Research Article

Post-Pandemic Prevalence of SARS-CoV-2 RNA in the Saliva of HIV/AIDS Patients in Jos, Nigeria

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Abstract | On May 5, 2023, the World Health Organization (WHO) cautiously declared COVID-19 no longer a global health emergency, because the causative agent, SARS-CoV-2, continues to evolve and is no longer a serious public health threat. This makes surveillance essential, especially for high-risk groups like HIV/AIDS patients. Consequently, this study assessed the post-pandemic prevalence of SARS-CoV-2 RNA in the saliva of HIV/AIDS patients in Jos, Nigeria. This cross-sectional study was conducted across two HIV treatment centers in Jos, where 200 consenting HIV/AIDS patients were recruited using convenience sampling. Eligible participants were aged 18 years and above, unvaccinated against COVID-19, asymptomatic, and had not tested for COVID-19 in the past. From each participant, 2 ml of saliva was collected and processed for RNA extraction using the QIAamp Viral RNA Mini Kit. SARS-CoV-2 RNA detection was performed with the QIAGEN Artus SARS-CoV-2 Pre-amp UM Kit on a Bio-Rad CFX96 RT-PCR platform. Associations between demographics and SARS-CoV-2 status were tested using Chi-square. SARS-CoV-2 RNA was detected in 4 participants (2.0%, with 95% CI), with Ct values of 28.01, 29.57, 29.81, and 33.68. The mean age of the study population was 47.64 ± 10.559 years. All the positive cases occurred among middle-aged groups (38–57 years). No significant associations were found between SARS-CoV-2 status and age (p = 0.824) or sex (p = 0.716). These finding highlight the need to continually monitor the SARS-COV-2 for future evolution and prevalence in the community especially in the context of concurrent infections.

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Introduction

Nigeria began the battle against COVID-19 on February 27, 2020, when it confirmed its index case in a 44-year-old Italian visitor in Lagos State. This occurred shortly after the initial outbreak of the disease in Wuhan, China, in December 2019 (Nigeria Centre for Disease Control and Prevention, 2020).

The aetiological agent, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), rapidly spread worldwide and was declared a pandemic by the WHO on March 11, 2020 (WHO Director-General's Opening Remarks at the Media Briefing on COVID19, 2020). The pandemic claimed more than seven million lives, leaving behind profound and lasting global consequences (WHO COVID-19 Dashboard, 2023; Cawley et al., 2021).

Throughout the pandemic, numerous controversies were aroused, initiating the challenges of unnoticed and undiagnosed cases, vaccine hesitancy, prevention disputes, widespread misinformation, and health inequality (Caceres et al., 2022; Babatope et al., 2023; Johansson, 2021; Ojo et al., 2023). For instance, Nigeria achieved only half (30.5%) of the WHO's 70% vaccination target during the pandemic era, and COVID-19 tests uptake was low (Statement of the Independent Allocation of Vaccines Group (IAVG) of COVAX, 2021). As of April 2, 2024 when global reports were no longer centralized, Nigeria had conducted only 5,708,974 COVID-19 tests for a country of over 200 million people, according to Worldometer data (Worldometers, 2024). While the initial gold standard for SARS-CoV-2 testing relied on oral and nasopharyngeal swab, saliva is generally convenient alternative for symptomatic and asymptomatic cases (Chopoorian et al., 2023; Yoon et al., 2020; Carrouel et al., 2023).

The global response to COVID-19 was swift, involving the rapid development and deployment of vaccines such as RNA-based (Pfizer-BioNTech, Moderna), viral vector (AstraZeneca, Johnson and Johnson), and protein subunit vaccines (Novavax). These efforts changed the course of the pandemic, convincing the WHO to declare the pandemic ended on May 5, 2023. Nonetheless, the WHO called for continued vigilance and monitoring of the virus, even as the world transitioned into a phase of long-term management (Statement on the fifteenth meeting of the IHR (2005), Emergency Committee on the COVID-19 pandemic, 2023), due to concerns about viral RNA persistence, latency, resurgence, and re-infection (Chopoorian et al., 2023; Joukar et al., 2021; Lau et al., 2021; Chen et al., 2023).

