Wastewater-Based Epidemiology as a Strategy for Tracking and Tracing COVID-19 at a University

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the illness responsible for the global pandemic of COVID-19, declared by the World Health Organization (WHO) in late 2019. This virus causes respiratory illnesses in humans; its spread is typically via direct airborne routes from inhaling droplets of saliva or sputum containing viral particles, or indirectly via surfaces contaminated by individuals infected with the virus. Upon its outbreak, researchers focused their efforts to identify positive cases of the illness worldwide. At the University of Miami (UM), wastewater-based epidemiology (WBE) was a strategy employed to detect the viral RNA shed from individuals as it can also be shed within wastewater from various input sources of a building (i.e., toilet, shower, sink, clothes & dish washing water). WBE, a growing strategy in the epidemiological field for assessing the risk of public health against COVID-19, was instilled with methodology targeting a rapid-turnaround assessment, under 48 hours, so that University officials could inform the community for targeted testing on a week-by-week basis to contain the spread amongst the University's faculty and students. Methodology utilized included pretreatment of sewage prior to concentration with electronegative filtration. Subsequent viral RNA extraction and quantitative polymerase chain reaction (qPCR) assays were performed for the nucleocapsid region of the SARS-CoV-2 genome, in addition to other microbiological targets for inferencing phenomenon such as percent recovery of viral particles, molecular inhibition, and contribution from human sources. In this two-year study, research was used to track the rate of infections, and predictions were made to supplement results from clinical testing within the University's campus and Hospital. This was novel as those who were pre- or asymptomatic rarely sought after reported testing, skewing the effectiveness of human-reliant surveillance. Results of the rapid-turnaround assessment allowed for effective communication from laboratory analysis to policy decision for the University. From there, sub-studies to assess relationships between clinical cases, hospitalizations, variant analyses, methodology optimization, and effective reporting of SARS-CoV-2 have branched from this work. WBE is expanding as a tool that is independent of human testing, demographics, and can provide real-time data for the protection of community health.

Methods/Materials

Sampling occurred weekly on Wednesday mornings from 7:30 am to 10:30 am, starting on September 30, 2020, and continuing through the summer of 2022. Both clusters & individual buildings were sampled, all collected either from manholes or from lift stations (Figure 1 & 2). At each site, a bottle-on chain approach was used to retrieve the wastewater. An ISCO peristaltic pump auto-sampler was used at two individual building scale manholes in order to collect a weekly 24-hour composite sample (Figure 3).

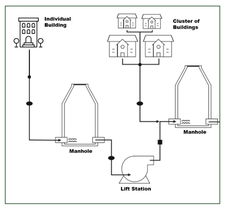


Figure 1: Building scales

Figure 2: Lift station sampling

Figure 3: ISCO auto-sampler

Methods/Materials

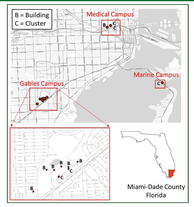


Figure 4: University of Miami sites

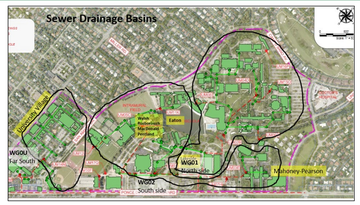


Figure 5: University of Miami, Gables campus sewer drainage basins

Upon receipt at the laboratory, an aliquot of 10mL was removed from the sample bottle into a sterile centrifuge tube for further processing for fecal indicator bacteria by culture, as a means of confirming fecal inputs to the sampling site. Fecal coliform were analyzed by membrane filtration using mFC agar by culturing 1 mL and 0.1 mL aliquots from 100:1 dilutions in sterile phosphate buffered saline. Colonies with characteristic blue color were quantified (Figures 6, 12, 13). A separate wastewater aliquot was then spiked with OC43 and then salted & acidified; once pretreated, the aliquot underwent electronegative filtration and the concentrated filter was sent to colleagues for further analysis (Figure 6).

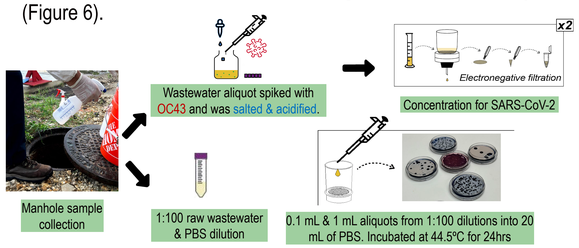


Figure 6: field-to-lab overview

Results

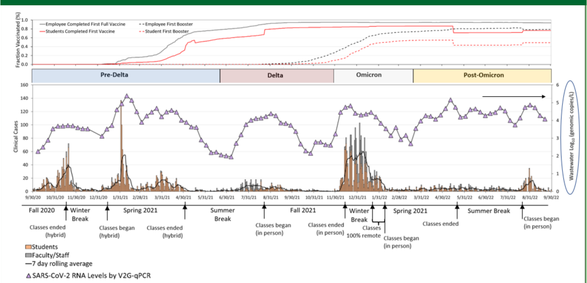


Figure 7: SARS-COV-2 Positive Cases Among Faculty/Staff and Students from Fall 2020 to September 2022

