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Coinfections with SARS-CoV-2 variants and influenza virus during the 2019 Coronavirus disease pandemic in Burkina Faso: A surveillance study

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Abstract

Background and Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) particularly the variants of concern coinfections with influenza is a public health concern in Africa. We aimed to characterize the SARS-CoV-2 variants and determine the rate of coinfections with influenza in Burkina Faso.

Methods: COVID-19 surveillance study was conducted between August 2021 and January 2022 using reverse transcription polymerase chain reaction (RT-PCR). Positive specimens were further screened for SARS-CoV-2 variants using the multiple variants real-time PCR kits. In addition, influenza virus strains were detected by RT-PCR in SARS-CoV-2 positive specimens using the CDC primers, probes, and protocols.

Results: Of 324 specimens assessed, the Omicron and Delta variants of SARS-CoV-2 were the most prevalent with 27.2% [95% confident interval (CI): 22.5–32.4] and 22.2% [95% CI: 17.9–27.2], respectively. The Beta and Gamma variants were detected in 4.3% [95% CI: 2.4–7.1] and 0.3% [95% CI: 0.0–1.7], respectively. Coinfections of Omicron and Beta variants were reported in 21.3% [95% CI: 17.0–26.2], Omicron and Delta variants in 1.2% [95% CI: 0.3–3.1] of specimens, and the Omicron–Gamma variants' coinfections in 0.6% [95% CI: 0.1–2.2]. One COVID-19 specimen with an undetected SARS-CoV-2 variant was also tested positive for the seasonal influenza A (H_3N_2) virus. No cases of pandemic influenza A (H_1N_1) pdm09, seasonal A/H_1N_1, and influenza B were detected.

Conclusions: The current World Health Organization SARS-CoV-2 variants of concern were prevalent and their coinfections with influenza were uncommon. Continuous surveillance of both pathogens is, however, needed because of their public health implications.

KEYWORDS
Burkina Faso, COVID-19, influenza, mutation, SARS-CoV-2, variant

1 | INTRODUCTION

Simultaneous infections with more than one respiratory virus are not uncommon in human, and is associated with an increased risk of hospitalization, admission to intensive care unit and death.[1] In sub-Saharan Africa, an estimated 10% of patients with lower respiratory tract infections are coinfected with one or more viruses.[1] The occurrence of the novel Coronavirus disease 2019 (COVID-19) caused by a virus termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), phylogenetically related to the SARS-CoV[2,3] has significantly modified the landscape of respiratory infectious diseases, particularly, in sub-Saharan Africa.[4,5] SARS-CoV-2 has many variants with different infectivity properties, and the World Health Organization (WHO) variants of concern (VOC) are the biggest threat to the global population.[6,7] In Africa, 51 of the African Union member states (AU MS) reported the presence of the Alpha (45 MS), Beta (41 MS), Delta (43 MS), and/or Gamma (2 MS) VOC by December 9, 2021. The Omicron VOC was identified in 14 MS (Botswana, DR Congo, Ghana, Malawi, Mozambique, Namibia, Nigeria, Senegal, Sierra Leone, South Africa, Tunisia, Uganda, Zambia, and Zimbabwe) as of December 13, 2021, reported the presence of the Omicron VOC.[8] The disease commonly starts with nonspecific upper respiratory tract symptoms, making it challenging to distinguish from other respiratory diseases, in particular, influenza virus infections.[9] Indeed, the coinfections of SARS-CoV-2, particularly, the most virulent variants, with influenza viruses,[10,11] could jeopardize the control efforts because of their immunological interactions, and change in the disease pathogenicity.[12] As of March 12, 2022, globally, 452,201,564 confirmed cases of COVID-19 (6,029,852 deaths),[13] including 20,858 cases and 382 deaths in Burkina Faso were reported.[14]

