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April 21, 2022

Surveillance for COVID-19 Using **W**astewater and **A**dvancing **N**asal **S**elf-Collection of **S**pecimens
(SWANSS) in an Atlanta Jail

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Rollins School of Public Health of Emory University
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Abstract

Surveillance for COVID-19 Using Wastewater and Advancing Nasal Self-Collection of Specimens (SWANSS) in an Atlanta Jail

By Lindsay Saber

Background: Correctional facilities historically house some of the most vulnerable persons in our society. With crowded living conditions, decreased access to quality medical care, and limited resources, infectious disease outbreaks can be extremely dangerous, particularly for highly transmissible diseases like COVID-19. Wastewater-Based Surveillance is a low- cost, highly sensitive, non- invasive method that can provide an early warning of COVID-19 surges in the community and outbreaks in institutions, but this has not yet been applied in correctional facilities.

Objective: The study's main objective was to examine if WBS is a practical and sensitive strategy to surveil for new COVID-19 outbreaks in a large jail setting.

Methods: The study period was from June 15, 2021 to March 16, 2022 (39 weeks). COVID-19 diagnostic tests were administered to jail residents and analyzed on a weekly basis—rapid diagnostic test data was collected daily by the jail administration, and 16 mass PCR testing events were conducted by the study team. Wastewater samples were collected via Moore swabs on 28 unique weeks and analyzed for SARS-CoV-2 by realtime RT-qPCR. Temporal and correlation analysis were applied to wastewater and COVID-19 diagnostic test results to examine the association between the prevalence of COVID-19 identified by diagnostic testing and detection of SARS-CoV-2 RNA in the wastewater.

Results: The efficiency of diagnostic testing increased with repeated trials and improved staffing. During the study period, COVID-19 test positivity ranged from 0% to 29.5%. SARS-CoV-2 RNA was detected in the wastewater samples from 25 of the 28 weeks with samples. Wastewater collection and analysis was feasible for a team with a designated sampling and lab team. Stronger RT-PCR signals for SARS-CoV-2 in the jail wastewater preceded rises in the number of COVID-19 cases in the jail, and regression analysis indicated a strong relationship between SARS-CoV-2 RNA concentrations in wastewater samples and positivity rates of COVID-19 diagnostic testing.

Conclusions SARS-CoV-2 RNA detection in wastewater collected at the jail was associated with COVID-19 diagnostic test results in the jail population. Wastewater based surveillance is a practical strategy to surveil for new COVID-19 outbreaks in a jail setting.

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Chapter 1: Introduction

In the United States, there have been over 80 million cases of Coronavirus disease 19 (COVID-19) with just under 1 million of those cases resulting in death (CDC, 2022c). Higher transmission of SARS-CoV-2 (the virus that causes COVID-19) is attributable to close living conditions, the inability to quarantine, and lack of Personal Protective Equipment (PPE) (WHO, 2021). It is reported that at least 4.9 million people are arrested and jailed in the United States every year, amounting to about 644 people per 100,000 (PP1c). The CDC has released guidelines with suggestions on how correctional facilities should handle COVID-19 prevention and outbreaks, yet it is still a threat in correctional facilities due to overcrowding, decreased access to preventative care, and movement within/ between facilities (Hagan et al., 2021; Kirbiyik et al., 2020). These conditions have led to prisons and jails having a COVID-19 incidence rate five times higher than the surrounding communities (Marusinec et al., 2022).

In the past, relying on mass diagnostic testing to stop outbreaks has been a norm; but this can be costly, time consuming, and difficult to reach all residents in a correctional facility (Hagan et al., 2021; Hagan et al., 2020; Njuguna et al., 2020; Tompkins et al., 2021; Tsoungui Obama et al., 2021; Zawitz et al., 2021). This poses the need for a surveillance system that is implemented on the institutional level where an entire prison or jail can be surveilled for SARS-CoV-2 rather than administering individual diagnostic tests. Wastewater based surveillance has shown promise in detecting SARS-CoV-2 at an institutional level, and if implemented in jails, could potentially save time, resources, and lives (Harris-Lovett et al., 2021).

Institutional- level WBS allows for samples to be taken from manholes downstream of a building or small group of buildings, with the potential of surveilling for SARS-CoV-2 from any

wastewater that exits an institution. Several studies have provided evidence that WBS may be used as a sensitive, low-cost, non-invasive surveillance tool for early detection of COVID-19 cases on an institutional level (Betancourt et al., 2021; Gonzalez et al., 2020; Karthikeyan et al., 2021; Wang et al., 2022). Nevertheless, these methods have never been tested in a jail setting. Given the need for early outbreak detection because of the crowded setting and limited resources in correctional facilities, WBS methodology is promising (Kirbiyik et al., 2020). There is a need to conduct further research in utilizing WBS for early detection of SARS-CoV-2 in jails to prevent outbreaks of COVID-19.

Chapter 2: Literature Review

COVID-19 in United States Correctional Facilities

In the United States, there have been over 80 million cases of Coronavirus disease 19 (COVID-19) with just under 1 million of those cases resulting in death (CDC, 2022c). In Georgia alone, there have been 2.5 million cases and 37,500 deaths due to COVID-19 (CDC, 2022c). Higher transmission of SARS-CoV-2 (the virus that causes COVID-19) is attributable to close living conditions, the inability to quarantine, and lack of Personal Protective Equipment (PPE) (WHO, 2021).

It is reported that at least 4.9 million people are arrested and jailed in the United States every year, amounting to about 644 people per 100,000 (PP1c). Georgia's incarceration rate, however, is even higher, with 968 people incarcerated per 100,000 in the population (PP1a). Given the large number of residents¹ cycling through the system each year, it is essential that their health be prioritized. The CDC has released guidelines with suggestions on how correctional facilities should handle COVID-19 prevention and outbreaks, including: testing residents at intake and whenever symptoms arise, quarantining infected residents for 10 days, vaccination of residents, use of advanced masking, physical distancing strategies. Though these suggestions are in place, COVID-19 is still a threat in correctional facilities due to overcrowding, decreased access to preventative care, and movement within/ between facilities (Hagan et al., 2021; Kirbiyik et al., 2020). These conditions have led to prisons and jails having a COVID-19 incidence rate five times higher than the surrounding communities (Marusinec et al., 2022).

¹ Residents refer to incarcerated persons

Since March of 2020, there have been hundreds of studies examining all aspects of COVID-19 in correctional facilities, such as disease transmission patterns, prevention strategies, treatment options and more. These studies utilized previous knowledge on conducting infectious disease research in correctional facilities to characterize the COVID-19 pandemic within jail and prison walls. Therefore, it is important to understand what was known about infectious diseases in correctional facilities prior to March of 2020, to contextualize the research and response that was seen with the introduction of COVID-19.

Infectious Diseases in Correctional Facilities

Jails and prisons house some of the most vulnerable people in our communities, and those who are admitted to these facilities are historically considered to have drastically reduced accessibility to quality medical care (PPIb). Residents are exposed to unsanitary living conditions, overcrowding, and high-risk activities, contributing to an array of medical concerns seen in these facilities. Infectious diseases are of particular concern because they can spread rapidly and do so at a rate two to ten times higher in prisons and jails than they do in the surrounding populations (Weinbaum et al., 2005). With this increased risk and lack of medical attention, health outcomes for correctional facility residents are bleak, at best; even if they are released, many don't have the resources to seek medical attention on their own.

Previous to the onset of the COVID-19 pandemic, infectious diseases of greatest concern in correctional facilities were ones that were transmitted sexually, or through needle sharing; therefore, these diseases have been studied the most (Gough et al., 2010; Huber et al., 2019; Weinbaum et al., 2005; Woznica et al., 2021; Yanes-Lane et al., 2020). While there are studies on respiratory viruses such as Tuberculosis (Cords et al., 2021; Grenzel et al., 2018; Parvez et al.,

2010; Seri et al., 2017), H1N1 (Chao et al., 2017; Guthrie et al., 2012), and influenza (Awofeso et al., 2001; Besney et al., 2017; Centers for Disease & Prevention, 2012; Finnie et al., 2014) the majority of research on infectious diseases in correctional facilities are mostly focused on STIs or infections from sharing blood. Those diseases that are mentioned in infectious disease studies include: influenza, adenovirus, Tuberculosis, varicella, measles, and mumps (Beaudry et al., 2020). Most studies were located outside the United States, making it harder to generalize to correctional facilities in this country.

With limited funding for correctional facility studies, it is difficult to draw definitive conclusions regarding the history of respiratory virus prevention and treatment in jails. If respiratory infection surveillance is necessary in a correctional facility, CDC recommends that there should be a specific emphasis on screening, contact tracing, and isolating infected individuals in order to control the disease (CDC, 2000). Surveillance needs to be more than just symptomatic screening as well; because of the stigma surrounding infections, the possibility of isolation, and/ or the lack of trust residents may have in medical or other facility staff, symptomatic screening alone can miss a significant number of cases (Wallace, Hagan, et al., 2020; Wallace, Marlow, et al., 2020). In recent years, the literature has also begun to address how these disease control measures need to be weighed against the potential negative mental health consequences for residents (Hewson et al., 2020). Strong interagency communication between groups such as prison administration and healthcare staff, local and state health departments, public health laboratories, and hospital services is essential, in order to stay up to date on best practices and the current health status of the facility residents (Venkat et al., 2019). These relationships are hard to maintain, however, when there are limited resources. On

a yearly basis, the state of Georgia spends \$3,610 on health care per inmate, which is 37% less than the \$5,720 per inmate country average (McKillop, 2017). There are only 6 other states that spend less on their inmate healthcare (McKillop, 2017).

COVID-19 in Correctional Facilities

The COVID-19 pandemic revealed a dangerous lack of preparedness for health emergencies in correctional facilities and because of this, there has been heightened interest in basing studies in jails and prisons. Recent research has focused on describing COVID-19 outbreak characteristics, spatial and temporal transmission trends in correctional facilities, and more importantly, using this information to develop guidelines on how correctional facilities should handle COVID-19 (CDC, 2022a).

One major point of emphasis in the correctional health field is that jails and prisons are not isolated from the surrounding community. Jails tend to have shorter stay times than penitentiaries, on average 26 days vs 2.7 years nationally, indicating that residents are cycled through faster (NCSL, 2021). This is cause for concern because infections that spread in jails will eventually make their way into the surrounding communities once infected residents are released. The movement in and out of corrections is known as jail- community cycling. One study found that jail- community cycling was a significant predictor of COVID-19 case rates; zip codes with higher rates of arrested and released individuals also had significantly higher SARS-CoV-2 infections (Reinhart & Chen, 2020). This same study estimated that in Chicago, for every one individual who is released from jail, 2.2 additional COVID-19 cases are reported per capita (Reinhart & Chen, 2020). Generally, people in correctional facilities also have an increased prevalence of underlying health conditions and come from predominantly marginalized

communities, so spreading disease amongst these communities' places burden on those who may already be struggling to access basic health services (Beaudry et al., 2020).

For the reasons above, measures to prevent the transmission of SARS-CoV-2 to avoid outbreaks in correctional facilities is essential. The CDC has recommendations for transmission prevention measures such as medically isolating those who are confirmed and suspected to have COVID-19, providing residents with PPE, and encouraging vaccination. Diagnostic screening is emphasized, including the frequency of testing and test types (CDC, 2022a).

COVID-19 Diagnostics Methods

Since the emergence of SARS-CoV-2, there have been several testing methods developed to confirm COVID-19 cases. The two major categories being diagnostic and serology (or antibody) tests. Diagnostic testing is used to determine if an individual is currently infected with SARS-CoV-2 while serology tests determine if SARS-CoV-2 antibodies are present in the immune system (FDA, 2022a). Serology tests cannot determine if there is a current infection, rather if an individual has had an immune response to the virus, either through previous infection or a vaccine (FDA, 2022a).

