Journal of Community Medicine and Primary Health Care. 36 (1): 91-104
https://dx.doi.org/ 10.4314/jcmphc.v36i1.8

JOURNAL OF COMMUNITY MEDICINE AND PRIMARY HEALTH CARE

ORIGINAL ARTICLE

Diagnostic Accuracy of Antigen-Based Rapid Test during the Fourth Wave of COVID-19 in Edo State, Nigeria

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Keywords
COVID-19,
Diagnostic accuracy,
Antigen-based RDT,
Edo State,
Nigeria

ABSTRACT

Background: Since the use of rapid test kits for diagnosis of COVID-19, diagnostic accuracy has been of concern, particularly in resource-limited settings. This study assessed diagnostic accuracy of Abbot Panbio antigen-based rapid diagnostic test (RDT) for COVID-19 using polymerase chain reaction (PCR) as the gold standard in Edo State, Nigeria.

Methodology: We conducted a descriptive cross-sectional study during the 4th wave of the pandemic in Edo State from January 5 – February 4th, 2022. Nasopharyngeal and oropharyngeal swabs were collected from 240 consenting participants at 10 sample collection sites. The index test (RDT) and gold standard test (real-time reverse transcription PCR) were performed simultaneously. Sensitivity, specificity, predictive values, and ROC analysis were conducted on the RDT compared to RT-PCR using SPSS version 26.0 software.

Results: Thirty-six (15.0%) participants tested positive on RDT and 34 (14.2%) tested positive on PCR. RDT had a sensitivity of 73.5% (95% CI: 66.0–81.0) and specificity of 94.7% (95% CI: 91.6-97.8) with a positive predictive value of 69.4% (95% CI: 55.8-80.7), and negative predictive value of 95.6% (95% CI: 91.8-98.0). The area under the curve (AUC) of the ROC for the RDT against PCR was 84.1% (75.1% - 93.1%).

Conclusion: The diagnostic accuracy of the Panbio rapid antigen test for SARS-CoV-2 was below WHO standards. However, they remain useful tools for continued surveillance of the disease. Rigorous evaluations combining the results of rapid antigen tests with other clinical information would prove useful for prompt diagnosis and surveillance of COVID-19 in Nigeria.

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INTRODUCTION

Since its emergence in 2019, the COVID-19 pandemic has posed a significant threat to global public health, with the World Health Organization (WHO) declaring it a pandemic in March 2020.[1,2] As the virus spread, the demand for testing increased, and the gold standard for

COVID-19 diagnosis became the polymerase chain reaction (PCR) test.[3] However, PCR tests require specialized laboratories and equipment, which can limit access in resource-limited settings. To address this issue, the WHO approved the use of antigen-based COVID-19 test kits in September 2020 (with a recommended minimum sensitivity of ≥80% and specificity ≥97%).[4] Antigen-based tests are less expensive, faster, and easier to perform than PCR tests, making them an attractive alternative for COVID-19 diagnosis, especially in low-income countries.[5, 6]

Nigeria, like many other countries in sub-Saharan Africa, was heavily impacted by the COVID-19 pandemic.[7,8] As of December 2022, Nigeria recorded 266,145 cases of the disease and 3,155 deaths since the outbreak began.[9] However, inadequate infrastructure and expertise limited the use of the gold standard PCR test for COVID-19 diagnosis.[10] Consequently, rapid antigen tests were prioritized as alternative diagnostic tests.

The accuracy of these tests has been a concern, with several studies reporting lower sensitivity and specificity than PCR particularly in resource-limited settings such as Nigeria.[11,] Antigen-based RDT's sensitivity varied significantly by location and time, ranging from 0% to 94%, with an average of 56.2% (95% CI 29.5 to 79.8%) and an average specificity of 99.5% (95%CI 98.1% to 99.9%). [12]

Since the use of rapid test kits has become rampant in sub-Saharan Africa, Nigeria inclusive, it is important to evaluate their diagnostic accuracy in African settings. Especially given that extreme environmental conditions, such as hot and humid weather (specific to parts of sub-Saharan Africa), could affect the performance of these tests and lead to inaccurate results.[13,14] Such evaluations remain few and far in between in the Nigerian setting.