Several COVID-19 treatment approaches have been extensively evaluated during the past years,[15,16] and while most of them have failed to show efficacy, herd immunity is encouraged to control the pandemic.[17,18] Vaccination using different platforms and nonpharmaceutical public health interventions remain the most promising approach for the control of the COVID-19 pandemic.[19,20] Although several vaccines have been authorized for use, there are still many concerns regarding their accessibility, hesitancy in communities, most importantly, their efficacy in preventing infection with emerging virus variants.[21] The apparition of different SARS-CoV-2 variants combined to their association with other respiratory viral infections could further threaten the vaccine efficacy and alters control efforts.[21] Preparedness is a key component for disease control efforts and response measures during an outbreak.[22] Therefore, continued surveillance is needed to provide sufficient epidemiologic data that will guide capability development to support control efforts and the WHO recommends each country to monitor circulating SARS-CoV-2 variants and influenza virus in its territory.[23]

In Burkina Faso, less than 10% of the population received at least one dose of the COVID-19 vaccine, and yet no study has assessed neither the circulating variants of SARS-CoV-2 nor their association with other respiratory viral infections, particularly, influenza viruses. Therefore, Burkina Faso National Influenza Reference Laboratory (NIRL), which is in charge of influenza and COVID-19 surveillance, developed a protocol to screen SARS-CoV-2 variants circulating in the country and their coinfections with influenza virus between August 2021 and January 2022 so that proactive prevention measures can be initiated.

2 | MATERIALS AND METHODS

2.1 | Specimens' management

Nasopharyngeal or oropharyngeal swabs were collected from COVID-19 suspected cases, contacts cases, and travelers and were stored in universal transport medium (Copan Diagnostics). Specimens were shipped to the NIRL for analysis.

2.2 | RNA extraction

The viral RNA was automatically extracted from 200 µl of the nasopharyngeal or oropharyngeal swabs using the KingFisher Flex instrument (Thermo Scientific Inc.) according to the manufacturer's instructions. The MS2 Phage Control provided in the kit was used to check the efficacy of the specimen preparation and the absence of inhibitors in the reverse transcription polymerase chain reaction (RT-PCR). For that purpose, it was recommended to add 5 µl to each sample well and to the negative control just before extraction. Nuclease-free water (not DEPC-treated) containing MS2 Phage Control was used as the negative control. Approximately 50 µl of the total nucleic acid eluate was recovered into elution plate and either tested immediately or stored at −70°C until use.

2.3 | RT-PCR assay for SARS-CoV-2

The RT-PCR assay was performed using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Applied Biosystems). Briefly, each 25 µl of reaction mixture contained 7.5 µl of nuclease free water, 6.25 µl of ™ 1-Step Multiplex Master Mix, 1.25 µl of COVID-19 Assay Multiplex and 10 µl of RNA. Thermal cycling was performed at 25°C for 02 min, 53°C for 10 min, and 95°C for 02 min, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. Each run included one TaqPath™ COVID-19 positive control and one negative control. The multiplex RT-PCR assay targets ORF1ab, S and N genes and was performed using the

QuantStudio™ 5 real-time-reverse transcriptase PCR (real-time PCR) instrument (Applied Biosystem). Validation of results was performed automatically by the Applied Biosystems™ COVID-19 Interpretive Software based on the performance of the positive and negative controls. Specimens with a cycle threshold (C_t) value < 37 for one or more SARS-CoV-2 gene targets were considered positive for SARS-CoV-2.

2.4 | Screening of SARS-CoV-2 variants and influenza virus in COVID-19 patients

For influenza, all SARS-CoV-2 positive specimens were analyzed by real-time RT-PCR in ABI 7500 Fast thermal cyclers (Applied Biosystems) using CDC, Atlanta, primers, probes, and protocol[24] for influenza A and influenza B detection followed by influenza A subtyping for H_1N_1pdm09, and seasonal H_1N_1.

For SARS-CoV-2 variant detection, three multiplex real-time qualitative RT-PCR assays targeting some of the most widespread mutations were used on positive specimens for rapid and presumptive detection of the SARS-CoV-2 variants. The multivariant panel consisted of the following assays:

The SNPsig® SARS-CoV-2 (EscapePLEX) kit allows simultaneous discriminatory identification of four clinically significant mutations, E484K, K417N, K417T, and P681R in a first step and confirmation of a SARS-CoV-2 positive specimen in a second step.

