

## Surveillance of Respiratory Syncytial Virus, Influenza A and B viruses in COVID-19 Negative Individuals in Oyo State, Nigeria

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### ABSTRACT

**Introduction:** The COVID-19 pandemic highlighted the need for robust surveillance of other respiratory viruses, as their overlapping clinical presentations with SARS-CoV-2 can complicate accurate diagnosis. This retrospective study investigated the prevalence of Respiratory Syncytial Virus (RSV) and Influenza A and B infections among SARS-CoV-2-negative individuals in Oyo State, Nigeria. **Methods:** We collected nasopharyngeal samples (n = 206) between January 2023 and June 2023 from adults and children who tested negative for COVID-19 using RT-qPCR. These samples underwent analysis via a standardized protocol, including viral RNA extraction and a multiplex one-step RT-qPCR assay, with data analyzed using IBM SPSS Statistics (version 25). **Results:** Among individuals who tested negative for COVID-19, RSV was detected in 2.8% of children and 2.0% of adults, Influenza B was found in 2.8% of children and 1.0% of adults, and Influenza A was shown in 1.0% of adults but in no children. The mean age was 2.97 years for children and 33.51 years for adults. **Conclusion:** Although detection rates were low, our findings underscore the continued need to screen for RSV and Influenza A and B in individuals with respiratory symptoms, even after a negative SARS-CoV-2 test. Sustained surveillance is critical to understand respiratory viral epidemiology, including circulation dynamics, geographic variations, and outbreak hotspots, guiding evidence-based public health and clinical strategies. Future prospective sentinel surveillance studies could further elucidate the complexities of respiratory viral co-infections and reveal nuanced spatiotemporal and demographic patterns.

### INTRODUCTION

The 1918 influenza pandemic, caused by Influenza A (H1N1) virus and commonly known as the Spanish flu, had a devastating impact on Nigeria, resulting in an estimated 500,000 fatalities. This figure represented approximately 3% of the nation's population at that time, estimated at 16–20 million individuals. The 1918 influenza pandemic starkly demonstrated Nigeria's susceptibility to widespread respiratory infections, particularly those of viral etiology. Since then, Nigeria has experienced recurrent epidemics of various respiratory pathogens, notably RSV and Influenza A and B viruses, all of which are recognized to cause significant morbidity and mortality, particularly in vulnerable populations. These recurring outbreaks underscore the persistent need for robust and sustained surveillance systems to inform

evidence-based public health interventions and effectively mitigate future outbreaks [1].

RSV and Influenza A and B viruses are prevalent respiratory pathogens frequently implicated in acute lower respiratory tract infections (LRTIs). These infections disproportionately affect vulnerable populations, including young children, particularly those under five years of age, older adults (aged  $\geq 65$  years), and individuals with compromised immune systems or underlying comorbidities such as chronic obstructive pulmonary disease (COPD), asthma, and cardiovascular disease [2]. The clinical manifestations of these viral infections can range from mild upper respiratory tract illnesses to severe respiratory distress requiring hospitalization. Globally, these infections contribute significantly to morbidity and mortality, impacting both

temperate and tropical regions, albeit with discernible variations in epidemiological patterns and disease burden [3]. While the seasonal epidemiology of RSV and influenza is well-established in temperate regions, their timing and drivers remain poorly defined in sub-Saharan Africa due to limited data. In this setting, unique environmental and demographic factors, including climatic variations and rapid urbanization, likely influence distinct patterns of viral circulation [2, 4]. This complexity highlights the crucial importance of localized surveillance efforts and the development of context-specific public health strategies within the region.

Influenza A and B viruses are major respiratory pathogens responsible for seasonal epidemics in human populations. These negative-sense, single-stranded RNA viruses belong to the *Orthomyxoviridae* family and maintain a natural reservoir in wild aquatic birds. Influenza A and B viruses spread primarily through human-to-human transmission via respiratory droplets, though zoonotic spillover from wild aquatic birds can introduce novel strains [5]. Influenza viruses, belonging to the *Orthomyxoviridae* family, are enveloped viruses possessing a pleomorphic morphology, with particle diameters ranging from 50 to 120 nm. Their genomes are comprised of segmented, negative-sense, single-stranded RNA. Significantly, the viral surface features two key glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which protrude as distinctive spikes and are critical for viral attachment to and entry into host cells [6]. The genomes of Influenza A and B viruses are composed of eight distinct segments of single-stranded RNA, each encoding one or more proteins. These viral proteins play critical roles in various stages of the viral life cycle, including attachment, entry, replication, transcription, and translation, as well as assembly and budding, further contributing to the overall virion structure [6].

