer of 2022. As mentioned earlier, all prevalence values of COVID-19 on campus were thus normalized by the daily population values.

During the four different waves of the collected campus wastewater data, the slope value of the Delta wave period (4.0 cases per 10,000 per $\log_{10}$ gc/L) was similar to that of the Pre-Delta wave period (3.9), while the slope value of the Omicron wave period went up to 13.6 cases per 10,000 per $\log_{10}$ increase in gc/L (for non-normalized data), and its value during the Post-Omicron period was reduced (3.1) back to levels comparable to the periods prior to the Omicron period (Figure 2a). The change in slope may also indirectly reflect the corresponding transmission and possibly virulence differences between the different variants. Although the slope data will also be affected by external factors such as vaccines. For example, vaccines were fully provided by the community during the Delta wave period, likely contributing towards the relatively low slope value during this period compared to Omicron.

3.2. Wastewater vs. Clinical Cases in Miami-Dade County

The regional demographic changes that occur in universities result in the number of recorded cases being highly influenced by academic schedules. On the contrary, due to the wide range of data collection and uniform population distribution, the changes in county-level values can better reflect the replacement of different variants. The spread of different variants has a major influence on clinical and wastewater trends, but the use of vaccines also had a very significant effect, depending upon the current VOC, throughout the pandemic.[23, 25, 31] When the Delta wave began in March 2021 in Florida, vaccinations were limited to healthcare professionals, people over 40, and people at high risk for serious illness, such as the immunocompromised.[27] On April 5, vaccine eligibility was expanded to individuals over 18 years of age, and the US Food and Drug Administration approved Pfizer's COVID-19 vaccine on May 12 for individuals 12 years of age or older.[27] In Miami-Dade County, vaccinations began in January 2021 and coverage rates rose rapidly, which likely led to a decline in the number of clinical cases after the first peak during the Pre-Delta wave (Figure 1b). From January 2021 onwards, a relatively steady decline until June 2021 was noticed; however, there was a very small wave of increase at the end of March 2021 (Figure 1b). It is speculated that this is related to the initial spread of the Delta variant, which is consistent with the conclusion in the Cella et al. 2022[27] study that the Delta variant spread in Florida starting in March 2021.

Both SARS-CoV-2 RNA concentration and human health cases showed relatively high correlations, Spearman r-values above 0.68 for all variant waves without normalization by PMMoV or B2M (Table S-4). These high r values were observed for the UM composite samples, UM grab samples and for the MD-WASD samples. At the community scale results show no statistical differences between UM grab and UM composite samples ($p = 0.34$) and both sample sets followed similar temporal trends relative to case data. However, the MD-WASD SARS-CoV-2 levels were higher ($p < 0.001$) by about an order of magnitude (Table 2), although showing the same temporal trends. The differences between the UM data and the MD-WASD data is likely due to differences in sample preparation and analysis which resulted in an offset in the levels observed.

The levels of SARS-CoV-2 RNA observed in Miami-Dade County are comparable to those observed in other parts of the world. To compare the results, the SARS-CoV-2 data was normalized by WWTP flow rate and population served as described by Gonzalez et al. 2020[61] for wastewater plants in Virginia and Monteiro et al. 2022[62] for wastewater plants in Portugal. On average over the period of record, using the mean flow rate and population served, we compute 5.4 Log_{10} genomic copies per person (gc/person) released into the wastewater using the UM values and 6.3 Log_{10} gc/person using the MD-WASD values. Overall, these results compare well with those observed by Gonzalez et al. and Monteiro et al. who report ranges from detection limits of 4.5 to high values of 8 Log_{10} gc/person within the WWTPs in their respective communities.[61, 62]

At the Miami-Dade County level, the normalization of the SARS-CoV-2 signal by PMMoV and B2M did not have consistent positive effects on the correlation and some normalization results reduced the r-value.[44] Among them, the analysis results of UM Lab composite samples and grab samples show that the relationship between wastewater data and clinical cases becomes weaker with normalization during the Pre-Delta, and Omicron waves. In the data collected by MD-WASD, it was shown that the normalization with PMMoV improved the correlations during the Pre-Delta wave, Delta wave, and Omicron waves but decreased correlations during the Initial and Post-Omicron waves (Table S-4). Overall, at the large county scale, correlations between wastewater SARS-CoV-2 levels and clinical cases were strong without normalization; normalization did not consistently improve the correlations. In general, normalization with PMMoV at the community scale did not provide consistent improvements in the correlations between SARS-CoV-2 RNA levels in wastewater and COVID-19 cases.

The data analysis of UM Lab shows that the change of slope was consistent with the trends observed on at the UM Gables campus (Figure 2b). The value of the slope, as given by both the UM and MD-WASD data, was very similar between the Initial, Pre-Delta, Delta, and Post-Omicron waves showing between 5 to 11 cases county-wide per 10,000 population per $\log_{10}$ change in SARS-CoV-2 levels in wastewater (Table S-4). For all sample sets, the slope increased significantly during the Omicron wave to values between 30 to 46 cases per 10,000 population per $\log_{10}$ change in SARS-CoV-2 (Table S-4). The slope of the Omicron variant was highest among all different variant wave periods in the Miami-Dade County data analysis, which is consistent with the UM campus results. The Omicron variant is by far the most highly mutated of all VOC, which causes enhanced transmissibility and partial resistance to COVID-19 vaccine-induced immunity,[34, 63] which resulted in an infection rate that was four times that of the wild type.[34] Many factors can affect the transmission rate of the Omicron variant; mutations of the spike protein in Omicron increases binding affinity to receptors on infected cells, which is a major factor for increased viral load per infected patient, making the Omicron variant more infectious towards the host.[32] This high immune escape and immune evasion ability is also reflected in our data analysis results, via direct qPCR measurements which show marked increases in human case data during the Omicron wave.

Comparison of the slope values between the campus data and the county level data shows that the campus values (3 to 11 cases per 10,000 population per gc/L) were lower than the county values (11 to 46 cases per 10,000 population per gc/L). This difference may be associated with the different demographics of the campus population skewed towards younger University-aged adults and the higher vaccination rates of the campus employees and students as listed above (78% for student to 91% for employees at the end of August 2021) compared to the general county population (e.g., 61% at the end of August 2021).