This study assessed the diagnostic accuracy of the Abbot Panbio rapid COVID-19 antigen test device using PCR as the gold standard in Edo State, Nigeria. The study specifically determined the sensitivity, specificity, positive and negative predictive values of the antigen-based test kit by comparing it with PCR in the diagnosis of COVID-19.

METHODOLOGY

Study Area: This study was done in Edo State, Nigeria located in the South-South geopolitical zone of Nigeria, covering an area of approximately 19,187 square kilometres. The state has Benin City as its capital, and it is further divided into three senatorial districts with a population of over 4 million people.15

Edo State recorded its first case of COVID-19 in February 2020. As of December 2022, the state had recorded a total of 7,927 confirmed cases, with 322 deaths and 4 epidemic waves.9 The state government implemented various measures to combat the spread of the disease, including testing (at 10 sites: 5 hospitals, 2 schools, 2 churches and 1 Civil service building), and vaccination campaigns. The use of antigen-based rapid test commenced in the second wave of the pandemic in Edo State and COVID-19 vaccination began in March 2021.

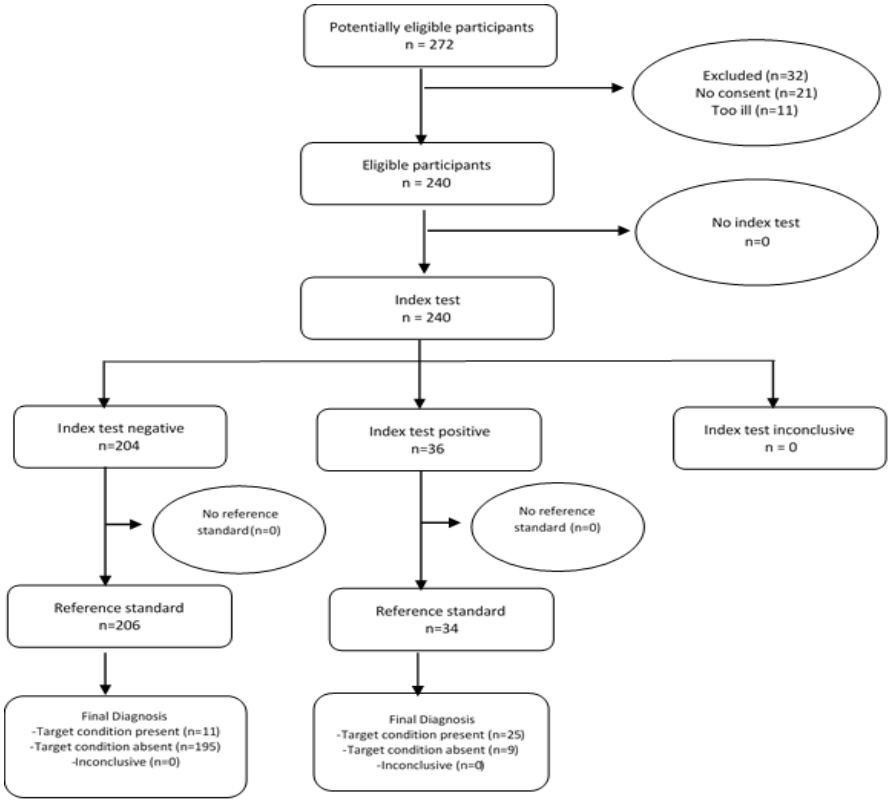


Figure 1. STARD diagram to report flow of participants through the study.

Table 1: Age, Sex and Vaccination status Distribution of Participants

Characteristics	Frequency (n = 240)	Percent
Age Group (years)*		
0-9	11	4.6
10-19	8	3.3
20-29	96	40.0
30-39	51	21.3
40-49	29	12.1
50-59	18	7.5
60-69	13	5.4
70-79	11	4.6
80-89	3	1.3
Sex		
Male	143	59.6
Female	97	40.4
Vaccination Status		
Vaccinated	28	11.7
Unvaccinated	212	88.3

**Median (range); 30 (2 to 87) years*

Study design: A descriptive cross-sectional study was done. The index test and the gold standard test (real-time reverse transcription PCR) were performed on the study participants at the same time.

