

**Original Article****Open Access****A one-year genomic surveillance of SARS-CoV-2 in patients from two sites in Burkina Faso**

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Abstract:**Background**

Despite the availability of vaccines against SARS-CoV-2, mortality due to COVID-19 continues to increase, with nearly 7 million deaths reported globally. Continued analysis of the evolution and genomic diversity can provide necessary information to timely inform public health responses. We report the dynamics of SARS-CoV-2 lineages in Burkina Faso between August 2021 and September 2022.

Methodology: A total of 188 patients, whose nasopharyngeal and/or oropharyngeal swabs tested positive for SARS-CoV-2 by RT-PCR at the National Influenza Reference Laboratory (NIRL) of the Institut de Recherche en Sciences de la Santé (IRSS) in Burkina Faso, were sequenced, and the whole genomes of the virus annotated and screened for mutations using the ARTIC protocol and Oxford Nanopore Technology. Subsequently, different SARS-CoV-2 lineages were assigned using NextClade. The socio-demographic characteristics of the study participants were collected. Descriptive statistics on the data were conducted using R software version 4.3.2. Comparisons were made using the Chi-square test for qualitative variables, and statistical significance was set at $p < 0.05$.

Results: Majority of the 188 COVID-19 positive cases lived in urban areas (85.1%), travelling from high-risk transmission zones. Of 134 SARS-CoV-2 genomes successfully sequenced and submitted to the NextClade database, the Delta (50.7%, $n=68$) and Omicron (42.5%, $n=57$) variants were predominant. The most frequent lineages detected were B.1.617.2 (20.1%, $n=27$), BA.1.13 (17.2%, $n=23$), and AY.133 (14.2%, $n=19$). Temporal trend showed that the pandemic was driven by the Delta variant which was progressively replaced by the Omicron variant of SARS-CoV-2.

Conclusion: Although heterogeneity was seemingly not important during the study period, the Delta variant which was the most predominant variant in the country, was progressively replaced by the Omicron variant. Continuous genomic surveillance might be necessary to timely detect the virulent variants.

Keywords: SARS-CoV-2; sequencing; ONT; Delta variant; Omicron variant; Burkina Faso

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Surveillance génomique du SARS-CoV-2 sur une période d'un an chez des patients de deux sites au Burkina Faso

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Résumé:

Contexte: Malgré la disponibilité de vaccins contre le SRAS-CoV-2, la mortalité due au COVID-19 continue d'augmenter, avec près de 7 millions de décès signalés dans le monde. L'analyse continue de l'évolution et de la diversité génomique peut fournir les informations nécessaires à la mise en place de mesures de santé publique en temps opportun. Nous rapportons la dynamique des lignées de SRAS-CoV-2 au Burkina Faso entre août 2021 et septembre 2022.

Méthodologie: Un total de 188 patients dont les écouvillons nasopharyngés et/ou oropharyngés ont été testés positifs pour le SRAS-CoV-2 par RT-PCR au Laboratoire national de référence de la grippe (LNRG) de l'Institut de recherche en sciences de la santé (IRSS) au Burkina Faso, ont été séquencés, et les génomes entiers du virus annotés et criblés de mutations en utilisant le protocole ARTIC et la technologie Oxford Nanopore. Par la suite, différentes lignées de SARS-CoV-2 ont été assignées à l'aide de NextClade. Les caractéristiques socio-démographiques des participants à l'étude ont été recueillies. Les statistiques descriptives des données ont été réalisées à l'aide du logiciel R version 4.3.2. Les comparaisons ont été effectuées à l'aide du test du chi carré pour les variables qualitatives, et la signification statistique a été fixée à $p < 0,05$.

Résultats: La majorité (85,1%) des 188 cas COVID-19 positifs vivaient dans des zones urbaines et provenaient de zones de transmission à haut risque. Sur les 134 génomes de SARS-CoV-2 séquencés avec succès et soumis à la base de données NextClade, les variants Delta (50,7%, n=68) et Omicron (42,5%, n=57) étaient prédominants. Les lignées les plus fréquemment détectées étaient B.1.617.2 (20,1%, n=27), BA.1.13 (17,2%, n=23) et AY.133 (14,2%, n=19). La tendance temporelle a montré que la pandémie était alimentée par le variant Delta qui a été progressivement remplacé par le variant Omicron du SARS-CoV-2.