RSV, currently classified within the genus *Orthopneumovirus*, family *Pneumoviridae* (formerly genus *Pneumovirus*, family *Paramyxoviridae*), is an enveloped virus that contains a single-stranded, non-segmented, negative-sense, linear RNA genome. This genome comprises approximately 15,222 nucleotides (nt) in length and encodes 11 proteins [7].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded, positive-sense RNA virus classified within the family *Coronaviridae* [8], originated in Wuhan, China, in December 2019, where it caused a cluster of pneumonia cases. The virus rapidly disseminated globally, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern (PHEIC) on January 30, 2020, which was subsequently elevated to a pandemic on March 11, 2020 [9]. COVID-19 infections may manifest as asymptomatic or symptomatic. Symptomatic individuals can present a diverse array of clinical manifestations, including fever, cough, headache, dyspnea, tachypnea, myalgia, anosmia, ageusia, nausea,

vomiting, rhinorrhea, and diarrhea, with each symptom exhibiting variable degrees of severity [10]. Severe outcomes of COVID-19 infection, including mortality, are more frequently observed in individuals with pre-existing comorbidities such as cardiovascular disease, diabetes mellitus, chronic respiratory diseases, or immunocompromising conditions [10].

The emergence of the COVID-19 pandemic in early 2020 significantly shifted public health priorities in Nigeria, potentially reducing surveillance of influenza and RSV despite their significance and co-infection risks with SARS-CoV-2 [11].

Influenza viruses are recognized for their significant zoonotic potential, with well-established patterns of transmission to humans. Interspecies transmission has been extensively modeled and confirmed through numerous studies, underscoring the critical importance of surveillance efforts to mitigate zoonotic transmission risks [12, 13]. The sporadic transmission of influenza viruses from wild avian species to domestic poultry, followed by zoonotic spillover to humans, can precipitate severe outbreaks, thereby underscoring the necessity of robust surveillance at the animal-human interface. Influenza viruses, alongside RSV and SARS-CoV-2, threaten vulnerable groups, particularly children under five and adults over 51, with co-infections increasing hospitalization risks [14]. This burden is particularly pronounced in sub-Saharan Africa, largely due to resource limitations within public health systems, encompassing constrained surveillance capacity, limited data availability, and challenges in healthcare access [15].

This study sought to determine the prevalence of RSV and Influenza A and B infections among individuals testing negative for SARS-CoV-2 in Oyo State, Nigeria, utilizing real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Specifically, the study focused on two distinct age cohorts: pediatric participants under the age of five years and adult participants in three age groups. The findings of this study are expected to contribute valuable data to inform evidence-based public health interventions aimed at mitigating the impact of RSV and influenza in Nigeria.

## MATERIAL AND METHODS

**Study design and sample collection.** This retrospective study employed nasopharyngeal swab samples collected between January and June 2023 in Oyo State, Nigeria. Upon collection, all respiratory samples underwent testing for SARS-CoV-2 via RT-qPCR. This study included samples from participants with respiratory symptoms who tested negative for SARS-CoV-2 to avoid confounding by concurrent infection. A total of 206 samples, accompanied by their corresponding clinical data, were selected based on the following inclusion criteria: (1) age <5 years (pediatric participants) or adult participants; and (2) absence of a prior laboratory-confirmed diagnosis of influenza virus or RSV infection.

Conversely, samples were excluded if participants had a documented history of laboratory-confirmed influenza or RSV infection prior to the study period.

**RNA extraction.** Respiratory samples, previously stored at  $-80^{\circ}\text{C}$ , were thawed and RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, LOT 154050800), following the manufacturer's recommended protocol [16]. All procedures were conducted under sterile conditions at an RNase- and DNase-free workstation, adhering to standard biosafety level II practices. Extracted RNA was immediately stored at  $-80^{\circ}\text{C}$  until further processing via RT-qPCR.

