Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i8.7340

# WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN A HEALTHCARE COMPLEX: A CROSS-SECTIONAL STUDY AT UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA

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**ABSTRACT**

**BACKGROUND:** The COVID-19 pandemic, driven by SARS-CoV-2, has posed significant global health challenges since late 2019. Efficient monitoring of the virus's spread is crucial for controlling outbreaks and implementing effective public health measures. Wastewater-based epidemiology (WBE) has emerged as a promising method for surveillance by detecting viral RNA in sewage, indicating community-level infection rates. This study aims to evaluate the presence of SARS-CoV-2 RNA in wastewater samples from various residential buildings within the University College Hospital (UCH) complex in Ibadan, Nigeria.

**METHODS:** Conducted as a cross-sectional study, wastewater samples were collected from residential buildings housing medical staff and students within the UCH complex. A total of 180 untreated wastewater samples were systematically collected and analyzed using reverse transcription-polymerase chain reaction (RT-PCR) to detect SARS-CoV-2 RNA. Factors such as building type, population density, and proximity to medical facilities were examined for their influence on the presence of viral RNA.

**RESULT:** Among the 180 wastewater samples analyzed, 3 (1.9%) tested positive for SARS-CoV-2 RNA. Most samples (98.1%) did not contain detectable viral RNA. A preliminary pilot study demonstrated the methodology's capability to detect the virus in wastewater, irrespective of the building type. The distribution of positive samples suggests the potential of WBE for early detection and monitoring of COVID-19 outbreaks within densely populated healthcare settings.

**CONCLUSION:** The findings underscore the feasibility of employing WBE as a robust surveillance tool for tracking SARS-CoV-2 in complex urban environments like UCH. The low prevalence of detectable viral RNA highlights the effectiveness of current public health measures and the importance of continuous monitoring. This study contributes valuable insights into the dynamics of viral shedding and transmission, supporting the implementation of WBE in other healthcare and urban settings for pandemic surveillance and response.

**KEYWORDS:** COVID-19, SARS-CoV-2, wastewater-based epidemiology, surveillance, public health, RT-PCR, University College Hospital, Ibadan, Nigeria.

## INTRODUCTION

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to unprecedented global health challenges since its emergence in late 2019. As the virus rapidly spread across the world, it brought about significant morbidity and mortality, stressing healthcare systems and prompting widespread public health interventions [1]. Monitoring the spread of the virus is crucial for controlling outbreaks and implementing effective public health measures. One emerging method for surveillance is wastewater-based epidemiology (WBE), which involves analyzing sewage for the presence of viral RNA as an indicator of community-level infection rates [2].The University College Hospital (UCH) in Ibadan, Nigeria, represents a microcosm of a larger urban environment, hosting a diverse population that includes medical staff, students, and other employees. The UCH complex encompasses not only medical facilities but also various residential and commercial units, making it an ideal study area for wastewater surveillance. Given its size and diversity, UCH provides a unique opportunity to assess the feasibility and effectiveness of WBE for tracking SARS-CoV-2 in a densely populated healthcare setting. This study aims to evaluate the presence of SARS-CoV-2 RNA in wastewater samples collected from various residential buildings within the UCH complex. By doing so, it seeks to establish the utility of WBE as a tool for early detection and monitoring of COVID-19 outbreaks. Understanding the spatial distribution of viral RNA within the hospital complex can provide valuable insights into infection patterns and inform targeted public health interventions. The research is designed as a cross-sectional study involving the collection and analysis of untreated wastewater from different buildings within UCH. The study focuses on residential blocks housing medical staff and students, as these populations are directly involved in healthcare delivery and are at higher risk of exposure to SARS-CoV-2. By integrating environmental sampling with molecular diagnostic techniques, this research aims to contribute to the growing body of evidence supporting the use of WBE in pandemic surveillance and response. In addition to detecting SARS-CoV-2 RNA, this study will also examine various factors that may influence viral presence in wastewater, such as building type, population density, and proximity to medical facilities. These factors are crucial for understanding the dynamics of viral shedding and transmission within a complex urban setting. The findings of this research will not only enhance our knowledge of WBE but also provide practical guidance for implementing similar surveillance systems in other healthcare and urban environments. In conclusion, this study addresses a critical need for innovative surveillance methods in the fight against COVID-19. By leveraging the unique characteristics of the UCH complex and employing rigorous scientific methodologies, it aims to demonstrate the potential of wastewater analysis as a reliable and scalable tool for monitoring viral outbreaks and protecting public health.