3.3. Wastewater vs. Hospitalization in Miami-Dade County

Hospitalization rates for Miami-Dade County also showed high correlations with wastewater SARS-CoV-2 levels, but normalization did not help to consistently improve the correlations. Since the hospitalization data provided by the CDC starts at the end of July 2020, only a portion of the curve for the Initial wave is shown in Figure 1b (gray dashed line). The Delta wave and Omicron wave respectively showed obvious hospitalization wave peaks, and the change in the trend of the number of hospitalizations was basically the same as that of the wastewater detection results, while the changes in the number of hospitalizations in the other waves were not as high (Figure 1b). In both the UM and MD-WASD datasets, the raw wastewater detection data and hospitalization were significantly correlated. Correlations between hospitalizations and SARS-CoV-2 wastewater levels without normalization by PMMoV or B2M were all higher than r = 0.62 with some correlations as high as r = 0.96 (Table S-5). In the comparison of hospitalization and wastewater data, the relative slopes in different periods were different from what was seen with the comparison of clinical cases and wastewater data. Considering the non-normalized SARS-CoV-2 data for MD-WASD which has the longer period of record, a decreasing trend in the slope over time was evident. Among them, the Initial wave period had the highest slope 6.2 hospitalization per 10,000 per $\log_{10}$ gc/L), which corresponded to the early times during the pandemic when COVID-19 symptoms were severe. The slope then dropped sharply during the Pre-Delta wave (to values 1.9), and then rebounded to a certain extent in the following Delta wave (5.0), but the slope gradually decreased during Omicron (3.3) and post-Omicron (1.8) (Table S-4).

This trend can also be directly shown in Figure 2c. The slope value decreased from the Initial wave to the Pre-Delta wave period. This decrease may be related to the community’s initial uptake of vaccines during the Pre-Delta wave (Figure 1b). During the Delta wave period the slope increased likely due to the higher pathogenicity, viral load, and transmissivity of the Delta variant. After the Delta wave the slopes decreased possibly due to the combined effects of decreased virulence, increased vaccination rates and improved immune response of the public to severe symptoms. By comparing these results with those from the clinical case data from the prior section (results section 3.2), the relatively steep slope for hospitalization during the Initial wave emphasized the lack of vaccines at the time, the increased virulence of COVID-19, and the lack of tests available early during the pandemic. For those getting ill, there was a higher proportion requiring hospitalization and testing was restricted to those who were symptomatic. This was reflected in the slope for hospitalization (6.2) being close to the slope for cases (6.6) during the initial phase.

4.0. Conclusions

Wastewater surveillance is an important tool for tracking COVID-19, able to build up the relationship between wastewater SARS-CoV-2 levels and clinical cases based on mutation dynamics of SARS-CoV-2 and vaccine prevalence. Since the virulence characteristics and transmission speed of each variant have been observed to be different, data analysis through wastewater monitoring can also reflect the characteristics of the virus and the effectiveness of the corresponding vaccine, to a certain extent. This can bring some inspiration for continued research, severe-illness prevention, and continued development of vaccines of COVID-19 variants. For example, after the first dose of the vaccine, the difference in vaccine effectiveness was pronounced, with vaccine effectiveness being 48.7% for the Alpha variant and only 30.7% against the Delta variant. After two doses of the vaccines, the effectiveness against the Delta variant rose to around 67% to 88%.[23] Hospitalizations rates per given levels of SARS-CoV-2 in wastewater decreased over time as the populations became more highly vaccinated. This supports the community maximizing vaccine intake among vulnerable populations, regardless of the VOC to increase protection against severe COVID-19 illness.[23]

In this study, the correlations between wastewater SARS-CoV-2 RNA concentration and human health fluctuated accordingly by variant. In the five different pandemic waves, both the campus and Miami-Dade County clinical cases and wastewater detection results showed high correlations. Slope changes in different periods was also significantly related to the prevalence of the different strains given concentrations found in wastewater. In campus-level case tracking and wastewater surveillance, changes in SARS-CoV-2 correlated simultaneously with school holidays, population numbers and densities. The early observation of Omicron at the campus scale coincided with the end of the Thanksgiving holiday break when many students traveled back to campus. At the community level, the UM lab which analyzed CDWWTP samples and the MD-WASD lab which analyzed samples from all three WWTPs, had the steepest slopes during the Omicron wave. The campus data also showed its steepest slope during the Omicron wave, but the absolute value of the slope was lower for the campus data relative to the county level data likely due to differences in population demographics and vaccination rates. The hospitalization data were more closely matched to the wastewater SARS-CoV-2 RNA concentration data, with Spearman correlation coefficients above 0.9 in some cases.

Normalization of SARS-CoV-2 levels by PMMoV and B2M generally improved correlations between wastewater measures and clinical cases at the campus level. However, normalization did not improve correlations for either the clinical cases or hospitalizations at the county scale in this study. Thus, normalization is recommended for only smaller sewershed scales subject to greater variability in local human inputs. It is not recommended for the larger community scale based on the analysis done here.

Overall, the relationship between wastewater SARS-CoV-2 levels and COVID-19 cases within the corresponding sewershed did vary during the different variant waves. The largest change was observed during the Omicron wave, when the slope of the relationship steepened by a factor of 2 to 3 suggesting that SARS-CoV-2 shedding from this variant was not as high compared to preceding variants. For hospitalizations, the steepest slope was observed during the Initial wave during a time when vaccines were not yet available, and the virulence of SARS-CoV-2 was high.