**Molecular detection.** Extracted RNA was subjected to a multiplex real-time RT-qPCR assay for the simultaneous detection of RSV, Influenza A, and Influenza B. The Allplex™ Respiratory Panel 1 Assay kit (Seegene Inc., South Korea) was employed for the amplification and detection of target viral nucleic acid sequences. This assay utilizes virus-specific primers and probes and incorporates fluorescence-based technology to achieve sensitive and specific detection of each target virus.

**RT-qPCR protocol.** The Allplex™ Respiratory Panel 1 Assay kit comprises a lyophilized master mix containing

target-specific primers and probes (Table 1), reaction buffer, deoxyribonucleotide triphosphates (dNTPs), magnesium ions ( $\text{Mg}^{2+}$ ), Taq DNA polymerase, and reverse transcriptase, provided in a single vial (1X) [16, 17]. The kit also incorporates a lyophilized positive control containing quantified, inactivated Influenza A virus, Influenza B virus, and RSV nucleic acids, supplied in a single tube (1X), and a negative control consisting of 0.25 mL of sterile normal saline solution, also supplied in a single tube (1X). The QIAamp® diluent was reconstituted with 1.40 mL of molecular-grade water according to the manufacturer's instructions, and supplied in a single tube (1X). All primers and probes were reconstituted according to the manufacturer's guidelines, adhering to strict cold chain maintenance principles.

Reverse transcription was performed at  $50^{\circ}\text{C}$  for 30 min, followed by an initial denaturation step at  $95^{\circ}\text{C}$  for 10 min. Amplification was achieved through 45 cycles, with each cycle consisting of a denaturation phase at  $95^{\circ}\text{C}$  for 30 seconds, an annealing phase at  $60^{\circ}\text{C}$  for 30 seconds, and an extension phase at  $72^{\circ}\text{C}$  for 60 seconds. A final extension step was conducted at  $72^{\circ}\text{C}$  for 5 min, and the reaction was subsequently held at  $4^{\circ}\text{C}$ .

**Table 1.** Primer and probe sequences used in the multiplex RT-qPCR assay [17, 18]

Virus	Target Gene	Primer/Probe Sequence (5' to 3')
Influenza A	Matrix protein (M)	<b>Forward:</b> CAA GAC CAA TCG TGT CAC CTC <b>Reverse:</b> GCA TTY TGG ACA AAV CGT CTA CG <b>Probe:</b> TGC AGT CCT/ZEN/CGC TCA CTG GGC AGC
Influenza B	Nonstructural protein (NS)	<b>Forward:</b> TCC TCA AYT CAC TCT TCG AGC AGC G <b>Reverse:</b> CGG TGC TCT TGA CCA AAT TGG <b>Probe:</b> CCA ATT CGA /ZEN/GCA GCT GAA ACT GCG GTG
RSV (Subgroup A)	Nucleoprotein (N)	<b>Forward (A21):</b> GCTCTTAGGAAAAGTCAAGTTGAA <b>Reverse (A102):</b> TGCTCCGTTGGATGGTGTATT <b>Probe:</b> AACTCAACAAAAGATCAACTTCTGTATCCAGC
RSV (Subgroup B)	Nucleoprotein (N)	<b>Forward (B17):</b> GATGGCTCTTAGCAAAGTCAAGTTAA <b>Reverse (B120):</b> GTCAATATTATGCTGTACTACGTTGAA <b>Probe:</b> TGATACATTAATAAGGATCAGCTGCTGTATCCA

**Ethical consideration.** This retrospective study received ethical approval from the University of Ibadan/University College Hospital Joint Research Ethics Committee (UI/EC/23/0607) and the Oyo State Ethics Committee Secretariat, Ibadan. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH-GCP) guidelines.

The research utilized biorepository samples obtained from participants who had previously provided written informed consent for future research use. The informed consent process encompassed comprehensive pre-research education regarding the study's nature and purpose, the voluntary nature of participation and the right to withdraw consent at any time without penalty, and data protection measures. All samples were anonymized prior to analysis to ensure participant confidentiality.

Furthermore, samples from individuals without documented consent were excluded from the study.

**Data analysis.** Prior to formal analysis, the dataset underwent a rigorous data cleaning process to identify and address any logical inconsistencies. This included verifying the congruence between reported symptoms and corresponding laboratory findings, as well as scrutinizing date and time entries for any improbable values. Statistical analyses were performed using IBM SPSS Statistics, version 25. Descriptive statistics, specifically means with accompanying standard deviations (SD), were employed to summarize the age distribution of the study participants. Virus detection rates were expressed as percentages, often accompanied by 95% confidence intervals (CIs) to provide a measure of precision. Associations between categorical variables were assessed using the *chi-square* ( $\chi^2$ ) test. The threshold for statistical significance was set at  $P < 0.05$ .