Diagnostic testing includes two different types of tests: molecular and antigen tests. Molecular tests, such as a PCR test, are the most accurate form of diagnostic test (FDA, 2022a). These assays analyze specimens such as nasopharyngeal swabs for the presence of SARS-CoV-2 RNA, but results may take longer to process since the samples are normally sent to a laboratory for PCR analyses (FDA, 2022a). While antigen tests are less accurate and less sensitive, they are typically more accessible and can be performed on site. A simple saliva or nasopharyngeal swab sample is required and they may only take a few minutes to provide a result; hence a more

common term “rapid tests” (FDA, 2022b). These tests may be single target, which are designed to detect one antigen on the spike protein, or multiple target, designed to detect more than one section of the SARS-CoV-2 spike protein (FDA, 2022b).

Since serology tests do not detect current infection, the best method to prevent the spread of COVID-19 is diagnostic (molecular or antigen) tests. High throughput PCR tests are ideal because of their high sensitivity and specificity. The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test, for example, is a novel test and uses an end-point reverse transcription polymerase chain reaction (RT-PCR) assay that is intended for the qualitative detection of nucleic acids from SARS-CoV-2 in direct nasal swabs from anterior nares (LGC, 2021). This test detects 100% of positive samples at a concentration of 250 genome copies/ swab and 80% of samples at a concentration of 125 genome copies/ swab; ultimately determined to be highly sensitive and specific (LGC, 2021).

COVID-19 Diagnostics in Jails

Diagnostic testing in jails is essential to confirm COVID-19 outbreaks. In the past, relying on mass diagnostic testing to stop outbreaks has been a norm; but this can be costly, time consuming, and difficult to reach all residents in a correctional facility (Hagan et al., 2021; Hagan et al., 2020; Njuguna et al., 2020; Tompkins et al., 2021; Tsoungui Obama et al., 2021; Zawitz et al., 2021). But asymptomatic cases are less likely to receive a diagnostic test, especially when diagnostic supplies are limited, leaving the incarcerated population even more vulnerable. This poses the need for a surveillance system that is implemented on the institutional level where an entire prison or jail can be surveilled for SARS-CoV-2 rather than administering individual diagnostic tests. Wastewater based surveillance has shown promise in

detecting SARS-CoV-2 at an institutional level, and if implemented in jails, could potentially save time, resources, and lives (Harris-Lovett et al., 2021).

Wastewater Based Surveillance for COVID-19

Overview

Alternative forms of surveillance include Wastewater Based Surveillance (WBS), a surveillance tool that was originally created in 1948 to detect *Salmonella* for the purpose of disease surveillance in a sewage system (Barrett et al., 1980). It has since evolved to detect other diseases such as *Vibrio Cholerae*, *Rotavirus*, *Hepatitis A* and *Poliovirus* (Adefisoye et al., 2016; Tao et al., 2010). WBS is a low cost, noninvasive, sensitive, simple, and quick method to survey the wastewater from different target populations for the presence and concentration of specific gene sequences (Liu et al., 2022). This method may be utilized when infected individuals shed the virus in their feces, regardless of symptoms. Community level surveillance can then be enacted: sampling wastewater from manholes, then sending samples to the laboratory to determine the presence and/ or concentration of the virus from the sample (Chen et al., 2020). WBS proves to be most useful with diseases that are rapidly spread, have non-specific symptoms, and where asymptomatic cases are common because the true burden of these infections is often under-estimated. Given all these characteristics are true of the COVID-19 pandemic, and the SARS-CoV-2 virus was shown to shed in fecal matter, WBS is a practical and effective means of mass surveillance (Chen et al., 2020; Jones et al., 2020).

Wastewater Based Surveillance Methods

Samples for WBS are normally collected from a manhole from which the general origin of the wastewater is the intended population for surveillance. Access to actively flowing

wastewater is best practice, to ensure accurate sampling (CDC, 2022b). Two WBS sampling methods exist, the Moore swab and grab sample. Moore swabs are a gauze pad tied with string which is then placed in flowing wastewater downstream of the targeted surveillance area. Wastewater flows over the swab for a period of time, the swab is then collected and sent back to the laboratory where the liquid is squeezed out and tested for SARS-CoV-2 (Sikorski & Levine, 2020). A grab sample, on the other hand, is a cross sectional sample; a bottle is filled with the flowing wastewater which is then sent back to the laboratory to determine the viral load of SARS-CoV-2 in the sample (Sikorski & Levine, 2020).

The two different WBS sampling methods both have their strengths and weaknesses. Grab samples measure the wastewater cross sectionally because they are collected at one specific point in time; the wastewater that is flowing through the manhole at that moment will be sent back to the laboratory. By no means is a single grab sample representative of an entire community, it is purely representative of the wastewater in that location, at that point in time (Sikorski & Levine, 2020). In the lab, grab samples can be processed to provide information on the concentration of viral RNA. Following Manual Nanotrap Concentration, or Nanotrap KingFisher Concentration techniques, Real Time PCR is used to determine the viral load of the sample (Cavallo et al., 2022; Sablon et al., 2022). The viral load is represented as a Concentration threshold (Ct) value. Ct values indicate how many copies of a particular gene sequence need to be made before the PCR can detect the RNA sequence (APHL, 2021). Therefore, the lower the Ct Value, the less copies are needed for PCR to detect the RNA, and the higher the original concentration of the target sequence.

The Moore swab method allows for a continuous longitudinal sample to be collected, capturing microorganisms from all wastewater that flows over the swab (Sikorski, 2020). While representative of a period of time, the results from the Moore swab method are not as precise. The samples have the liquid squeezed out of them to have the virus concentrated by either Skim Milk Flocculation, Manual Nanotrap Concentration, or Nanotrap KingFisher techniques, and the nucleic acid is extracted using a Qiagen or MagMax kit (Cavallo et al., 2022; Dunbar et al., 2022; Sablon et al., 2022). The nucleic acid is analyzed for SARS-CoV-2 gene targets using quantitative real-time reverse transcription PCR (RT-qPCR) (Cavallo et al., 2022; Dunbar et al., 2022; Sablon et al., 2022). While this method provides a Ct value, the results are reported as either negative, weakly positive, strongly positive for SARS-CoV-2 RNA. This is because the Moore swab method does not collect all wastewater in a given time period, it just collects a sample. Therefore, the exact concentration of SARS-CoV-2 over a period of time is not able to be measured by the Moore swab method, and the Ct values are more so estimates than true representations of viral load (Wilson et al., 2022).

Swab samples have demonstrated more sensitivity than traditional grab samples, with a PPV=82% and NPV=88.9% (Betancourt et al., 2021). One study claimed that when there is a low number of COVID-19 cases, and therefore less SARS-CoV-2 shed into the wastewater, the Moore swab method is more likely to detect a positive signal over a grab sample (Wang et al., 2022). However, autosamplers, devices which automatically collect grab samples, may be just as effective if programmed to be collect wastewater every 5-30 minutes (Gibas et al., 2021; Liu et al., 2022; Rafiee et al., 2021; Wang et al., 2022). Depending on the budget and location of

costly to purchase and require electricity. There are studies that examine each method of WBS sampling,

Previous Studies on Wastewater Surveillance

The COVID-19 pandemic offered a real-world example of needing a surveillance system that is large enough to test entire communities and complexes in a quick and easy manner. Since the emergence of SARS-CoV-2 in November of 2019, the literature on WBS has grown significantly with many research groups, governments, and organizations dedicating resources to understanding how to best utilize WBS for COVID-19 surveillance. As such, the literature has grown and now offers a wealth of information on COVID-19 WBS.

Fecal Shedding Characteristics

Initially, there was an effort to understand how SARS-CoV-2 was shed in feces. Studies examined individual fecal shedding patterns for people that are symptomatic, asymptomatic, mildly- and pre- symptomatic for COVID-19 (Chen et al., 2020; Jones et al., 2020; Park et al., 2021; Wang et al., 2020). These studies were able to characterize fecal shedding on an individual basis and provide evidence that WBS had the potential to be effective at the community level. Fecal shedding characteristics were collected on an individual level to understand basic viral shedding behavior such as how many days, on average, a person sheds the virus and how this relates temporally to respiratory tests. Fecal shedding characteristics are important to understand before discussing the results of a population level study, because trends on an individual level may relate to trends on a community level.

Several studies examined the temporal relationship between SARS-CoV-2 fecal shed and diagnostic test results. It was found that anywhere from 16.7-88.9% (pooled detection rate of

43.7%) of positive COVID-19 cases shed SARS-CoV-2 in their feces up to 50 days after they received a positive respiratory specimen test (Park et al., 2021; Wong et al., 2020). The lag time between detection of SARS-CoV-2 in a stool sample and clinical diagnosis is between 2-5 days, which is also the average reported lag time from exposure to the virus and onset of symptoms (Guan et al., 2020; Lauer et al., 2020; Li et al., 2020; Wu et al., 2022). On rare occasions, virus is shed in fecal matter when a respiratory test is negative; One study reported that 60% of participants continued to shed virus in their feces after a negative nasal swab, regardless of disease severity (Chen et al., 2020; Ling et al., 2020; Zhang et al., 2020)

SARS-CoV-2 has also shown to multiply in the gut, giving a possible reason to why stool samples come back positive even after respiratory samples are negative (Jones et al., 2020). It has been suggested that fecal viral shedding peaks around day 2 of infection, but there have been instances where a second peak was detected, even after there is a decline of virus in respiratory samples; this is additional reason to think there is viral multiplication occurring in the gut (Cevik et al., 2021; Wu et al., 2022).

Wastewater Surveillance at Institutions

With evidence that the SARS-CoV-2 virus sheds in stool, and a basic understanding of individual shedding patterns, the next step was to examine feasibility of scaling up WBS to a community-wide surveillance system. Studies have observed elevated levels of SARS-CoV-2 in WBS samples up to 8 days prior to a similar rise in community case rates (Liu et al., 2022; Peccia et al., 2020). These findings have been so promising that the CDC has created a National Wastewater Surveillance System (CDC, 2022b), where they provide guidelines as to how WBS can be implemented in a community (CDC, 2022b).

WBS has been used at the community level to survey larger populations, in an attempt to connect COVID-19 prevalence in specific populations with SARS-CoV-2 detection in wastewater samples originating from these same populations. Some experiments have been conducted at a wider community level, sampling from wastewater treatment plants, while others are more focused on institutional level surveillance, such as university dormitories or hospitals (Betancourt et al., 2021; Gibas et al., 2021; Gonzalez et al., 2020; Karthikeyan et al., 2021; Wang et al., 2022; Wu et al., 2022). Institutional- level WBS allows for samples to be taken from manholes downstream of a building or small group of buildings, with the potential of surveilling for SARS-CoV-2 from any wastewater that exits an institution. Community level surveillance is useful to detect spikes in community COVID-19 trends prior to spikes in diagnostic case count numbers, to prepare hospitals for a rise in patients, for example. Institutional level WBS can provide a warning to those in a building or cluster of buildings that SARS-CoV-2 is present, and perhaps individuals should take a diagnostic COVID-19 test.

One study at the University of California- San Diego (UCSD) utilized autosamplers to surveil for SARS-CoV-2 on a daily basis (Karthikeyan et al., 2021). During the study period, 59 COVID-19 cases on UCSD's campus were confirmed through diagnostic testing, 50 of which (84.5%) were preceded by positive wastewater samples. SARS-CoV-2 presented in the wastewater either in the days prior to or the day of diagnostic testing that confirmed the positive COVID-19 cases, indicating high sensitivity in the WBS system.

Another study examined the temporal relationship between WBS and confirmed COVID-19 cases on Emory University's campus (Wang et al., 2022). Using both Moore swabs and grab samples, they were able to detect SARS-CoV-2 in the wastewater up to 2 weeks prior to surges

of cases detected by diagnostic testing. This temporal relationship between positive COVID-19 diagnostic tests and detection of SARS-CoV-2 in the wastewater is evidence that the WBS system was sufficiently sensitive to provide early warning of COVID-19 outbreaks.

