Spring 2022

Wastewater Surveillance of SARS-CoV-2 on American University Campuses: A Comparison of Responses to the COVID-19 Pandemic

Emily Gosnell
University of South Carolina

Wastewater Surveillance of SARS-CoV-2 on American University Campuses: A Comparison of Responses to the COVID-19 Pandemic

Emily Gosnell

University of South Carolina

South Carolina Honors College

Senior Thesis

Abstract

Wastewater surveillance has been used for a variety of purposes but, in recent years, has most notably been utilized during the COVID-19 pandemic. Many universities in the United States used it as a means of monitoring levels of the SARS-CoV-2 virus on their campuses. The University of South Carolina, University of Arizona, and University of North Carolina- Charlotte were three such schools. An analysis of data published by these three schools has been used to synthesize a proposed list of best methods to be used by other universities during the reemergence of SARS-CoV-2 or during the outbreak of a new pathogen. Additionally, factors such as supply chain issues and burnout have been evaluated to determine their effects on laboratory efficiency and the sustainability research projects in the midst of a pandemic.

Keywords: pandemic, COVID-19, wastewater surveillance, university

Contents

Wastewater Surveillance of SARS-CoV-2 on American University Campuses: A Comparison of Responses to the COVID-19 Pandemic

Introduction

What influences researchers to brave the unpleasantness of the sewer system to collect samples of raw wastewater? The answer is simple: the desire to improve the public's health. Wastewater, defined by the Centers for Disease Control and Prevention (CDC) as "water from household/building use (i.e., toilets, showers, sinks) that contain human fecal waste, as well as water from non-household sources (e.g., rainwater and industrial use)," is an invaluable public health tool (CDC, 2021c). Municipal sewage collection systems handle almost 80 percent of all households in the United States, anonymously summarizing the health of citizens (CDC, 2021c). The collection and processing of raw sewage, analyzed in a laboratory, is used to quantify the viral load or amount of other infectious agents present in the population represented by the sample (Ahmed, 2020a). This form of environmental epidemiology has been deemed wastewater surveillance (Gosnell, 2021).

Wastewater surveillance is a population-level tactic, meaning that it can provide information on the prevalence of an infectious disease within a specific geographical region (Hokajärvi, 2021). The technique of wastewater surveillance was initially developed as a means of monitoring the use of illicit drugs by certain groups but was quickly modified to analyze disease prevalence on a population level (Daughton, 2020). This allows it to serve as an early detection system for infectious diseases by providing evidence of a virus before clinical evidence is noticed by officials (CDC, 2021c). The value of such a methodology is tremendous. Individual consent, for example, is not required for population-level testing. Moreover, everyone produces human waste, so all persons located in a given geographical area will be involved in the testing

pool. Although no sample collected will contain waste from the entire population, it will provide an adequate representation of the prevalence of the disease or substance being tested (Gosnell, 2021).

While this methodology has many applications, this thesis will focus on the usage of wastewater surveillance on university campuses during the COVID-19 pandemic. The SARS-CoV-2 virus is shed in human feces and can thus be detected in sewage, provided that samples are collected and analyzed in a timely manner because of the rapid degradation of the virus, particularly under warm, basic, and/or acidic conditions (Ahmed 2020b). Wastewater surveillance is often used as a predictive factor for imminent outbreaks of disease, such as those of the SARS-CoV-2 virus. It allows researchers to pinpoint specific regions of the broader testing area that have the highest viral load of the SARS-CoV-2 virus. Often, individuals are infectious and shed the virus before exhibiting viral symptoms that indicate testing is needed. Asymptomatic patients do not develop symptoms but are still capable of shedding the virus (Hill, 2020). Thus, wastewater surveillance is an effective tool that many universities have implemented during the COVID-19 pandemic to track levels of the SARS-CoV-2 virus. These data are used to effectively target testing and treatment efforts towards specific locations on campuses (Horn, 2020). This paper investigates the relative successes of several universities' approaches to wastewater surveillance and proposes the best methodology for future disease outbreaks.

Wastewater Surveillance for Poliovirus

Wastewater surveillance has been used to detect infectious diseases prior to the COVID-19 pandemic. As described by the CDC, poliomyelitis "is a disabling and life-threatening disease caused by the poliovirus," known for causing paralysis (CDC, 2021e).

Polioviruses are a type of enteroviruses, small and non-enveloped RNA viruses of the genus Enterovirus, family Picornaviridae. They can spread via oral or aerosol routes and often clinically present as asymptomatic infections. This class of virus is shed in stool, meaning poliovirus can be detected in wastewater, either as the result of an active infection or from an individual vaccinated with a live poliovirus vaccine. Characteristically, enteroviruses have moderate resistance to temperature and pH, allowing them to remain infectious and detectable in wastewater for an extended period of time (Ivanova, 2019; Gosnell, 2021).

Russia was one of the first nations to realize the scientific value of the poliovirus's presence and longevity in human fecal matter. Starting in the middle of the 20th century, they began to test wastewater to determine the effectiveness of their existing wastewater treatment system and to monitor enteroviruses. Russia's National Poliomyelitis Eradication Program was implemented in 1996 to monitor poliovirus via wastewater surveillance. Russia also started a national program, "Epidemiological surveillance and prophylaxis of non-polio enterovirus infection," to detect and monitor the presence of non-polio enteroviruses. Both programs utilize the recommendations from the World Health Organization to correctly identify the viruses. (Ivanova, 2019; Gosnell, 2021).

Russia is not the only nation to utilize wastewater surveillance to monitor poliovirus. Israel has had a similar sewage surveillance system since 1989. It involves weekly collection and analysis of sewage from the sewer system and wastewater treatment plants. In 2013, researchers used it to detect poliovirus, prompting the government to spring into action and vaccinate their citizens. This may have prevented the occurrence of a severe polio outbreak. Furthermore, researchers were able to isolate the virus and examine its molecular characteristics. They were able to learn that "the virus originated in Pakistan, then traveled into the region, diverging into

Egypt, Israel, and Syria (Eisenberg, 2018).” Researchers at the University of Michigan’s School of Public Health claim that “as the world approaches the final stages of polio eradication, environmental surveillance becomes key. Looking for poliovirus in sewage is more sensitive than counting up cases of [accute flaccid paralysis],” a condition caused by poliovirus and used to monitor its transmission (Eisenberg, 2018). Additionally, wastewater surveillance can “detect virus shed in the feces of non-paralyzed people infected with polio- … the silent circulation of polio” (Eisenberg, 2018). The same research team argued that the scope of wastewater surveillance should be extended beyond poliovirus and used to detect other pathogens that are shed into wastewater (Eisenberg, 2018; Gosnell, 2021).

India has also had great success with wastewater surveillance as a protective public health measure. According to an article published by the Thomas Reuters Foundation, their wastewater-based epidemiology served as an early warning system by detecting a strain of the poliovirus in the city of Hyderabad in 2016. They had been “declared polio free by the World Health Organization in March 2014 after an almost two-decade long, multi-million dollar effort -- lauded as one of the country’s biggest public health achievements in recent times” (Bhalla, 2016). Being deemed “free” of a virus does not mean that an infectious disease cannot reemerge, though, as indicated by the detection of polio in India’s wastewater. Thus, wastewater surveillance can serve as a maintenance tool of sorts. In this case, its use allowed vaccination efforts to be targeted at Hyderabad and Rangareddy, the areas that were deemed high risk for transmission of the virus (Bhalla, 2016). The remainder of this paper will shift its focus from poliovirus and move to the use of wastewater surveillance to monitor a now-infamous virus, severe acute respiratory syndrome coronavirus 2.