Participants and Eligibility: Participants in this study included individuals who were suspected of having COVID-19 based on clinical symptoms, individuals who had been exposed to a known COVID-19 case(s), or individuals who were asymptomatic but at high risk of contracting the virus (e.g., healthcare workers or frontline workers, the elderly) who presented to any of the accredited sample collection and RDT testing sites in Edo State from 5th January 2022 to 4th February 2022 during the 4th wave of the pandemic in the State. We excluded anyone who did not consent to the study, or anyone with a prior positive RT-PCR test for SARS-CoV-2 within one month or who had received a COVID-19 vaccine within two weeks.

Sample size and Sampling Technique: using the Buderer formula for calculating the required sample size for a diagnostic accuracy study,[17] we estimated that a minimum of 202 participants will be required to yield a 12% width of a two-sided 95% Confidence interval, for a sensitivity of 80%,[4] with a COVID-19 sero-prevalence of 23.3%,[18] after accounting for 10% attrition rate. However, the study included 240 participants recruited consecutively from 10 accredited sample collection and RDT testing sites distributed across the three senatorial districts (Edo South - 3 hospitals and the State civil service building; Edo Central - 1 hospital, 1 school and 1 church; Edo North - 1 hospital, 1 school and 1 church) of Edo State. Samples were collected from 5th January 2022 to 4th February 2022 at the 10 sample collection sites in Edo state.

Index Test: The index test was the PanBio (Abbott Diagnostic GmbH, Jena, Germany) COVID-19 antigen-based rapid test kit. It is an in-vitro diagnostic test used for the qualitative detection of SARS-CoV-2 nucleocapsid antigen in human nasopharyngeal swab specimens. The test is manufactured by Abbott, a global healthcare company.[19] The Abbott PanBio COVID-19 Antigen Rapid Test Device was introduced in August 2020 for use in COVID-19 screening and diagnosis.[20] Since then, the test is widely used around the world for the rapid detection of SARS-CoV-2 antigens in people with suspected COVID-19. The manufacturer reported sensitivity and specificity of the test when compared to molecular (PCR) testing methods were 92.9% and 99.4% respectively.[19]

Gold standard: The gold standard used was the real-time reverse transcription PCR (RT-PCR), which is a variation of PCR that allows for the detection and quantification of RNA. A positive result for the COVID-19 RT-PCR test was defined as the detection of the viral gene targets at cycling threshold (Ct) of 40 or less in participants' samples. A negative result means that the virus was not detected in the sample.

Procedures: Nasopharyngeal and oropharyngeal swabs were collected from each subject at the respective RDT Testing Centers (Subjects were sampled twice). Nasopharyngeal swab was processed according to the manufacturer's instruction using the Abbot PanBio RDT and the results were recorded accordingly.[19]. The results were read visually after 15 minutes. A positive result was

indicated by the presence of a colored line in both the test and control regions. A negative result was indicated by the absence of a colored line in the test region. The absence of a coloured line in the control region signified an invalid result.

For the PCR testing, nasopharyngeal and oropharyngeal swab samples were collected from the patient, triple packaged and transported to any of the three Nigeria Centre for Disease Control (NCDC) accredited molecular laboratories in the state for testing. Laboratory personnel working in both the Ag-RDT testing team and the RT-PCR laboratory were always blinded to the results of the other test.

Table 2: RDT and PCR Test Results of Participants

Characteristics	Frequency (n = 240)	Percent
RDT Test		
Positive	36	15.0
Negative	204	85.0
PCR Test		
Positive	34	14.2
Negative	206	85.8

Data collection: A data collection form was used to record the results of the index and gold standard tests. The age, sex, and vaccination status of the participants were also collected. The testing site where samples were collected and the time of collection were documented.

Statistical analysis: We presented the STARD diagram to report the flow of participants through the study (Figure 1). Analysis of the demographic characteristics of the participants using appropriate summary statistics was done and the vaccination status was summarized using frequency and percentages. The kappa statistics was used to estimate the degree of agreement between the index test and the gold standard. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the antigen-based test kit compared to RT-PCR using standard methods. We also conducted a receiver operating characteristic (ROC) curve analysis to assess the overall performance of the antigen-based test kit. Multivariable logistic regression analysis was performed with the outcomes of PCR and Ag-RDT (separately) to identify independent associations between age, sex, and vaccination status with test outcomes. For all statistical analyses done, the significance threshold was set at a two-sided alpha value of 5% and the confidence interval was constructed at the 95% limit. We used the IBM SPSS statistical software (version 25.0) to perform the analyses.