## RESULTS

**Study population and viral detection rates.** A total of 206 individuals were enrolled in this study, comprising

106 children and 100 adults. Within the pediatric cohort (1–5 years), 3 (2.8%) tested positive for RSV in children aged 1, 2, and 5 years, and 3 (2.8%) tested positive for Influenza B in children aged 1, 3, and 5 years (Table 1).

**Table 1.** Prevalence of respiratory virus infections among children by age group in Oyo State, Nigeria

Age group (years)	Number of participants (%)	Influenza A positive n (%)	Influenza B positive n (%)	RSV positive n (%)
1	30 (28.3)	0 (0.0)	1 (3.3)	1 (3.3)
2	16 (15.1)	0 (0.0)	0 (0.0)	1 (6.3)
3	21 (19.8)	0 (0.0)	1 (4.8)	0 (0.0)
4	6 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)
5	33 (31.1)	0 (0.0)	1 (3.0)	1 (3.0)
Children total	<b>106 (100.0)</b>	<b>0</b>	<b>3 (2.8)</b>	<b>3 (2.8)</b>

**Note:** Chi-square analyses revealed no statistically significant association between age group and the detection of influenza B virus ( $P = 0.916$ ) or RSV ( $P = 0.827$ ). The mean age of the pediatric participants was 2.97 years.

**Distribution of infections by sex.** Stratifying by sex, our analysis revealed that 1 of 58 (1.7%) male children and 2 of 48 (4.2%) female children tested positive for RSV, indicating a numerically higher prevalence in

female children. However, this difference did not reach statistical significance. Within the pediatric group, three male children (5.2%) tested positive for influenza B virus and a child (1.7%) tested positive for RSV (Table 2).

**Table 2.** Prevalence of respiratory viruses by sex among children in Oyo State, Nigeria

Sex	No. of participants (%)	Influenza A positive n (%)	Influenza B positive n (%)	RSV positive n (%)
Male	58 (54.7)	0	3 (5.2)	1 (1.7)
Female	48 (45.3)	0	0	2 (4.2)

**Note:** Statistical chi-square analyses for the association between sex and the detection of influenza B virus and RSV in children yielded  $P$ -values of 0.218 and 0.302, respectively. Influenza A virus was not detected in this pediatric cohort.

Generally, this study determined an overall prevalence of 2.8% for RSV and 2.8% for influenza B virus among

children. Notably, influenza A virus was not detected in this pediatric age group.

**Table 3.** Overall prevalence of respiratory viruses among children in Oyo State, Nigeria

Virus	Positive n (%)	Negative n (%)
Influenza A	0	106 (100.0)
Influenza B	3 (2.8)	103 (97.2)
RSV	3 (2.8)	103 (97.2)

**Note:** Total number of children = 106.

In adults, the prevalence rates were 1.0% for influenza A virus, 1.0% for influenza B virus, and 2.0% for RSV (Table 4). It is important to note that age data were unavailable for five adult participants due to religious beliefs. However, among these individuals, within the female subgroup ( $n=45$ ), 1 (2.2%) tested positive for influenza B virus, and 2 (4.4%) tested positive for RSV. On the other hand, adult participants were stratified into three age groups: 15–35 years, 36–50 years, and  $\geq 51$  years. In the 15–35 year age group, influenza B virus and RSV were each detected in 1 participant (2.0%). The 36–50 year age group exhibited the detection of influenza A

virus in 1 participant (4.0%) and RSV in 1 participant (2.4%). Conversely, no viral detections were observed in the  $\geq 51$  year age group. Table 5 presents the detection rates of tested agents stratified by age group among adults, while Table 6 summarizes detection rates by gender, including individuals for whom age data were unavailable.

Table 7 details the prevalence of respiratory virus infections by senatorial district in Oyo State, specifying the distribution of positive cases for each virus across the three districts.