The SNPsig® VariPLEX (COVID-19) real-time PCR Assay is a CE marked in vitro diagnostic real-time PCR multiplex assay intended for the allelic discrimination of SARS-CoV-2 VOC 20I/501Y.V1, 20H/501Y.V2, 20J/501Y.V3, and 20C/S.452R, as well as biologically significant mutations N501Y and E484K.

SARS-CoV-2^E Spike Delta/Omicron TaqMan Typing cibles the following targets SARS Spike ins214EPE, SARS Spike del157/158 SARS E gene, and UBC (human gene).

All three kits were used and their results interpreted according to the manufacturer's instructions (Supporting Information: Appendix 1). The SNPsig® SARS-CoV-2 (EscapePLEX) identifies the Beta, Gamma, or Delta variants, the SNPsig® VariPLEX (COVID-19) real-time PCR identifies the wild type (WT), E484K pos, Alpha, S484, N501+Ve, and S452 variants, and, the SARS-CoV-2^E Spike Delta/Omicron TaqMan Typing identifies the Omicron and Delta variants.

2.5 | Statistical analysis

Data were entered into an Excel database and transferred onto R statistical software (R Development Core Team, R Foundation for Statistical Computing) for statistical analysis. Proportions were calculated for categorical variables and summarized on frequency tables. Means and standard deviations were calculated for continuous variables. χ^2 or the Fisher exact tests were used to compare proportions, and the Student test to compare means. The binomial test was used to calculate the 95% confidence interval of the proportions. The significance level was set at 5% (two-sided).

3 | RESULTS

3.1 | Characteristics of the study participants

A total of 324 specimens collected from COVID-19-confirmed cases in Burkina Faso were included in the current study. The majority of study participants were male 69.3% (224/323) with a mean age of 39.2 (±13.2) years. Nearly 70% (225/321) were aged between 30 and 59 years, and the participants aged less than 30 years and over 60 years represented 25.2% (81/321) and 4.7% (15/321) of the study participants, respectively. Cases were mostly collected during the cold season (December and January; 79.4% [257/324]). Majority of the participants (87.6%, 134/153) were from the urban area in Ouagadougou and 11.8% (18/153) from Bagassi (a rural area). Specimens were collected from nine sites and the majority of them were from the country main international airport (43.5%, 141/324) and from the NIRL (26.5%, 86/324) (Table 1). Nearly all specimens were oropharyngeal and nasopharyngeal with 51.2% and 47.8%, respectively.

3.2 | Prevalence of SARS-CoV-2 variants in the study area

Of the total specimens analyzed using the EscapePLEX kit, the most common detected SARS-CoV-2 variant was the Beta in 26.5% (86/324) of specimens, then the Delta variant in 21.3% (69/324) and the Gamma variant in 0.9% (3/324) of the specimens. Variants coinfections were detected in 0.3% (1/324) and 0.6% (2/324) of specimens for Beta-Gamma and Beta-Gamma/Delta, respectively.

Of the 105 specimens tested with the SNPsig® VariPLEX kit, 3.8% (4/105) were negative to any variants. The Beta variant was detected in 1.9% (2/105) samples. The WT of SARS-CoV-2 was reported in 75.2% (79/105) samples, and the other mutations (not yet classified as variant) included the N501Y-VE, detected in 16.2% (17/105). There were coinfections of the N501Y-VE mutation with the Beta variant (1.9%, 2/105), and the WT was detected in 1.0% (1/105) of the tested specimens.

For the Delta/Omicron TaqMan Typingkit, the most detected variants were the Omicron (50.6%, 164/324), and the Delta (21.3%, 69/324). The Delta-Omicron co-coinfection was detected in 0.3% (1/324) of specimens.