## METHODOLOGY

### 3.1 STUDY AREA

The study was conducted using samples obtained from blocks and buildings housing medical staff and students in The University College Hospital (UCH). The hospital complex lies between 7.4019° N and 3.9021° E in the largest city in sub-Saharan Africa, Ibadan, Oyo State, Nigeria. The University College Hospital (UCH) is a sizeable heterogeneous community with various independently functional units and corporate organizations such as guest houses, primary schools, churches, mosques, banks, a fuel station, a supermarket, residential blocks, and halls of residences aside from the hospital unit and its extensions. The hospital unit has a capacity of 1229 bed spaces and 163 examination couches. Most of the people living in the various residential buildings within The University College Hospital Complex are medical staff, medical students, or individuals who are employed at the hospital.

## 3.2 STUDY DESIGN

This is a cross-sectional study that involves the collection of wastewaters from the UCH environment.

## 3.3 STUDY POPULATION

The study population comprised the residential blocks and buildings housing medical students and staff within the University College Hospital complex, Ibadan, Oyo State.

## 3.4 SAMPLE SIZE DETEMINATION

In untreated hospital wastewater samples, Gonçalves et al. (2021) reported 13.4% positive samples without any concentration method (with a 5% level of significance). Adopting the reported values by Gonçalves et al. (2021) as the hypothesized prevalent rate for this study, the sample size was calculated as a total of 180 wastewater samples which was collected for this study [3].

## 3.5 SAMPLING TECHNIQUE

A simple random sampling technique was employed in the selection of buildings.

## 3.6 INCLUSION CRITERIA

Blocks and buildings within the University Hospital complex housing medical students or staff.

## 3.7 EXCLUSION CRITERIA

a) Blocks and buildings that don’t have a drainage system.
b) Blocks and buildings that haven’t been inhabited in at least two weeks.
c) Blocks and buildings that don’t house medical students or staff.
d) Blocks and buildings where residents don’t consent to the collection of samples.

## 3.8 DATA COLLECTION PROCEDURE

An informed consent form was signed by the individual in charge of the building where samples were collected, and a specimen investigation form developed by the investigator was used to get information about the nature of the sample.

## 3.9 SAMPLE COLLECTION AND TRANSPORTATION

Effluent untreated grab wastewater samples (50 mL/sample) were collected from the drain or water cistern of buildings included in the study. The samples were labelled serially with a study number, date, and time of collection. They were transported on ice within 6 hours of collection to the biorepository laboratory for processing. Samples were stored at -20°C until processed.

## 3.10 MATERIALS AND REAGENTS FOR SAMPLE COLLECTION AND RT-PCR

Tube rack, 50 mL Falcon tubes, Nitrile gloves, clean plastic containers, Syringe, CFX96 Real-time PCR machine, RNA extraction kit, Master mix (containing DNA polymerase, dNTP, probes and primers of the target gene and internal reference), Biosafety cabinet, Vortex mixer, Microcentrifuge machine, and Elution buffer

## 3.11 LABORATORY PROCEDURE

### 3.11.1 SAMPLE COLLECTION

A total of 180 grab samples of untreated wastewater (50 mL/sample) were collected in Falcon tubes from the wastewater drainage/sewage management system associated with each building and transported on ice in a Giostyle box to the laboratory. Samples were stored at -20°C until further procedures were carried out [4].

### 3.11.2 RNA EXTRACTION FROM WATEWATER SAMPLE

SARS-CoV-2 RNA was extracted from wastewater samples using the QIAamp Viral RNA Mini Kit by Qiagen, following the manufacturer's instructions and good laboratory safety practices. Approximately 140 µL of each specimen was used for the extraction. The entire kit's contents were defrosted, vortexed, and briefly centrifuged. The extraction process involved mixing 560 µL of lysis solution with 140 µL of the sample and 140 µL of elution buffer, followed by vortexing and incubation. The mixture was then processed through a series of centrifugation steps involving wash buffers and spin columns to purify the RNA. Finally, 60 µL of elution buffer was used to elute the RNA, which was either amplified immediately or stored at -80°C for later use [5].