Overview of COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA viral agent that causes COVID-19. Coronaviruses are identifiable as enveloped, positive-strand, RNA viruses that are capable of infecting vertebrates. SARS-CoV-2 belongs to the subgenus Sarbecovirus of the family Coronaviridae (Coronaviridae, 2020). Symptoms of COVID-19, according to the Centers for Disease Control and Prevention (CDC), include fever, chills, shortness of breath, cough, difficulty breathing, fatigue, muscle and body aches, headache, the new loss of taste and smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea. Individuals who are elderly or have underlying medical conditions, such as heart disease, lung disease, and diabetes, are at a higher risk of developing a severe case of COVID-19. This often manifests as breathing difficulties, with the most critical cases resulting in respiratory and organ failure, organ dysfunction, septic shock, and/or death (CDC, 2021a; Gosnell, 2021).

The CDC also states that the "estimated incubation period is between 2 and 14 days with a median of 5 days. It is important to note that some people become infected and do not develop any symptoms or feel unwell"(CDC, 2021a) The presence of asymptomatic cases during the COVID-19 pandemic highlights the need for wastewater surveillance. Although there are some exceptions, many people only feel the need to get tested for COVID-19 if they are having symptoms. Thus, wastewater surveillance may capture cases of COVID-19 that would not have been otherwise caught, providing a more holistic image of viral load in a population than clinical testing alone. Humans shed SARS-CoV-2 in feces, making it an ideal candidate for wastewater surveillance (CDC, 2021c; Gosnell, 2021).

Methods for Wastewater Surveillance of COVID-19

The specifics of the methodology of wastewater collection, processing, and analysis depend on the exact purpose of the study. The CDC has released a testing methods overview in which recommendations are made for the surveillance of SARS-CoV-2 in wastewater. They are summarized below (CDC, 2021b; Gosnell, 2021).

I. Wastewater samples are collected.
   A. Samples may come from a wastewater treatment plant for a homogenous representation of a sewershed, wastewater coming from a specific geographical area that feeds into a certain wastewater treatment plant.
   B. Samples may come from a point source, such as a sewer that contains wastewater coming from a single, specific building (CDC, 2021b; CDC, 2021c).

II. Wastewater samples are processed, ideally within 24 hours of collection to minimize degradation of SARS-CoV-2.
   A. Sample preparation
      1. Samples are stored at 4℃ if processing occurs within 24 hours of collection. Samples that cannot be processed within this time frame should be spiked with a matrix recovery control and refrigerated at 4℃ or frozen at -20℃ or -70℃.
      2. Samples are mechanically mixed to homogenize liquid and solid components. Homogenization may include inversion, sonication, or other mechanical methods.
      3. Samples are clarified by large pore size filters or centrifugation to remove large solids (CDC, 2021b).

B. Sample Concentration

1. Ultrafiltration, ultracentrifugation, polyethylene glycol precipitation, filtration through an electronegative membrane after pre-treatment with $MgCl_2$ or acidification, and skim milk flocculation are all methods that can be used to concentrate SARS-CoV-2.
2. One of the options above should be selected via the following criteria
    a) Sample type: Untreated samples can be concentrated through several options, but primary sludge should be concentrated via centrifugation.
    b) Sample volume: Large untreated volumes may need to be divided into smaller volumes before filtration, and volumes greater than 5L may need to be pre-concentrated via large cartridge ultrafiltration or a similar means.
    c) Potential supply chain issues: Membrane filters, ultrafiltration cartridges, and other commercial filtration products may not always be readily available.
    d) Sample processing time: Membrane filtration can be a lengthy process. Lab personnel must have the facilities and availability if this is a possibility.
    e) Availability of laboratory equipment: Centrifuge volumes, force capacity of centrifuges, and membrane filtration unit availability should be considered (CDC, 2021b).

C. RNA extraction

1. Nucleic acids are extracted and purified. Extraction protocols can vary but should be specific for RNA, produce high purified nucleic acid extracts, and use RNase denaturants before lysis steps. Extracts should be placed into separate tubes and stored at -70℃ to prevent degradation during multiple freeze-thaw cycles (CDC, 2021b).

D. RNA measurement methods (CDC, 2021b)

1. SARS-CoV-2 RNA quantification can be performed via reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or by reverse transcription- droplet digital polymerase chain reaction (RT-ddPCR). One-step and two-step reactions are suitable for both forms of PCR.
2. SARS-COV-2 N (N1 and N2) are good targets for primers and probes (CDC, 2021b).

The data collected via the methods above can be analyzed in many ways at the researcher's discretion. It is commonplace to utilize laboratory controls to ensure that the data collected is accurate. Matrix recovery control, human fecal normalization, quantitative measure controls, inhibition assessment, and negative controls are all recommended by the CDC to allow for reliable comparison between the concentration of SARS-CoV-2 RNA in wastewater samples. (CDC, 2021b; Gosnell, 2021).

Wastewater surveillance is not a form of research that only entails a laboratory. Rather, it relies on support from a variety of external, community sources. Epidemiologists and environmental health specialists are needed to obtain and analyze the data, but access to wastewater treatment plants and/or sewer systems is crucial to sample collection (CDC, 2021c).

It is crucial for researchers to have the support and understanding of their community. Adequate research cannot be performed without the help of individuals whose work involves wastewater treatment, sewershed maintenance, and/or plumbing (Gosnell, 2021).

Drawbacks of Using Wastewater Surveillance during the COVID-19 Global Pandemic

Wastewater surveillance, as discussed above, can serve as a remarkable public health tool. However, it has its downfalls. The data collected from sewage testing is indicative of a population's general viral load, but a means of accurately using this data to determine the number of cases of COVID-19 within the said population has not yet been developed (CDC, 2021c). Some wastewater treatment plants use measures, such as pre-treatment of sewage, that prevent accurate data from being obtained. Furthermore, certain outliers cannot be captured with wastewater surveillance. This includes prisons, universities, and hospitals that treat their own waste on-site. Houses with septic tanks/ systems also fall into this category. As with any form of research, it is not possible for researchers to be 100% valid and accurate. Testing methods may not be sophisticated enough to detect low levels of SARS-CoV-2, and this limit of detection is still not entirely understood (CDC, 2021c; Gosnell, 2021).