Ethical considerations: This study was approved by the Health Research Ethics Committee (HREC) of the University of Benin Teaching Hospital and was conducted following the principles outlined in the Declaration of Helsinki.

RESULTS

Table 1 shows the age, sex, and vaccination status distribution of the 240 participants included in the study. The median age of participants was 30 years (range of 2 to 87 years) with most participants in the age group of 20-29 years (40.0%), followed by those in the age group of 30-39 years (21.3%). The least represented age group was 80-89 years, with only 3 participants (1.3%). There were more male participants (59.6%) than female participants, and only 28 participants (11.7%) were vaccinated.

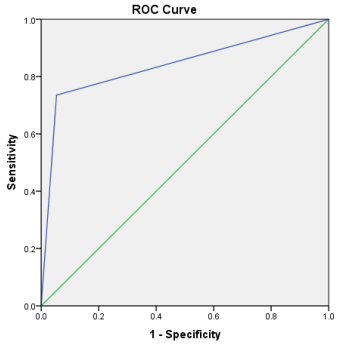
Table 2 presents the RDT and PCR test results of study participants. Of the total number, 36 (15.0%) participants tested positive with the RDT, 34 (14.2%) participants tested positive on the PCR test. These results indicate that the prevalence of positive test results was higher for the RDT than for the PCR test.

Table 3: Diagnostic Accuracy and degree of agreement of RDT against PCR results of participants

	PCR Test (%)		Validity (95% CI)		Kappa	p-value
RDT Tests	**Positive**	**Negative**	**Sensitivity**	**Specificity**	**Statistics**	
Positive	25 (73.5)	11 (5.3)	73.5 (66.0–81.0)	94.7 (91.6-97.8)	0.66*	<0.001
Negative	9 (26.5)	195 (94.7)				
Total	34 (100.0)	206 (100.0)				
	PCR Test (%)		**Predictive Value (%)**			
RDT Test	**Positive**	**Negative**	**Positive**	**Negative**		
Positive	25 (69.4)	11 (30.6)	69.4 (54.5-84.3)	95.6 (91.3-99.9)		
Negative	9 (4.4)	195 (95.6)				
Total	34 (14.2)	206 (85.8)				

degree of agreement between PCR and RDT test results,* ***p-value**; measure of statistical significance association between PCR and RDT test results.*

Diagnostic accuracy and degree of agreement of RDT against PCR results of participants are presented in Table 3. Among the 240 participants, the PCR test confirmed the presence of the disease in 34 individuals while the remaining 206 tested negative for it. Out of the 34 individuals with the disease, the index test correctly identified 25 as positive (true positives) and missed 9 (false negatives). Out of the 206 individuals without the disease, the index test correctly identified 195 as negative (true negatives) and incorrectly identified 11 as positive (false positives). This gave a sensitivity of 73.5% (95% CI: 66.0–81.0) and a specificity of 94.7% (95% CI: 91.6-97.8) for detecting the disease. The positive predictive value of the test was 69.4% (95% CI: 55.8-80.7), which means that among all individuals who tested positive, 69.4% actually had the disease. The negative predictive value of the test was 95.6% (95% CI: 91.8-98.0), meaning that among all individuals who tested negative, 95.6% were disease-free. The kappa statistic for the agreement between the RDT and PCR tests was 0.66, with a p-value of 0.001, indicating a substantial level of agreement between the two tests



AUC of the ROC = 84.1% (75.1% to 93.1%)

Figure 2: Area Under the Curve (AUC) of the ROC of RDT against PCR test results of the participants

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve is illustrated in Figure 2. The AUC of the ROC for the RDT against PCR test results of the participants was 84.1% (75.1% to 93.1%).

