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# SARS-CoV-2 Variants Screening in Burkina Faso

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ABSTRACT

**Introduction**: In Sub-Saharan Africa, the data on the mutations and variants of circulating SARS-CoV-2 is limited. This study aimed to screen specific mutations and variants of SARS-CoV-2 circulating in Burkina Faso. **Methods**: This study included symptomatic and asymptomatic individuals who underwent diagnostic testing for SARS-CoV-2 by RT-PCR on nasopharyngeal and oropharyngeal swabs from 7 December 2021 to 12 January 2022. Samples from individuals with a Ct value ≤ 33 were selected for the variants-specific mutation screening. The screening was performed using two kits, "SNPsig® SARS-COV-2 (Escape PLEX)" and "SNPsig® VariPLEX™ (COVID-19) Real-Time PCR Assay". **Results**: SARS-CoV-2 prevalence was 18.9% (332/1758). A total of 113 samples (34.04%) had a Ct value less than (≤ 33), with only 20.35% (23/113) belonging to symptomatic patients. The mean age was 39.01±13 years. The Beta variant (B.1.351) was the most detected one comprising 78.8% (89/113) of variants. Gamma and Delta variants were detected at a low proportion of 0.9% (1/113). No mutation or variant was detected in seven (6.2%) samples. **Conclusion**: Specific mutation screening detected Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) variants of SARS-CoV-2 circulating in Burkina Faso. The absence of mutations in some samples might suggest variants other than those detected.

## INTRODUCTION

Coronavirus 2019 disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2], is considered one of the most significant threats to humans in recent centuries [3]. On 16 June 2022, over 537 million people worldwide were infected, with more than 6 million deaths [4].

SARS-CoV-2 is a single-stranded RNA virus with positive polarity [5]. It belongs to the family *Coronaviridae*, genus *Betacoronavirus*, which includes SARS-CoV-1 and MERS-CoV, among others [6]. The virus has four main structural proteins, including spike protein (S), an envelope protein (E), matrix protein (M), and nucleocapsid protein (N) [7]. The S protein is responsible for binding host angiotensin-converting enzyme 2 (ACE 2) to the host cell and determining host tropism via receptor binding domains (RBDs). SARS-CoV-2, like other RNA viruses, is prone to genetic evolution while adapting to its new human hosts with the development of mutations over time, resulting in the emergence of multiple variants that may have different characteristics compared to its ancestral strains [8]. Indeed, under selective immune pressure, SARS-CoV-2 undergoes mutations and antigenic variations [9, 10].

The maximum number of mutations in the S protein alters the rate of infection, severity, and affinity to the host's ACE 2 receptor, as well as the potential to alter neutralizing antibody efficacy and vaccine effectiveness [11, 12]. Various SARS-CoV-2 mutants emerged during



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the pandemic, and some spread widely. Classification systems for genetic variants were established by the Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) independently to distinguish between emerging variants of concern (VOC), variants of interest (VOI), and variants under surveillance (VUM) [13].

The most detected VOCs are associated with increased transmissibility or virulence, reduced neutralization by antibodies obtained by natural infection or vaccination, ability to escape detection, and decreased therapeutic or vaccine efficacy [13, 14]. Up to now, five variants have been classified as VOC (WHO label/Pango lineage), namely Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1 .1.529) that emerged in the United Kingdom (September 2020), South Africa (May 2020), Brazil (November 2020), India (October 2020), and then in several countries (November 2020), and rapidly spread worldwide [10, 15]. These variants have other names according to the GISAID and Nextstrain clade: 20I/501Y.V1 (Alpha); 20H/501Y.V2 (Beta); 20J/501Y.V3 (Gamma); 21A (Delta), 21K, 21I, 21M (Omicron) [16].

PCR screening methods are available for detecting specific mutations and variants to facilitate surveillance of SARS-CoV-2 variation [17]. Screening allows more reactive monitoring of the variants spread carrying these mutations of interest at the national level and in the most affected countries. Several countries have adopted this strategy because it reduces sequencing costs and is easy to deploy in resource-limited settings.

Limited data on SARS-CoV-2 variants circulating in West Africa, particularly in Burkina Faso, is available. Given that new emerging variants can potentially spread and alter viral infectivity, transmissibility, and pathogenicity, this study aimed to perform a PCR screening of specific mutations and VOCs in that context.

## MATERIAL AND METHODS

**Study Population.** The study was conducted at the Biomedical Research Laboratory (LaReBio) of the "Institut de Recherche en Sciences de la Santé (IRSS)" in Burkina Faso. The study population consisted of travelers, suspected, follow-up, and contact cases, both symptomatic and asymptomatic, diagnosed with SARS-CoV-2 by RT-PCR using nasopharyngeal and/or oropharyngeal swabs.