Several factors influence the course and severity of SARS-CoV-2 infection, including the strength of host immune response (Lau et al., 2021; Vibholm et al., 2021), viral load, body fluid type used for diagnosis (Chopoorian et al., 2023; Carrouel et al., 2022; Joukar et al., 2021), and the presence of underlying health conditions (Yoon et al., 2020; Ambrosioni et al., 2021). People living with HIV (PLWH) represent a particularly vulnerable group due to their immunocompromised status (Johansson, 2021; Ambrosioni et al., 2021), hence, this study sought to assess the post-pandemic prevalence of SARS-CoV-2 RNA in the saliva of HIV/AIDS patients receiving care and treatment at two HIV clinics in Jos, Plateau State, Nigeria.

Materials and Methods

Study design, participants and area

This study adopted a cross-sectional design involving 200 adult HIV/AIDS patients receiving care and treatment at two HIV Centers in Jos, Plateau State, Nigeria: The Aids Prevention Initiative in Nigeria (APIN) and Faith Alive Foundation (FAF).

Ethical considerations

The study protocol was reviewed and approved by the Jos University Teaching Hospital Health Research Ethics Committee (JUTH/DCS/IREC/127/XXXI/599) as well as the Institutional Review Boards (IRBs) of the selected study Centers. Participants were informed about the study and assured of confidentiality. They were requested to complete a consent form voluntarily, with the full right to refuse or withdraw participation. Only those who gave consent to participate were included in the study.

Estimation of study sample size

The formula for cross-sectional study below was used to estimate the minimum sample size (Pourhoseingholi et al., 2013).

$$n = \frac{p(1-p)Z^2}{E^2}$$

Where, n = required sample size, p = (11.5% = 0.115) expected prevalence from another study (Peluso et al., 2021), E = precision (5% = 0.05), and Z = Z statistic at desired confidence level (at confidence level 95%, Z = 1.96). Given these parameters, 156.39 was arrived at. However, considering potential attrition rate, the sample size was increased to 200.

Inclusion criteria

Only patients who met all the following criteria were included in the study: HIV positive, 18 years or older, show no obvious symptoms related to Covid-19, unvaccinated and without previous COVID-19 diagnosis or test. Preferred participants must have not brushed their teeth or haven't taken a meal or have unavoidably done so at least 30 minutes prior to sample collection.

Sampling and data collection

A convenience sampling technique was employed to recruit participants who provided informed consent for both verbal and physical interviews. Participant information was recorded using a researcher-administered structured questionnaire, based on predefined inclusion criteria. Approximately 2 mL of saliva (not mixed with sputum) was collected from each participant into sterile universal containers without preservatives, buffers, or additives. Samples were immediately stored at -80˚C until laboratory analysis. The study commenced on February 28, 2024, and was completed on June 6, 2024.

Laboratory protocol

The laboratory procedures were conducted in two stages at the Genomics and Postgraduate Research Laboratory, Faculty of Clinical Science, College of Health Sciences, University of Jos, Nigeria.

Stage 1– RNA extraction from saliva samples

RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen), following the silica spin column protocol as outlined by the manufacturer (QIAGEN, 2023).

Frozen saliva samples were retrieved from storage and allowed to thaw completely at room temperature. Each sample was homogenized by gentle pipetting. A 500 µL aliquot of each homogenized sample was transferred into individually labeled 1.5 mL Eppendorf tubes and centrifuged at 4˚C for 1 hour at 14,000 rpm. After centrifugation, 360 µL of the supernatant was carefully discarded. To the remaining 140 µL pellet, 540 µL of AVL lysis buffer and 5 µL of β-mercaptoethanol (BME) were added. The mixture was vortexed briefly and incubated at 56˚C for 15 minutes in a thermomixer to inactivate the virus.

Next, 560 µL of absolute ethanol was added to the lysate, followed by another brief vortexing. A 630 µL volume of the resulting mixture was transferred to a silica spin column and centrifuged at 8,000 rpm. The column was then placed into a new 2 mL collection tube, and the process was repeated for the remaining volume. Flow-throughs were discarded after each centrifugation.

The column was washed sequentially: First, with 500 µL of buffer AW1 (reconstituted wash buffer), centrifuged at 8,000 rpm for 1 minute. Second, with 500 µL of buffer AW2, centrifuged at 14,000 rpm for 3 minutes. After discarding the final flow-through, the column was transferred to a labeled 1.5 mL Eppendorf tube.

Finally, 60 µL of elution buffer AVE was added to the column, followed by a 1-minute incubation at room temperature. The column was then centrifuged at 8,000 rpm for 1 minute, and the eluted RNA was immediately stored at –80˚C until further use.