A third study collected daily grab samples from dormitories around the University of Arizona campus in August 2020 to survey for SARS-CoV-2 in the first week of classes (Betancourt et al., 2021). When one sample came back positive, the study team returned to the collection site the next day and sampled the wastewater every 5 minutes between 8-8:50am, a time they determined to be peak wastewater flow. Each of these samples were positive, triggering a mass testing event of all residents of the dorm. Of all 311 residents, one symptomatic individual and one asymptomatic individual tested positive with antigen tests. This study exemplifies the sensitivity of WBS and how it can be used to trigger mass diagnostic testing events before an outbreak occurs. Other studies on university campuses have come to similar conclusions, twenty- five of which are summarized in Harris-Lovett et al. (2021).

Wastewater Based Surveillance in a Jail

The evidence presented above suggests that WBS may be used as a sensitive surveillance tool for early detection of COVID-19 cases on an institutional level. Nevertheless, these methods have never been tested in a jail setting. Given the need for early outbreak detection because of the crowded setting and limited resources in correctional facilities, WBS methodology is promising (Kirbiyik et al., 2020). There is a need to conduct further research in utilizing WBS for early detection of SARS-CoV-2 in jails to prevent outbreaks of COVID-19.

Chapter 3: Manuscript

Introduction

In the United States, there have been over 80 million cases of Coronavirus disease 19 (COVID-19) with just under 1 million of those cases resulting in death (CDC, 2022c). Higher transmission of SARS-CoV-2 (the virus that causes COVID-19) is attributable to close living conditions, the inability to quarantine, and lack of Personal Protective Equipment (PPE) (WHO, 2021). It is reported that at least 4.9 million people are arrested and jailed in the United States every year, amounting to about 644 people per 100,000 (PP1c). The CDC has released guidelines with suggestions on how correctional facilities should handle COVID-19 prevention and outbreaks, yet it is still a threat in correctional facilities due to overcrowding, decreased access to preventative care, and movement within/ between facilities (Hagan et al., 2021; Kirbiyik et al., 2020). These conditions have led to prisons and jails having a COVID-19 incidence five times higher than the surrounding communities (Marusinec et al., 2022).

In the past, relying on mass diagnostic testing to stop outbreaks has been the norm; but this can be costly, time consuming, and difficult to reach all residents in a correctional facility (Hagan et al., 2021; Hagan et al., 2020; Njuguna et al., 2020; Tompkins et al., 2021; Tsoungui Obama et al., 2021; Zawitz et al., 2021). This poses the need for a surveillance system that is implemented on the institutional level where an entire prison or jail can be surveilled for SARS-CoV-2 rather than administering individual diagnostic tests. Wastewater based surveillance has shown promise in detecting SARS-CoV-2 at an institutional level, and if implemented in jails, could potentially save time, resources, and lives (Harris-Lovett et al., 2021).

Institutional- level WBS allows for samples to be taken from manholes downstream of a building or small group of buildings, with the potential of surveilling for SARS-CoV-2 from any wastewater that exits an institution. Several studies have provided evidence that WBS may be used as a sensitive, low-cost, non- invasive surveillance tool for early detection of COVID-19 cases on an institutional level (Betancourt et al., 2021; Gonzalez et al., 2020; Karthikeyan et al., 2021; Wang et al., 2022). Nevertheless, these methods have never been tested in a jail setting. Given the need for early outbreak detection because of the crowded setting and limited resources in correctional facilities, WBS methodology is promising (Kirbiyik et al., 2020). There is a need to conduct further research in utilizing WBS for early detection of SARS-CoV-2 in jails to prevent outbreaks of COVID-19.

Research Objectives

There is a need for low- cost, sensitive, non-invasive COVID-19 surveillance in correctional facilities, and Wastewater Based Surveillance could fill that need. This study aimed to:

Aim 1: Administer weekly COVID-19 diagnostic tests to a large portion of the jail population

Aim 2: Conduct weekly Wastewater Based Surveillance (WBS) by collecting wastewater samples from several collection points on the jail grounds and analyzing them for SARS-CoV-2 RNA.

Through these aims, the study's main objective can be achieved: to examine if WBS is a practical and sensitive strategy to surveil for new COVID-19 outbreaks in a large jail setting.

Rationale

There is evidence to suggest that WBS may be used as a sensitive surveillance tool for early detection of COVID-19 cases on an institutional level. While tested in dormitory and hospital setting, these wastewater surveillance methods have never been tested in a jail setting. Given the need for early outbreak detection because of the crowded setting and limited resources in correctional facilities, WBS methodology is promising (Kirbiyik et al., 2020). There is a need to conduct further research in utilizing WBS for early detection of SARS-CoV-2 in jails to prevent outbreaks of COVID-19.

Methods

COVID-19 Diagnostic Testing

PCR Nasal Swab Collection

Concurrent with the wastewater monitoring, a COVID-19 diagnostic testing cross-sectional study was conducted in which any resident in the Fulton County jail from October 2021 to January 2022 may be selected for participation. The primary goal of this study was to measure the weekly overall prevalence of COVID-19 in the jail by laboratory-confirmed PCR diagnostic testing. The secondary goal was to assess the feasibility of these self-collected nasal swabs for routine mass COVID-19 diagnostic testing. A tertiary goal was to measure process improvement of our systematic testing methodology.

The Emory University Institutional Review Board (IRB) determined that this data collection and analysis was exempt from the requirement for IRB review. The study was determined to be non-human subject research because the data was public health practice and deidentified before examined by researchers. Information about the tests and study was offered to jail residents verbally, and PCR testing consent was obtained with a verbal opt-out offer of testing. Our approach to PCR diagnostic testing was novel due to the self-administration of the nasal swabs by the residents themselves. The areas of the jail that were tested each week were either randomly selected, or targeted areas based on known existing outbreaks, often upon request of the jail.

On dates which nasal swab collection was performed, a full roster of residents was sent to the researchers and was sorted by floor, wing, zone and last name of resident before printing two copies. A group of researchers arrived at the jail and were then split up into anywhere

between one to eight swab collection teams. Each team of three was equipped with a cart filled with supplies for nasal swab collection: two printed resident rosters for the area in the jail they were responsible for sorted by floor, wing, zone, and last name of resident; sufficient nasal swabs for all residents in their area; a rack to hold collected swab samples; labeling supplies for racks; personal protective equipment for researchers; laptop; ethernet cord; barcode scanner. Each team would have a minimum of two correctional officers to escort them around the area of the jail for which the team was responsible for.

Depending on the day and time the research team arrived, the residents would either be out in the common area of their zone, or in their cells with the door closed. If the former was true, the research team would demonstrate the nasal swab procedure to everyone and repeat the explanation when asked by residents. If residents were in their cells, researchers would demonstrate the procedure upon access to the cells. Researchers identified each resident on the printed roster after they provided their last name and date of birth. Residents would then answer five preliminary questions and handed their self-administered nasal swab to a researcher who placed it in the holding rack, and the position of their swab in the rack was recorded next to their name on the roster. After all the samples were collected in one zone, one researcher took the laptop, ethernet cord, and barcode scanner to an area with connectable internet and linked the nasal swab samples and answers to preliminary questions to the residents through the Northwell Health system portal. The swabs were then shipped overnight to Northwell Health Laboratory in Lake Success, New York or RT-PCR analysis.

RT-PCR Test Procedure

The Northwell Health Laboratory used an LGC, Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test (BT-SCV2-UHTP-EP) to detect positive nasal swab samples. This novel technology is highly sensitive with a very low Limit of Detection (LoD) of 250 copies/ nasal swab (Biosearch Technologies, 2021). The specificity is also extremely high with 100% of people who are positive for COVID-19 will result in a positive test (Biosearch Technologies, 2021). Specific reagents and materials required for this method are specified in LGC, Biosearch Technologies, *Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test Protocol* (Biosearch Technologies, 2021).

Rapid Test Collection

Rapid COVID-19 diagnostic tests were administered by the jail healthcare personnel every weekday and non-holiday. The rapid test collection was a part of the jail's standard operating procedure, the study team did not collect rapid tests from jail residents. All residents were administered a rapid test at the time of intake and if an individual requested one and was exhibiting symptoms of COVID-19. BinaxNOW rapid tests were used from the start of the study until February 1, 2022, at which time, the jail switched antigen tests to the QuickVue brand. The BinaxNOW tests are a lower nostril nasal swab which has a sensitivity of 64.2% for specimens from symptomatic persons and 35.8% for specimens from asymptomatic persons and a near 100% specificity of the BinaxNOW test in both symptomatic and asymptomatic cases (Prince-Guerra et al., 2021). The QuickVue tests are a nasal pharyngeal swab sample with highly sensitive (Percent Positive Agreement= 96.6%) and even more highly specific (NPA=99.3%) (Quidel). All rapid test data came from these tests administered by the jail.

Wastewater Based Surveillance

Sample Collection

A sampling team collected weekly wastewater samples from Fulton County Jail (weather permitting) from June 15 2021 through March 16 2022. Both Moore swab and grab sampling methods were used in all three manholes on the jail property, a fourth manhole was later found farther from the main building and was then added to the sampling routine (Figure 1). On day one of sample collection, the team would assemble the Moore swabs, made of gauze held together by fishing line, prior to arrival at the jail (Sikorski & Levine, 2020; VanTassell et al., 2022). Once there, with an escort from a contracted maintenance team, the sampling team would open the designated manholes, place swabs into the wastewater streams, and secure the fishing line inside the manhole. After 24 +/- 6 hours, the sampling team returned to collect the Moore swab samples placed the previous day. The swabs were placed in labeled Ziploc bags and put on ice for transportation. When the manhole was open, grab samples were collected with a metal bucket that was lowered into the wastewater flow, filled, and brought back up; the wastewater was then transferred into a sterile, polyethylene one-liter bottle. This was repeated until the bottle was filled. It was then sealed and put on ice. Both of these methods are further described in VanTassell, et al. *Moore Swab Sample Collection* (VanTassell et al., 2022). At least one Moore swab sample was collected weekly from each of the three (and later four) sites near to the main jail building, and grab samples were collected at sites where the team could successfully lower a bucket and collect wastewater from a stream from which they knew the wastewater origin.

Laboratory Analyses

The wastewater samples were then brought to the Center for Global Safe WASH Environmental Microbiology laboratory where virus concentration, RNA extraction, and real time, quantitative reverse transcription-polymerase chain reaction (RT-qPCR) were performed. Wastewater was squeezed from the swabs manually; getting as much liquid out of each swab with a potato ricer. Virus was concentrated from the swab wastewater using Nanotrap particles (Ceres Nanosciences, Manassas, VA) and a KingFisher robot (Thermo Fisher Scientific, Weltham MA) as described in Sablon et al. (2022). Bovine Respiratory Syncytial Virus (BRSV) was added to every sample to serve as a process control. The end product of the concentration was 400 μ L of lysate which was then used for the RNA extraction procedure with the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Bengaluru India). PCR was conducted as explained in Sablon et al (2022).

After viral RNA extraction from the wastewater, RT-qPCR was performed in order to detect the SARS-CoV-2 N1 gene. Standard curve aliquots for SARS-CoV-2 were also prepared in order to quantify the CT values through qPCR. For a detailed protocol of Singleplex qPCR for SARS-CoV-2 N1 and BRSV, refer to Svezia et al (2022).

Interpreting Results

The amount of SARS-CoV-2 viral RNA present in a sample is measured by the RT-qPCR cycle threshold (Ct) value. This is defined as the number of cycles required for the fluorescent signal to cross the threshold and are normally inversely proportional to the actual amount of RNA in the sample (eg. a low Ct value means there is more RNA present in the sample).