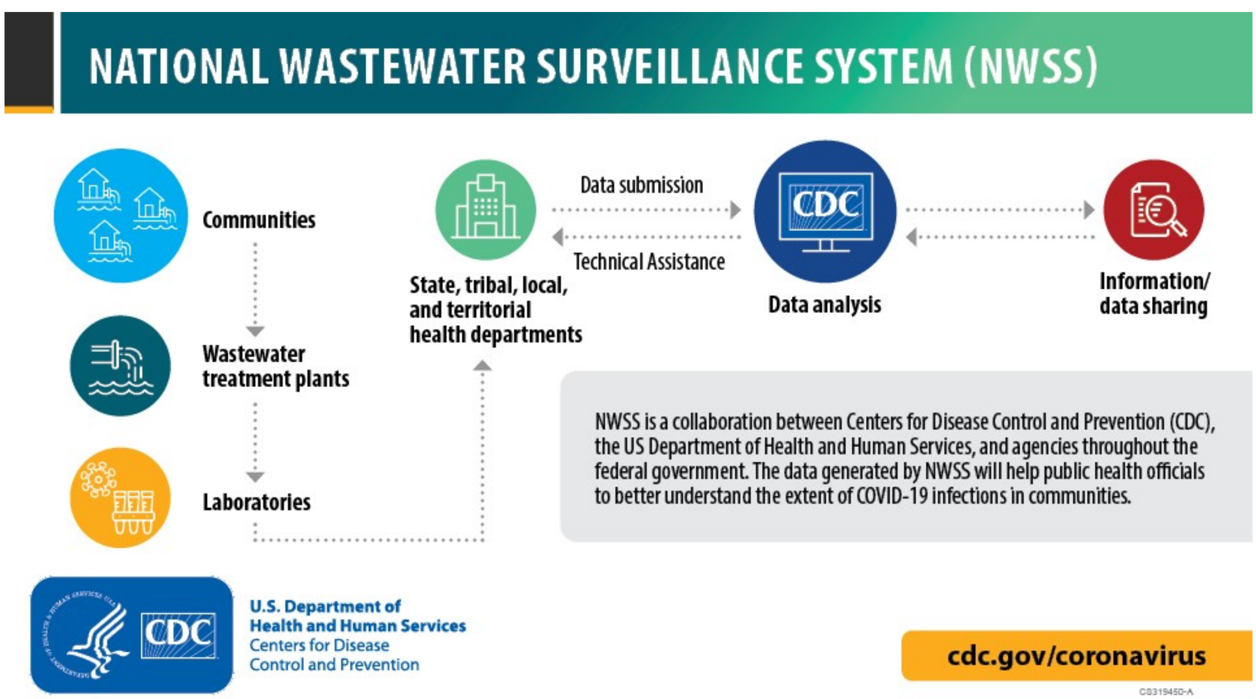
Targeted wastewater surveillance is a means of obtaining wastewater samples that are upstream from the wastewater treatment plant. While more specific than testing from an entire sewershed, targeted wastewater surveillance also has disadvantages. Some desired locations may not be possible to use as a testing site, depending on the infrastructure of the sewer lines themselves (CDC, 2021c). Researchers must pay careful attention to all of these factors and create their experimental design accordingly (Gosnell, 2021).

Implementation of the National Wastewater Surveillance System

The Centers for Disease Control and Prevention are currently developing the National Wastewater Surveillance System (NWSS) to track data obtained from wastewater surveillance at state, tribal, local, and territorial levels (CDC, 2021c). This will create a cohesive database that can be used for future applications of wastewater surveillance. Many colleges and universities already have online dashboards that document data regarding the COVID-19 pandemic (See: "Wastewater Surveillance of SARS-CoV-2 on College and University Campuses" section). The NWSS aims to do something similar on a national scale by compiling and publishing wastewater surveillance data (CDC, 2021c). The flowchart below depicts the exchange stream of information regarding COVID-19 infections. (Gosnell, 2021).

Figure 1

NWSS Flow Chart (CDC, 2021d)



The value of the NWSS is that communities will be able to compare their own response to the COVID-19 pandemic with the responses of other communities. These responses, with the right analytics, can be compared with case numbers and viral load in the wastewater and used to determine which measures taken were effective. Already, many locations across the nation have adopted wastewater surveillance to monitor the viral load of SARS-CoV-2. Although we may be able to end the COVID-19 pandemic, it does not mean that SARS-CoV-2 will be eradicated at the same time. Once established, the NWSS can be used to continue to monitor SARS-CoV-2 in the case of future outbreaks without the need to restart wastewater surveillance with each new outbreak of COVID-19. It is expected that there will be concerns about "privacy, stigma, and potential negative repercussions of sharing these data," for some have already expressed apprehension about the publication of data from wastewater sampling at the college and university level (CDC, 2021d). However, "the community-wide, non-individualized nature of the technology mitigates [any] potential legal and ethical issues" by ensuring anonymity (CDC, 2021d). Thus, objections to the NWSS in the realm of ethical and privacy concerns will likely be quickly refuted by reassurances that wastewater surveillance cannot link an infection to any one individual (Gosnell, 2021).

Wastewater Surveillance of SARS-CoV-2 on College and University Campuses

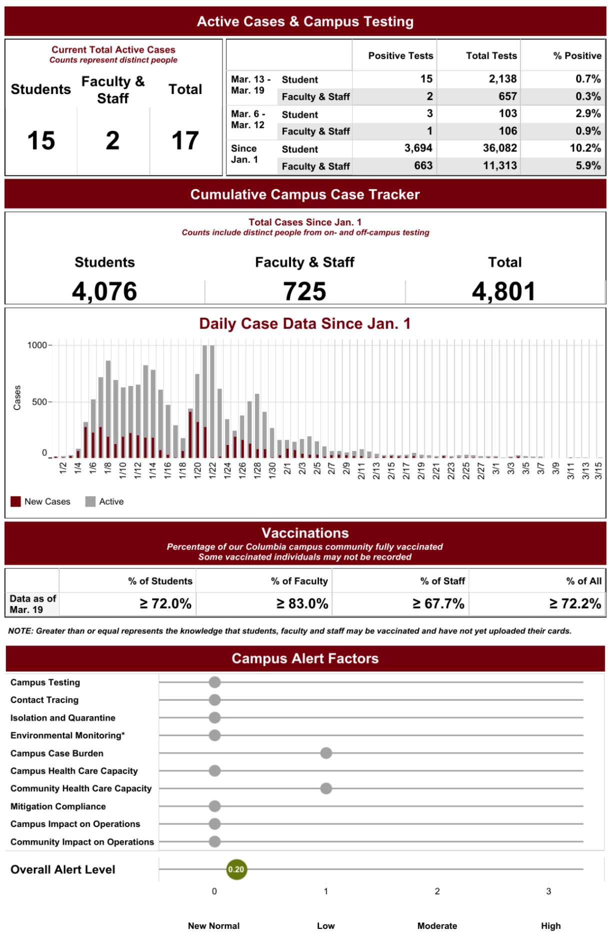
There has been a particularly notable usage of wastewater surveillance during the COVID-19 pandemic, monitoring levels of SARS-CoV-2 on college and university campuses (Harris-Lovett, 2021). Many schools began wastewater sampling efforts in the fall semester of 2020. Some had classes in person, some had virtual classes, and some had a hybrid of both, but most colleges and universities had some population of students living on campus. The University of Arizona (UArizona) became one of the most prominent schools in the media after

"announcing that it had detected genetic material from SARS-CoV-2 in the wastewater from a student dormitory" (Harris-Lovett, 2021). Wastewater surveillance quickly became a promising public health tool to keep colleges and universities safe by serving as a predictive measure for SARS-CoV-2 outbreaks. By January 2021, over "210 colleges around the world had begun monitoring wastewater for SARS-CoV-2," providing a large influx of data to be analyzed regarding the effectiveness of wastewater surveillance in the fight against the COVID-19 pandemic. (Harris-Lovett, 2021; Gosnell, 2021).

Many colleges and universities have published public, online dashboards to document levels of SARS-CoV-2 on campus as the semester progresses. The University of South Carolina Columbia (UofSC) is one such school as demonstrated in Figure 2. Their dashboard displays the following data: number of current active cases, number of clinical tests performed, number of positive tests, and vaccination rates. These numbers are also displayed as a cumulative case tracker. The university then assesses the following campus alert factors: campus testing, contact tracing, isolation and quarantine, environmental monitoring, campus case burden, campus health care capacity, community health care capacity, mitigation compliance, campus impact on operations, and community impact on operations. These factors are combined to synthesize the overall alert level, a scale that ranges from new normal (0) to high (3), with low (1) and moderate (2) as the levels in the middle (University of South Carolina, 2021; Gosnell, 2021).