Table 4 displays the unadjusted and adjusted predictors of RDT and PCR test results of the participants. The predictors included in the model for RDT are sex, age group, and vaccination status. No statistically significant associations were found between RDT results and sex, age group, or vaccination status. However, the adjusted OR for sex was 0.95 (95% CI: 0.46-1.96), and the adjusted ORs for age groups 0-29 and 30-59 were 1.66 (95% CI: 0.44-6.21) and 1.33 (95% CI: 0.37-4.87), respectively. The adjusted OR for vaccination status was 2.53 (95% CI: 0.88-7.22), but the p-value was not statistically significant (p=0.115). The results for predictors of PCR test outcomes, showed that there was no statistically significant difference in the odds of having a positive PCR result between males and females. For age group, the odds of having a positive PCR result were significantly lower for participants aged 0-29 years compared to those aged 60-89 years. However, there was no significant difference in the odds of having a positive PCR result between participants aged 30-59 years and those aged 60-89 years. For vaccination status, there was no statistically significant difference in the odds of having a positive PCR result between vaccinated and unvaccinated participants.

DISCUSSION

From January 2021, antigen-based RDTs were widely deployed in Nigeria to complement testing with the gold standard RT-PCR. However, limited information on their performance in the Nigerian environment exists to back up this widespread use. In this diagnostic accuracy study, we evaluated the Abbot PanBio COVID-19 antigen-based rapid test kit, one of two rapid antigen tests recommended for use and supplied to states by the Nigeria Centre for Disease Control (NCDC) for the diagnosis and public health surveillance of COVID-19. The study population was largely unvaccinated, and the prevalence of COVID-19 based on the reference PCR test was 14.2%. At sensitivity of 73.5% and specificity of 90.4%, the index test fell short of both the

manufacturer's claims[19] and the WHO recommendation for RDTs to possess sensitivity of at least 80% and specificity of at least 97%.[4]

The Panbio kit is a popular brand within and outside the African continent.[21-27] From previous evaluations conducted in other countries, its reported sensitivity varies widely from 41.3%-91.7% and the performance in our study falls within this bracket. Compared to our observation, some researchers within Africa found better sensitivities of 76.9% and 81% in Libya and Ethiopia respectively.[21,22] In contrast, some other studies report poorer performance for the kit than we found. For instance, in South Africa, the sensitivity was 69%, in Kenya, it was 46.9% and in Mozambique, it was as low as 41.3%.[23-25] These disparities may be attributable to several factors such as the presence of symptoms, duration of symptom onset and viral load. A recent systematic review identified higher sensitivity of rapid antigen tests amongst symptomatic versus asymptomatic individuals.[28] The sensitivities recorded would therefore depend on the mix of participants in relation to these factors. Other factors such as virus variant and environmental conditions such as temperature and humidity have also been proposed to affect performance but there are conflicting schools of thought regarding these.[28]

Table 4: Unadjusted and Adjusted Predictors of RDT and PCR test results of participants

	RDT Results			Unadjusted OR	Adjusted OR (95%
Predictors*	**Positive**	**Negative**	**p-value**	**(95% CI)**	**CI)⁺**
Sex					
Male	21 (14.7)	122 (85.3)	0.868	0.94 (0.46 – 1.93)	0.95 (0.46 – 1.96)
Female	15 (15.5)	82 (84.5)		1	1
Age group (years)⁺					
0 – 29	18 (15.7)	97 (84.3)	0.962	1.07 (0.33 – 3.46)	1.66 (0.44 – 6.21)
30 – 59	14 (14.3)	84 (85.7)		0.96 (0.29 – 3.19)	1.33 (0.37 – 4.87)
60 – 89	4 (14.8)	23 (85.2)		1	1
Vaccination status					
Vaccinated	7 (25.0)	21 (75.0)	0.115	2.10 (0.82 – 5.39)	2.53 (0.88 – 7.22)
Unvaccinated	29 (13.7)	183 (86.3)		1	1
	PCR Results				
Predictors**	**Positive**	**Negative**			
Sex					
Male	20 (14.0)	123 (86.0)	0.922	0.96 (0.46 – 2.02)	1.00 (0.47 – 2.14)
Female	14 (14.4)	83 (85.6)		1	1
Age group (years)					
0 – 29	11 (9.6)	104 (90.4)	0.006	0.21 (0.08 – 0.58)	0.21 (0.07 – 0.64)
30 – 59	14 (14.3)	84 (85.7)		0.33 (0.13 – 0.89)	0.33 (0.12 – 0.94)
60 – 89	9 (33.3)	18 (66.7)		1	1
Vaccination status					
Vaccinated	6 (21.4)	22 (78.6)	0.241	1.79 (0.67 – 4.81)	0.97 (0.32 – 2.97)
Unvaccinated	28 (13.2)	184 (86.8)		1	1