**Specimen and data collection.** Stored nasopharyngeal and oropharyngeal swabs were used from participants. Socio-demographic data were collected. Samples were collected between 7 December 2021 and 12 January 2022, corresponding to the second wave of the COVID-19 pandemic in Burkina Faso [18]. This period is characterized by the dry and dusty northeasterly trade wind and is associated with respiratory illnesses and even easy dissemination of viral particles.

The Ministry of Health of Burkina Faso approved this study and authorized using the stored samples from people for SARS-CoV-2 variant screening.

**Detection of SARS-CoV-2 by real-time RT-PCR.** RNA extraction from samples was performed using the "QIAamp® viral RNA mini Kit" (QIAGEN, USA), and amplification and detection were performed using the "FAST PLEX™ SARS-COV-2 detection kit (RT-PCR)" (PreciGenome LLC, San Jose, CA) on the QuantStudio5 Real-Time PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. It is an efficiently designed tool for detecting two SARS-CoV-2 genes: *ORF1ab* and *N*, with a detection limit of 285.7 copies/mL and a Ct threshold of 39.

**Selection of SARS-CoV-2 positive samples.** Only SARS-CoV-2 positive samples were used to screen mutations and VOCs after the RT-PCR assays. Positive samples with a Ct value ≤33 for *ORF1ab* or *N* genes were then selected. This value of 33 reflects high and medium viral load, and Ct values >33 were considered as low viral load and, therefore, not very contagious [19, 20].

**Detection of circulating SARS-CoV-2 variants.** SARS-CoV-2 variants detection was performed simultaneously with two kits according to the manufacturer's protocol. The first one, the "SNPsig SARS-COV-2 (Escape PLEX) kit" (Primerdesign, UK), allows the identification of mutations specific to Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) (E484K, K417N/ K417NT, and P681R). The particularity is that it allows simultaneous detection of the presence of SARS-CoV-2 (detection of *ORF1ab* and *M* genes) and mutations. The amplification program included one cycle of 55 °C for 10 min, one cycle of 95 °C for 2 min, followed by 45 cycles comprising 95 °C for 10 sec and 60 °C for 60 sec. The second kit, the "SNPsig® VariPLEX™ (COVID-19) Real-Time PCR Assay," is a multiplex PCR assay that detects the 20I/501Y.V1 (Alpha), 20H/501Y.V2 (Beta), 20J/501Y.V3 (Gamma); and 20C/S.452R (Delta), as well as biologically significant mutations N501Y and E484K/M. The amplification was done on QuantStudio 5 using the same PCR program mentioned above.

**Data analysis.** Data were uploaded into Excel 2019, and statistical analyses were performed using IBM SPSS Statistics V21.0. Fisher's exact test was used for comparison, and the significance level was set at $P \leq 0.05$. Mutations and variants were interpreted according to the kit manufacturers' procedures and WHO guidelines [21].

## RESULTS

**Characteristics of participants.** From 7 December 2021 to 12 January 2022, 1758 swabs samples were collected, and 332 individuals became positive by RT-PCR, representing a positivity rate of 18.9%; 113 samples (34.04%) had a Ct value ≤ 33 and were used for variant screening. The travelers were most represented



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(55.75%), and those who resided in urban areas represented 96.5%. Only 20.35% (23/113) of participants were symptomatic. The participants' age ranged from 1 to 77 years, with a mean of 39.01±13 years. Of the 113 samples, 53.1% belonged to men, and the sex ratio (M/F) was 1.13 (Table1).

**Table 1**. Socio-demographic and clinical characteristics of patients

| | No. (n=113) | Percentage (%) |
|---|---|---|
| **Sex** | | |
| Males | 60 | 53.1 |
| Females | 53 | 46.9 |
| **Status** | | |
| Contact cases* | 20 | 17.7 |
| Suspect cases** | 22 | 19.5 |
| Follow-up cases*** | 8 | 7.1 |
| Travelers | 63 | 55.7 |
| **Clinical symptoms** | | |
| Yes | 23 | 20.4 |
| No | 90 | 79.6 |
| **Residence** | | |
| Urban | 109 | 96.5 |
| Rural | 4 | 3.5 |

**Note :**
*****Contact case is a person who has been in contact with a SARS-CoV-2 positive person
****** Suspect case is a person who has clinical symptoms of SARS-CoV-2
*** Follow-up case is a person who tested positive for SARS-CoV-2 and then tested after seven days or 14 days for control.