### 3.11.3 SARS-COV-2 DETECTION AND QUANTIFICATION USING REAL-TIME POLYMERASE CHAIN REACTION

For the detection of SARS-CoV-2 in wastewater samples, RT-qPCR assays were conducted using the GeneFinder COVID-19 Plus RealAmp Kit by OSANG Healthcare, following the manufacturer's instructions and adhering to good laboratory safety practices. This one-step reverse transcription real-time PCR kit detects SARS-CoV-2 by targeting three viral genes: RdRp, envelope (E), and nucleocapsid (N). Compatible with the CFX96 PCR model, the kit allows for the qualitative detection of COVID-19 through RNA reverse transcription and PCR amplification. The RT-PCR process denaturation of the RNA at 95°C to separate its strands, annealing of the primers to specific sequences on the single-stranded DNA at 50°C, creating two distinct DNA strands, and extension at 72°C, DNA polymerase extends the primers, creating new complementary DNA strands. This cycle repeats 40 to 50 times, exponentially amplifying the DNA to produce over one billion copies. Following RNA extraction, the RNA was reverse transcribed into DNA, which was then amplified through these PCR cycles for analysis [6].

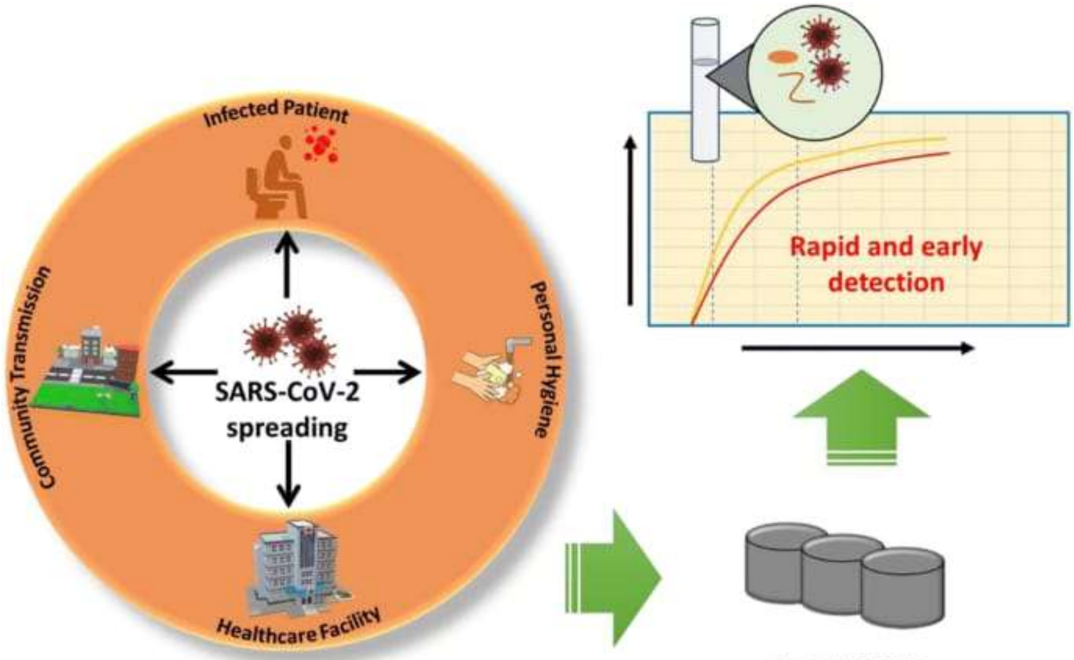


**FIGURE 1:** A schematic portrayal of the SARS-CoV-2 transmission cycle and the job of wastewater reconnaissance in fast and early discovery. The graph features the pathways of viral spread through local area transmission, medical services offices, and individual cleanliness. The coordination of sewage treatment plants (STPs) and wastewater treatment plants (WWTPs) into the observing framework works with early identification of viral presence, supporting convenient general wellbeing mediations.