Conclusion: Bien que l'hétérogénéité n'ait apparemment pas été importante au cours de la période étudiée, la variante Delta, qui était la variante la plus prédominante dans le pays, a été progressivement remplacée par la variante Omicron. Une surveillance génomique continue pourrait être nécessaire pour détecter à temps les variants virulents.

Mots clés: SARS-CoV-2; séquençage; ONT; variant Delta; variant Omicron; Burkina Faso

Introduction:

Coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 in Wuhan, China (1,2). Since then, the virus has spread rapidly throughout the world causing almost 776,007,137 confirmed cases of COVID-19 including 7,059,6127 deaths reported by WHO, as of 18th August 2024 (3). The same report shows that the African continent remains less affected in terms of number of cases with 9,600,000 cases and mortality of 175,000, compared to other regions of the world. In Burkina Faso, the first positive case was reported in March 2020 (4). To date, around 22,114 cases have been reported by the WHO with 400 deaths as of 13th April 2024 (5).

Previous studies on COVID-19 seroprevalence show a high population exposure to SARS-CoV-2 up to 55% in Burkina Faso (6). Since 2020, several SARS-CoV-2 variants have emerged and are associated with higher transmissibility, immune escape, or increased pathogenicity in many countries around the world because of mutations within the genome, notably in the spike region (7). Successively, five

major SARS-CoV-2 lineages were previously described as variant of concern (VOC), including Alpha (B.1.1.7), first reported in the United Kingdom; Beta (B.1.351), first reported in South Africa; Delta (B.1.617.2) first seen in India; Gamma (P.1) first seen in Japan and Brazil; and Omicron (B.1.1.529) first reported in South Africa (8–13). Thus, emergence of VOC and variants of interest (VOI), presenting different epidemiological and clinical characteristics, remain an important threat to the global control of the pandemic.

Genomic surveillance is a powerful tool to monitor novel SARS-CoV-2 importations, to detect transmission chains for contact tracing, and enables rapid identification of novel VOC and can provide early-warning insight of new variants circulating in communities. However, by consulting the database of the Global Initiative on Sharing All Influenza Data (GISAID), there is very little sequence data of SARS-CoV-2 shared on GISAID by African countries and in particular in Burkina Faso. This study is the first, using Oxford Nanopore technology, to identify circulating SARS-CoV-2 variants in symptomatic and asymptomatic participants Burkina Faso in the surveillance.

Materials and method:

Study setting and ethical consideration:

This work was part of a routine surveillance data performed by the Ministry of Health in collaboration with the National Influenza Reference Laboratory (NLIR) of the Institut de Recherche en Sciences de la Santé (IRSS) in Burkina Faso. There was no requirement for formal ethical approval. However, patients were included following the Helsinki recommendations. In addition, all data were fully anonymized to protect patients' identities and data usage was done in accordance with ethical regulations. The results were shared with the Ministry of Health, Public Hygiene and Welfare as part of the routine surveillance of SARS-CoV-2 variants. Only the laboratory number was recorded to ensure confidentiality.

Study population and participants:

COVID-19 testing was carried out on travelers, suspected cases, control cases, screening cases and contact cases (who are the individuals listed as close contacts of a confirmed case) according to WHO definition (14) from two sites in Burkina Faso, Bagassi and Ouagadougou. Samples were collected in Bagassi located in the "Boucle du Mouhoun" region in the north-west of Burkina Faso and in Ouagadougou, the capital city.

Patients were subdivided into symptomatic and asymptomatic groups. We defined symptomatic case as an individual who had tested positive for SARS-CoV-2 using real time RT-PCR test with at least one of the following signs and/or symptoms; fever, cough, tiredness, loss of taste or smell, sore throat, headache, aches and pains, diarrhea, a skin rash, or discoloration of fingers or toes, difficulty breathing or shortness of breath, loss of speech or mobility or confusion, chest pain. Asymptomatic cases were individuals who tested positive for SARS-CoV-2 using a real time RT-PCR test but had no symptoms that were consistent with COVID-19 and were largely travelers (15).

