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Original Article

Detection of SARS-CoV-2 and Absence of Influenza A/B Among Acute Symptomatic Respiratory Cases in Khartoum, Sudan

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ABSTRACT

Background: Upper Respiratory Tract Infections (URTIs) are among the most common causes of morbidity worldwide, particularly in low-resource settings. Viral pathogens such as severe acute respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B often present with overlapping clinical symptoms, complicating diagnosis and management. Aim This study aimed to assess the prevalence of SARS-CoV-2, influenza A, and influenza B among symptomatic patients in Khartoum, Sudan, using a multiplex Real-Time Polymerase Chain Reaction (RT-PCR) assay. **Materials and Methods:** Nasopharyngeal Aspirates (NPAs) were collected from 35 patients presenting with acute respiratory symptoms of ≤3 days duration. Multiplex RT-PCR was performed to detect SARS-CoV-2, influenza A, and influenza B viruses. **Results:** Results of the 35 patients, 42.8% tested positive for SARS-CoV-2, while no cases of influenza A, influenza B, or viral co-infection were detected. **Conclusion:** These findings underscore the dominance of SARS-CoV-2 and highlight the utility of multiplex RT-PCR for differential diagnosis in Sudan's surveillance efforts. The absence of influenza may reflect seasonal variation, public health interventions, or limited sample size.

Keywords: SARS-CoV-2, Influenza virus, Co-infection, Multiplex RT-PCR, Nasopharyngeal aspirates, Respiratory viral infections, Khartoum State, Sudan, Acute respiratory symptoms, Viral RNA detection.

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INTRODUCTION

Respiratory viruses remain a significant global health concern, particularly in low-resource settings where diagnostic infrastructure and surveillance systems are limited. Among the most impactful are coronaviruses and influenza viruses, both of which have demonstrated pandemic potential and high transmissibility. Severe acute respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19), emerged in late 2019 in Wuhan, China, and rapidly escalated into a global pandemic (Huang, 2020; Zhou, 2020). By mid-2025, over 700 million confirmed cases and nearly 7 million deaths had been reported worldwide, with estimates suggesting that more than 3 billion individuals have been infected at least once (Mishra, 2025).

SARS-CoV-2, a Betacoronavirus within the Coronaviridae family, has the largest known RNA genome (~30 kb) (Gorbalenya, 2020). Coronaviruses infect both humans and animals, causing a spectrum of diseases including respiratory, enteric, renal, and neurological disorders (Matoba, 2018). Three major zoonotic outbreaks, SARS (2002), MERS (2012), and COVID-19 (2019), have highlighted the pandemic potential of coronaviruses. (Hu, 2024; Joint WHO-China Study Team, 2021; Zaki, 2012). Influenza viruses, belonging to the Orthomyxoviridae family, are characterized by segmented negative-sense RNA genomes (Angius, 2024). The two primary genera infecting humans are Alpha influenzavirus (Influenza A) and Beta influenzavirus (influenza B). Birds serve as a critical reservoir for these viruses, particularly for influenza A. Influenza A viruses are classified into 18 HA and 11 NA subtypes, while influenza B comprises Victoria and Yamagata lineages. Highly pathogenic avian influenza strains such as H5N1 have demonstrated mortality rates approaching 50% in humans, with over 100 million birds affected across

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Asia, Africa, and the Middle East since 2003 (Sesifredo, 2025). Historically, influenza has caused significant global morbidity and mortality. The 1918 H1N1 pandemic resulted in an estimated 40 million deaths worldwide (Clayville, 2011). Seasonal influenza epidemics typically occur in winter in temperate regions but can persist year-round in tropical climates (Shi, 2025). Seasonal influenza causes up to 500,000 deaths annually, with year-round circulation in tropical regions. (Clayville, 2011). Both viruses spread via droplets and aerosols and present with overlapping symptoms such as fever, cough, and fatigue, complicating diagnosis. However, distinct features of SARS-CoV-2 are associated with hematological abnormalities, while influenza often causes severe myalgia (Du, 2020; Marofi, 2021; To, 2020; Schuit, 2020; Uyeki, 2019).