**Table 4.** Overall prevalence of respiratory viruses among adults in Oyo State, Nigeria

Virus	Positive n (%)	Negative n (%)	Total
Influenza A	1 (1.0)	99 (99.0)	100
Influenza B	1 (1.0)	99 (99.0)	100
RSV	2 (2.0)	98 (98.0)	100

**Note:** Total number of adults = 100.

**Table 5.** Prevalence of respiratory viruses by age group among adults in Oyo State, Nigeria

Age group	No. of participants (%)	Influenza A positive n (%)	Influenza B positive n (%)	RSV positive n (%)
15-35	49 (49.0)	0 (0.0)	1 (2.0)	1 (2.0)
36-50	41 (41.0)	1 (4.0)	0 (0.0)	1 (2.4)
≥51	10 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)

**Note:** Statistical chi-square analyses for the association between age group and the detection of influenza A virus, influenza B virus, and RSV among adults yielded *P*-values of 0.387, 0.789, and 0.534, respectively. The mean age of the adult participants was 33.5 years.

**Table 6.** Prevalence of respiratory virus infection by sex among adults in Oyo State, Nigeria

Sex	No. of participants (%)	Influenza A positive n (%)	Influenza B positive n (%)	RSV positive n (%)
Male	55 (55.0)	1 (1.8)	0 (0.0)	0 (0.0)
Female	45 (45.0)	0 (0.0)	1 (2.2)	2 (4.4)

**Note:** Statistical chi-square analyses for the association between sex and the detection of influenza A virus, influenza B virus, and RSV among adults yielded *P*-values of 0.383, 0.247, and 0.100, respectively.

**Table 7.** Distribution of respiratory virus infections by senatorial district among adults in Oyo State, Nigeria

Senatorial district	No. of participants (%)	Influenza A positive (n, %)	Influenza B positive (n, %)	RSV positive (n, %)
Oyo North	5 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
Oyo Central	43 (43.0)	0 (0.0)	1 (2.3)	1 (2.3)
Oyo South	52 (52.0)	1 (1.9)	0 (0.0)	0 (0.0)

**Note:** Chi-square analyses revealed no statistically significant association between senatorial district and the detection of influenza A virus (*P* = 0.818) or influenza B virus (*P* = 0.633). However, a statistically significant association was observed between senatorial district and RSV detection (*P* = 0.049).

## DISCUSSION

This study enrolled 206 participants, comprising 106 children and 100 adults. Within the pediatric cohort, three (2.8%) tested positive for RSV. These positive cases were identified in children aged 1 year (3.3% prevalence), 2 years (6.3% prevalence), and 5 years (3.0% prevalence). Furthermore, three children (2.8%) tested positive for influenza B virus, with age-specific prevalence rates of 3.3%, 4.8%, and 3.0% for children aged 1, 3, and 5 years, respectively. Influenza A virus was not detected within the pediatric cohort. Children showed a slight male predominance (54.7%, *n* = 58) over females (45.3%, *n* = 48), as did adults (55.0%, *n* = 55 vs. 45.0%, *n* = 45). These findings underscore the ongoing circulation of RSV and influenza B virus within this population, even in the context of the COVID-19 pandemic. They highlight the continued importance of robust surveillance efforts to inform public health strategies and enhance the clinical management of these respiratory viral infections in Oyo State, Nigeria. Overall prevalence was 0%, 2.8%, and 2.8% for Influenza A, Influenza B, and RSV in children, and 1.0%, 1.0%, and 2.0% in adults, respectively.

A similar study conducted in the USA investigated the burden of respiratory infections associated with seasonal influenza in children under 5 years of age [18]. The authors reported that influenza viruses were responsible for an estimated 10.1 million cases of acute lower respiratory tract infection (ALRI), leading to 870,000 hospitalizations and 15,300 in-hospital deaths, and contributing to a total of up to 34,800 influenza virus-associated ALRI deaths. According to that study, influenza virus was responsible for 7% of ALRI cases, 5% of ALRI hospitalizations, and 4% of ALRI deaths in that

pediatric population. In comparison, our study, which focused on individuals negative for COVID-19 in Oyo State, Nigeria, and included both children and adults, differed slightly in study design from the aforementioned study by Wang *et al.* (2020) [18]. A key distinction is that our study included adults, whereas the study by Wang *et al.* (2020) [18] focused solely on children. Despite these methodological differences, our findings contribute to the understanding of the substantial global burden of influenza viruses and RSV across various age groups. This difference underscores the need for comprehensive surveillance of respiratory infections across all age groups to enhance our understanding of their epidemiology and inform effective public health interventions.