As SARS-CoV-2 continues to be considered a pandemic, detecting the variants early is critical for the protection of public health and for the defense mechanisms of current healthcare systems. Overall, wastewater monitoring can be used to estimate clinical COVID-19 cases and predict peak hospitalizations, providing more time and information for communities and healthcare facilities to respond to outbreaks. These relationships should be continuously checked over time as changes in population demographics, vaccination rates, and changes in the variant characteristics can alter the correlations between SARS-CoV-2 levels in wastewater and COVID-19 cases and hospitalizations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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5.0. References

1. (CDC), C. f. D. C. a. P. COVID Data Tracker. https://covid.cdc.gov/covid-data-tracker/#county-view?list_select_state=all_states&data-type=CommunityLevels (August 3, 2022).
2. Cucinotta D; Vanelli M, WHO Declares COVID-19 a Pandemic. Acta Biomed 2020, 91, (1), 157–160. DOI: 10.23750/abm.v91i1.9397 [PubMed: 32191675]
3. Medicine, J. H. U. o., COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) and Johns Hopkins University (JHU). https://coronavirus.jhu.edu/map.html (October 28, 2022).
4. Aleem A; Akbar Samad AB; Slenker AK, Emerging Variants of SARS-CoV-2 And Novel Therapeutics Against Coronavirus (COVID-19). In StatPearls, Treasure Island (FL), 2022.
5. Boehm E; Kronig I; Neher RA; Eckerle I; Vetter P; Kaiser L; Geneva Centre for Emerging Viral, D., Novel SARS-CoV-2 variants: the pandemics within the pandemic. Clin Microbiol Infect 2021, 27, (8), 1109–1117. DOI: 10.1016/j.cmi.2021.05.022 [PubMed: 34015535]
6. Karthikeyan S; Levy JI; De Hoff P; Humphrey G; Birmingham A; Jepsen K; Farmer S; Tubb HM; Valles T; Tribelhorn CE; Tsai R; Aigner S; Sathe S; Moshiri N; Henson B; Mark AM; Hakim A; Baer NA; Barber T; Belda-Ferre P; Chacon M; Cheung W; Cresini ES; Eisner ER; Lastrella AL; Lawrence ES; Marotz CA; Ngo TT; Ostrander T; Plascencia A; Salido RA; Seaver P; Smoot EW; McDonald D; Neuhard RM; Scioscia AL; Satterlund AM; Simmons EH; Abelman DB; Brenner D; Bruner JC; Buckley A; Ellison M; Gattas J; Gonias SL; Hale M; Hawkins F; Ikeda L; Jhaveri

H; Johnson T; Kellen V; Kremer B; Matthews G; McLawhon RW; Ouillet P; Park D; Pradenas A; Reed S; Riggs L; Sanders A; Sollenberger B; Song A; White B; Winbush T; Aceves CM; Anderson C; Gangavarapu K; Hufbauer E; Kurzban E; Lee J; Matteson NL; Parker E; Perkins SA; Ramesh KS; Robles-Sikisaka R; Schwab MA; Spencer E; Wohl S; Nicholson L; McHardy IH; Dimmock DP; Hobbs CA; Bakhtar O; Harding A; Mendoza A; Bolze A; Becker D; Cirulli ET; Isaksson M; Schiabor Barrett KM; Washington NL; Malone JD; Schafer AM; Gurfield N; Stous S; Fielding-Miller R; Garfein RS; Gaines T; Anderson C; Martin NK; Schooley R; Austin B; MacCannell DR; Kingsmore SF; Lee W; Shah S; McDonald E; Yu AT ; Zeller M; Fisch KM; Longhurst C; Maysent P; Pride D; Khosla PK; Laurent LC; Yeo GW; Andersen KG; Knight R, Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. Nature 2022, 609, (7925), 101–108. DOI: 10.1038/s41586-022-05049-6 [PubMed: 35798029]

7. Lin L; Liu Y; Tang X; He D, The Disease Severity and Clinical Outcomes of the SARS-CoV-2 Variants of Concern. Front Public Health 2021, 9, 775224. DOI: 10.3389/fpubh.2021.775224 [PubMed: 34917580]
8. Mercatelli D; Giorgi FM, Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Front Microbiol 2020, 11, 1800. DOI: 10.3389/fmicb.2020.01800 [PubMed: 32793182]
9. Schmedes SE; Azarian T; Cella E; Motes J; Tekin O; Weiss J; Miller N; Blanton J, Introduction and community transmission of SARS-CoV-2 lineage A.2.5 in Florida with novel spike INDELS. medRxiv 2021. DOI: 10.1101/2021.12.03.21266538
10. Dumonteil E; Herrera C, Polymorphism and Selection Pressure of SARS-CoV-2 Vaccine and Diagnostic Antigens: Implications for Immune Evasion and Serologic Diagnostic Performance. Pathogens 2020, 9, (7). DOI: 10.3390/pathogens9070584
11. Mengist HM; Kombe Kombe AJ; Mekonnen D; Abebaw A; Getachew M; Jin T, Mutations of SARS-CoV-2 spike protein: Implications on immune evasion and vaccine-induced immunity. Semin Immunol 2021, 55, 101533. DOI: 10.1016/j.smim.2021.101533 [PubMed: 34836774]
12. Meng B; Kemp SA; Papa G; Datir R; Ferreira I; Marelli S; Harvey WT; Lytras S; Mohamed A; Gallo G; Thakur N; Collier DA; Mlcochova P; Consortium C-GU; Duncan LM; Carabelli AM; Kenyon JC; Lever AM; De Marco A; Saliba C; Culap K; Cameroni E; Matheson NJ; Piccoli L; Corti D; James LC; Robertson DL; Bailey D; Gupta RK, Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. Cell Rep 2021, 35, (13), 109292. DOI: 10.1016/j.celrep.2021.109292 [PubMed: 34166617]
13. Tegally H; Wilkinson E; Lessells RJ; Giandhari J; Pillay S; Msomi N; Mlisana K; Bhiman JN; von Gottberg A; Walaza S; Fonseca V; Allam M; Ismail A; Glass AJ; Engelbrecht S; Van Zyl G; Preiser W; Williamson C; Petruccione F; Sigal A; Gazy I; Hardie D; Hsiao NY; Martin D; York D; Goedhals D; San EJ; Giovanetti M; Lourenco J; Alcantara LCJ; de Oliveira T, Sixteen novel lineages of SARS-CoV-2 in South Africa. Nat Med 2021, 27, (3), 440–446. DOI: 10.1038/s41591-021-01255-3 [PubMed: 33531709]
14. Davies NG; Abbott S; Barnard RC; Jarvis CI; Kucharski AJ; Munday JD; Pearson CAB; Russell TW; Tully DC; Washburne AD; Wenseleers T; Gimma A; Waites W; Wong KLM; van Zandvoort K; Silverman JD; Group CC-W; Consortium C-GU; Diaz-Ordaz K; Keogh R; Eggo RM; Funk S; Jit M; Atkins KE; Edmunds WJ, Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021, 372, (6538). DOI: 10.1126/science.abg3055
15. Zhan Q; Babler KM; Sharkey ME; Amirali A; Beaver CC; Boone MM; Comerford S; Cooper D; Cortizas EM; Currall BB; Foox J; Grills GS; Kobetz E; Kumar N; Laine J; Lamar WE; Mantero AMA; Mason CE; Reding BD; Robertson M; Roca MA; Ryon K; Schurer SC; Shukla BS; Solle NS; Stevenson M; Tallon JJJ; Thomas C; Thomas T; Vidovic D; Williams SL; Yin X; Solo-Gabriele HM, Relationships between SARS-CoV-2 in Wastewater and COVID-19 Clinical Cases and Hospitalizations, with and without Normalization against Indicators of Human Waste. ACS ES&T 2022, (Water). DOI: 10.1021/acsestwater.2c00045
16. Khandia R; Singhal S; Alqahtani T; Kamal MA; El-Shall NA; Nainu F; Desingu PA; Dhama K, Emergence of SARS-CoV-2 Omicron (B.1.1.529) variant, salient features, high global health concerns and strategies to counter it amid ongoing COVID-19 pandemic. Environ Res 2022, 209, 112816. DOI: 10.1016/j.envres.2022.112816 [PubMed: 35093310]
17. Boehm AB; Hughes B; Wolfe MK; White BJ; Duong D; Chan-Herur V, Regional Replacement of SARS-CoV-2 Variant Omicron BA.1 with BA.2 as Observed through Wastewater Surveillance.