Risk factors for severe disease in both infections include advanced age (>50 years), cardiovascular disease, metabolic disorders, obesity, psychiatric conditions, and immunosuppression (Morens,2011 and Mertz,2013). Complications of SARS-CoV-2 include Acute Respiratory Distress Syndrome (ARDS), secondary bacterial and fungal infections, and multi-organ involvement (Rawson, 2020; Hu, 2020). Influenza complications primarily involve viral and secondary bacterial pneumonia, with additional cardiovascular, renal, and neurological sequelae (Uyeki, 2019; Long, 2020). Coinfection with SARS-CoV-2 and influenza viruses has been documented globally, with a pooled prevalence of approximately 14% (Golpour, 2025). Although relatively uncommon, such coinfections are associated with increased disease severity, particularly in vulnerable populations such as children and the elderly. A recent meta-analysis of 38 studies estimated a global coinfection rate of 14%, with higher prevalence in Asia and Europe (Golpour, 2025). In children, coinfection is linked to more severe symptoms and prolonged hospitalization (Săsăran, 2025).

Understanding the virological and clinical interplay between SARS-CoV-2 and influenza viruses is essential for developing effective diagnostic and therapeutic strategies. Multiplex Real-Time Polymerase Chain Reaction (RT-PCR) assays offer a valuable tool for simultaneous detection of multiple respiratory pathogens, enabling timely and accurate diagnosis. In resource-limited settings like Sudan, such assays can enhance diagnostic capacity and inform public health responses. However, challenges remain, including cost, infrastructure, and training.

This study aims to assess the prevalence of SARS-CoV-2, influenza A, and influenza B among patients presenting with acute respiratory symptoms in Khartoum, Sudan, using a multiplex RT-PCR assay. By evaluating the circulation of these viruses and their clinical manifestations, we seek to contribute to the growing body of evidence on respiratory virus epidemiology in sub-Saharan Africa and support the development of targeted public health interventions.

MATERIALS AND METHODS

Study Design and Population

This descriptive cross-sectional study was conducted at a private diagnostic laboratory in Khartoum State, Sudan, from January to March 2023. The study targeted patients aged 18 years and older who presented with acute respiratory symptoms of ≤3 days duration, including fever, cough, sore throat, nasal congestion, and fatigue. Patients with chronic respiratory conditions or recent antibiotic use within the past month were excluded to minimize confounding factors.

A total of 35 patients meeting the inclusion criteria were enrolled. Each participant was asked to complete a structured questionnaire capturing demographic and clinical data, including age, gender, occupation, symptom onset, clinical presentation, response to antibiotics, and location of sample collection.

Ethical Considerations

Ethical approval for the study was obtained from the Al-Neelain University Research Committee, Khartoum State, Sudan. Written informed consent was secured from all adult participants.

Sample Collection and Processing

Nasopharyngeal Aspirates (NPAs) were collected from all 35 patients using sterile regular flocked swabs (Cat. No. 520CS01, Copan Diagnostics Inc., Murrieta, CA, USA). Each swab was placed into viral transport medium and maintained under cold chain conditions. Samples were stored at 4°C for short-term use (≤72 hr), at −20°C for up to one week, or at −80°C for long-term preservation until RNA extraction.

RNA Extraction

Total viral RNA was extracted from the NPAs using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol.

Multiplex RT-PCR Assay

A commercial Respiratory Multiplex RT-PCR kit (LABSYSTEMS DIAGNOSTICS, Finland) was used to detect SARS-CoV-2, influenza A, influenza B, and Respiratory Syncytial Virus (RSV A/B). The assay targets the following viral genes: SARS-CoV-2: ORF1ab, Influenza A: M gene, Influenza B: HA gene, RSV A/B: RSV-specific gene, and Internal control: Human β-globin gene