## RESULTS

### TABLE 4.1: CHARACTERISTICS DATA OF THE BUILDING AND ITS OCCUPANTS

Table 4.1 reveals the distribution of population sizes within the surveyed buildings. The largest proportion of buildings (38.5%) housed populations between 101 and 200 people, followed by 23.1% of buildings housing 21 to 50 occupants. Smaller populations, specifically those with 0-10 and 51-100 occupants, each represented 15.4% of the sample, while buildings with 11-20 occupants were the least common, at 7.7%. This distribution indicates that a significant number of buildings house relatively large populations. Buildings accommodating 101-200 people are the most common, suggesting that these facilities are likely designed to support substantial occupancy, potentially indicating multi-family residential buildings or large institutional housing. The overwhelming majority of samples were collected from water cisterns (92.3%), with only one sample (7.7%) taken from a drain. This study had more samples from water cisterns, due to their relevance in water quality and sanitation studies. The minimal sampling from drains indicates a lower presence of open drains in the surveyed area for our research. Most buildings (92.3%) featured a closed drainage system, with only one building (7.7%) having an open drainage system. The prevalence of closed drainage systems indicates a generally modern and controlled approach to waste management in the research area, which have significant implications for sanitation and public health. Closed systems are typically more efficient in preventing environmental contamination and reducing the spread of waterborne diseases. Most buildings surveyed were storey buildings (76.9%), with bungalows comprising the remaining 23.1%. This suggests a dominance of multi-storey structures, which may be due to space optimization in urban settings where the research is carried out and the buildings serving multiple purposes such as residential and few commercial activities. The prevalence of storey buildings also reflects economic and developmental factors in the study area. Most buildings (92.3%) were located less than a kilometer from the main hospital area, with only one building (7.7%) being approximately one kilometer away. The proximity of most buildings to the hospital area implies easy access to healthcare services for residents, which is beneficial for public health. This close distance also influences the sanitation practices and infrastructure of the buildings, as proximity to medical facilities often necessitates higher standards of hygiene and safety. Only one building (7.7%) reported having cats, while the remaining buildings (92.3%) had no pets. The low incidence of pets, particularly cats, suggests that pet ownership is not common among the surveyed buildings' occupants. This is due to cultural factors, building policies, or economic considerations. The absence of pets might also reduce the risk of zoonotic diseases in these buildings.

**Table 4.1:** Characteristics data of the building and its occupants

| Variable | Frequency | Percentage (%) |
|---|---|---|
| **Population**<br>0 – 10 | 2 | 15.4 |
| 11 – 20 | 1 | 7.7 |
| 21 – 50 | 3 | 23.1 |
| 51 – 100 | 2 | 15.4 |
| 101 – 200 | 5 | 38.5 |
| **Sample Type**<br>Water cistern | 12 | 92.3 |
| Drain | 1 | 7.7 |

| | | |
|---|---|---|
| **Drainage System**<br>Open | 1 | 7.7 |
| Closed | 12 | 92.3 |
| **Type of building**<br>Bungalow | 3 | 23.1 |
| Storey building | 10 | 76.9 |
| **Distance of building to the hospital area**<br>Less than a kilometer 12 | | 92.3 |
| Approximately 1 kilometer 1 | | 7.7 |
| **Pets**<br>Cats 1 | | 7.7 |
| None 12 | | 92.3 |

## 4.2 HISTORY OF PREVIOUS COVID-19 INFECTIONS

More than half of the participants (53.8%) have a history of previous COVID-19 infections. A notable proportion (23.1%) either did not have a previous infection or were uncertain about their infection history. All individuals 7 (100.0%) who reported a previous COVID-19 infection experienced it over a year ago. Most of the individuals with a history of COVID-19 infections reported mild5 (71.4%) cases, with a smaller proportion experiencing moderate 2 (28.6%) severity. All 13 (100.0%) participants in the study were vaccinated against COVID-19. Vaccination doses are evenly split between two 6 (46.2%) and three 6 (46.2%) doses for most individuals, with one person having received five 1 (7.7%) doses.

| **Variable Frequency** | | **Percentage (%)** |
|---|---|---|
| **History of previous COVID-19 infections**<br>Yes 7 | | 53.8 |
| No 3 | | 23.1 |
| Unknown 3 | | 23.1 |
| **Time of Infection**<br>Over a year ago 7 | | 100.0 |
| **Severity of infection**<br>Mild 5 | | 71.4 |
| Moderate 2 | | 28.6 |
| **COVID-19 Vaccination History**<br>Vaccinated | **y**<br>13 | 100.0 |
| **Doses Received**<br>2 Doses | 6 | 46.2 |
| 3 Doses | 6 | 46.2 |
| 5 Doses | 1 | 7.7 |

### 4.3 SARS-COV-2 RNA PRESENCE

The overwhelming majority of samples 157 (98.1%) did not contain detectable SARS-CoV-2 RNA, while a very small fraction 3 (1.9%) tested positive for the presence of the virus.