Figure 2

UofSC Columbia COVID-19 Dashboard as of March 27th, 2022 (University of South Carolina, 2021).



As discussed above, wastewater surveillance (described as environmental monitoring by UofSC) is only one aspect of a campus's efforts to contain and monitor SARS-CoV-2. This is important to note, not to discount the value of wastewater surveillance, but rather to understand the complexity of the public health response of which it is only a part. Wastewater surveillance programs on college and university campuses have been most often initiated by engineering departments, but biology, math, environmental health, and epidemiology departments have also

been the catalyst for the implementation of these programs. Once this was undertaken, many schools assembled multidisciplinary teams to accomplish their research, with "faculty, facilities staff, and student health professionals [collaborating] to sustain the effort"(Harris-Lovett, 2021). In addition to recognizing the diversity within the research teams, there is a wide range of beneficiaries of this research effort. These include the entire research team, college administrators, students, the local public health department, and members of the local community (Harris-Lovett, 2021; Gosnell, 2021).

It must be recognized that the local community plays a role in both the response to the COVID-19 pandemic and in data collection. Colleges and universities tend to have a constantly changing population of students, faculty, and the general public. There is also the movement of campus visitors within buildings open to the public, such as visitor's centers and libraries. Thus, depending on the locations colleges and universities choose as their testing sites, surveillance may reveal SARS-CoV-2 that was not shed by members of the school. This could skew the results of sample collection and the consequential public health response to the data. Many students utilize on-campus academic buildings and resources but live off-campus. There are also some students who will choose to get tested at off-campus doctor's offices and pharmacies out of convenience or appointment availability. The data collected by wastewater surveillance and on-campus clinical testing may then not be representative of the entire student body. Additionally, anyone who does not live on campus, whether a student or not, may abide by mask mandates and social distancing requirements while on-campus but not in the rest of their daily routine. This may undermine the apparent effectiveness of COVID-19 containment efforts on campus. The lack of research in these areas offers an opportunity for retrospective studies that

investigate external factors that may influence the validity and reliability of wastewater surveillance on college and university campuses (Gosnell, 2021).

University-Specific Data and Responses

University of Arizona

As mentioned above, UArizona was a frontrunner in the effort to implement wastewater surveillance on campuses. They began their efforts in the summer of 2020 and published a paper on the topic in July 2021. It is important to note that with UArizona beginning sampling so early into the pandemic, its wastewater surveillance technology was not as readily acquired. Its research team's first set of published data was thus collected via grab samples. This method uses a "pole/dipper" technique to obtain samples from the sewer system at a specific location and time (Betancourt, 2021). UofSC also utilized grab samples in the beginning stages of their surveillance but switched over to autosamplers as soon as they were available. The rationale behind this switch in methodology was to reduce the margin for human error and to increase the validity and reliability of testing by using composite samples. Unlike grab samples, composite samples represent the population at multiple points in time. Autosamplers function by using a hose to draw up a programmed volume of sewage on a fixed schedule. For example, UofSC programmed their autosamplers to collect 30mL of sewage every 15 minutes over 24 hours, giving each sample 96 data points. This increases the likelihood that fecal matter from infected individuals will be captured. Homogenization of samples allows for a uniform and random blending of all 96 data points (Sellers, 2022).

Despite not having access to autosamplers, UArizona modeled initial grab sample methodology to resemble that of composite sampling. The researchers described their sampling schedule as follows:

> Samples were collected at 8:00 am on August 18 and 20 during the week that students moved into the dorm. Daily samples were collected from August 25–31 to monitor SARS-CoV-2 RNA in wastewater during the first week of classes. On August 26, five samples were collected five minutes apart between 8:30–8:50 am when peak flow occurred indicating maximum restroom usage to determine sample variation during sample collection. On August 27, two more samples were collected from Dorm A, one in the morning (9:30 am) and afternoon (1:15 pm) to ensure the presence or absence of the virus at different times of the day (Betancourt, 2021).

The data collected on August 26th mirrors that of a composite sampling, for sewage was collected multiple times from one location. However, it does not seem that the samples were compiled into one sample. Rather, they were individually tested in order to determine the degree to which variation occurred. This functioned as a control measure of sorts, with the researchers able to analyze how reliable their results were. The concentration of N2 (copies/L) remained constant between all samples taken on August 26th, but the concentration of N1 (copies/L) demonstrated fluctuation. N1 and N2 are genes found in SARS-CoV-2 and are commonly used as targets for testing. The researchers briefly commented on these findings, stating that it was believed that these "variations in the efficiency of the methods are predominantly associated with the complexity of this environmental matrix" (Betancourt, 2021). The team also performed a recovery analysis, with matrix spikes demonstrating "an average recovery of 14 ± 16% which indicated low and highly variable efficiencies of recovery of HCoV 229 E in wastewater as observed in studies with other coronaviruses used for the same purpose" (Betancourt, 2021) They attributed the low efficiency of recovery to the environmental matrix as well (Betancourt, 2021). This information, when simplified, indicates that the data collected at UArizona was not

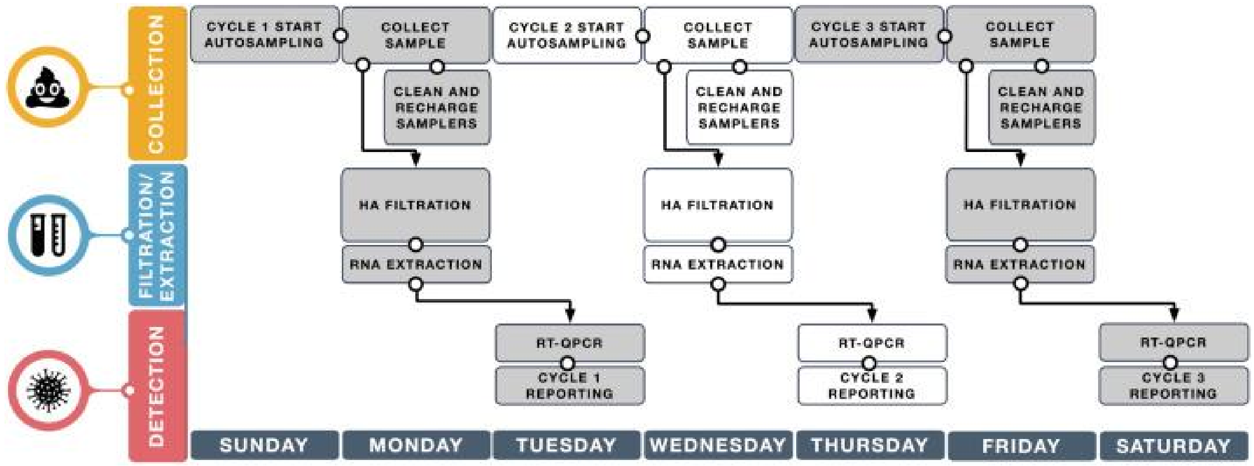
always consistent, and researchers believed it was a result of the complexity of the sewershed, building infrastructure, and other variations that may have made it difficult to standardize sampling.