Generally speaking, the limit of detection is defined as the measured concentration of SARS-

CoV-2 RNA that produces at least 95% positive replicates, which is the standard cutoff (Forootan et al, 2017; Borchardt et al, 2021). This cutoff may vary between methodology and the selected sample. With Singleplex PCR, in the Environmental Microbiology laboratory a concentration of 10 SARS-CoV-2 N1 gene copies/ well could be detected 95% of the time (Svezia et al, 2022). This concentration has a Ct value of 35.6, and was defined as Strong Positive, any value > 35.6 and <40 was defined as a Weak Positive, and a Ct value of 40 and above was considered to be Negative or undetected.

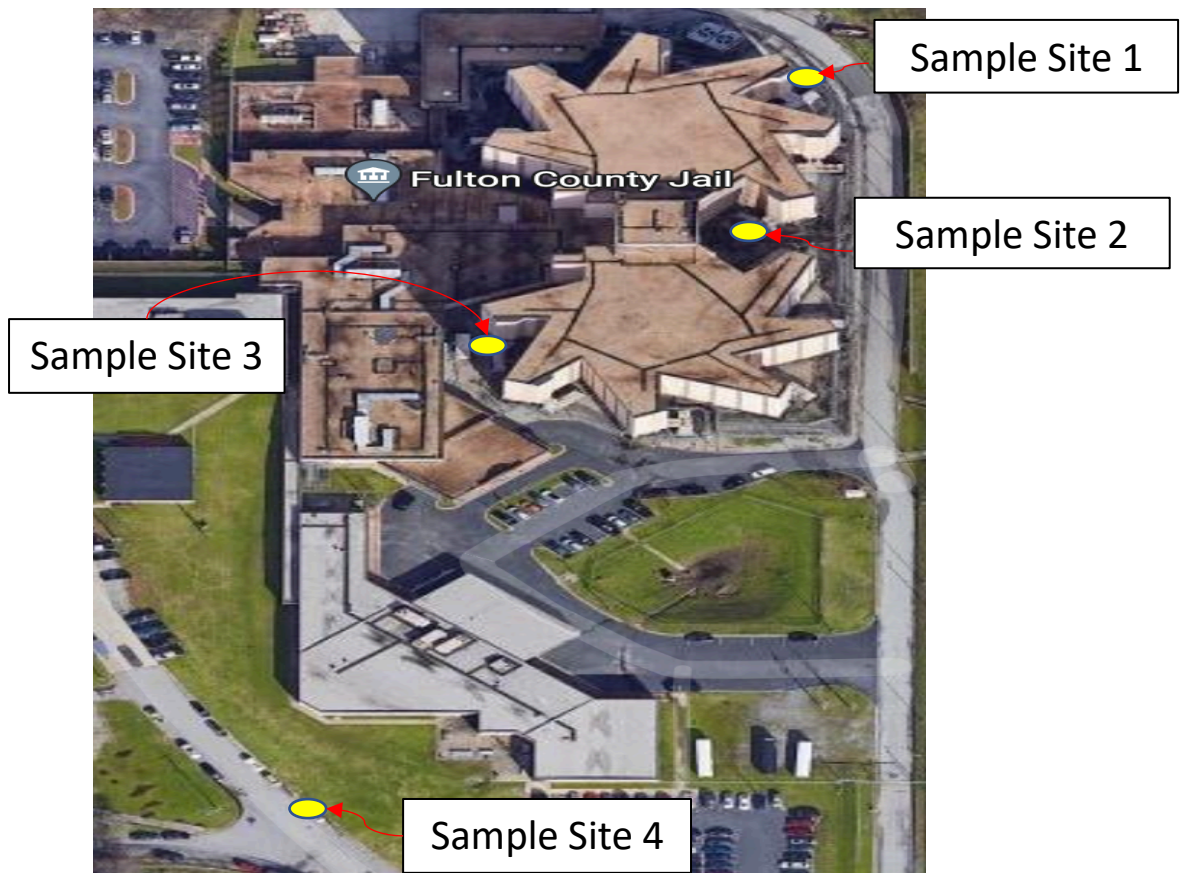


Figure 1. A map of the manhole locations on the Fulton County Jail property.

Data Analysis

All data was stored and managed in Microsoft Excel and all analysis was completed either through Microsoft Excel or R Studio. All r values are Pearson's correlation coefficients.

COVID-19 Diagnostic Testing Analysis

All the COVID-19 diagnostic testing data (both the PCR tests done as part of the study and the rapid tests that were administered by the jail) were aggregated into weekly COVID-19 prevalence estimates. Most variables used for analysis were derived directly from the data, but some involved additional sources or manipulation:

PCR Tests- There were three possible results from the PCR diagnostic test; the SARS-CoV-2 virus was either: detected, not detected, or the swab was invalid. The invalid outcome was reported when an individual provided a nasal swab sample, but there was not enough specimen on the swab to conduct an accurate PCR test. All conclusions were drawn from confirmed positive test results, rather than possible negatives, so invalid results were considered to be negative for analysis purposes.

Jail Population- The total resident population of the Fulton County Jail was determined through the resident count on weeks that the study team received rosters from jail personnel (point count of the jail). On the weeks that no rosters were received, we used public records from the Georgia Department of Community Affairs (DCA) (Georgia Department of Community Affairs, 2022) to estimate the jail population. The population data on the DCA website is reported by county, rather than jail. There is only one other county jail facility in Fulton County, a smaller women's jail, which was holding approximately 250 women each month, based on our sources. Therefore, to estimate the population at the Rice Street Facility when we did not have

a roster from the jail, we subtracted 250 from the reported average jail population for the month in which the majority of the week fell. The percent of the jail population that was tested was calculated using these numbers: total diagnostic tests results in a week divided by the jail population for the same week. COVID-19 prevalence was estimated as the total number of positive diagnostic tests for that week divided by the jail population for the same week.

Positivity Rate- The total number of diagnostic tests (PCR + rapid) that resulted positive in a week divided by the total number of tests administered in the same week.

Percent of the Jail Tested- The total number of diagnostic tests (PCR + rapid) administered in a week divided by the jail population in the same week.

Wastewater Data Analysis

Only Moore Swab data were used for WBS analysis because of the increased sensitivity and longitudinal nature of the results in comparison to grab samples, which are a purely cross-sectional sample. The Ct values of Moore swab samples were determined in the laboratory and were then categorized into signal strength categories (Negative, Weak Positive, Strong Positive), as explained previously. The *Saber Score* metric was created in order to standardize the categorical WBS results across all collection sites for a given day. The score was calculated from the results of all the samples collected on the same day, and only one sample result was used from each collection site². First the categorical data was converted into a signal strength rating for each individual sample (Negative= 0, Weak Positive= 1, Strong Positive= 2). All of these values were then summed together and divided by the number of collection sites, resulting in the Saber Score.

² Collection site may also be referred to as manhole

Results

The study period ran from June 15, 2021 to March 16, 2022 (39 weeks), during which time all diagnostic tests and WBS samples were collected and processed. There were 16 mass diagnostic PCR testing events in the jail which resulted in a total of 3,827 self- collected swabs that were tested by RT-PCR, and rapid COVID-19 diagnostic test results were collected every week (39 weeks), totaling 10,176 over the study period. The total number of diagnostic tests administered over the entire study period was 14,003 tests. When weather conditions permitted, wastewater was sampled on a weekly basis. The 28 collection days resulted in 110 unique specimens collected. Demographic information is presented in Table 1, a vast majority of residents being male and Black.

| <i>Demographic Summary for Residents at the Fulton County Jail</i> | |
|--|------------------------------|
| <i>June 15, 2021- March 16, 2022</i> | |
| Sex | Percent of Population |
| Male | 98.4 |
| Female | 1.6 |
| Race | |
| Black | 88.8 |
| White | 10.3 |
| Hispanic | <1% |
| Native Hawaiian | <1% |
| Asian | <1% |
| Middle Eastern | <1% |
| Multiracial | <1% |
| Other | <1% |
| Unavailable | <1% |

Table 1. Demographic summary for all residents in the Fulton County Jail from June 15, 2021 to March 16, 2022.

COVID-19 Diagnostic Testing

Diagnostic testing results from June 15, 2021 to March 16, 2022 are summarized in Table 2. The overall median number of diagnostic tests conducted each week was 346 tests, and the majority of these were the rapid tests that were administered by the jail health authorities. The median number of weekly rapid diagnostic tests was 306 compared to the number of PCR diagnostic tests that were administered during specific mass testing events by the research study (mass testing event median = 189). The total percentage of positive diagnostic tests peaked at 29.5%, with a mean of 4% (SD= 5.6%).

Figures 3 through 6 represent the number of weekly diagnostic tests administered and the percent of those that were positive over the study period. The total percent of the jail population that was tested fluctuated over time, but there was an overall positive trend; as the study went on, the total percent of the jail population who received diagnostic tests per sampling period generally increased (Figure 2). The total number of tests administered, seen in (Figure 3) was mostly comprised of the rapid tests, and the weeks with maximum diagnostic testing were those where both rapid and PCR tests were administered. While the rapid tests were collected by the jail consistently every weekday, the PCR tests were the result of 16 mass testing events between October 20, 2021 and March 16, 2022. Figures 1 and 2 demonstrate that between November 17, 2021 and January 5, 2022, the number of tests administered, and the percentage of the jail population tested peaked.

Figure 4 shows is the total number of COVID-19 diagnostic tests administered and indicates the number of positive tests. There were two peaks in the overall number of positive

tests³; one on the week of August 10, 2021 with a total of 25 positive tests and a second on the week of January 5, 2022, with a total of 135 positive tests. Figure 5 depicts the positivity rate⁴ of all diagnostic tests over the course of the study. The peaks in positivity rates were similar, but not identical, to those of the overall positive tests. There was a positivity rate peak on the week of August 17, 2021 with 19.7% of tests resulting in a positive outcome, and on the week of December 28, 2022 with 29.5% of all tests resulting in a positive outcome. Both of these diagnostic test positivity rate peaks are one week following the peaks in the overall number of positive diagnostic tests.

The weekly positivity rate moderately correlates with the total percentage of the jail tested ($r=0.43$). This relationship can be seen, for example, in the week of January 5, when the highest percentage of the jail population was tested (38.2%) (Figure 2). The previous week, December 28, 2022, yielded the highest percentage of positive diagnostic tests (29.5%) out of all diagnostic testing weeks (Figure 5).

Figure 6 describes the positivity rate for both types of tests administered⁵. Overall, the PCR tests consistently had a higher positivity rate than rapid tests per week. During the large midwinter surge, particularly on the week of December 28, 2021, there was a much higher proportion of positive PCR tests (63.5%) compared to the proportion of positive rapid tests (24.4%) performed during the same week. However, there was still a very strong correlation

³ Overall positive tests refer to the raw number of positive diagnostic tests resulted in a given week

⁴ The positivity rate is calculated by dividing the number of resulted positive tests by the total number of tests administered in a given week

⁵ The positivity rate for each type of test is the number of positive resulted tests for one method divided by the total number of administered tests for that same method

between PCR positivity and rapid test positivity results, on the weeks when they were both administered ($r= 0.91$).

| Descriptive Statistics for Diagnostic Tests | | | | | |
|---|--------------|----------|------------|---------------|----------------------------|
| Dates: 6/15/2021- 3/16/2022 | | | | | |
| COVID-19 Diagnostics Weekly | Mean (SD) † | Median † | Min, Max † | Missing (%) ‡ | Totals over entire study • |
| All Diagnostic Tests | 373 (224) | 346 | 17, 961 | 0 | 14,003 |
| Rapid Tests | 282 (121) | 306 | 17, 533 | 0 | 10,176 |
| PCR Tests | 232 (174) | 189 | 20, 591 | 74 (60.7%) | 3,827 |
| % of Jail Tested † | 0.63 (1.20) | 0.12 | 0, 5.36 | 0 | |
| Overall Test Positivity ‡ | 0.04 (0.056) | 2.2 | 0, 29.5 | 0 | |

- † Mean and SD of the indicated variable between weeks
- Median of the indicated variable between weeks
- † Minimum and maximum of the indicated variable between weeks
- ‡ Number of (and percent of total) weeks that the variable was not measured
- Total of variable over the entire study period
- † Numerator is the number of positive tests in a given week, denominator is the jail population for the week
- ‡ Numerator is the number of positive tests in a given week, denominator is the total tests for the same week

Table 2. Summary of all diagnostic testing results. All diagnostic test results from the same week were consolidated into one datapoint.