**Presence of SARS-CoV-2 in wastewater collected from residential buildings in UCH**

| SARS-CoV-2 RNA | Frequency | Percentage (%) |
|---|---|---|
| Not present | 157 | 98.1 |
| Present | 3 | 1.9 |

Table 4.4 provides odds ratios (OR) and confidence intervals (C.I.) for various variables and their significance levels, indicating the likelihood of certain outcomes based on these variables. The wide confidence interval suggests a lack of precision in the odds ratio estimate, indicating that the population size has no significant effect on the outcome. The significance value (0.997) further supports this, suggesting no statistically significant association. Like the sample type, the high odds ratio implies a strong association between the type of building and the outcome. However, the significance value (0.998) indicates that this association is not statistically significant. The odds ratio suggests a strong association, but with no confidence interval, this is difficult to interpret accurately. The significance value (0.998) indicates no statistically significant association. No significant association is found for students, as indicated by the significance value of 1.000. No significant association is found for medical doctors and dentists, as indicated by the significance value of 1.000. The odds ratio of 0.000 indicates no association, and the significance value (0.997) supports this finding. The high odds ratio suggests a strong association, but the lack of a confidence interval and the significance value (0.999) suggest no statistically significant association. Logistic regression for the predictors of the presence of SARS-CoV-2 in waste water

| Variable | Significance | OR | 95% C.I OR |
|---|---|---|---|
| **Population** | 0.997 | 0.744 | 0.000 - $6.808 \times 10^{57}$ |
| **Sample Type**<br>Water cistern<br>Drain | 0.999 | $4.774 \times 10^{13}$ | - |
| **Type of building**<br>Bungalow<br>Storey building | 0.998 | $4.185 \times 10^{26}$ | - |
| **Distance of building to the hospital area**<br>Less than a kilometer<br>Approximately 1 kilometer | 0.998 | $5.026 \times 10^{36}$ | - |
| **Occupation of occupants**<br>Students<br>Medical doctors & Dentists | 1.000 | - | - |
| Physiotherapist | 0.997 | 0.000 | - |
| Others | 0.999 | $1.154 \times 10^{8}$ | - |

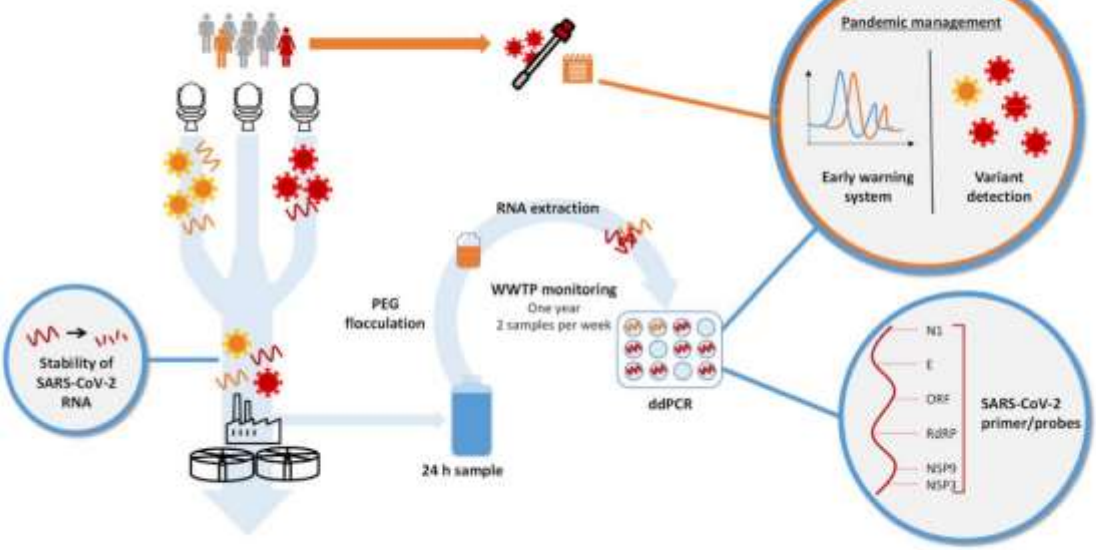


**FIGURE 2:** The most common way of checking SARS-CoV-2 in wastewater. This flowchart delineates the means associated with wastewater observation, including Stake flocculation for concentrating viral particles, RNA extraction, and discovery utilizing ddPCR. The cycle adds to pandemic administration by giving early admonitions and recognizing variations.