Stage 2– Molecular detection of SARS-CoV-2 RNA virus using BIORAD CFX 96 real-time PCR machine

SARS-CoV-2 AMP UM Kit (Qiagen) was used for detection. Reagents were removed from the freezer and allowed to thaw at room temperature. The SARS-CoV-2 primers and internal control (IC) mix volumes were adjusted according to the number of samples.

15µl of the reaction mix was dispensed into each well of the PCR plate. Nuclease-free water was loaded into wells designated for negative extraction control (NEC) and the no template control (NTC), and mixed by pipetting up and down. 10 µL of the SARS-CoV-2 positive control (PC) was added to the appropriate well. Similarly, 10 µL of each unknown sample was transferred into its designated well and mixed gently. The PCR plates were sealed with caps to prevent cross-contamination and briefly centrifuged to collect the contents at the bottom of the wells.

A 25ul RT-PCR reaction protocol was performed as follows: Reverse transcription was initiated at 50˚C for 10 minutes, followed by PCR initial heat activation at 95˚C for 2 minutes. Denaturation occurred at 95˚C for 5 seconds, and annealing/extension at 58˚C for 30 seconds. The program ran for 40 cycles at a ramp rate of 1.60/sec in each step. FAM, HEX, and Cy5 reporter channels were used for fluorescence detection. After the amplification, results were analyzed using CFX Manager Dx software.

Statistical analysis

IBM SPSS Statistics software version 27.0 was used to analyze the data. We used summary measures to determine the number and percentage of cases within each demographic category. Associations between age, sex, and SARS-CoV-2 status were explored using the Chi-square test. Statistical significance was set at $p \leq 0.05$, corresponding to a 95% confidence interval (CI).

Results

Out of the 200 saliva samples analyzed for the presence of SARS-CoV-2 RNA, 4 (2.0%; 95% CI) tested positive, while 196 (98.0%) tested negative. The four positive cases were observed at threshold cycles (Ct) of 28.01, 29.57, 29.81, and 33.68, respectively. These findings are illustrated in Figure 1. The mean age of the study population was 47.64 ± 0.747 years, with an age range spanning from 18 to 77 years. Notably, all four positive cases occurred among middle-aged groups, suggesting a clustering of SARS-CoV-2 RNA detection in this demographic group. However, this association was not statistically significant (p = 0.824). Gender distribution within the study population showed a predominance of females (n = 133; 66.5%) compared to males (n = 67; 33.5%). However, this difference did not reach statical significance (p = 0.716). This suggest that, within the limits of this sample size, gender may not be a major determinant of SARS-CoV-2 RNA detection in saliva among HIV/AIDS patients. Table 1 summarizes the demographic distribution of the study population in relation to SARS-CoV-2 RNA status.

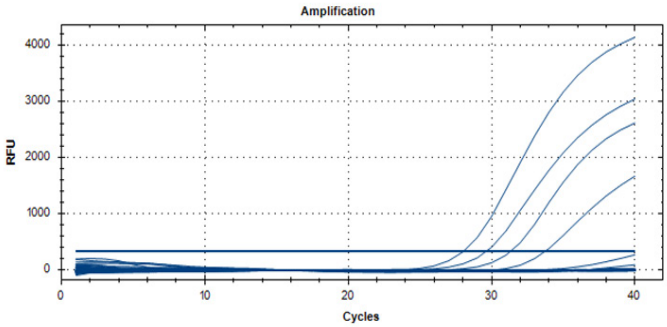

Figure 1: *Real-time PCR Amplification of SARS-CoV-2 RNA in Saliva Samples from Study Participants (Bio-Rad CFX96).*

Discussion

We report a 2.0% prevalence of asymptomatic SARS-CoV-2 RNA in the saliva of unvaccinated adult HIV/AIDS patients in Jos, Plateau State, Nigeria, thirteen months after the WHO declared COVID-19 no longer a global emergency on May 5, 2023. This finding is lower than the 11.5% prevalence in saliva reported by Peluso and colleagues among 70 COVID-19 survivors at 53 days post-infection (Peluso et al., 2021). However, our finding among PLWH in the post-pandemic context projects a different opinion, showing that PLWH could be faced with Covid-19 complications. On the other hand, 2.0% in our study is a higher prevalence compared to the 1.5% prevalence reported by Mike-Ogburia and colleagues among 200 incarcerated persons in Port-Harcourt, Nigeria (Mike-Ogburia et al., 2023). In contrast to our molecular post-pandemic study, Mike-Ogburia and colleagues used rapid diagnostic testing, conducted during the waning stages of the pandemic (Mike-Ogburia et al., 2023).