Environ Sci Technol Lett 2022, 9, (6), 575–580. DOI: 10.1021/acs.estlett.2c00266 [PubMed: 35711323]

18. Liu Y; Rocklov J, The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. J Travel Med 2021, 28, (7). DOI: 10.1093/jtm/taab124
19. Vo V; Tillett RL; Papp K; Shen S; Gu R; Gorzalski A; Siao D; Markland R; Chang CL; Baker H; Chen J; Schiller M; Betancourt WQ; Buttery E; Pandori M; Picker MA; Gerrity D; Oh EC, Use of wastewater surveillance for early detection of Alpha and Epsilon SARS-CoV-2 variants of concern and estimation of overall COVID-19 infection burden. Sci Total Environ 2022, 835, 155410. DOI: 10.1016/j.scitotenv.2022.155410 [PubMed: 35469875]
20. Li B; Deng A; Li K; Hu Y; Li Z; Shi Y; Xiong Q; Liu Z; Guo Q; Zou L; Zhang H; Zhang M; Ouyang F; Su J; Su W; Xu J; Lin H; Sun J; Peng J; Jiang H; Zhou P; Hu T; Luo M; Zhang Y; Zheng H; Xiao J; Liu T; Tan M; Che R; Zeng H; Zheng Z; Huang Y; Yu J; Yi L; Wu J; Chen J; Zhong H; Deng X; Kang M; Pybus OG; Hall M; Lythgoe KA; Li Y; Yuan J; He J; Lu J, Viral infection and transmission in a large, well-traced outbreak caused by the SARS-CoV-2 Delta variant. Nat Commun 2022, 13, (1), 460. DOI: 10.1038/s41467-022-28089-y [PubMed: 35075154]
21. Pater AA; Bosmeny MS; Barkau CL; Ovington KN; Chilamkurthy R; Parasrampuria M; Eddington SB; Yinusa AO; White AA; Metz PE; Sylvain RJ; Hebert MM; Benzinger SW; Sinha K; Gagnon KT, Emergence and Evolution of a Prevalent New SARS-CoV-2 Variant in the United States. bioRxiv 2021. DOI: 10.1101/2021.01.11.426287
22. Prasek SM; Pepper IL; Innes GK; Slinski S; Betancourt WQ; Foster AR; Yaglom HD; Porter WT; Engelthaler DM; Schmitz BW, Variant-specific SARS-CoV-2 shedding rates in wastewater. Sci Total Environ 2023, 857, (Pt 1), 159165. DOI: 10.1016/j.scitotenv.2022.159165 [PubMed: 36195153]
23. Lopez Bernal J; Andrews N; Gower C; Gallagher E; Simmons R; Thelwall S; Stowe J; Tessier E; Groves N; Dabrera G; Myers R; Campbell CNJ; Amirthalingam G; Edmunds M; Zambon M; Brown KE; Hopkins S; Chand M; Ramsay M, Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. N Engl J Med 2021, 385, (7), 585–594. DOI: 10.1056/NEJMoa2108891 [PubMed: 34289274]
24. Mlcochova P; Kemp SA; Dhar MS; Papa G; Meng B; Ferreira I; Datir R; Collier DA; Albecka A; Singh S; Pandey R; Brown J; Zhou J; Goonawardane N; Mishra S; Whittaker C; Mellan T; Marwal R; Datta M; Sengupta S; Ponnusamy K; Radhakrishnan VS; Abdullahi A; Charles O; Chattopadhyay P; Devi P; Caputo D; Peacock T; Wattal C; Goel N; Satwik A; Vaishya R; Agarwal M; Indian, S.-C.-G. C.; Genotype to Phenotype Japan, C.; Collaboration, C.-N. B. C.-.; Mavousian A; Lee JH; Bassi J; Silacci-Fegni C; Saliba C; Pinto D; Irie T; Yoshida I; Hamilton WL; Sato K; Bhatt S; Flaxman S; James LC; Corti D; Piccoli L; Barclay WS; Rakshit P; Agrawal A; Gupta RK, SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature 2021, 599, (7883), 114–119. DOI: 10.1038/s41586-021-03944-y [PubMed: 34488225]
25. Zhang M; Liang Y; Yu D; Du B; Cheng W; Li L; Yu Z; Luo S; Zhang Y; Wang H; Zhang X; Zhang W, A systematic review of Vaccine Breakthrough Infections by SARS-CoV-2 Delta Variant. Int J Biol Sci 2022, 18, (2), 889–900. DOI: 10.7150/ijbs.68973 [PubMed: 35002532]
26. Kannan SR; Spratt AN; Cohen AR; Naqvi SH; Chand HS; Quinn TP; Lorson CL; Byrareddy SN; Singh K, Evolutionary analysis of the Delta and Delta Plus variants of the SARS-CoV-2 viruses. J Autoimmun 2021, 124, 102715. DOI: 10.1016/j.jaut.2021.102715 [PubMed: 34399188]
27. Cella E; Ali S; Schmedes SE; Rife Magalis B; Marini S; Salemi M; Blanton J; Azarian T, Early Emergence Phase of SARS-CoV-2 Delta Variant in Florida, US. Viruses 2022, 14, (4). DOI: 10.3390/v14040766
28. Ai Y; Davis A; Jones D; Lemeshow S; Tu H; He F; Ru P; Pan X; Bohrerova Z; Lee J, Wastewater SARS-CoV-2 monitoring as a community-level COVID-19 trend tracker and variants in Ohio, United States. Sci Total Environ 2021, 801, 149757. DOI: 10.1016/j.scitotenv.2021.149757 [PubMed: 34467932]
29. Bolze A; Luo S; White S; Cirulli ET; Wyman D; Dei Rossi A; Machado H; Cassens T; Jacobs S; Schiabor Barrett KM; Tanudjaja F; Tsan K; Nguyen J; Ramirez JM 3rd; Sandoval E; Wang X; Wong D; Becker D; Laurent M; Lu JT ; Isaksson M; Washington NL; Lee W, SARS-CoV-2 variant