From the overall analysis, the most detected SARS-CoV-2 variants were the Omicron (27.2%, 88/324) and the Delta (22.2%, 72/324). The most common coinfections was the Omicron–Beta (21.3%, 69/324) (Table 2). One COVID-19 specimen with an undetected SARS-CoV-2 variant was coinfected with the seasonal influenza A (H_3N_2) virus. No case of influenza A (H_1N_1)pdm09, seasonal A/H_1N_1 and influenza B was detected.

TABLE 1 Characteristic of the included participants ($n = 324$)

Characteristics	Number of cases	%
Sex		
Male	224	69.3
Female	99	30.7
Missing[a]	1	
Age group (in years)		
<30	81	25.2
30–59	225	70.1
60 and more	15	4.7
Missing[a]	3	
Residency		
Bagassi	18	11.8
Ouagadougou	134	87.6
Sabcé	1	0.7
Missing[a]	171	
Types of sampling		
Nasopharyngeal	155	47.8
Oropharyngeal	166	51.2
Mix	3	0.9
Sampling sites		
Airport	141	43.5
Bissa-Gold Site	15	4.6
NIRL	86	7.7
Military camp	25	26.5
Local United Nations health facility	13	4.0
Orezone mining site	17	5.2
Roxgold mining site	27	8.3
Period		
August 2021	16	4.9
September 2021	28	8.6
October 2021	7	2.2
November 2021	16	4.9
December 2021	28	8.6
January 2022	229	70.7
Status		
Contact case	51	16.2
Suspected case	42	13.3
Spontaneous screening	7	2.2
Control case	51	16.2
Travelers	164	52.1
Missing[a]	9	

[a]Missing values were not included in calculations, but only presented as number.

TABLE 2 Distribution of circulating SARS-CoV-2 variants and influenza A in Burkina Faso during the COVID-19 pandemic ($n = 324$)

SARS-CoV-2 variants	Number of cases	Percentage [95% CI]
Beta	14	4.3 [2.4–7.1]
Delta	72	22.2 [17.9–27.2]
Delta-Gamma	2	0.6 [0.1–2.2]
Gamma	1	0.3 [0.0–1.7]
Omicron	88	27.2 [22.5–32.4]
Omicron-Beta	69	21.3 [17.0–26.2]
Omicron-Delta	4	1.2 [0.3–3.1]
Omicron-Gamma	2	0.6 [0.1–2.2]
No variants detected	71	22.0 [17.5–26.8]
Influenza A (H_3N_2)	1	0.3 [0.01–1.7]
Influenza A (H_1N_1)	0	-
Influenza A (H_1N_1)pdm09	0	-
Seasonal influenza A/H_1N_1	0	-
Seasonal influenza B	0	-

Abbreviation: CI, confident interval.

4 | DISCUSSION

Burkina Faso is a landlocked country located in west Africa,[25] and has reported 20,858 cases of COVID-19 (including 382 fatal cases) by April 14, 2022.[14] Four different waves of COVID-19 outbreaks were recorded in the country respectively in march 2020, October 2020, May 2021, and November 2021.[8] Each wave was caused by a different SARS-CoV-2 variant with different transmission potential, severity and response to vaccines and therapeutics. This study aimed to describe the SARS-CoV-2 variants that were circulating in the country during the last two waves and to assess their coinfections with other respiratory viral infections. In the AU MS, 51 have reported the presence of the Alpha (45 MS), Beta (41 MS), Delta (43 MS), and/or Gamma (2 MS) VOC by December 9, 2021. As of December 13, 2021, 14 MS (Botswana, DR Congo, Ghana, Malawi, Mozambique, Namibia, Nigeria, Senegal, Sierra Leone, South Africa, Tunisia, Uganda, Zambia, and Zimbabwe) reported the presence of the Omicron VOC.[8] The next generation sequencing (NGS) were the preferred method to reconstruct the full genome of SARS-CoV-2 and study its properties.[26–28] These methods, however, require expensive advanced technologies, and well-trained human resource, not usually available in resource-limited settings. The use of the rapid in vitro real-time PCR test for SARS-CoV-2 variants detection appeared as valid alternative in these areas to accomplish adequate surveillance.[29] While the EscapePLEX kit was key to the identification of the Beta, the Gamma, and Delta variants of SARS-CoV-2 and their respective coinfections, the Delta/Omicron TaqMan Typingkit detected the Omicron and the Delta variants and their coinfections. The SNPsig®