University of North Carolina Charlotte

The University of North Carolina at Charlotte (UNCC) implemented wastewater surveillance in the Fall of 2020 as well. Their research team "was able to detect single asymptomatic individuals in dorms with resident populations of 150-200" through the utilization of wastewater surveillance on a thrice-weekly schedule (Gibas, 2021). The university's overall approach was described as "four-pronged" and used "symptomatic testing, daily health checks, and in-hour contact tracing" in addition to wastewater testing (Gibas, 2021). They identified six areas in which they had to decide which methodology would yield the best results: "1) where to sample, 2) how often to sample, 3) what kind of sample to collect, 4) what type of sample concentration protocol to use, 5) what kind of RNA extraction protocol to use, and 6) what type of detection method to use"(Gibas, 2021). These considerations are crucial, for they can influence the quality of data collected. It is important to note that their article stresses the short time frame of eight weeks that the research team had to make these decisions, on top of limited equipment and protocols available. The figure below shows the sampling, collection, processing, and reporting schedule that UNCC's team used (Gibas, 2021).

Figure 3

UNCC's weekly wastewater surveillance schedule (Gibas, 2021).

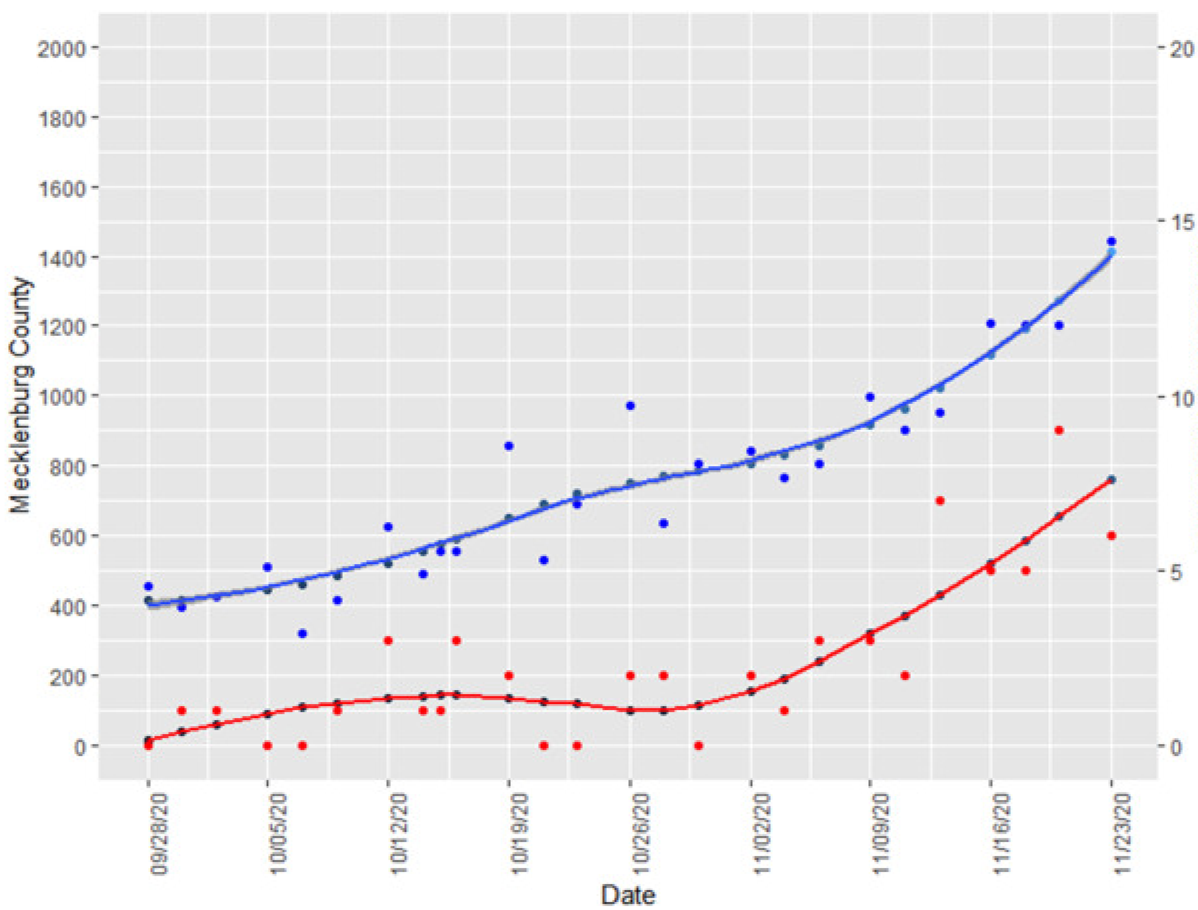


UNCC used an electronegative filtration process, HA, as opposed to physical filtration (Gibas, 2021). Dr. Cynthia Gibas, a professor for the Department of Bioinformatics and Genomics and a member of UNCC's COVID-19 wastewater surveillance team, explained the rationale for this choice of methodology by stating, "Our collaborator Mariya Munir was already using HA filtration as part of the statewide NC wastewater monitoring effort so it was easy to get up and running. Later we switched to InnovaPrep Cp Select concentrators because they are faster" (Gibas, C., personal communication, March 27, 2022). Efficiency was a driving factor for UNCC's testing protocol. By the spring semester of 2021, they were processing their samples on the same day that they collected them, yielding same-day results (Gibas, 2021). According to Dr. Gibas, UNCC "tried using ddPCR instead of qPCR, but the ddPCR runs take too long for the reporting schedule [they] are on" (Gibas, C., personal communication, March 27, 2022). Unique to UArizona and UofSC, UNCC implemented surge clinical testing at dormitories that had a positive signal for SARS-CoV-2 that could not be explained by a recently identified and quarantined positive case or otherwise accounted for. They used clinical testing as a means of finding the source of the positive signal to rapidly isolate the infected individual(s). Thus, they

needed to ensure wastewater samples were processed and analyzed as quickly as possible to prevent the further spread of the virus. The figure below displays the "total number of SARS-CoV-2 positive wastewater samples from UNCC dormitories (red, right axis) and daily new positive cases in Mecklenburg county (blue, left axis) from September 28 to November 23, 2020" **(**Gibas, 2021).

Figure 4

Comparison between the wastewater signals from UNCC and clinically-proven cases in Mecklenburg, the county in which UNCC is located **(**Gibas, 2021).

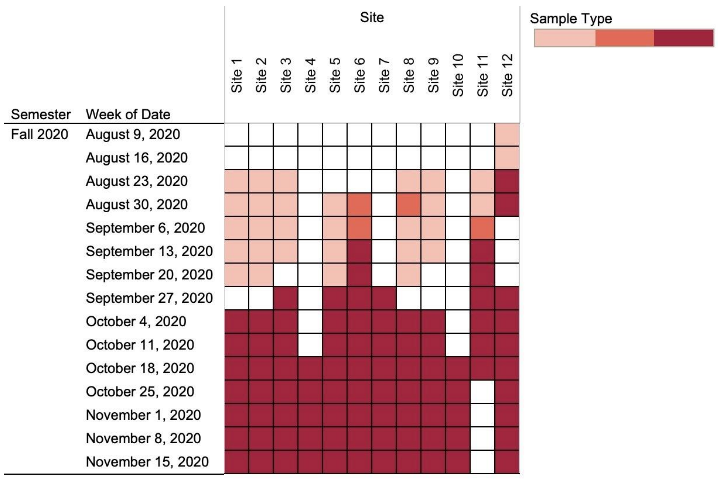


University of South Carolina

In the Fall of 2020, UofSC began wastewater surveillance in an effort to mitigate the effects of COVID-19 on a university that had not switched to fully-online instruction. The first month was filled with trial and error, as no one at the university had performed wastewater sample collection prior to the pandemic. UofSC, as mentioned above, was able to switch over to ISCO autosamplers later in the Fall of 2020. Information, collected before the samplers were obtained, was still reported to the university but will not be discussed in this paper for the sake of both consistency and accuracy. Figure 5 displays the gradual transition researchers made between grab and composite samples. By September 27th, all samples were being collected via autosamplers. The team modified locations throughout the semester as they found certain sewer access points easier to deploy an autosampler in than others. Figure 6 shows the finalized list of locations that were tested throughout the course of the Fall 2020 semester, and Figure 7 shows the data collected from these sites (Sellers, 2022).