Each reaction consisted of 8 µL of RT-PCR buffer and enzyme mix, 2 µL of respiratory prime-probe mix, and 15 µL of extracted RNA, yielding a final volume of 25 µL. Amplification was performed using the Applied Biosystems 7500 Real-Time PCR System. The thermal cycling protocol included reverse transcription at 45°C for 20 min, initial denaturation at 95°C for 3 min, followed by 44 amplification cycles of denaturation at 95°C for 15 sec and annealing/extension at 59°C for 20 sec. Fluorescence detection

was activated in the FAM, HEX, TEXAS RED, CY5.5, and CY5 channels, with "none" selected for passive reference dye. Positive controls containing detection sequences for all target viruses and an internal control were included in each run to verify assay performance. Negative controls were used to confirm the absence of contamination.

Statistical Analysis

All collected data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS), version 12.0. Descriptive statistics were used to summarize demographic and clinical variables. Associations between symptom distribution and demographic factors (age group, gender) were assessed using Fisher's Exact Test where appropriate. A p-value≤0.05 was considered statistically significant. No significant differences in symptom distribution were observed between age groups or genders (p>0.05 for all comparisons).

RESULTS

Demographic Characteristics

A total of 35 patients were enrolled, with a mean age of 39.2 years (range: 18-80). Of these, 20 (57.1%) were males and 15 (42.9%) were females. The majority (53.3%) were aged 18-40 years, while 46.7% were aged 41-80 years.

Clinical Presentation

All patients presented with fever (100%), followed by cough (86.7%), sore throat (73.3%), and nasal congestion (60%). Fatigue and myalgia were reported in (40%) and (33.3%) of cases, respectively, as illustrated in Figure 1. No significant differences in symptom distribution were observed between age groups or genders, as illustrated in Figure 2.

RT-PCR Findings

RT-PCR analysis was conducted on 35 Nasopharyngeal Aspirates (NPAs). Of these, 15 samples (42.8%) tested positive for SARS-CoV-2. None of the samples showed evidence of influenza A or influenza B infection, and no cases of viral co-infection were detected. Among the SARS-CoV-2-positive patients, 60% were male, and 53.3% were between the ages of 18 and 40 years. All individuals in this group reported experiencing fever. Additionally, 86.7% presented with cough, while 73.3% reported having a sore throat.

DISCUSSION

The findings of this study reflect the continued predominance of SARS-CoV-2 among patients presenting with acute respiratory symptoms in Khartoum, Sudan. Of the 35 nasopharyngeal aspirates analyzed, 42.8% tested positive for SARS-CoV-2, while no cases of influenza A, influenza B, or viral co-infection were detected. This pattern aligns with global observations during the COVID-19 pandemic, where influenza circulation declined significantly due to widespread public health interventions such as masking, social distancing, travel restrictions, and improved hygiene practices (Liang, 2024; Haseeb, 2023). Several studies have documented similar trends across different regions. In Saudi Arabia, a retrospective study conducted between 2020 and 2022 found that SARS-CoV-2 was the dominant pathogen among patients with respiratory symptoms, with influenza A and B accounting for less than 5% of cases during peak COVID-19 waves (AlBahrani, 2024; Al-Dorzi, 2020). In Yemen, where diagnostic infrastructure is limited due to ongoing conflict, surveillance data from Aden and Sana'a showed sporadic influenza detection but consistent SARS-CoV-2 positivity among symptomatic individuals (Al-Waleedi, 2020). These findings suggest that reduced influenza activity may be a regional phenomenon influenced by both behavioral and structural factors. A recent meta-analysis of 38 studies from Asia, Europe, and the Middle East estimated the global prevalence of SARS-CoV-2 and influenza co-infection at 14% (95% CI: 8-20%) (Golpour, 2025). Subgroup analysis showed that Asia had the highest co-infection rate for influenza A (17%), while Europe exhibited a higher prevalence for influenza B (20%) (Golpour, 2025). In contrast, studies from Arab countries such as Egypt, Jordan, and the United Arab Emirates reported co-infection rates below 10%, with most cases occurring among younger adults and healthcare workers (Golpour, 2025; Saleh, 2024; Alzahrani, 2025). These regional differences may be due to variations in testing capacity, vaccination coverage, seasonal virus circulation, and the degree of exposure. In our study, the absence of influenza A and B may be attributed to several factors. First, the sampling period coincided with a low influenza season in Sudan, which typically peaks between November and February. Second, public health measures implemented during the COVID-19 pandemic, such as masking, social distancing, and travel restrictions, likely suppressed influenza transmission. Third, the small sample size and single-center design may have reduced the likelihood of detecting co-infections. Similar trends were observed in Singapore, where researchers reported a significant decline in influenza activity during the pandemic, attributing it to both non-pharmaceutical interventions and decreased surveillance