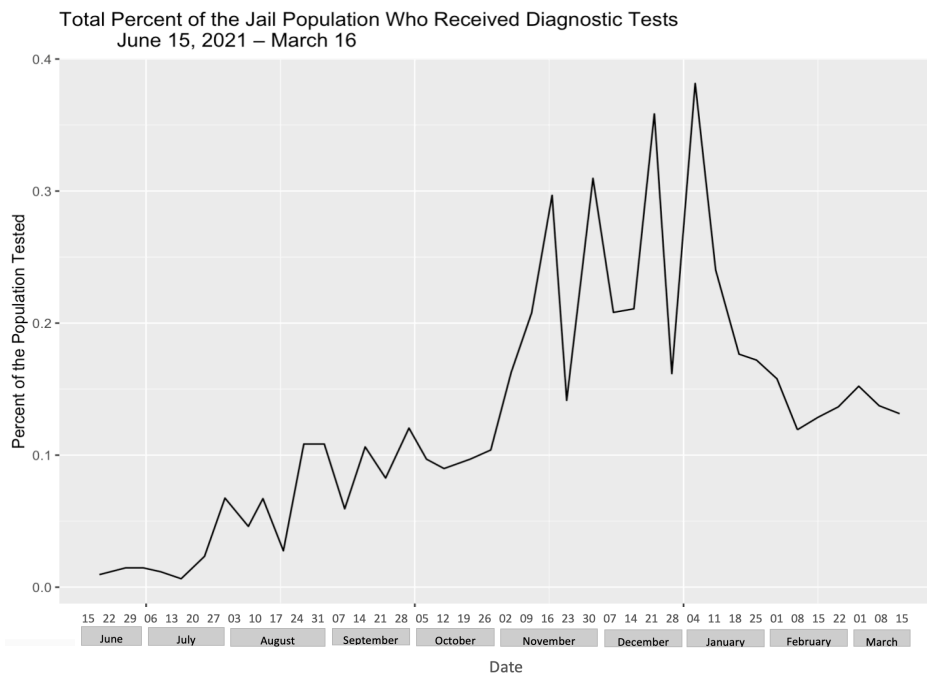


Figure 2. Total percentage of the jail residents who received a COVID-19 diagnostic test by week, June 15, 2021 – March 16, 2022.

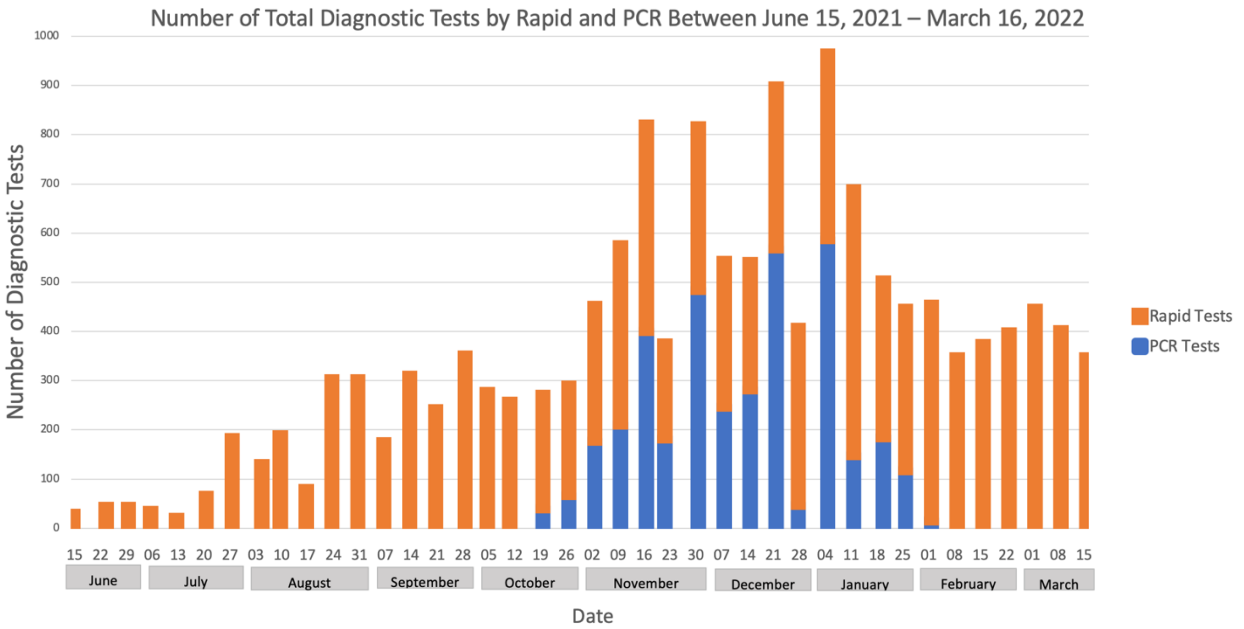


Figure 3. Total number of COVID diagnostic tests by week and type of test, June 15, 2021 – March 16, 2022.

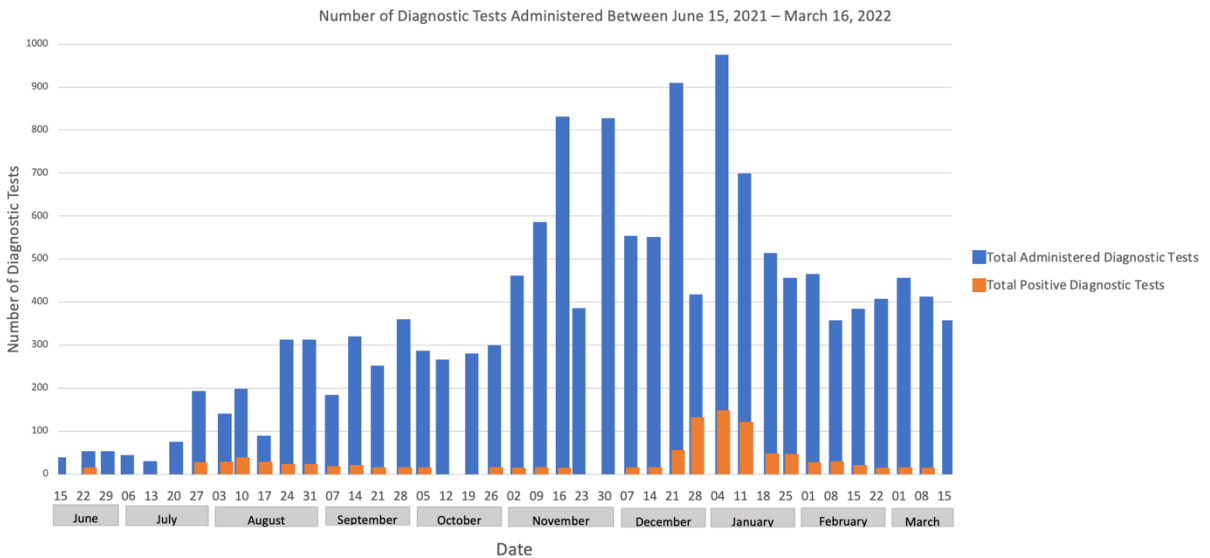


Figure 4. Total number of COVID-19 diagnostic tests of jail residents and total number of positive tests by week, June 15, 2021 – March 16, 2022.

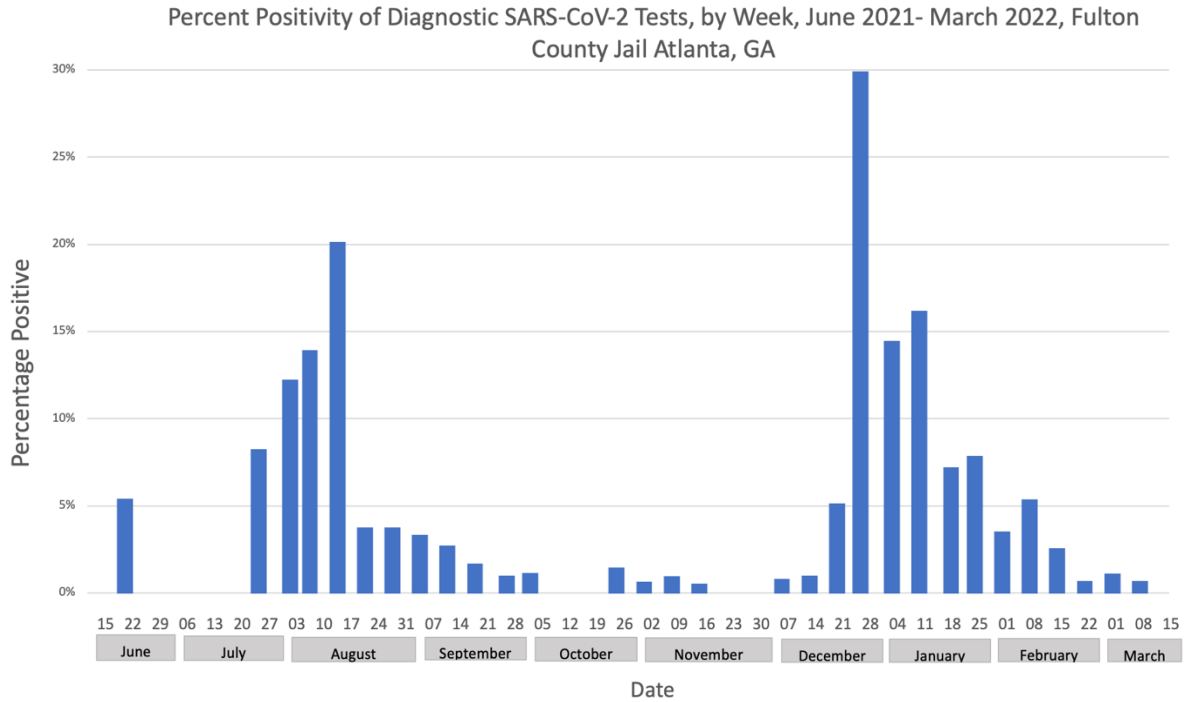


Figure 5. Of the tests administered in Figure 4, those that resulted as positive by week, June 15, 2021 – March 16, 2022.