Figure 5

The transition between grab samples and composite samples in the Fall 2020 semester (Sellers, 2022).



Note. The colors of the squares represent the following types of samples: pink, grab; orange; one day of grab and one day of composite; red, composite.

Figure 6

UofSC Fall 2020 semester on-campus sampling locations (Sellers, 2022).

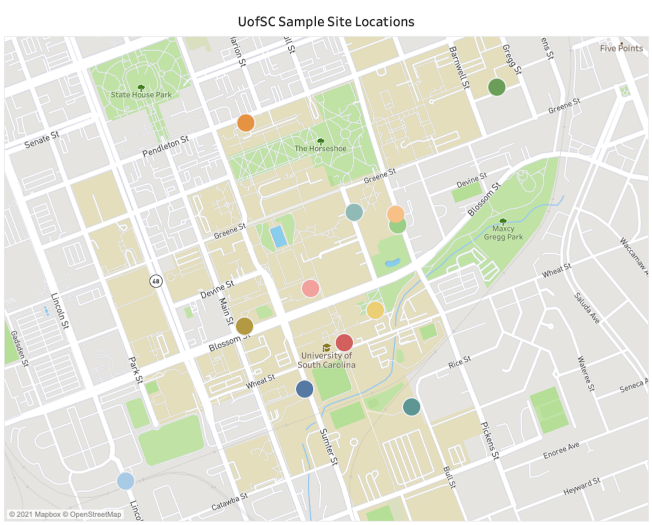
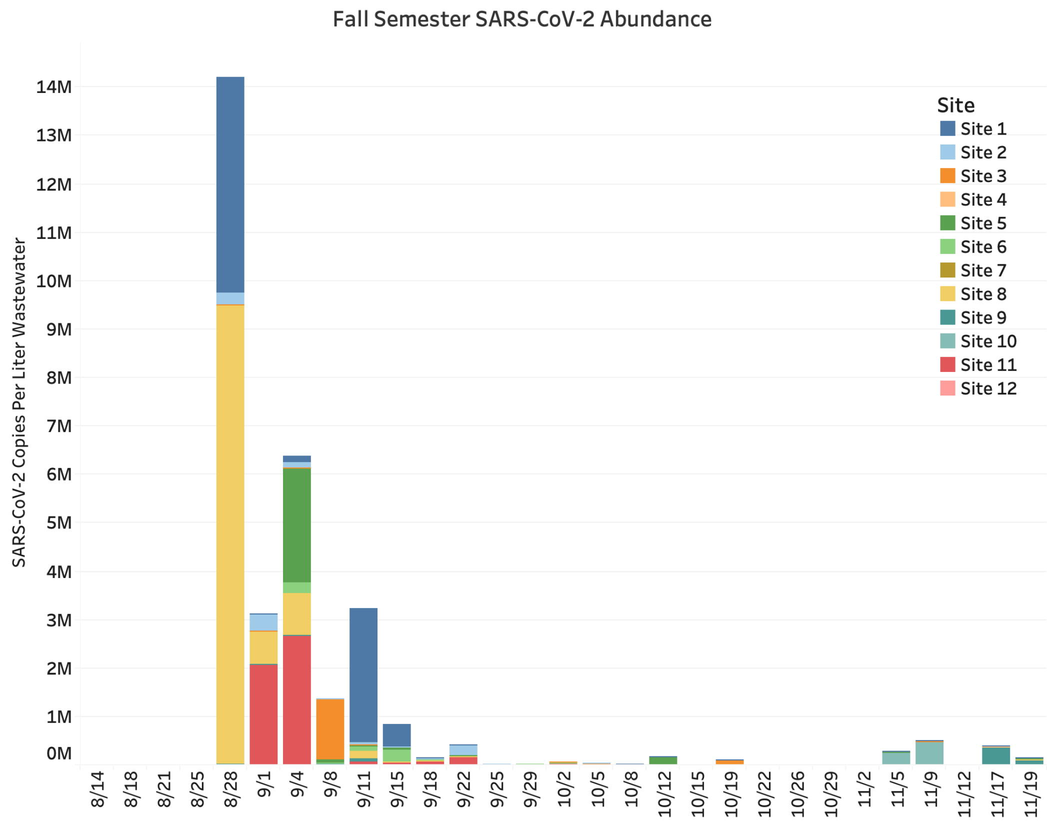


Figure 7

The relative concentration of SARS-CoV-2 in wastewater (Sellers, 2022).



The protocol used by UofSC was as follows: samples were collected twice weekly, with autosamplers deployed on Mondays and Thursdays. The composite samples were collected on Tuesdays and Fridays and promptly processed. Homogenization was accomplished by blending each sample for ten minutes. The samples were then physically filtered to a volume of 400 µL via Amicon Ultrafilters. 200 µL of each sample was stored at -80℃ while the other 200 µL was used for RNA extractions. The viral load in each sample was quantified using RT-qPCR until the end of December 2020. These data were then relayed to university personnel to determine which areas on campus would most benefit from targeted pop-up testing sites (Sellers, 2020).

At the start of the Spring 2021 semester, the team gained access to Droplet Digital PCR (ddPCR) technology and implemented it into their protocol instead of RT-qPCR. A 2017 study "conclude[d] that for sample/target combinations with low levels of nucleic acids ($Cq \geq 29$) and/or variable amounts of chemical and protein contaminants, ddPCR technology will produce more precise, reproducible and statistically significant results required for publication-quality data" in comparison to qPCR (Taylor, 2017). Thus, the team felt that ddPCR would provide more reliable and accurate results (Sellers, 2022).

Unlike UNCC, UofSC was not able to implement surge testing of specific dormitories. University ethical and legal concerns led to this decision, for not every dormitory was feasible as a testing location. Students living in non-testable dormitories would have consequently been placed at a disadvantage if they did not receive the same access to surge testing. The university did implement pop-up testing sites, though. After receiving data from the research team, UofSC placed clinical testing locations on areas of campus that had high signals for SARS-CoV-2 (Sellers, 2022).

Challenges

Supply Chain Issues

One difficulty experienced by the scientific community during COVID-19 did not concern the science itself. Instead, it involved widespread supply chain issues. The American Society for Microbiology noted that both diagnostic and non-diagnostic testing were affected. According to them, pandemic shortages included, but were not limited to, "culture and transport media, swabs, pipettes, pipette tips, and collection tubes" (Hagen, 2020). The research team at UofSC experienced this first-hand with autosamplers. At the start of the Fall 2020 semester, ISCO autosamplers were ordered with the intention of collecting composite samples starting in

September. However, due to problems with the supply chain, the team did not receive their autosamplers until the end September of 2020. UofSC researchers also experienced a similar issue with tubing for autosamplers at the start of the Spring 2021 semester. When they were unable to obtain it directly from the autosampler company, they bypassed the supply chain by purchasing tubing of the same material and diameter from a local hardware store. Additionally, the author and other members of the research team performing wastewater surveillance on UofSC's campus had difficulty obtaining the following supplies during the peak of the pandemic:

- Amicon Ultrafilters
- 200 µL pipette tips
- gloves
- bleach
- KN95 and N95 masks
- ddPCR Mastermix
- N1 and N2 assays
- conical tubes (Sellers, S., personal communication, March 24, 2022).