VariPLEX kit detected few cases of the Beta variant, the WT (variant with no evidence of any impact on disease severity or increased transmission) and the mutation N501Y-VE (mutation not yet ranked as variant), thus could be key for early detection of emerging potential variants. The combination of the three molecular assays showed that the Omicron, Delta variants, and Omicron-Beta coinfections were the most represented in the country during the study period and mainly occurred during the cold and dry season (December and January). Our study showed that the current WHO VOC including the Omicron and Delta are predominant among circulating variants in the country. Thus, continuous surveillance is required to prevent new waves of outbreaks and their severe impact on the health systems.[23] Also, previous VOC, such as the Gamma and Beta types were detected in small numbers and could be indicative of a progressive replacement of previous variants. However, the presence of high proportions of the Beta and Omicron variants' coinfections suggests that the new variants do not only replace previous one, but cohabit with more severe effects. The presence of high proportions of the WT with the SNPsig® VariPLEX kit indicates the high occurrence of mutations although not yet classified either as VOC, variant of interest (VOI) nor variant under monitoring (VUM) and highlighted the variability of the SARS-CoV-2 genome.[30] The occurrence of several mutations in short period of time justified the need for continuous surveillance of SARS-CoV-2. SARS-CoV-2 coinfection with influenza virus was, however, uncommon and this could be related to the low prevalence of influenza infection as testing was conducted among patient with no evidence of influenza-like illnesses and thus explain the low prevalence of the coinfections.[31] Indeed, study conducted in the country in subjects with symptoms reported a higher prevalence of influenza A and B infections, thus studies should assess the populations and areas more at risk for targeted interventions.[31]

Some limitations are worth noting in the interpretation of the study results as reagents were available for 105 samples using the SNPsig® VariPLEX kit and results interpretation for this kit were performed on the available test results.

5 | CONCLUSION

This study showed that current VOC (Omicron, Delta), and previous VOC (Gamma, Beta) were circulating in Burkina Faso during the last two waves of COVID-19 outbreak, but SARS-CoV-2 coinfections with the influenza virus was uncommon. In addition, several mutations were observed and suggest the need for continuous surveillance to timely detect variants that may cause new waves of uncontrolled outbreaks in the country.

AUTHOR CONTRIBUTIONS

Moussa Lingani: Data curation; formal analysis; investigation; methodology; resources; software; validation; visualization; writing – original draft. **Assana Cissé**: Conceptualization; data curation; investigation; methodology; validation; visualization; writing – review and editing. **Dieudonné Tialla**: Data curation; investigation; validation; writing – review and editing. **Abdoul Kader Ilboudo** and **Madi Savadogo**: Investigation; methodology; visualization; writing – review and editing. **Catherine Sawadogo**: Investigation; validation; visualization; writing – review and editing. **Sandrine Gampini**: Data curation; investigation; methodology; resources; validation; visualization; writing – review and editing. **Grissoum Tarnagda**: Investigation; validation; writing – review and editing. **Maria Tao**: Methodology; validation; visualization; writing – review and editing. **Serge Diagbouga**: Investigation; methodology; supervision; validation; visualization; writing – review and editing. **Sanata Bamba**: Investigation; methodology; supervision; visualization; writing – review and editing. **Zekiba Tarnagda**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – review and editing. All authors have read and approved the final version of the manuscript. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This was a pandemic response/surveillance data and the study protocol ethical clearance was obtained from the national ethics committee of health research of Burkina Faso (clearance certificate number CERS-2020-7-126). In addition, data were fully anonymized to protect participants' identities and personal data. Usage was done in accordance with ethical regulations.

TRANSPARENCY STATEMENT

The lead author Moussa Lingani affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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