**Detection of SARS-CoV-2 mutations and variants.** The SNPsig® VariPLEX™ (COVID-19) kit detected the 20H/501Y.V2 (B.1.351) variant as the predominant variants (75.2%, n=85). Eleven participants (9.7%) had simultaneous infection with 20H/501Y.V2 (B.1.351) and 20J/501Y.V3 (P.1) variants. Of note, with this kit, no 20I/501Y.V1 (B.1.1.7) variant was detected, and no variant was detected among 16 participants in this study (Tables 2 and 4). On the other hand, the SNPsig ® SARS-CoV-2 kit (EscapePlex) identified mainly Beta (B.1.351) (56.6%) while detecting the Gamma (P.1) and Delta (B.1.617.2) variants in only one patient (0.9%). Beta (B.1.351) and Gamma (P.1) variants were detected simultaneously among three patients (2.7%) with COVID-19. Five patients (4.4%) simultaneously had Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) variants. This kit detected no variants among 39 participants (34.5%) (Tables 3 and 4).

**Table 2.** Mutations and variants detected with the SNPsig ® VariPLEX™ kit (COVID-19) kit

| Mutations | Variants | No. (n=113) | Percentage (%) |
|---|---|---|---|
| **20H/501Y.V2.MUT, N501Y.MUT** | 20H/501Y.V2 (South African)* | 85 | 75.2 |
| **20H/501Y.V2 MUT, 20J/501Y.V3 MUT, N501Y.MUT** | 20H/501Y.V2 (South African) 20J/501Y.V3 (Brazilian)** | 11 | 9.7 |
| **N501Y.MUT** | South African/ Brazilian /California | 1 | 0.9 |
| **None** | None | 16 | 14.2 |

**Note :**
***** 20H/501Y.V2 (South African), also named Bêta (B.1.351)
** 20J/501Y.V3 (Brazilian), also named Gamma (P.1)

**Table 3**. Mutations and variants detected with the SNPsig ® SARS-CoV-2 kit (EscapePlex)

| Mutations | Variants | No. (n=113) | Percentage (%) |
|---|---|---|---|
| K417N | Beta (B.1.351) | 64 | 56.6 |
| K417N, K417T | Beta (B.1.351), Gamma (P.1) | 3 | 2.7 |
| K417N, K417T, P681R | Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) | 5 | 4.4 |
| K417T | Gamma (P.1) | 1 | 0.9 |
| P681R | Delta (B.1.617.2) | 1 | 0.9 |
| None | None | 39 | 34.5 |

Table 4 shows the results of the simultaneous use of both Variplex and EscapePlex kits. The Beta variant (B.1.351) with 78.8% was the main identified variant (89/113). However, the number of unidentified variants decreased to 7 (6.2%) as opposed to 16 and 39 when both kits were used independently. Gender, status, clinical signs, and Ct were significantly related to the identified variants ($P$<0.001) (Table 5). The Beta (B.1.351) variant was found most among travelers (n=49), asymptomatic patients, and those with a Ct value ≤ 25.



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**Table 4**. Variants detected using both kits simultaneously

| Variants | No. (n=113) | Percentage (%) |
|---|---|---|
| Beta (B.1.351) | 89 | 78.8 |
| Beta (B.1.351), Gamma (P.1) | 10 | 8.8 |
| Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) | 5 | 4.4 |
| Delta (B.1.617.2) | 1 | 0.9 |
| Gamma (P.1), | 1 | 0.9 |
| None | 7 | 6.2 |

**Table 5**. Bivariate analysis of socio-demographic and clinical characteristics and variants

| | | Identified variants | | | | | *P*-value |
|---|---|---|---|---|---|---|---|
| | Beta | Beta, Gamma | Beta, Gamma, Delta | Delta | Gamma | Know | |
| **Sex** | Female | 42 (79.2) | 6 (11.3) | 3 (5.7) | 0 (0.0) | 1 (1.9) | |
| | Male | 47 (78.3) | 4 (6.7) | 2 (3.3) | 1 (1.7) | 0 (0.0) | <0.001 |
| **Status** | Contact cases | 16 (80) | 0 (0.0) | 2 (10) | 0 (0.0) | 0 (0.0) | |
| | Suspected cases | 18 (81.8) | 4 (18.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | <0.001 |
| | Followed-up cases | 6 (75) | 1 (12.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| | Travelers | 49 (77.8) | 5 (7.9) | 3 (4.8) | 1 (1.6) | 1 (1.6) | |
| **Clinical symptoms** | Yes | 21 (75) | 2 (7.1) | 5 (17.9) | 0 (0.0) | 0 (0.0) | |
| | No | 68 (80) | 8 (9.4) | 0 (0.0) | 1 (1.2) | 1 (1.2) | <0.001 |
| **Threshold cycle (Ct)** | ≤ 25 | 39 (76.5) | 5 (9.8) | 1 (1.9) | 1 (1.9) | 0 (0.0) | |
| | 26 - 29 | 37 (88.1) | 4 (9.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.011 |
| | 30 - 33 | 13 (65) | 1 (5) | 4 (2) | 0 (0.0) | 1 (5) | |