Dr. Gibas identified the following supply shortages experienced by UNCC, some of which overlap with those of UofSC's.

- autosamplers
- pipette tips
- primers for targeted amplicon sequencing and other reagents (Gibas, C., personal communication, March 27, 2022).

Both universities experienced great difficulty with supply shortages at the beginning of testing in the Fall 2020 semester. Gloves and bleach are often purchased in bulk from laboratory sources, and while these items can also be found at hardware and grocery stores, supply-chain shortages might also affect these alternative sources.

Burnout

Although unrelated to supply chains, there was another shortage that many research teams experienced during their surveillance of COVID-19: a shortage of research personnel as individuals became ill or infected. The research team at UofSC primarily consisted of three undergraduate students in the semester of Fall 2020. As various members of this small group fell ill and were quarantined, master's and post-doctorate students had to fill in to keep testing on a regular schedule. This was a huge strain on the lab, for these graduates were already performing sample processing of wastewater for the South Carolina Department of Health and Environmental Control (Sellers, S., personal communication, March 24, 2022).

When asked if burnout was also experienced by UNCC's research team, Dr. Gibas replied:

> Oh yes. The 3x/week schedule is tough on everyone. We have gone through four different field collection people since that is the most physically demanding job, and we've had about a dozen different part timers during the project, but partly that's just because students graduate in the natural course of things. We've had five PhD students involved in different aspects of method optimization, 12 Masters students, and 13 undergraduates involved in lab work (some as volunteers and some paid). Plus three full time lab personnel. We had to have a lot of redundancy because even when we were being

maximally careful people would be out with COVID from time to time (Gibas, C., personal communication, March 27, 2022).

Dr. Gibas touched on the unique challenge that COVID-19 brought to research labs- the possibility of laboratory staff becoming isolated or quarantined when infected or exposed to the SARS-CoV-2 virus.

UofSC experienced this very issue. With only three undergraduate students, one lab director, and two members of maintenance tasked with sample collection and processing, COVID-19 had a great impact on the availability of staff. Sarah Sellers, one of the undergraduates, remembers a time when both of the other undergraduates were sick with COVID-19 one after the other. She explained, “that was especially difficult because we were still new to the process so it wasn’t quite as easy…. I think a big issue that anyone working with COVID would say is that there’s a lot of burnout when you experience all of these shortages and then are still expected to keep going…” (Sellers, S., personal communication, March 24, 2022). The very nature of a pandemic lends itself to burnout. There is no freedom to take a break from wastewater surveillance, for the health of students, faculty, and staff depends on it. Increasing the number of research staff may help to mitigate the problem of burnout by having a rotation of team members that can take turns collecting and processing samples. Each member should also be trained in all steps of the process so that they can readily step in if a coworker falls ill. Increasing the number of researchers and knowledge of each employee will initially be time-consuming and costly but will pay off in the long run in terms of the mental health and stamina of employees.

Results Comparison

It is difficult to concretely say one university's methodology was better than the others. It is, however, feasible to analyze which approaches are most appropriate for university-level sampling based on testing schedules, building locations, and the implementation of control measures. These factors are discussed in the following section. This paper initially aimed to look at published data concerning the correlation between positive detections of SARS-CoV-2 and positive clinical testing. However, there is a severe shortage of such data in published literature.

Dr. Gibas provided the following information regarding the correlational analysis of UNCC's data: "we've got all sorts of data that we're working on publishing -- everything from clinical sequencing that matches up with cases on specific dates in buildings to overall building population and case count data. We did an IRB with the university that allowed us to access aggregated case, contact tracing, symptom reporting, and vaccination data from the university's COVID registry and we are working on analyzing that now" (Gibas, C., personal communication, March 27, 2022). UofSC is in the midst of using a biostatistical approach to provide adjusted, correlational data between positive cases on campus and positive wastewater signals. The author could not obtain a statement from researchers at UArizona but speculates that their data is also similarly being analyzed. Therefore, further comparison and analysis between these three universities should be conducted after all of these data are available. This may further support claims in this paper regarding appropriate methodologies.

Future Directions

One keyword comes to mind when considering America's initial reaction to the COVID-19 pandemic: unprepared. It is extremely likely, almost certain, that we will experience another pandemic of this magnitude in our lifetimes. Thus, we must take measures now to

prepare our nation's universities for an epidemic similar in magnitude to the COVID-19 pandemic. Although a supply chain shortage cannot be easily avoided, precautionary measures can still be taken. Laboratories that anticipate performing wastewater surveillance during a future pandemic should keep extra supplies on hand at all times, such as gloves, bleach, pipette tips, autosamplers, and autosampler tubing. These are not perishable supplies, so long-term storage of them should not be an issue. At the first sign of the rapid spreading of an emerging or reemerging infectious disease, laboratories should order necessary perishable supplies, such as primers and reagents so that they will be ready to start wastewater surveillance as soon as it is needed.

An established universal protocol should be readily available for the next pandemic, even if it will require adjustment. Each institution can modify the protocol according to its testing and materials availability. However, the following recommendations can be made after analyzing UArizona's, UNCC's, and UofSC's methodology and data and speaking with their staff. The parentheticals contain the institution(s) that have already utilized that methodology.

- Bovine coronavirus can be used as a process control to see how effective testing is (Sellers, 2022; Gibas, 2021).
- Data loggers are devices that can be placed in sample collection containers to track their temperature throughout the collection period and initial processing. This will demonstrate whether or not samples are maintained at the ideal temperature to minimize the risk of viral degradation (Sellers, 2022).
- Perform Building Information Modeling (BIM) should be used when selecting testing sites and determining whether to use an internal or external access point (Gibas, 2021).
- Autosamplers should be used to collect composite samples. Collection timing and volume can be adjusted based on flow rate and volume at each school. However, composite

samples should be considered the gold standard in comparison to grab samples (Sellers, 2022; Gibas, 2021).

- Steel strainers should be placed on the end of autosampler tubing to prevent clogging and maximize sample collection (Sellers, 2022; Gibas, 2021).
- A drafted list of items that can be purchased locally if backordered, such as tubing from hardware stores (Sellers, 2022).
- Standardized usage of dd-PCR (Sellers, 2022).
- Cooperation of university administration to allow for surge testing (Gibas, 2021).
- Large numbers of laboratory staff to prevent burnout and maximize efficiency (Gibas, 2021).

In addition to the above methods, there are other measures that the nation should take to prepare universities for the next pandemic. Funding should be secured and used to establish equipment for wastewater surveillance for as many universities as possible. This includes all of the non-perishable supplies discussed previously. Perishable supplies should not be purchased, but funding should be set aside to be used for the purchase of such items when necessary. This preemptive approach will benefit universities by taking away the initial struggle of finding the money necessary for sample collection and processing. It is acceptable for each laboratory to have a preference for a particular brand of extraction kits and other materials; however, staff should have a list of suitable alternatives, should supply chain issues hinder the acquisition of their typical choice of supplies.