## DISCUSSION

The characteristics of the buildings and their occupants provide valuable information into the living conditions and environmental factors of the surveyed area. The predominance of larger populations in multi-storey buildings with closed drainage systems indicates a relatively urbanized setting with modern infrastructure. The focus on water cisterns for sampling highlights the importance of water quality in the study, potentially reflecting concerns over waterborne pathogens or contaminants. The proximity of most buildings to the hospital area suggests that residents have good access to healthcare, which is crucial for maintaining public health standards. However, this also underscores the need for stringent sanitation measures to prevent hospital-acquired infections from spreading into the community. Overall, the data highlights the importance of understanding building characteristics and occupant demographics in addressing public health issues. The findings suggest that future interventions should focus on maintaining and improving water and sanitation systems, particularly in densely populated areas, to prevent the spread of diseases and ensure a healthy living environment for all residents [7]. The significant number of individuals with a history of COVID-19 infections may indicate a high exposure rate to the virus in the past. The presence of a substantial "Unknown" category suggests gaps in knowledge or record-keeping regarding past infections, which could impact the accuracy of infection history and subsequent analysis of immunity or vulnerability. The timing indicates that these individuals have had ample time to recover and potentially develop immunity, either naturally or through vaccination. This period may also influence their current health status and response to vaccinations or re-infections. The predominance of mild cases aligns with the general distribution of COVID-19 severity in the broader population. However, the presence of moderate cases highlights the variability in disease impact, which may have implications for post-infection health and vaccine response [8]. The universal vaccination coverage among participants suggests a high level of compliance with vaccination recommendations, which is essential for community protection and reducing transmission rates. This factor is crucial for understanding the potential impact of vaccination on infection rates and severity among the study group. The distribution of vaccine doses indicates varied adherence to booster dose recommendations. The equal number of individuals with two and three doses suggests a transition phase in vaccination schedules, where some have received an additional booster [9]. The single instance of five doses is unusual and may indicate special circumstances, such as higher risk status requiring more frequent boosting. The low prevalence of SARS-CoV-2 RNA in the sampled population is indicative of either a low current infection rate or successful containment and mitigation measures in place at the time of sampling. This could be attributed to several reason such as the high vaccination coverage that likely contributed to the reduced viral load and transmission. With a significant portion of the population possibly having prior exposure to the virus and subsequent recovery, combined with vaccination efforts, herd immunity may be playing a role in reducing the prevalence of the virus. Continued adherence to public health guidelines such as mask-wearing, social distancing, and hygiene practices can significantly lower the spread of the virus, contributing to the low detection rate of SARS-CoV-2 RNA. The accuracy and sensitivity of the testing methods used to detect SARS-CoV-2 RNA are crucial [10]. A low false-negative rate would suggest that the findings are reliable, and the actual prevalence of the virus in the population is indeed low.

The results suggest that current control and prevention measures are effective in managing the spread of SARS-CoV-2 within the population. Public health strategies should continue to focus on maintaining high vaccination rates and encouraging booster doses where necessary to sustain immunity levels. Despite the low prevalence, ongoing surveillance is essential to detect any new outbreaks promptly. This is especially important with the emergence of new variants that may have different transmission characteristics or vaccine escape potential. The low detection rate may allow for the reallocation of resources to other areas of need, such as the management of long COVID or other healthcare services that were deprioritized during the pandemic. These findings can be used to reassure the public about the effectiveness of vaccines and public health measures [11]. However, it is also important to communicate the need for continued vigilance to prevent complacency. The results indicate that none of the variables analyzed show statistically significant associations with the outcomes measured. The wide and often implausibly large confidence intervals for odds ratios, where provided, further suggest that the estimates are not precise. The significance values close to 1.000 across all variables indicate a lack of statistical significance in the associations tested. The lack of significant associations could be due to a small sample size, which reduces the statistical power to detect true associations. Unmeasured confounding variables might be influencing the outcomes, obscuring the true associations. The findings suggest that the variables analyzed do not have a significant impact on the outcomes of interest in this study. This may guide researchers to consider other variables or larger sample sizes in future studies to better understand the factors influencing the outcomes. The need for more precise measurement and control of confounding variables should also be considered to improve the validity of future research.

## CONCLUSION

This study provides information into the historical and vaccination-related characteristics of the study group. A significant portion of individuals had previous COVID-19 infections, predominantly mild, and all are vaccinated with varied doses. This information is critical for assessing the population's immunity landscape and guiding public health strategies to prevent future outbreaks and manage vaccine distribution effectively. Continuous monitoring and updating of vaccination status and infection history are recommended to maintain an accurate understanding of community immunity levels. The detection of SARS-CoV-2 RNA in only a small fraction of the samples indicates a successful containment of the virus within the study population. High vaccination rates, possible herd immunity, and effective public health measures likely contribute to this positive outcome. Ongoing surveillance and maintaining public health interventions remain crucial to prevent potential outbreaks and manage the pandemic effectively.

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