## DISCUSSION

This study aimed to simultaneously detect SARS-CoV-2 variants circulating in Burkina Faso by RT-PCR using two ARS-CoV-2 mutation detection kits. Most individuals who tested positive for SARS-CoV-2 were asymptomatic, confirming that the virus can infect without causing clinical manifestations [22]. During the study period, we detected three variants circulating in Burkina Faso, including Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), with a predominance of the Beta (B.1.351) variant. Nevertheless, by performing them on an individual basis, the Beta variant (B.1.351) was detected in 75.6% with the SNPsig® VariPLEX™ kit (COVID-19) kit versus 56.6% with the SNPsig® SARS-CoV-2 kit (EscapePlex) kit. This difference means that the SNPsig® VariPLEX™ (COVID-19) kit could detect some variants not detected by the SNPsig® SARS-CoV-2 (EscapePlex) kit and vice versa. This phenomenon is corroborated by detecting no mutation in 34.5% of patients with the SNPsig® SARS-CoV-2 (EscapePlex) kit versus 14.2% with the SNPsig® VariPLEX™ (COVID-19) kit. These results demonstrated the need for complementary tests when using the two kits. Indeed, the combined use of the two kits allowed the detection of the Beta variant (B.1.351) in 78.8% of the samples, the most prevalent one in Africa. This variant, alongside others, has contributed to the second severe wave of the COVID-19 pandemic in many countries [23]. This variant was also reported in Malawi, with an increase in COVID-19 seroprevalence to 64.9% in May 2021 among blood donors [24]. Even the Harmattan period is critical in viral particle spread; this study occurred when the country experienced a resurgence of SARS-CoV-2 positive cases despite the vaccination campaign instituted on 2 June 2021 [25]. The predominance of the Beta (B.1.351) variant would be due to its capacity for rapid transmission and resistance to neutralizing antibodies induced by natural infection and vaccines [23, 26-28].

In the present study, double infections with the Beta (B.1.351) and Gamma (P.1) variants were detected in 8.8% of specimens, and triple infection with the Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) variants in 4.4%. These co-infections were evidenced due to detecting mutations specific to each variant, e.g., K417N, N501Y for Beta, K417T, E484K for Gamma, and P681R for Delta. These mutations are located on the spike protein and increase the binding affinity to the ACE receptor [8]. The risk of reinfection with Beta (B.1.351) is about 5%, according to Shinde *et al*. (2021) [29]. In January 2021, two individuals were simultaneously infected with two different coronavirus variants, the Beta (B.1.351) and a local variant called VUI-NP13L in Brazil [30]. The possibility of co-infection with the Beta (B.1.351) variant adds a new factor to the complex interplay between immune response systems and mutations on the spike protein.

In the present study, the kits detected no mutations among 6.2% of specimens, which might be due to the wild-type SARS-CoV-2 infection, but also to variants other than Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) targeted by the screening kits used in this study. For these specimens, using other screening



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kits capable of searching for other variants of concern, such as Omicron (B.1.1.529), which was already circulating in several countries [16] at the time of this study (December 2021 to January 2022), could further clarify the viral status.

The present study showed a relationship between the detected variants with gender, status, clinical symptoms, and Ct value. Asymptomatic patients were most likely to have the Beta variant. A similar study conducted in Singapore found that symptomatic patients (fever, cough, dyspnea) were most likely to have the Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2) variants [31]. Delta (B.1.617.2) variant was associated with a higher probability of pneumonia, and, in contrast, Alpha (B.1.1.7) and Beta (B.1.351) were significantly less likely to be associated with pneumonia [31]. These three variants were the most detected in travelers, symptomatic individuals, and those with low Ct values. Indeed, the association of VOCs with lower Ct values and the longer duration of the viral load has shown important implications for transmissibility [31].

In the present study, using specific mutation screening kits for variants did not reveal with certainty all the circulating variants because of the high genetic variability of SARS-CoV-2, which could limit the specificity of the kits used for the screening. Sequencing the virus's complete genome would then confirm the identified variants. In addition, the somewhat restricted window of the study period (December 2021 to January 2022) did not allow us to establish the exact period of entry of these variants in Burkina Faso.

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## CONFLICT OF INTEREST

The author declares that there are no conflicts of interest associated with this manuscript.

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