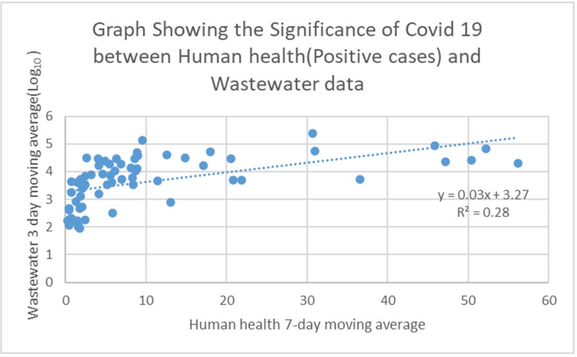


Figure 8: Positive Cases at the University of Miami against the 3-day moving average of Wastewater Data

Results

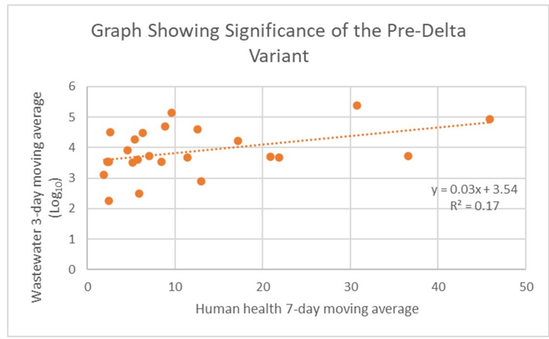


Figure 9: Correlation of Pre-Delta Variants between Wastewater and Human Health Data at the University of Miami

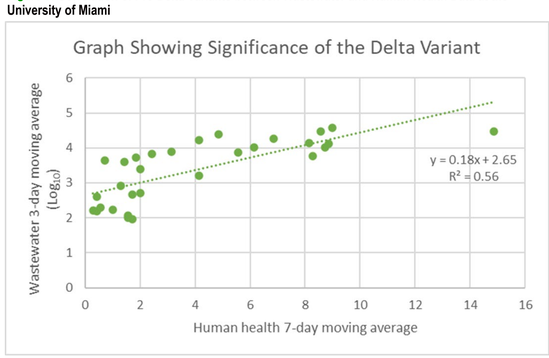


Figure 10: Correlation of Delta Variant between Wastewater and Human Health Data at the University of Miami

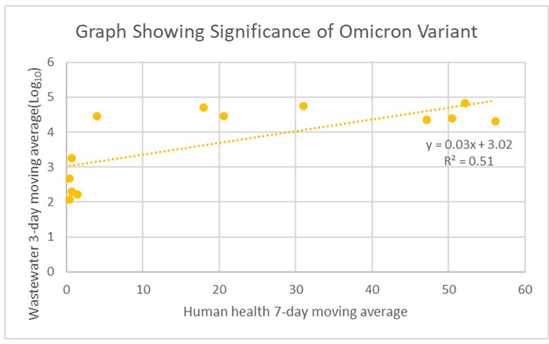
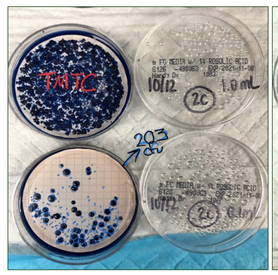


Figure 11: Significance of Omicron Variant between Wastewater and Human Health Data at the University of Miami



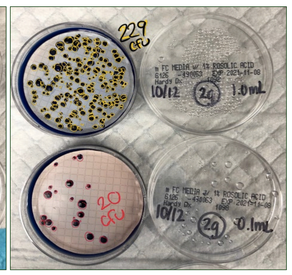


Figure 12: mFC agar plates with resulting Fecal coliform growth post-incubation. Top plate showing "Too many to count" (TMTC). Bottom plate showing acceptable CFU counting conditions.

Figure 13: mFC agar plates with resulting Fecal coliform growth post-incubation. Top and bottom plates showing acceptable CFU counting conditions

Discussion

Wastewater data from individual building & clusters of buildings were analyzed and sent to the appropriate University personnel. If the copies/L of sewage were high, the residential colleges or other campuses were required to undergo mandatory testing, which returned some positive results, depending on the location. It is possible that with daily sampling instead of weekly, the early warning period could be shorter than 4 days allowing more time to identify positive subjects and thereby possibly reducing disease transmission.

Conclusion

- This study showed that the challenges with tracking disease outbreaks associated with the COVID-19 pandemic can be met through a multi-pronged approach that integrates comprehensive human surveillance of the disease with environmental surveillance of the virus.
- In the case of COVID-19, the RNA of the etiologic agent of disease, SARS-CoV-2, was found to be excreted in urine and feces of both symptomatic and asymptomatic people.
- Although COVID-19 is a respiratory disease, and it would be expected in respiratory fluids, it also has been found in wastewater, allowing for an alternative approach to detecting early onset of outbreaks by measuring markers of the pathogen in wastewater.
- There was an observed presence of SARS-CoV-2 that causes COVID-19 that shows up in wastewater samples and the positivity rates on all campuses for both faculty and students.

Future Directions

- Work should focus on expanding techniques and protocols for environment monitoring of infectious agents for the purpose of tracking disease outbreaks.
- Compare the significance of the different variants of public schools versus University of Miami cases.
- Explore post-omicron trends.

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