The cornerstone of the scientific community is knowledge. This should never be gatekept, particularly in the midst of a deadly pandemic when people's lives are at stake. The establishment of an online database for laboratory teams to share best methods could allow for

the streamlining of wastewater surveillance on a national level to compile a collaborated, national protocol for wastewater surveillance. This could be readily used by all universities and allow for faster activation of response during a pandemic. Such a system would be particularly useful for new employees or schools that have not performed wastewater surveillance before.

Limitations

This paper has explored three universities and the available data associated with their COVID-19 responses. It is important to note that this thesis was limited by published material. The author attempted to bypass this by contacting researchers at UofSC, UArizona and UNCC. Researchers at UArizona could not be reached for further information. The author was privy to a greater degree of information for UofSC, as she was a member of the school's COVID-19 wastewater surveillance research team and a co-author of the team's pending research paper. Thus, some unconscious biases may have occurred while evaluating the research methodology of the other two schools.

Conclusions

There is no way of knowing in what form or when the next epidemic will take place. The COVID-19 pandemic has been filled with scientific trial and error as researchers have pushed boundaries to protect our nation. Wastewater surveillance is a prime example of one of these scientific endeavors, allowing universities to monitor the status of SARS-CoV-2 on campuses. Correlational research needs to be published before a supported best methods list can be compiled for multiple, specific settings. Although a conclusive statement cannot be yet made, it is likely that wastewater surveillance has saved the health, if not lives, of many members of our universities.

References

Ahmed et al., 2020a. 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*(2020), 138764. https://doi.org/10.1016/j.scitotenv.2020.138764

Ahmed et al., 2020b. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. *Environ. Res. 191*(2020), 110092. https://doi.org/10.1016/j.envres.2020.110092

Betancourt, W. Q., Schmitz, B. W., Innes, G. K., Prasek, S. M., Pogreba Brown, K. M., Stark, E. R., Foster, A. R., Sprissler, R. S., Harris, D. T., Sherchan, S. P., Gerba, C. P., & Pepper, I. L. (2021). COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. *The Science of the Total Environment*, *779* (146408), pp. 1-8.. https://doi.org/10.1016/j.scitotenv.2021.146408

Bhalla, N. (2016, June 15). *India to vaccinate 300,00 children after polio strain found in sewage.* Reuters. https://www.reuters.com/article/us-india-health-polio/india-to-vaccinate-300000-children-after-polio-strain-found-in-sewage-idUSKCN0Z11Z5

Centers for Disease Control and Prevention. (2021a, February 26). *COVID-19 overview and infection prevention and control priorities in non-US healthcare settings.* https://www.cdc.gov/coronavirus/2019-ncov/hcp/non-us-settings/overview/index.html

Centers for Disease Control and Prevention. (2021b, June 23). *Developing a wastewater surveillance sampling strategy.* https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html

Centers for Disease Control and Prevention. (2021c, July 21). *National wastewater surveillance system (NWSS).* https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html

Centers for Disease Control and Prevention. (2021d, September 1). *Wastewater Surveillance Data Reporting and Analytics.* https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/data-reporting-analytics.html

Centers for Disease Control and Prevention. (2021e). *What is polio?* https://www.cdc.gov/polio/what-is-polio/index.htm#:~:text=Polio%2C%20or%20poliomyelitis%2C%20is%20a,move%20parts%20of%20the%20body

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol 5,* 536–544. https://doi.org/10.1038/s41564-020-0695-z

Daughton, C. (2020). Wastewater surveillance for population-wide COVID-19: The present and future. *Science of the Total Environment, 736* (139631). https://doi.org/10.1016/j.scitotenv.2020.139631

Eisenberg, M., Brouwer, A., Eisenberg, J. (2018). *Sewage surveillance is the next frontier in the fight against polio.* University of Michigan School of Public Health. https://sph.umich.edu/pursuit/2018posts/sewage-surveillance-polio.html

Gibas, C., Lambirth, K., Mittal, N., Juel, M.A.I, Visva, B.B., Brazell, L. R., Hinton, K., Lontai, J., Stark, N. Young, I., Quach, C., Russ, M., Kauer, J. Nicolasi, B. Chen, D., Akella, S., Tang, W., Schlueter, J., & Munir, M. (2021, August 15). Implementing building-level

SARS-CoV-2 wastewater surveillance on a university campus. *Science of the Total Environment, 782* (146749). DOI: 10.1016/j.scitotenv.2021.146749

Gosnell, E. (2021). *Wastewater surveillance: Past, present, and future uses.* [Unpublished manuscript]. University of South Carolina.

Hagen, A. (2020, October 15). *Laboratory supply shortages are impacting COVID-19 and non-COVID diagnostic testing.* American Society for Microbiology. https://asm.org/Articles/2020/September/Laboratory-Supply-Shortages-Are-Impacting-COVID-19

Harris-Lovett, S., Nelson, K., Beamer, P., Bischel, H. N., Bivins, A., Bruder, A., Butler, C., Camenisch, T. D., De Long, S. K., Karthikeyan, S., Larsen, D. A., Meierdiercks, K., Mouser, P., Pagsuyoin, S., Prasek, S., Radniecki, T. S., Ram, J. L., Roper, D. K., Safford, H., Sherchan, S. P., … Korfmacher, K. S. (2021). Wastewater surveillance for SARS-CoV-2 on college campuses: Initial efforts, lessons learned and research needs. *medRxiv : the preprint server for health sciences*, 2021.02.01.21250952. https://doi.org/10.1101/2021.02.01.21250952

Hill et al., 2020. SARS-CoV-2 known and unknowns, implications for the water sector and wastewater-based epidemiology to support national responses worldwide: early review of global experiences with the COVID-19 pandemic. *Water Qual Res J*. (2020), 10.2166/wqrj.2020.100

Hokajärvi, et al., 2021. The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland. *The Science of the total environment*, *770*, 145274. Advance online publication. https://doi.org/10.1016/j.scitotenv.2021.145274

Horn, C., 2020. Coronavirus in wastewater treatment plants potential pathway to population testing. *The University of South Carolina.* https://www.sc.edu/uofsc/posts/2020/05/covid_in_sewage_research.php#.YEVXqpNKhQI

Ivanova, O. E., Yarmolskaya, M. S., Eremeeva, T. P., Babkina, G. M., Baykova, O. Y., Akhmadishina, L. V., Krasota, A. Y., Kozlovskaya, L. I., & Lukashev, A. N. (2019). Environmental Surveillance for Poliovirus and Other Enteroviruses: Long-Term Experience in Moscow, Russian Federation, 2004-2017. *Viruses*, *11*(5), 424. https://doi.org/10.3390/v11050424

Pruden, A., Vikesland, P.J, Davis, B.C, Mariade, A., Husman, R. (2021). Seizing the moment: now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments. *Current Opinion in Microbiology, 64,* 91-99. *https://doi.org/10.1016/j.mib.2021.09.013*

Sellers, S. C., Gosnell, E. A., Bryant, D., Belmonte, S., Self, S., McCarter, M. S. J., Kennedy, K., Norman, S. (2022). *Building-level wastewater surveillance of SARS-CoV-2 and targeted clinical testing in a university setting.* Unpublished manuscript.

Taylor, S.C., Laperriere, G., Germain, H. (2017, May 27). Droplet Digital PCR verus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data. *Springer Nature.* https://www.nature.com/articles/s41598-017-02217-x

University of South Carolina. (2021). *COVID-19 Dashboard.* https://sc.edu/safety/coronavirus/dashboard/index.php