Delta rapidly displaced variant Alpha in the United States and led to higher viral loads. Cell Rep Med 2022, 3, (3), 100564. DOI: 10.1016/j.xcrm.2022.100564 [PubMed: 35474739]

30. Mostafavi E; Dubey AK; Teodori L; Ramakrishna S; Kaushik A, SARS-CoV-2 Omicron variant: A next phase of the COVID-19 pandemic and a call to arms for system sciences and precision medicine. MedComm (2020) 2022, 3, (1), e119. DOI: 10.1002/mco2.119
31. Torjesen I., Covid-19: Omicron may be more transmissible than other variants and partly resistant to existing vaccines, scientists fear. BMJ 2021, 375, n2943. DOI: 10.1136/bmj.n2943 [PubMed: 34845008]
32. Araf Y; Akter F; Tang YD; Fatemi R; Parvez MSA; Zheng C; Hossain MG, Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. J Med Virol 2022, 94, (5), 1825–1832. DOI: 10.1002/jmv.27588 [PubMed: 35023191]
33. Callaway E., Heavily mutated Omicron variant puts scientists on alert. Nature 2021, 600, (7887), 21. DOI: 10.1038/d41586-021-03552-w [PubMed: 34824381]
34. Garcia-Beltran WF; St Denis KJ; Hoelzemer A; Lam EC; Nitido AD; Sheehan ML; Berrios C; Ofoman O; Chang CC; Hauser BM; Feldman J; Roederer AL; Gregory DJ; Poznansky MC; Schmidt AG; Iafrate AJ; Naranbhai V; Balazs AB, mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell 2022, 185, (3), 457–466 e4. DOI: 10.1016/j.cell.2021.12.033 [PubMed: 34995482]
35. Minka SO; Minka FH, A tabulated summary of the evidence on humoral and cellular responses to the SARS-CoV-2 Omicron VOC, as well as vaccine efficacy against this variant. Immunol Lett 2022, 243, 38–43. DOI: 10.1016/j.imlet.2022.02.002 [PubMed: 35131373]
36. Galani A; Markou A; Dimitrakopoulos L; Kontou A; Kostakis M; Kapes V; Diamantopoulos MA; Adamopoulos PG; Avgeris M; Lianidou E; Scorilas A; Paraskevis D; Tsiodras S; Dimopoulos MA; Thomaidis N, Delta SARS-CoV-2 variant is entirely substituted by the omicron variant during the fifth COVID-19 wave in Attica region. Sci Total Environ 2022, 159062. DOI: 10.1016/j.scitotenv.2022.159062 [PubMed: 36181801]
37. Gregory DA; Trujillo M; Rushford C; Flury A; Kannoly S; San KM; Lyfoung DT; Wiseman RW; Bromert K; Zhou MY; Kesler E; Bivens NJ; Hoskins J; Lin CH; O'Connor DH; Wieberg C; Wenzel J; Kantor RS; Dennehy JJ; Johnson MC, Genetic diversity and evolutionary convergence of cryptic SARS- CoV-2 lineages detected via wastewater sequencing. PLoS Pathog 2022, 18, (10), e1010636. DOI: 10.1371/journal.ppat.1010636 [PubMed: 36240259]
38. Smyth DS; Trujillo M; Gregory DA; Cheung K; Gao A; Graham M; Guan Y; Guldenpfennig C; Hoxie I; Kannoly S; Kubota N; Lyddon TD; Markman M; Rushford C; San KM; Sompanya G; Spagnolo F; Suarez R; Teixeiro E; Daniels M; Johnson MC; Dennehy JJ, Tracking cryptic SARS-CoV-2 lineages detected in NYC wastewater. Nat Commun 2022, 13, (1), 635. DOI: 10.1038/s41467-022-28246-3 [PubMed: 35115523]
39. Ahmed W; Harwood VJ; Gyawali P; Sidhu JP; Toze S, Comparison of concentration methods for quantitative detection of sewage-associated viral markers in environmental waters. Appl Environ Microbiol 2015, 81, (6), 2042–9. DOI: 10.1128/AEM.03851-14 [PubMed: 25576614]
40. Baaijens JA; Zuuli A; Ott IM; Petrone ME; Alpert T; Fauver JR; Kalinich CC; Vogels CBF; Breban MI; Duvallet C; McElroy K; Ghaeli N; Imakaev M; Mckenzie-Bennett M; Robison K; Plocik A; Schilling R; Pierson M; Littlefield R; Spencer M; Simen BB; Initiative YS-C-GS; Hanage WP; Grubaugh ND; Peccia J; Baym M, Variant abundance estimation for SARS-CoV-2 in wastewater using RNA-Seq quantification. medRxiv 2021. DOI: 10.1101/2021.08.31.21262938
41. Babler KM; Amirali A; Sharkey ME; Williams SL; Boone MM; Cosculluela GA; Currall BB; Grills GS; Laine J; Mason CE; Reding BD; Schurer SC; Stevenson M; Vidovic D; Solo-Gabriele HM, Comparison of Electronegative Filtration to Magnetic Bead-Based Concentration and V2G-qPCR to RT-qPCR for Quantifying Viral SARS-CoV-2 RNA from Wastewater. ACS ES&T Water 2022. DOI: 10.1021/acsestwater.2c00047
42. Galani A; Aalizadeh R; Kostakis M; Markou A; Alygizakis N; Lytras T; Adamopoulos PG; Peccia J; Thompson DC; Kontou A; Karagiannidis A; Lianidou ES; Avgeris M; Paraskevis D; Tsiodras S; Scorilas A; Vasiliou V; Dimopoulos MA; Thomaidis NS, SARS-CoV-2 wastewater surveillance data can predict hospitalizations and ICU admissions. Sci Total Environ 2022, 804, 150151. DOI: 10.1016/j.scitotenv.2021.150151 [PubMed: 34623953]