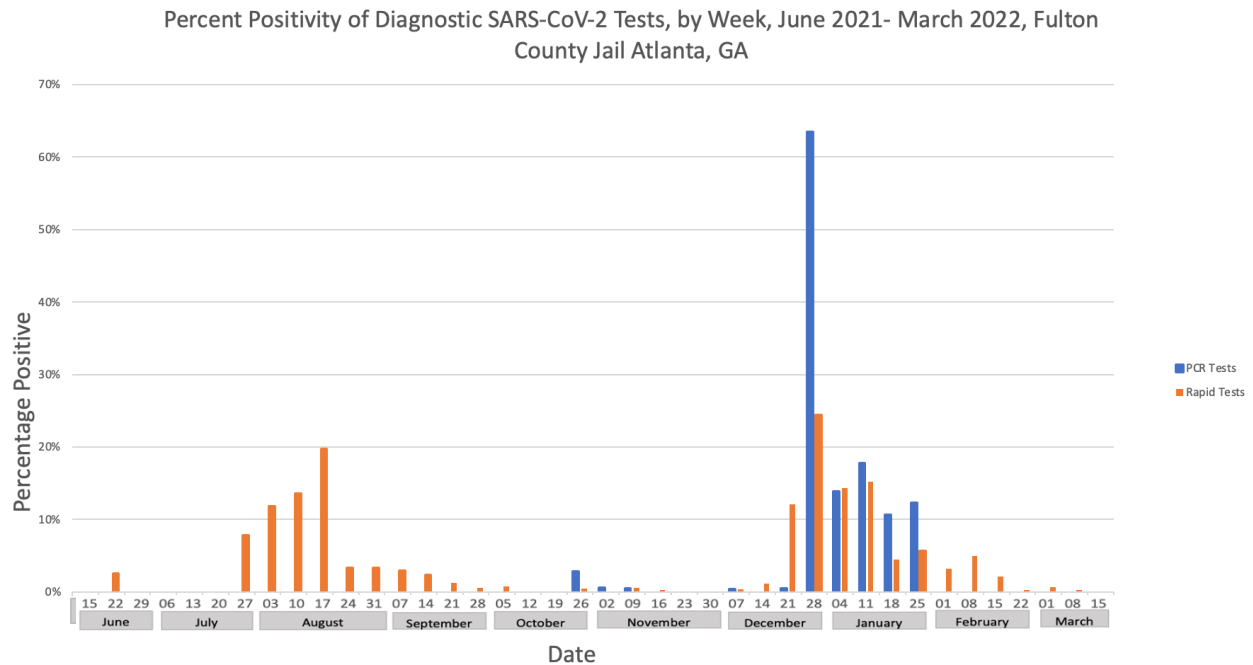


Figure 6. Of the tests administered in Figure 4, those that resulted as positive by week and type of test, June 15, 2021 – March 16, 2022.

Wastewater Testing Results

Wastewater results from 28 weeks between June 15, 2021 to March 16, 2022 are summarized in Table 3, stratified by the RT-PCR signal strength category (Negative, Weak Positive, Strong Positive). The mean and median of the RT-PCR Ct values for the Negative and Weak Negative categories are equal to each other (40 and 38, respectively), meaning that these two WBS categories have a normal distribution. The difference between the mean (31.88) and median (32.13) for the Strong Positive category is only 0.25, indicating that the Strong Positive category has a near normal distribution. There was a total of 13 weeks (33.3%) when all the wastewater samples collected were Strong Positives and 3 weeks (7.69%) when all wastewater samples collected were Negative. There were twelve weeks between June 15, 2021 and March 16, 2022, that wastewater testing was not conducted, due to inclement weather and holidays, totaling 9.8% of the time. The Ct values informed the signal strength categories, which in turn were used to calculate the Saber Score. Therefore, the correlation coefficients for all of these variables are very high.

The first evident peak in WBS positivity was between the dates of July 21, 2021-September 14, 2021, when the Saber Score was consistently 2 for all weeks (Figure 7). After this peak, there was a dip in the Saber Score for one week, but then it went back up to 2 until the week of October 20, 2021. Of major significance, there was a clear curve in WBS positivity starting on the week of December 1, 2021 and ending on the week of March 16, 2022. This curve starts from a Saber Score of 0, gradually rises to 2, plateaus for 5 weeks, then gradually dips back down to 0.25 on the week of March 16, 2022.

| Descriptive Statistics for Wastewater Samples | | | | | |
|---|------------|--------------|-----------|-----------------------|-------------|
| Dates: 6/15/2021- 3/16/2022 | | | | | |
| Wastewater n= 110 | n (%) | Mean Ct (SD) | Median Ct | Mean Saber Score (SD) | Missing (%) |
| Negative | 17 (13.9%) | 40(0) | 40 | 0(0) | 12 (9.8) |
| Weak Positive | 15 (12.3%) | 38.06(1.27) | 38 | 1.12(0.35) | 12 (9.8) |
| Strong positive | 78 (63.9%) | 31.88(2.68) | 32.13 | 1.85(0.30) | 12 (9.8) |

Table 3. Summary of all Wastewater sample results. Each signal strength category was analyzed individually.

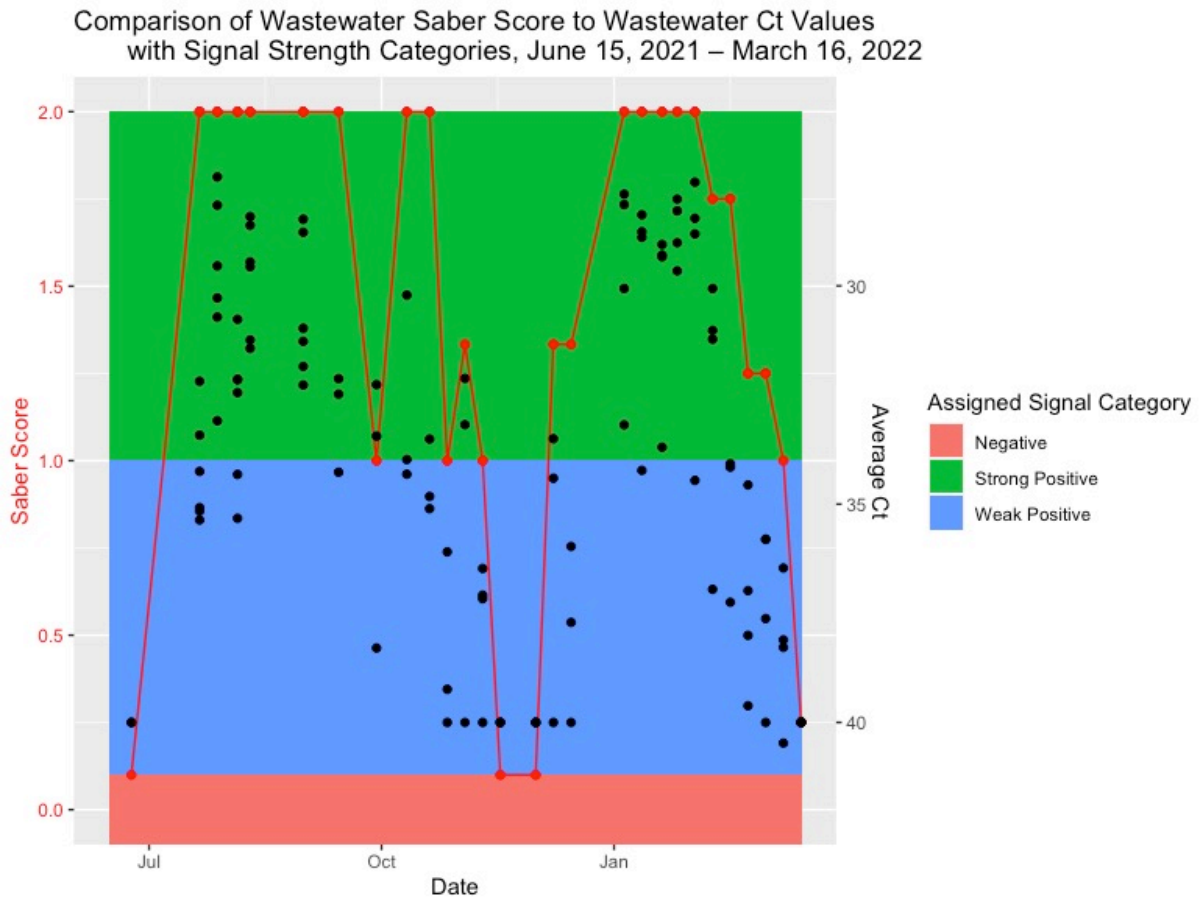


Figure 7. Ct values of all Moore swab samples between June 15, 2021 – March 16, 2022 (black dots, right Y-axis). The Saber score values, which were calculated from and representative of the Ct values, are in red (left Y-axis). The general thresholds assigned to the WBS signal strength categories are shown as background colors.

Relationship between COVID-19 Diagnostic Test Results and Wastewater Monitoring Results

The relationship between the diagnostic test results and WBS results were examined in several ways. It is evident that when the diagnostic test positivity rate was low for several weeks, (November 17, 2021- December 1, 2021) the Saber Score was also low – indicating that few of the wastewater samples were positive for SARS-CoV-2 RNA (Figure 8). A high Saber Score was observed when the diagnostic test positivity rate was high. For example, on January 5 and January 12, 2022 the diagnostic test positivity rates were 14% and 15.8%, respectively, and both dates had a Saber Score of 2. Overall, the total COVID-19 diagnostic test positivity rate had a strong correlation to the Saber Score ($r= 0.61$), and a moderate correlation to the total percent of the jail population that was tested ($r=0.43$) (Table 4).

Figure 9 represents the mean percent positivity of diagnostic tests for each of the WBS outcome categories. For all weeks that the WBS samples resulted in a Negative reading (no detection of SARS-CoV-2 RNA), the mean positivity rate for COVID-19 diagnostic tests taken in the same weeks was 0.66%, and for a WBS reading of Weak Positive (low concentration of SARS-CoV-2 RNA in wastewater sample) the mean diagnostic positivity rate was 0.81%. There was a strong increase in the mean diagnostic test positivity rate (5.67%) during the weeks when the wastewater samples had Strong Positive results. There was a moderate correlation between the weekly WBS signal strength results and the weekly total percent diagnostic test positivity, when both WBS and diagnostic test results were available ($r= 0.48$) (Table 4).

Because of the correlation between the RT-PCR Ct values for the wastewater samples and overall diagnostic positivity rate combined across time ($r=-0.68$), we performed a linear regression analysis matched by week (Figure 10). While there are some outliers, there was an

overall negative relationship between the two variables (slope= -0.0079), which is to be expected since a lower RT-PCR Ct value indicates a higher concentration of SARS-CoV-2 in the wastewater sample. However, the correlation resulted from the linear regression was very strong ($r^2=0.394$, $r= 0.628$).

The correlation between the wastewater Saber Score and the diagnostic test positivity rate was examined at different time intervals (weeks) between the wastewater sample collection and the COVID-19 diagnostic test result (Table 5). This analysis was focused on offsetting the Saber Score by one or two weeks, either ahead or behind the week when the tests were performed. While all the correlation coefficients in this analysis were very similar, the strongest coefficient was with the results from wastewater samples were collected and during the same week as the COVID-19 diagnostic tests were conducted (Saber Score displacement = 0 weeks, $r= 0.473$), meaning that the COVID-19 diagnostic test positivity rate is best predicted by the WBS samples from the same week. The next strongest correlation was between the wastewater sample results one week before the COVID-19 diagnostic test results (Saber Score displacement = -1 week, $r^2= 0.472$).

| Table of Correlation Coefficients | | | | |
|--|-----------------------------|-------------------------------|--|--------------------------------------|
| Wastewater | Ct Values | Signal Strength ⁶ | Saber Score | |
| Ct Values | 1.00 | -0.81 | -0.81 | |
| Signal Strength | | 1.00 | 0.80 | |
| Saber Score | | | 1.00 | |
| Diagnostics | % Positive PCR ⁷ | % Positive Rapid ⁸ | Total % Positive of all Tests ⁹ | Total % of Jail Tested ¹⁰ |
| % Positive PCR | 1.00 | 0.91 | 0.96 | 0.31 |
| % Positive Rapid | | 1.00 | 0.99 | 0.49 |
| Total % Positive of all Tests | | | 1.00 | 0.43 |
| Total % of Jail Tested | | | | 1.00 |
| Combined | % Positive PCR | % Positive Rapid | Total % Positive of all Tests | Total % of Jail Tested |
| Ct Values | -0.66 | -0.63 | -0.68 | 0.03 |
| Signal Strength | 0.46 | 0.45 | 0.48 | -0.22 |
| Saber Score | 0.59 | 0.57 | 0.61 | -0.26 |

⁶ The WBS category indicated from Ct Value: Negative, Weak Positive, or Strong Positive

⁷ Number of positive PCR tests in one week over the total number of PCR tests administered for the same week

⁸ Number of positive rapid tests in one week over the total number of rapid tests administered for the same week

⁹ Total number of positive diagnostic tests (PCR + rapid) in one week over the total number of diagnostic tests administered for the same week

¹⁰ Total number of tests administered in one week over the total population of the jail for the same week

Table 4. Table of Pearson R correlation coefficients for WBS and diagnostic variables compared within their variable groupings and then between variable groupings. Each datapoint is correlated with all other datapoints, none are grouped based on date or other variables.