**R^2 = 1.2% - 2.1%, **R^2 = 3.5% - 6.3%, Ref: reference category, OR: odds ratio, CI: confidence interval, +Adjusted for age, sex and vaccination status. ⁺age was re-categorized to improve statistical power*

Strikingly reword, the specificity of Panbio, in this study was lower than the 94-100% specificity recorded from most other studies evaluating the same and even other brands of rapid SARS-CoV-2 antigen tests.[21-27,29] For antigen tests with high specificity, a positive result does not require confirmation by rRT-PCR. In our context, the chances of encountering a false positive would be more common based on the relatively lower specificity. This has important implications for clinical decision making when using this kit in our context, because a positive test may still require confirmation using the PCR or a second validated RDT from a different manufacturer. As demonstrated during a study which investigated the incidence of false-positive results in a large sample of rapid antigen tests in Canada, false positivity could have resulted from manufacturing issues peculiar to one or more test lots used to conduct this study.[30] It could also have resulted from reading the tests after the recommended time or cross-contamination from other patients' samples.[30] Both these scenarios can occur when testing large batches without extra caution. It is prudent to have a quality assurance programme for rapid point of care tests which investigates issues like these and recommends corrective actions where necessary.

While our study did not find a significant gender-based difference in susceptibility to COVID-19, it did reveal distinct patterns of susceptibility across various age groups. Specifically, younger populations demonstrated a lower likelihood of testing positive for COVID-19 using PCR, aligning with existing literature that highlights significant variations in PCR results across age groups.[31,32] Previous studies have estimated that individuals under 20 years of age are approximately half as susceptible to infection as adults over 20 years old.[33]

The heightened vulnerability observed in the 60–89 age group may be linked to immunosenescence, while the decreased susceptibility in the 0–29 age group could be influenced by factors like a more robust immune response.[34] Notably, in countries characterized by younger population structures, such as many low- and middle-income countries, the expected per capita incidence of clinical cases would be lower than in countries with older population groups. Consequently, interventions targeting children might have a relatively modest impact on reducing SARS-CoV-2 transmission. These findings underscore the critical importance of considering demographic factors in pandemic response planning. They inform the need for targeted prevention efforts and the strategic allocation of resources to address the unique susceptibility patterns observed across age groups.

A limitation of this study is that the results may not be generalizable to other parts of the country due to varying prevalence of COVID-19. The results should also be interpreted with caution since the predictive value of the test would also be affected by disease prevalence. We conducted

the study towards the end of the fourth wave when the prevalence of COVID-19 in Nigeria was declining and transmission rates were low. The real-world context under which the study was conducted constitutes a strength because this is the setting where the majority of persons with mild symptoms and close contacts would be evaluated for COVID-19.

Although the World Health Organization pronounced that the COVID-19 pandemic ceased to be a public health emergency of international concern as of May 5, 2023, it continues to constitute a threat to public health.[35] In the event of future outbreaks, the results of this study can bolster preparedness and response by providing baseline information for prompt action in Nigeria and other countries with similar constraints to testing.

Conclusion

The sensitivity and specificity of the Panbio rapid antigen test for SARS-CoV-2 were below standards set by the WHO and results require interpretation based on clinical and epidemiological information. However, they remain useful tools for continued surveillance of the disease. Rigorous evaluations combining the results of rapid antigen tests with other clinical information (e.g., symptoms, physiological parameters, or imaging results) would prove useful. Quality assurance measures should be put in place to detect and address manufacturer dependent as well as implementation issues during the use of these tests.

Acknowledgements

The authors appreciate the efforts of the staff of Edo State Ministry of Health, and members of the Edo State COVID-19 Incidence Management Team.

Availability of data and materials

The information provided here is only to be used by us. And there is a prohibition on further use and disclosure of this information. All other data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interest.

Funding

No funding source.

Authors' contributions

GAO: Conceptualization, Project administration, Methodology, Data Curation, Formal analysis, Visualization, Writing - Original Draft. **LI**: Data collection, Project administration, **OII**: Validation, Writing - Review & Editing. **DEO**: Resources, Investigation, Writing - Review & Editing. All authors read and approved the final manuscript. The work reported in the paper has been performed by the authors. All authors provided critical feedback and helped shape the analysis and article.

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