43. Peccia J; Zulli A; Brackney DE; Grubaugh ND; Kaplan EH; Casanovas-Massana A; Ko AI; Malik AA; Wang D; Wang M; Warren JL; Weinberger DM; Arnold W; Omer SB, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nat Biotechnol 2020, 38, (10), 1164–1167. DOI: 10.1038/s41587-020-0684-z [PubMed: 32948856]

44. Sharkey ME; Kumar N; Mantero AMA; Babler KM; Boone MM; Cardentey Y; Cortizas EM; Grills GS; Herrin J; Kemper JM; Kenney R; Kobetz E; Laine J; Lamar WE; Mader CC; Mason CE; Quintero AZ; Reding AD; Roca MA; Ryon K; Solle NS; Schurer SC; Shukla B; Stevenson M; Stone T; Tallon JJ Jr.; Venkatapuram SS; Vidovic D; Williams SL; Young B; Solo-Gabriele HM, Lessons learned from SARS-CoV-2 measurements in wastewater. Sci Total Environ 2021, 798, 149177. DOI: 10.1016/j.scitotenv.2021.149177 [PubMed: 34375259]

45. Solo-Gabriele HM; Kumar S; Abelson S; Penso J; Contreras J; Babler KM; Sharkey ME; Mantero AMA; Lamar WE; Tallon JJ; Kobetz E; Solle NS; Shukla BS; Kenney RJ; Mason CE; Schurer SC; Vidovic D; Williams SL; Grills GS; Jayaweera DT; Mirsaeidi M; Kumar N, Predicting COVID-19 cases using SARS-CoV-2 RNA in air, surface swab and wastewater samples. Sci Total Environ 2023. 857, 159188. DOI: 10.1016/j.scitotenv.2022.159188 [PubMed: 36202365]

46. Gibas C; Lambirth K; Mittal N; Juel MAI; Barua VB; Roppolo Brazell L; Hinton K; Lontai J; Stark N; Young I; Quach C; Russ M; Kauer J; Nicolosi B; Chen D; Akella S; Tang W; Schlueter J; Munir M, Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus. Sci Total Environ 2021, 782, 146749. DOI: 10.1016/j.scitotenv.2021.146749 [PubMed: 33838367]

47. Vo V; Tillett RL; Chang CL; Gerrity D; Betancourt WQ; Oh EC, SARS-CoV-2 variant detection at a university dormitory using wastewater genomic tools. Sci Total Environ 2022, 805, 149930. DOI: 10.1016/j.scitotenv.2021.149930 [PubMed: 34536875]

48. Juel MAI; Stark N; Nicolosi B; Lontai J; Lambirth K; Schlueter J; Gibas A ; Munir M, Performance evaluation of virus concentration methods for implementing SARS-CoV-2 wastewater based epidemiology emphasizing quick data turnaround. Sci Total Environ 2021, 801, 149656. DOI: 10.1016/j.scitotenv.2021.149656 [PubMed: 34418628]

49. Zulli A; Pan A; Bart SM; Crawford FW; Kaplan EH; Cartter M; Ko AI; Sanchez M; Brown C; Cozens D; Brackney DE; Peccia J, Predicting daily COVID-19 case rates from SARS-CoV-2 RNA concentrations across a diversity of wastewater catchments. FEMS Microbes 2021, 2, xtab022. DOI: 10.1093/femsmc/xtab022 [PubMed: 35128418]

50. Schoen ME; Wolfe MK; Li L; Duong D; White BJ; Hughes B; Boehm AB, SARS-CoV-2 RNA Wastewater Settled Solids Surveillance Frequency and Impact on Predicted COVID-19 Incidence Using a Distributed Lag Model. ACS ES T Water 2022, 2, (11), 2167–2174. DOI: 10.1021/acsestwater.2c00074 [PubMed: 36380770]

51. Services, U. S. D. o. H. a. H. COVID-19 Reported Patient Impact and Hospital Capacity by Facility. https://healthdata.gov/Hospital/COVID-19-Reported-Patient-Impact-and-Hospital-Capa/anag-cw7u (October 2022).

52. Babler KM; Sharkey ME; Abelson S; Amirali A; Benitez A; Cosculluela GA; Grills GS; Kumar N; Laine J; Lamar W; Lamm ED; Lyu J; Mason CE; McCabe PM; Raghavender J; Reding BD; Roca MA; Schurer SC; Stevenson M; Szeto A; Tallon JJ Jr.; Vidovic D; Zarnegarnia Y; Solo-Gabriele HM, Degradation rates influence the ability of composite samples to represent 24-hourly means of SARS-CoV-2 and other microbiological target measures in wastewater. Sci Total Environ 2023, 867, 161423. DOI: 10.1016/j.scitotenv.2023.161423 [PubMed: 36623667]

53. (CDC), C. f. D. C. a. P. Science Brief: Omicron (B.1.1.529) Variant. https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-omicron-variant.html (December 16, 2021).