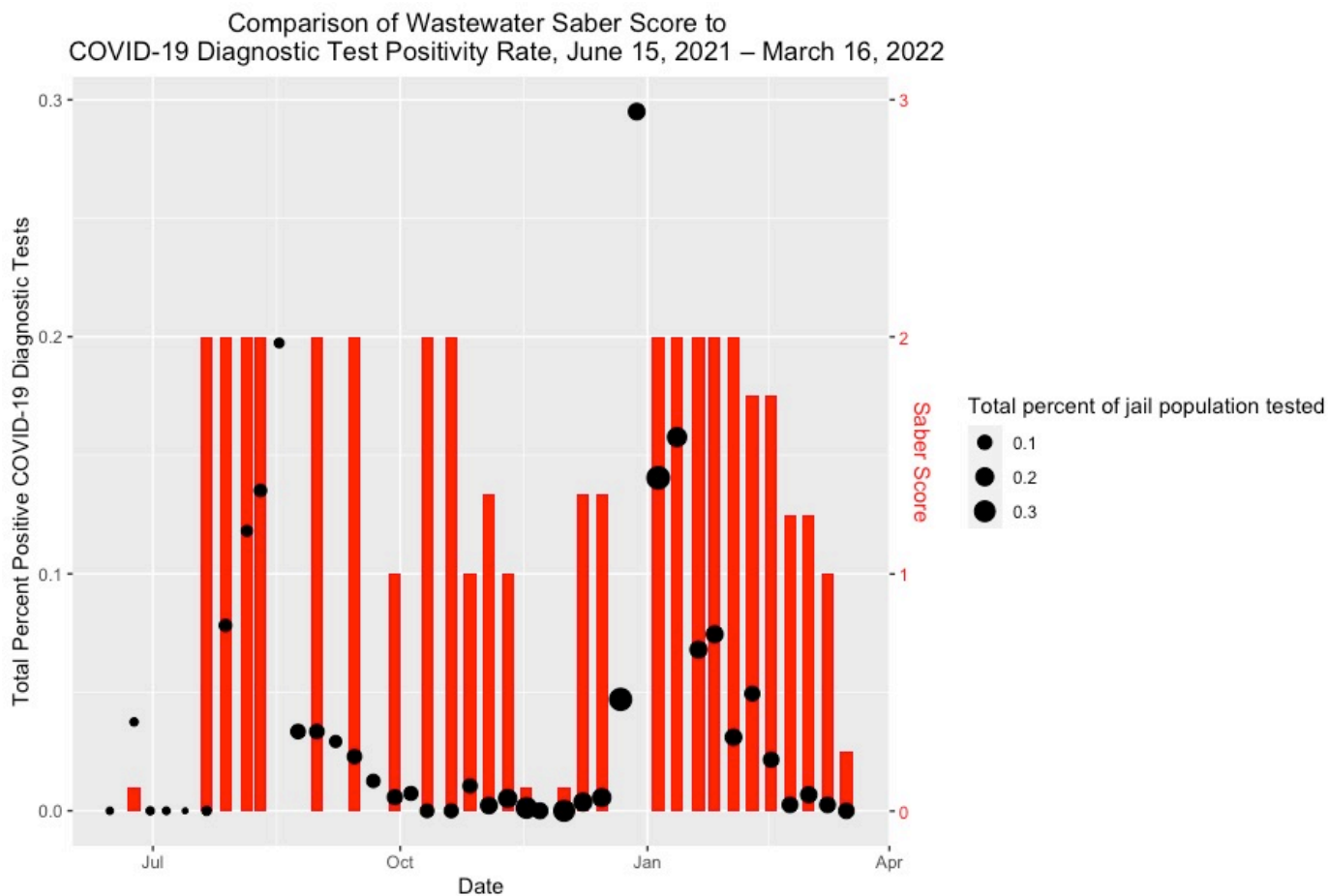


Figure 8. Saber Score (right Y-axis, red bars) and total percentage of positive COVID-19 diagnostic tests (left axis, black circles), with circle size reflecting the total percentage of the jail population that was tested for COVID-19, weekly between June 15, 2021, and March 16, 2022.

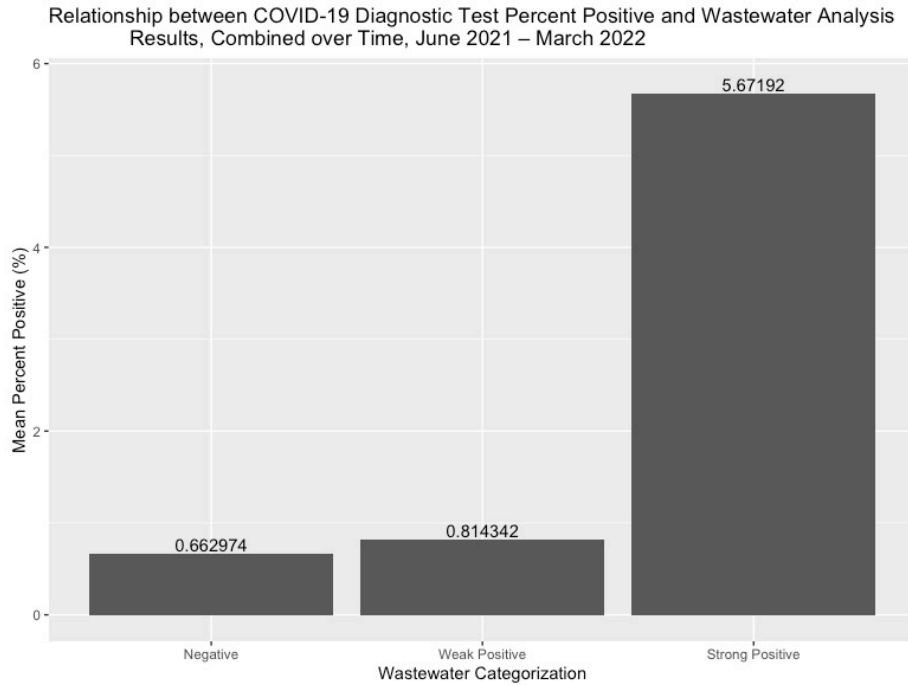
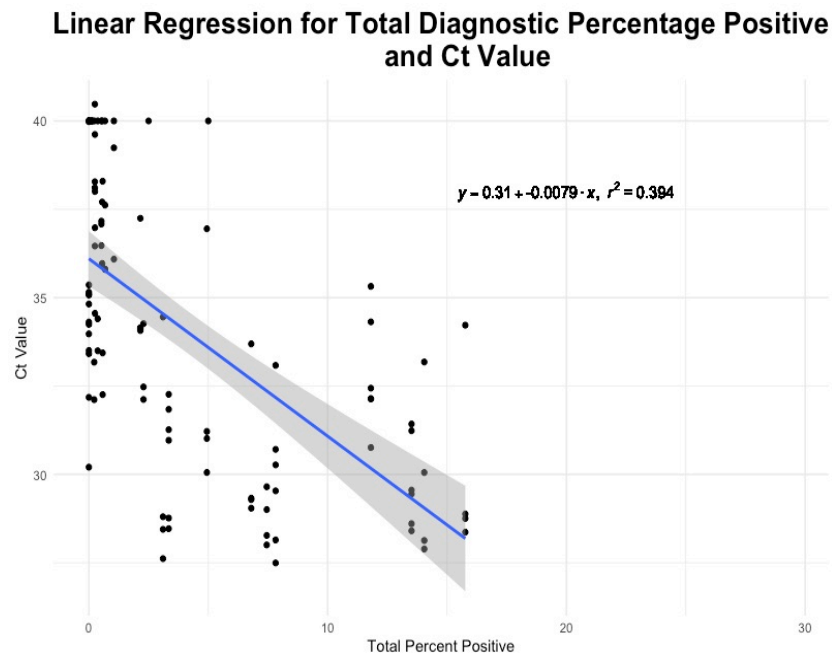


Figure 9. The mean percent positivity of all COVID-19 diagnostic tests by WBS signal strength category, between June 2021- March 2022.



*The mean percent positivity for each WBS signal strength category indicated at the top of each bar

Figure 10. Linear regression between the total percent positivity of the COVID-19 diagnostic tests and the wastewater RT-PCR Ct Values.

| Saber Score and Percent Positive correlation over time | | | | | |
|--|-------|-------|-------|-------|-------|
| Week Displacement ¹¹ | -2 | -1 | 0 | +1 | +2 |
| Correlation Coefficient ¹² | 0.458 | 0.472 | 0.473 | 0.461 | 0.459 |

¹¹ The Saber Score is the variable to shift in time (lags) while the percent positive stays constant.

¹² The correlation between these two continuous variables were analyzed with Pearson's R correlation coefficient.

Table 5. Correlation coefficients between Saber Score and percent positivity of COVID-19 diagnostic tests with time lags, offsetting the percent positivity.

Discussion

The main objective of this study was to gather evidence that WBS could represent a practical strategy to surveil for new COVID-19 outbreaks in a jail setting. We also determined the feasibility to conduct both COVID-19 diagnostic and WBS testing in a jail setting. There were two components in this study: the COVID-19 diagnostic testing aimed to test a large portion of the jail every week; while the WBS component aimed to examine wastewater samples from several collection points on the jail grounds for SARS-CoV-2 RNA. These two sets of results were first analyzed separately to examine temporal trends. We then compared the trends in time-matched results from the COVID-19 diagnostic tests and WBS. Overall, we found that our data supports the claim that WBS was a sensitive signal of when COVID-19 cases were present in the jail population.

Mass Diagnostic Testing in a Jail

One objective of this project was to determine the feasibility of conducting large scale diagnostic COVID-19 testing in a jail setting on a regular basis. Mass testing has been shown to be a practical method for surveillance outside of correctional facilities. We demonstrated that in a facility where residents are held in single or double cells, limited throughput in the testing process made mass screening challenging. Despite difficulties with testing logistics, over the fall of 2021, we saw an upward trend in the portion of the jail population that participated in the mass testing events (Figure 2 and Figure 4). This occurred as our routine for testing became more refined, the security personnel gained familiarity with our protocol, and we gained a better understanding of the jail culture and environment.

While our team had champions among the jail administrators, it was still extremely difficult to assemble both a full team of testing personnel and the same sized team of security escorts. The dynamic and unpredictable environment of a jail, which prevents prediction of how many security personnel will be available for non-emergent screening on a given day, made it difficult to plan ahead to ensure sufficient staffing.

To register the bar codes on the nasal swab samples into a cloud-based database, we needed a direct hook up via an ethernet cable to the internet. Cell phones and Wi-Fi were not available in the jail; ethernet outlets were often missing. When this occurred, the nasal swab samples had to be transferred to another floor scan the barcodes into the system. Additionally, the testing teams were not able to freely move around the jail without security. As non-jail personnel, the testing team needed two officers present with them at all times. The mechanics of collecting and registering the nasal swab samples and communicating with other testers was challenging. All of these aspects make mass COVID-19 diagnostic testing in a jail very different from other settings.

Previous studies have conducted mass diagnostic testing, but they were either one-time events, focused on testing officers, or the methods for their longitudinal testing of residents were not detailed in the publication. Hagan et al. (2021) and Tompkins et.al Tompkins et al. (2021) both report on conducting mass COVID-19 diagnostic testing in jail settings. Hagan et al. (2021) revealed that after their cross-sectional testing, the known COVID-19 cases increased by over twelvefold. Tompkins et al. (2021) used similar methods and emphasized the value of a mass testing event for the detection of asymptomatic cases compared to symptom- based testing. While the cross-sectional testing methodology is useful to determine the prevalence of

COVID-19 at a specific time, it does not allow us to understand the trends in positivity rate over time. As Thompkins et.al also emphasized, our mass testing events identified several asymptomatic cases that may not have otherwise been detected.