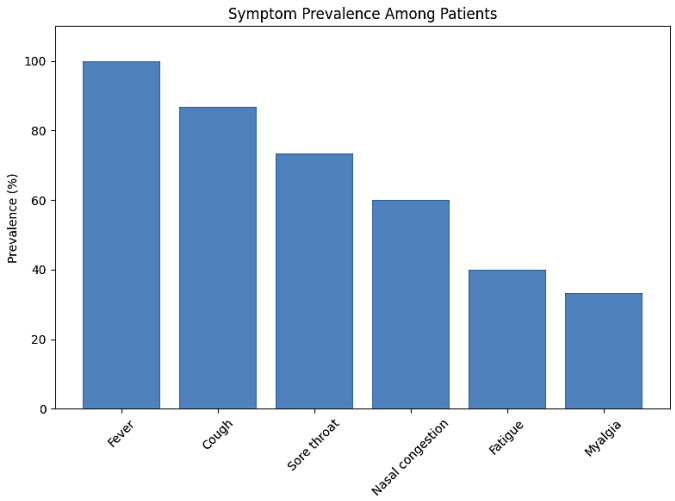

Figure 1: Symptom Frequency Among SARS-CoV-2 Positive Patients1.

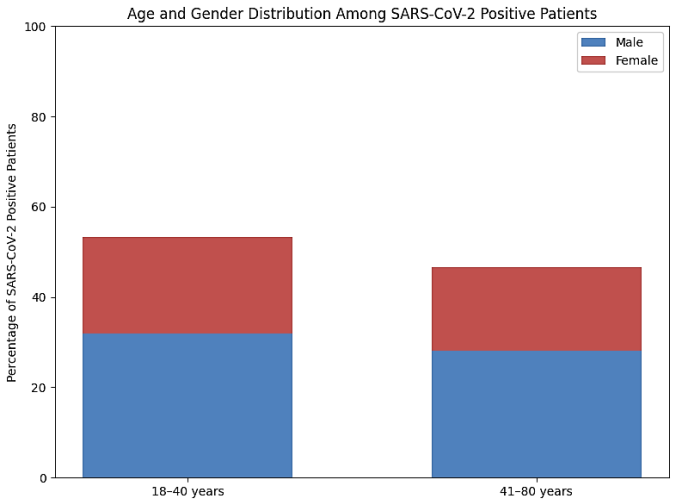

Figure 2: Age and Gender Distribution Among SARS-CoV-2 Positive Cases.

sensitivity (Ansah, 2021). In South Korea, national surveillance data showed that influenza virus detection sharply declined during COVID-19 waves, with authors noting that decreased testing capacity and prioritization of SARS-CoV-2 diagnostics led to underreporting of influenza cases (Lee, 2021). These findings emphasize the importance of robust, multi-pathogen surveillance systems, especially during overlapping epidemic seasons.

The demographic distribution of SARS-CoV-2 cases in our cohort, predominantly younger adults aged 18-40 years, mirrors global data showing higher infection rates among mobile and occupationally exposed populations. A study from the Mayo Clinic in the United States found that coinfected patients were relatively young (mean age: 26.7 years) and had fewer comorbidities than monoinfected COVID-19 patients (Pawlowski, 2022). In Saudi Arabia, younger males accounted for the majority of SARS-CoV-2 cases, a trend attributed to gender-based differences in healthcare-seeking behavior and occupational exposure (Alzahrani, 2025; Al-Hassinah, 2024).