54. Sharkey ME; Babler KM; Grills GS; Stevenson M; Solo-Gabriele HM, Development of a novel qPCR assay to detect SARS-CoV-2 RNA extracted from wastewater. In Advances in Genome Biology and Technology, Orlando, FL, USA, 2022.

55. Boehm AB; Hughes B; Duong D; Chan-Herur V; Buchman A; Wolfe MK; White BJ, Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. Lancet Microbe 2023, 4, (5), e340–e348. DOI: 10.1016/S2666-5247(22)00386-X [PubMed: 36965504]

56. Duvallet C; Wu F; McElroy KA; Imakaev M; Endo N; Xiao A; Zhang J; Floyd-O'Sullivan R; Powell MM; Mendola S; Wilson ST; Cruz F; Melman T; Sathyanarayana CL; Olesen SW; Erickson TB; Ghaeli N; Chai P; Alm EJ; Matus M, Nationwide Trends in COVID-19 Cases and SARS-CoV-2 RNA Wastewater Concentrations in the United States. ACS ES T Water 2022, 2, (11), 1899–1909. DOI: 10.1021/acsestwater.1c00434 [PubMed: 36380771]

57. Wu F; Zhang J; Xiao A; Gu X; Lee WL; Armas F; Kauffman K; Hanage W; Matus M; Ghaeli N; Endo N; Duvallet C; Poyet M; Moniz K; Washburne AA ; Erickson TB; Chai PR; Thompson J; Alm EJ, SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases. mSystems 2020. 5, (4). DOI: 10.1128/mSystems.00614-20

58. Ahmed W; Bivins A; Bertsch PM; Bibby K; Gyawali P; Sherchan SP; Simpson SL; Thomas KV; Verhagen R; Kitajima M; Mueller JF; Korajkic A, Intraday variability of indicator and pathogenic viruses in 1-h and 24-h composite wastewater samples: Implications for wastewater-based epidemiology. Environ Res 2021. 193, 110531. DOI: 10.1016/j.envres.2020.110531 [PubMed: 33249042]

59. Symonds EM; Sinigalliano C; Gidley M; Ahmed W; McQuaig-Ulrich SM; Breitbart M, Faecal pollution along the southeastern coast of Florida and insight into the use of pepper mild mottle virus as an indicator. J Appl Microbiol 2016, 121, (5), 1469–1481. DOI: 10.1111/jam.13252 [PubMed: 27501154]

60. Gussow D; Rein R; Ginjaar I; Hochstenbach F; Seemann G; Kottman A; Ploegh HL, The human beta 2-microglobulin gene. Primary structure and definition of the transcriptional unit. J Immunol 1987, 139, (9), 3132–8. DOI: 10.4049/jimmunol.139.9.3132 [PubMed: 3312414]

61. Gonzalez R; Curtis K; Bivins A; Bibby K; Weir MH; Yetka K; Thompson H; Keeling D; Mitchell J; Gonzalez D, COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Res 2020, 186, 116296. DOI: 10.1016/j.watres.2020.116296 [PubMed: 32841929]

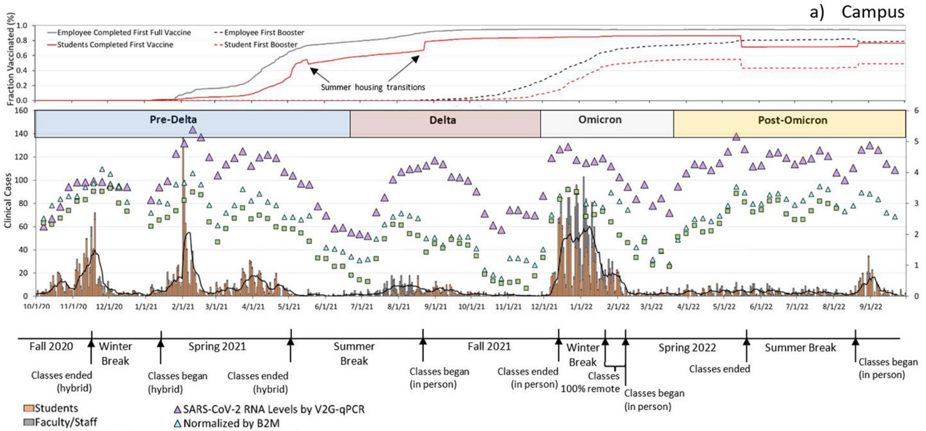
62. Monteiro S; Rente D; Cunha MV; Gomes MC; Marques TA; Lourenco AB; Cardoso E; Alvaro P; Silva M; Coelho N; Vilaca J; Meireles F; Broco N; Carvalho M; Santos R, A wastewater-based epidemiology tool for COVID-19 surveillance in Portugal. Sci Total Environ 2022, 804, 150264. DOI: 10.1016/j.scitotenv.2021.150264 [PubMed: 34798759]

63. (WHO), W. H. O. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern (December 16, 2021).

Synopsis:

SARS-CoV-2 levels in wastewater were significantly correlated with clinical cases and hospitalization within phases dominated by emerging variants during the COVID-19 pandemic.

a) Campus



b) County

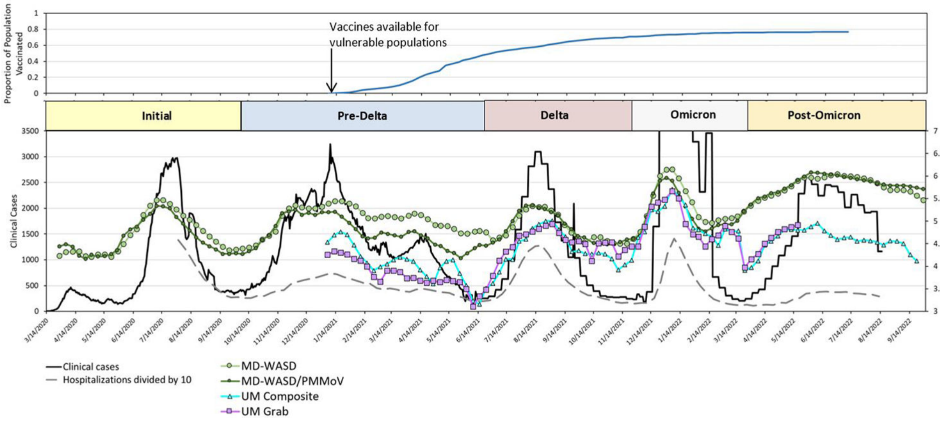


Figure 1:

Panel a) Time series of student and employee vaccination and clinical cases and wastewater SARS-CoV-2 levels at the University of Miami campus. Vaccination data is presented by completion of first full vaccination and first booster. Bar chart on bottom panel corresponds to stacked bar chart of clinical cases among students and employees. Heavy black line corresponds to the 7-day moving average. Wastewater data corresponds to the 3-week moving average of the SARS-CoV-2 signal plus SARS-CoV-2 normalized by PMMoV,and B2M. The 3-week moving average is the average data of SARS-CoV-2 concentration detected within every 3 weeks. Time frame of active semesters and breaks are emphasized in the bottom inset. Panel b) Time series of Miami-Dade County resident population that was vaccinated and clinical cases reported within the County and wastewater SARS-CoV-2 levels at the County wastewater treatment plants. Heavy solid line on bottom corresponds to clinical cases. Heavy dashed line corresponds to hospitalizations divided by 10. Wastewater values correspond to three-week moving average for SARS-CoV-2 levels as collected by WASD and UM (grab and composite samples) and SARS-CoV-2 normalized by PMMoV as measured by WASD). Figure 1 is an adaptation of a previously published figure within Zhan et al. 2022[15].

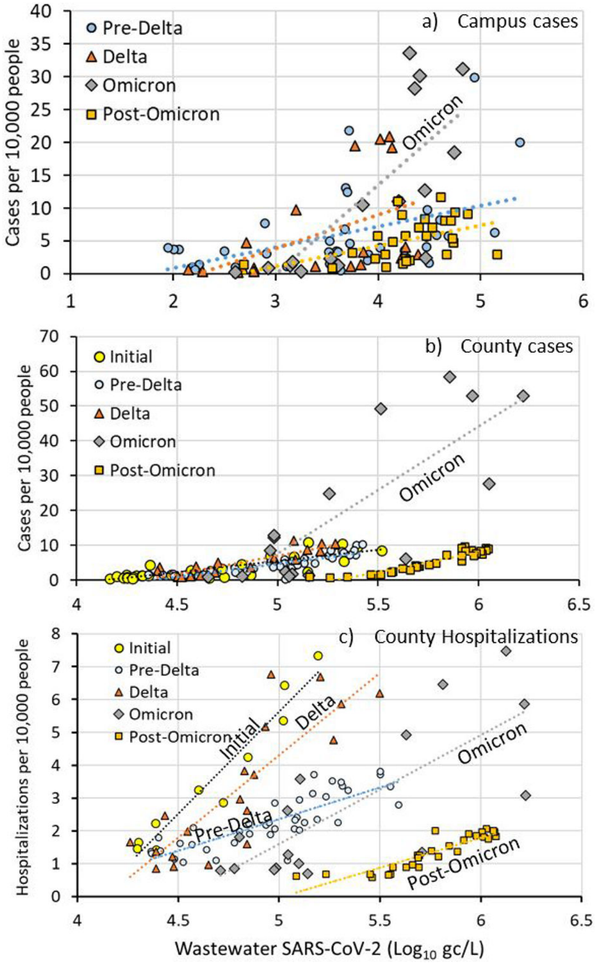


Figure 2:

Correlation plots for a) University of Miami Gables campus between wastewater SARS-CoV-2 ($\log_{10}$ gc/L) versus cases per 10,000 population within the sewershed, b) Miami-Dade County between wastewater SARS-CoV-2 ($\log_{10}$ gc/L) versus cases, and c) Miami-Dade County between wastewater SARS-CoV-2 ($\log_{10}$ gc/L) versus hospitalization. Best fit lines shown for the Pre-Delta wave (blue), Delta wave (brown), Omicron wave (gray), and post-Omicron wave (mustard).

Table 1:

Description of variant periods and the date range in which each occurred over the course of the COVID-19 pandemic; defined by the dominant variant seen throughout the community.

Variant Period	Date Range of Occurrence
Initial wave	Before Oct 1st, 2020
Pre-Delta wave	Oct 2nd, 2020 – June 7th, 2021
Delta wave	Jun 12th, 2021 – Nov 16th, 2021
Omicron wave	Nov 23rd, 2021 – March 9th, 2022
Post-Omicron wave	March 16th, 2022 – Aug 4th, 2022

Table 2:

Mean, standard deviation, range and positivity of raw (Log_{10} transformed) SARS-CoV-2 RNA for University of Miami samples at the campus scale and for MD-WASD at the community scale.

	Period of Record	No. samples	Mean (Log_{10} gc/L)	Standard deviation (Log_{10} gc/L)	Range (Log_{10} gc/L)	Positivity
UM, WG01	Sep. 20-Sep.22	102	4.36	4.74	BDL[a] - 5.48	76%
UM, WG02	Sep. 20-Sep.22	102	4.85	5.34	BDL - 6.21	84%
UM, WG0U	Sep. 20-Sep.22	96	4.48	6.56	BDL - 5.99	71%
UM, WC0Dc (Composite)	Jan. 21-Sep. 22	90	4.69[b]	4.94	BDL - 5.79	99%
UM, WC0Dg (Grab)	Jan. 21-May. 22	71	4.70	5.03	2.70 – 5.89	100%
MD-WASD (Central)	Mar.20-Sep. 22	170	5.62[c]	5.73	3.95 - 6.44	99%
MD-WASD (North)	Mar.20-Sep. 22	155	5.66	5.72	3.75 - 6.48	99%
MD-WASD (South)	Mar.20-Sep. 22	158	5.76	5.91	4.02 - 6.77	99%

[*] BDL = Below Detection Limits. Detection limit of 100 gc/L or Log_{10} = 2 gc/L.

[b] Using the mean flow rate for the CDWWTP and population served, the flow and population normalized mean genomic copies were computed as 2.23 x 10^5 gc/person or 5.4 Log_{10} gc/person.

[c] Similar to “b” above, the flow and population normalized mean genomic copies were computed as 1.89 x 10^6 or 6.3 Log_{10} gc/person.