While the median number of administered rapid and PCR tests differed greatly (117), the correlation between the positivity rate of the two different tests was extremely high ($r=0.91$). The high correlation suggests relatively accurate results from between both forms of diagnostic tests. This leads us to believe that during the weeks when there was an imbalance in PCR and rapid tests administered, we can assume that the positivity results would have been similar regardless of the testing method that was used.

There were two defined peaks of diagnostic test positivity over the duration of the study on August 17, 2021 and December 28, 2021 (Figure 5). The first peak occurred before PCR testing began in the jail, therefore all COVID-19 cases were detected using the rapid tests administered the jail health authorities. The August 17, 2021 peak (positivity rate=19.7%) precedes the COVID-19 peak in Fulton County by 17 days, which peaked on September 3, 2021 with an 493 average cases per day (Times, 2022). This finding is consistent with the report from Reinhart et al. (2020) that suggested that jail- community cycling is responsible for up to 55% of variance in COVID-19 case rates at a zip code level and that cycling jail residents through the correctional system may influence the community COVID-19 case rates. However, Wallace et al. (2021) observed a correlation between COVID-19 prevalence among the corrections staff and the prevalence of COVID-19 in jails and concluded that when the prevalence amongst staff was low, the prevalence within the jail mirrors that of the community. Unfortunately, this study did not collect COVID-19 prevalence data for the corrections staff and is not able to determine if

the August 17, 2021 peak was associated with an outbreak amongst staff. The Delta variant was at its peak in Georgia in August and September of 2021 (Times, 2022). While we do not have sequencing data from the COVID-19 tests administered at the jail, we hypothesize that the spike in COVID-19 cases in the jail around August 17, 2021 was due to the Delta variant.

The second peak, during the week of December 28, 2021 was the largest that we saw in the entire study period with a positivity rate of 29.5%. This spike in COVID-19 cases in the jail once again preceded the peak in reported COVID-19 cases in Fulton County of 2,253 average daily cases on January 5, 2022 and aligned with COVID-19 case surges nationwide due to the Omicron variant (Times, 2022). However, the lag from this peak in the jail to the community's peak in January was much shorter: just 8 days. According to Jansen et al. (2021), the incubation period for the Delta variant was about 4 days, while the incubation period for the Omicron variant was just 3 days. This variation may have contributed to the difference in time between jail and community peaks, but more data is needed to confirm this hypothesis.

WBS Testing in a Jail

The second objective of this study was to examine the efficiency of and ability to conduct WBS for SARS-CoV-2 in a jail setting. Many studies have examined the efficiency and effectiveness of WBS testing at an institutional level, but none to our knowledge have attempted WBS at a correctional facility. Of note, the institutions that have been studied in the past include campus dormitories, where turnover rate is low and students normally spend an entire semester, on average 105 days, living in the same room (Betancourt et al., 2021; Gibas et al., 2021; Karthikeyan et al., 2021; Liu et al., 2022; Moody, 2021; Wang et al., 2022). However, the average stay for someone charged with a misdemeanor in the Fulton County jail is much

shorter than that of a college dormitory and is reported to be 9.4 days in October of 2020 (Williams & F.C. Sheriff, 2020). This drastic difference between stay times may account for some variability in the WBS conclusions drawn from campus dormitory studies and this jail-based study.

The collection and processing of Moore Swab WBS samples at the jail was found to be much less expensive and faster than individual diagnostic testing of the jail populations, which aligns with the reports from the previous studies of campus residence halls (Liu et al., 2022). Previously reported from a WBS study in a dormitory, our lab was able to create ten Moore Swab samples for about \$12, with sample collection time averaging 30 minutes, a total processing time of 1.5 hours, and an overall turnaround time from sample collection to final lab results of being between 2 to 3 days for an on-campus dormitory experiment (Liu et al., 2022; Wang et al., 2022). In this jail study, the efficiency of the Moore Swab collection was less because of additional complications associated with getting access for sample collection at a jail. In total, for both Moore Swab setting and Moore Swab collection, the total duration for the sampling team was 2.5 hours +/- 0.5 hr. At times, the maintenance personnel were not available to escort the sampling team to the sample collection site upon arrival, which added up to 0.5 hours. However, adding the 4th sampling site to the study only added about 15 minutes to the overall procedure, indicating that gathering all the necessary personnel is the most time-consuming step. Laboratory processing times did not differ from the campus study reported by Liu et al. (2022).

Since the precise origin of the wastewater in the jail was unclear, and we could not find any records to provide further information, we created the Saber Score system to assign a value

of the aggregate WBS strength for all sites on a given day. To the author's knowledge, there have been no previous studies where researchers have combined WBS results from multiple collection sites. It is possible that other studies have known the exact origin of their wastewater, or the lack of this knowledge did not affect their analysis. A dye study is currently exploring the origin of the wastewater at different points in the jail facility in hopes of mapping out exactly which areas of the jail each manhole serves.

Using WBS to predict individual diagnostic cases

The primary objective of this study was to review the evidence that WBS will be an effective way to monitor for new COVID-19 outbreaks. This aim was accomplished by comparing the data from the first two objectives mentioned earlier. The Saber Score method is a novel approach to aggregate categorical RT-PCR outcomes from multiple WBS sites and comparing the Saber Score to the COVID-19 diagnostic test positivity rates is an innovative analysis strategy that has not been previously described.

Overall, we conclude that the COVID-19 diagnostic test positivity rate was strongly correlated with the concentration of SARS-CoV-2 RNA in the wastewater (Saber Score; $r=0.61$). While our analytical approach is unique, other studies have compared Ct (also notated as Cq) values and COVID-19 case counts at an institutional level and reported an association (Betancourt et al., 2021; Gibas et al., 2021; Karthikeyan et al., 2021; Liu et al., 2022; Wang et al., 2022). The analysis that we completed with RT-PCR Ct values showed very strong correlation with overall jail COVID-19 diagnostic test positivity rate (Figure 10). These studies also varied in their sampling methodology; some collected only grab samples, others combined swab and grab samples, and one study used autosamplers which collected composite wastewater

samples over time intervals. All of these studies, however, knew the exact origin from which their wastewater was flowing from. With this information, previous studies have been able to relate COVID-19 cases or diagnostic results in known population with specific WBS results. Since the plumbing infrastructure at the jail was not well characterized, we needed to compare the diagnostic test positivity rate for the jail population that was tested to an aggregate score for the jail's WBS results.

Nonetheless, our conclusions are not dissimilar from those in other studies. Our findings are consistent with those that have reported similar temporal trends in WBS results and COVID-19 case counts at an institutional level (Betancourt et al., 2021; Gonzalez et al., 2020; Karthikeyan et al., 2021; Wang et al., 2022). The Saber Score values were very consistent with the COVID-19 diagnostic test positivity rates (Figure 8). This is especially evident during the study period from December 28, 2021 to March 16, 2022. During the last week of December 2021, the COVID-19 diagnostic tests had a peak positivity rate, and as the COVID-19 test positive rate declined over time, so did the Saber Score. With current methods, WBS results cannot be utilized to directly estimate the number of COVID-19 cases in the jail due to unknown variance in the magnitude and duration of SARS-CoV-2 fecal shedding and unknown dilution from other wastewater (Wang et al., 2022). However, the positive correlation between the Saber Score and COVID-19 diagnostic test positivity rate suggests that WBS is an effective method to survey for SARS-CoV-2 in a jail setting.

Once we determined the association between the COVID-19 diagnostic test positivity rate and the Saber Score, we then analyzed the temporal relationship between the two variables. While the strongest correlation between the COVID-19 diagnostic test positivity rate

and Saber Score was between time-matched weeks ($r=0.473$), when the Saber Score results were offset by one week ahead ($r=0.461$) and behind ($r=0.472$) the positivity rate results, the correlations did not change much. This could be due to the fact that the COVID-19 cases may continue to shed virus in their fecal matter for up to 50 days after diagnosis (Park et al., 2021). Comparing one and two-week offsets may also be too large of a time gap between COVID-19 diagnostic positivity rates because of the dynamic nature of the population in the jail. It may be better to examine shorter offset periods of days rather than weeks.

Strengths

Our study had several strengths. This was a novel study conducted in a new setting: a correctional facility. Our study team had a good relationship with jail administration and frequent communication with the health authorities in the jail. The PCR diagnostic tests were one of the best techniques available and produced highly accurate results. While we only report the results from the Moore Swab samples, grab samples were also collected and processed in the laboratory using quantitative methods to estimate SARS-CoV-2 RNA concentration in the wastewater. WBS was conducted at every known manhole on the jail property to ensure wastewater samples from every part of the facility. The new Saber Score method allowed us to analyze all the WBS results collectively.

Limitations

The current study had several limitations. It was not possible to collect wastewater samples and PCR tests every week given a shortage in staff, weather patterns, and other difficulties. The diagnostic testing team did not have the capacity to regularly test the entire jail population, therefore, we are not certain how the weekly positivity rates observed in this study

can be generalized to the entire jail population. Diagnostic testing was not always conducted on a random basis. There were some weeks where PCR testing was targeted to certain areas in the jail, and rapid tests were mostly administered to new residents at intake rather than the general jail population. During mass PCR testing events, there were several steps that could result in human error, including matching the nasal swab samples in the collection racks with correct individual on the written roster, and possible data entry errors when scanning the barcodes for the samples into the Northwell website portal.

Furthermore, the precise origin of the wastewater in the jail is unclear. With an accurate sewerage map, we could possibly conduct further analysis of the COVID-19 diagnostic positivity rates in specific locations in the jail and examine spatial spread of COVID-19 based on building-specific wastewater samples. Given the nature of a jail, there are many people entering and leaving on a weekly basis. Therefore, it is not a closed system, and someone who sheds fecal matter containing SARS-CoV-2 on one day may be gone from the jail by the time a COVID-19 diagnostic test would be administered. Lastly, one major caveat to our analysis was that diagnostic testing was calculated on Sunday to Saturday, whereas wastewater testing was collected mostly on Wednesdays. We may have seen more of a temporal relationship with different timing of the wastewater sample collection.

Conclusion

Even with great effort, relationships, and resources, administering COVID-19 diagnostic tests to the entire Fulton County Jail on a weekly basis is not a feasible COVID-19 surveillance strategy. However, conducting WBS at the jail level was highly successful, and the WBS results aligned with the diagnostic test positivity rates. With our temporally matched wastewater and diagnostic data, we conclude that WBS could be an effective surveillance or monitoring tool for COVID- 19 in a correctional facility.

Chapter 4: Recommendations

Below are public health implications from this study, and recommendations for future research, insights into successes and challenges from our experience, and lessons learned:

COVID-19 Diagnostic Testing in a Jail

- It is essential to have good relationships with jail personnel—though our team had champions among the jail administrators, it was still extremely difficult to assemble a full team of testing personnel and security escorts
- Correctional settings are unpredictable, and plans change very quickly; flexibility is necessary.
- With Wi-Fi and cellphone access limited in a jail, web-based data entry systems are difficult to utilize

Wastewater-Based Surveillance in a Jail

- Sample collection can be more time consuming than other institutional- level WBS because extra security measures are in place in a jail.
- Understanding the sewerage system within the jail is essential for determining the origin of the wastewater. We suggest that if there is no map of sewerage system, it is useful to conduct dye tests to determine the origin of the wastewater. Dye testing helps to determine which manholes provide access to wastewater from specific areas in the jail.
- Utilize WBS in jails to inform COVID-19 diagnostic testing as a low- cost option

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