The absence of co-infection with influenza A and B and RSV in our cohort contrasts with findings from Asia, where co-infection rates were higher during the Omicron wave in late November 2021. A modeling study from China estimated that co-infection with SARS-CoV-2 and influenza A increased hospitalization duration and mortality risk, particularly among elderly and immunocompromised patients (Pan, 2023). In Europe, coinfection cases were associated with more severe symptoms, including fever, myalgia, and respiratory distress, although mortality rates did not differ significantly from monoinfected cases (Golpour, 2025). In addition to clinical severity, co-infection poses diagnostic challenges. Both viruses share overlapping symptoms, and without multiplex testing, misdiagnosis or delayed treatment may occur. Studies from the United States and South Korea have emphasized the importance of early differentiation, especially during peak respiratory seasons (Lee, 2021; Cuadrado-Payán, 2020). Moreover, co-infection may complicate antiviral therapy decisions, as treatment regimens differ for SARS-CoV-2 and influenza. Co-infection with other respiratory pathogens, beyond those detectable by the kit we used, was not addressed in this study, nor was the cause of illness in patients who tested negative for COVID-19.

The public health implications of these findings are significant. The suppressed circulation of influenza during COVID-19 waves may lead to reduced population immunity, increasing the risk of future outbreaks. WHO has warned of potential "immunity debt" in populations with low influenza exposure during the pandemic (Cohen, 2021). In Sudan, where influenza vaccination coverage remains low, this risk is particularly concerning. Strengthening seasonal influenza vaccination campaigns, especially among high-risk groups, is essential. Furthermore, the integration of respiratory virus surveillance into national health systems can improve outbreak preparedness. Countries like Singapore and South Korea have successfully implemented sentinel surveillance programs that monitor multiple pathogens simultaneously. Sudan could benefit from similar models, adapted to local infrastructure and epidemiological needs.

Environmental and climatic factors also influence the transmission of respiratory viruses. Studies from East Asia and North Africa have shown that temperature, humidity, and air pollution levels correlate with seasonal spikes in influenza and SARS-CoV-2 cases (Shi, 2025). In Khartoum, fluctuating climate conditions and urban density may contribute to virus spread, although further research is needed to quantify these effects. Socioeconomic disparities play a critical role in respiratory disease outcomes. Limited access to healthcare, diagnostic services, and vaccination disproportionately affects low-income populations (Bambra, 2020; Al-Mandhari, 2020). In Sudan, economic instability and healthcare fragmentation exacerbate these challenges. Addressing these inequities through targeted outreach, subsidized testing, and community education is vital for improving respiratory health outcomes. Strengthening diagnostic infrastructure, promoting vaccination, and enhancing public health awareness are critical steps toward improving respiratory virus management in Sudan and similar settings. As global health systems transition beyond the acute phase of the COVID-19 pandemic, integrated approaches to respiratory virus detection and prevention will be vital in mitigating future outbreaks and improving clinical outcomes.

CONCLUSION

This study highlights the predominance of SARS-CoV-2 among patients with acute respiratory symptoms in Khartoum, Sudan, during early 2023. No cases of influenza A, B, or viral co-infection were detected, reflecting global patterns of suppressed influenza circulation during COVID-19 waves. Multiplex RT-PCR assays proved effective for differentiating respiratory pathogens, supporting their use in clinical and surveillance settings. Younger adults and males were more frequently affected, consistent with regional data. Despite limitations, the findings offer valuable insight into respiratory virus epidemiology in a resource-limited context. Strengthening diagnostic infrastructure, expanding surveillance, and promoting seasonal vaccination are essential for public health preparedness. Future research should involve larger cohorts, cover full seasonal

cycles, and incorporate genomic and serological methods to inform targeted interventions and improve outbreak response.

DATA AVAILABILITY STATEMENT

The data underlying this study are available from the corresponding author upon reasonable request. Due to patient confidentiality and ethical restrictions, raw clinical data cannot be publicly shared.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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