Table 1: *Prevalence and distribution of SARS-CoV-2 RNA detection by age and sex.*

n = 200	SARS-CoV-2 RNA					
Parameter	**Positive**	**Negative**	**Total**	x^2	***df***	**p value**
Prevalence	4 (2.0%)	196 (98.0%)	200(100%)			
Age group (in years)				2.179	5	0.824
18 – 27	0	6	6(3.0%)			
28 – 37	0	29	29(14.5%)			
38 – 47	2	68	70(35.0%)			
48 – 57	2	59	61(30.5%)			
58 – 67	0	30	30(15.0%)			
68 – 77	0	4	4(2.0%)			
Total	4	196	200(100%)			
Sex				0.132	1	0.716
Male	1	66	67(33.5%)			
Female	3	130	133(66.5%)			
Total	4	196	200(100%)			

Mean age: 47.64 ± 10.559 years

We observed a higher prevalence in females (3) than in males (1). This difference could be attributed to the fact that the number of female participants was twice that of males. Two positive cases were detected in each of the age groups 39-48 and 49-58, corresponding with trends observed in other studies where younger and middle-aged groups often exhibit moderate to high vulnerability to SARS-CoV-2 infections and

are key drivers of transmission dynamics during pandemic phases (Carrouel et al., 2022; Monod et al., 2021). The mean age of the study population was 47.64 ± 10.559 years, contrasting with the median age of 52.0 years reported by Chen and colleagues (Chen et al., 2023). Chi-square revealed no influence of age or sex on SARS-CoV-2 RNA prevalence analysis ($p > 0.05$; 95% CI), consistent with initial observations (Carrouel et al., 2022).

The SARS-CoV-2 RNA positivity was detected at CT values of 28.01, 29.57, 29.81, and 33.68, as shown in Figure 1. Whether the virus is infective is not known in this study, but infectivity could be expected based on the CT values (Rabaan et al., 2021; Esteve et al., 2020). Furthermore, our study did not directly assess the specific predictive factors for SARS-CoV-2 persistence in HIV/AIDS patients, but other studies linked low CD4+ counts and high or uncontrolled HIV viral load to longer SARS-CoV-2 RNA persistence and risk of reinfection (Chen et al., 2023; Vibholm et al., 2021; Ambrosioni et al., 2021; Peluso et al., 2021; Maponga et al., 2023; Fernandez-de-las-Penas et al., 2024; Teran et al., 2023).

Conclusions

A low (2.0%) prevalence of asymptomatic SARS-CoV-2 RNA was detected in the saliva of unvaccinated adult HIV/AIDS patients in Jos, Nigeria, thirteen months after WHO declared the end of COVID-19 as a global emergency on May 5, 2023. An indication that SARS-CoV-2 remains clinically and epidemiologically relevant for post-pandemic public health planning in high-risk groups. It is important to sustain national surveillance capacity to keep track of SARS-CoV-2 evolution and ensure that immune susceptible populations remain central to post-pandemic health planning.

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Novelty Statement

This study represents one of pioneering investigation into the post-pandemic prevalence of SARS-CoV-2 RNA in the saliva of unvaccinated HIV/AIDS patients in Jos, Nigeria, filling a critical gap in understanding viral persistence in immunocompromised populations in sub-Saharan Africa.

Author's Contribution

LL conceptualized and developed the research proposal, performed data analysis, and drafted the manuscript. LL and OCS collected the samples. LL and RCN conducted the laboratory analyses. NTC and GEI supervised the research process. NTC, EB, and LN critically reviewed the manuscript. All authors read and approved the final version of the manuscript for publication.

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Generative AI and AI-assisted technology statement

The use of ChatGPT-5 was strictly limited to linguistic editing to enhance clarity, coherence, grammar, and punctuation. ChatGPT had no role whatsoever in the conception or design of the study, data acquisition, data analysis, interpretation of findings, validation of results, or any practical, clinical, or policy application of the results.

Conflict of interest

The authors have declared no conflict of interest.

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