In this study, the mean age of the children was 2.97 years, with an RSV prevalence of 2.8%. Conversely, the mean age of the adult participants was 33.5 years, with an RSV prevalence of 2.0%. The RSV prevalence observed in both children and adults in this study was considerably lower than that reported by Faneye *et al.* (2014) [19], who conducted a study among preschool children in the geographically proximate north-central region of Nigeria. Specifically, they reported an RSV prevalence of 85.7% in children and 23.3% in adults, compared to 2.8% and 2.0%, respectively, in this study. This substantial difference in prevalence rates may be attributed to methodological differences, such as the use of serological testing (IgG antibodies) by Faneye *et al.* (2014), which detected past exposure, compared to the RT-qPCR method used in this study that identifies active infections. Additional factors, such as differences in study population, seasonality of sample collection, or regional variations in RSV transmission dynamics, may also

contribute to the observed discrepancy. Generally, northern and north-central Nigeria have historically experienced challenges with vaccine hesitancy and refusal, resulting in lower vaccine uptake and potentially contributing to a higher burden of vaccine-preventable diseases, including RSV [20].

Co-circulating respiratory viruses, including those investigated in this study, are known to contribute to ALRIs in both children and adults [15, 21]. While a large study by Wang *et al.* (2020) in the US reported a significant association between age (0–59 months) and influenza virus-associated ALRI with hypoxemia ( $P < 0.05$ ), this study did not show any statistically significant association between age and either influenza B virus or RSV infection ( $P > 0.05$ ). Similarly, no statistically significant association was found between sex and influenza B virus or RSV infection among children ( $P > 0.05$ ) or between sex and any of the three viruses (influenza A virus, influenza B virus, and RSV) among adults ( $P > 0.05$ ). These findings underscore the critical importance of equitable vaccine access and administration, irrespective of sex, to enhance vaccine coverage and improve the prevention and control of RSV and influenza B virus infections across diverse populations. Such endeavors are crucial for strengthening our understanding of the epidemiology and outcomes associated with these infections.

Surveillance data regarding influenza A and B virus infections in Nigeria are limited, largely due to incomplete records maintained by the Nigeria Centre for Disease Control (NCDC) [21]. This study was conducted in Oyo State, a southwestern state in Nigeria, and enrolled participants with a diverse geographic distribution. Specifically, the geographic distribution of participants was as follows: 52% from Oyo South, 43% from Oyo Central, and 5% from Oyo North. Varying prevalence rates of respiratory virus infections were observed across the three senatorial districts. Among adults, the prevalence of influenza B virus and RSV in Oyo Central was 2.3% for both pathogens, while no cases of influenza B virus or RSV were detected in Oyo North or Oyo South. Among adults, the prevalence of influenza A virus was 1.9% in Oyo South and 0% in both Oyo North and Oyo Central. Although this study lacked data on endemic thresholds and historical trends for these viruses in the region, the observed prevalence rates suggest the ongoing circulation of these pathogens within Oyo State.

A chi-square test was performed to examine the association between age group (children *versus* adults) and respiratory virus infection status (positive or negative for RSV, influenza A virus, or influenza B virus). This analysis showed no statistically significant association between age group and infection status. However, it is crucial to acknowledge the inherent limitation of our study's statistical power due to the sample size. Consequently, while these findings suggest that age may not be a significant risk factor for infection with these

respiratory viruses within this specific study population, this conclusion warrants cautious interpretation. This contrasts with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, where advanced age is a well-established risk factor for severe disease [22].

In conclusion, this study elucidates the circulation of RSV, influenza A virus, and influenza B virus among individuals negative for SARS-CoV-2 in Oyo State, Nigeria, underscoring the co-circulation of these respiratory viral pathogens within the community. While RSV and influenza B virus were detected in both children and adults, influenza A virus was exclusively observed in adults. These findings underscore the critical importance of establishing and maintaining robust surveillance systems to accurately monitor the prevalence and circulation patterns of these viral pathogens. Furthermore, these results highlight the imperative for comprehensive public health interventions, including targeted vaccination campaigns and awareness programs, to effectively prevent and control the dissemination of these respiratory infections within Oyo State and across other regions of Nigeria.

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#### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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