The socio-demographic information collected from each participants included age, gender, place of residence, and COVID-19 status (suspect case and non-suspect case according to WHO).

Clinical sample collection and RNA extraction:

From August 2021 to September 2022, nasopharyngeal and/or oropharyngeal swabs were collected from 9247 patients and stored in universal transport medium (Copan Diagnostics). Samples were shipped to the NIRL for analysis. A total 188 of patients with confirmed COVID-19 were enrolled in this study. The viral RNA was automatically extracted from 200µL of the nasopharyngeal and oropharyngeal

specimens using the KingFisher Flex instrument (ThermoScientific Inc., USA) according to the manufacturer's instructions. Approximately 50µL of the total nucleic acid eluate was recovered into elution plate and either tested immediately or stored at -70°C until further analysis.

Real time RT-PCR assay:

The specimens were initially tested for SARS-CoV-2 using real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) assay. Two PCR kits were used depending on their availability; TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Applied Biosystems) and STANDARD M nCoV Real-Time Detection kit (SD Biosensor). PCR was performed on the QuantStudio5 Real-Time PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. This is an efficiently designed tool for detecting *ORF1ab*, *N*, and *S* genes of SARS-CoV-2 with TaqPath™ COVID-19 CE-IVD, and *ORF1ab* and *E* gene with STANDARD M nCoV Real-Time Detection. Validation of results was performed automatically by the Applied Biosystems™ COVID-19 Interpretive Software based on performance of the positive and negative controls.

SARS-CoV-2 library preparation & sequencing

Only SARS-CoV-2 positive samples with cycle threshold (Ct) value ≤32 for two or more SARS-CoV-2 gene targets were used for sequencing after the RT-PCR assays. Sequencing was carried out in the NIRL using the ARTIC protocol and Oxford Nanopore Technologies® (version MRT_9127_v110_revH_14 Jul2021). The reverse transcription PCR was done using the NEBNext® ARTIC SARS-CoV-2 Companion kit (Oxford Nanopore Technologies®, UK) from New England Biolabs, USA, with the ARTIC Network SARS-CoV-2 V3 primers.

The library was prepared using the Rapid Barcoding kit 96 (RBK11096.10.0023) kit from Oxford Nanopore Technologies®, UK. The library concentration was measured using the Qubit HS dsDNA assay kit (Thermo Fisher, USA). The MinIon MK1B and MK1C sequencers were used for whole genome sequencing with R9.4.1 flow cells (Oxford Nanopore Technologies®, UK). MinKNOW software was used to start the sequencing run for 12 hours.

Bioinformatic analysis:

NextClade was used for sequence quality control, clade assignment and lineage classifications to the sequences. NextClade was used to build the phylogenetic tree. Sequences with genome coverage ≥80% were included for analysis. Sequences that met quality control thresholds were deposited in the GISAID (<https://www.gisaid.org/>) database.

Statistical analysis:

Data were analyzed using R software version 4.3.2. A descriptive analysis according to socio-demographic and epidemiological data was carried out. Qualitative variables were presented with numbers and percentages, quantitative data are presented with medians and quartiles. Comparisons were made using the Chi-square test for qualitative variables. The statistical significance of tests was set at 0.05. The circulation of variants as a function of time has been graphically represented.

Results:**Socio-demographic characteristics of the study participants:**

A total of 9247 samples were collected from 9247 patients and 188 of them tested positive for SARS-CoV-2 during the surveillance period, generating 134 sequences. Majority of participants were males (68.7%, n=92),

and the median age was 35.0 (IQR 27.0-49.0) years. The difference was statistically significant ($p < 0.001$). Patients were mainly travelers from countries with high-risk of infection (44.7%, n=51), followed by confirmed cases that visited the NIRL site for control of their status (20.2%, n=23) and suspected cases that visited the centre for confirmatory testing (19.3%, n=22) (Table 1).

Diversity of SARS-CoV-2 lineages from whole-genome sequencing:

Lineage analysis of 134 whole-genome sequences was conducted with the Pangolin COVID-19 lineage assigner. The Delta variant was the most represented (50.7%, n=68), followed by the Omicron variant (42.5%, n=57) (Table 2). The predominant lineages were B.1.617.2 (20.1%, n=27), BA.1.13 (17.2%, n=23), and AY.133 (14.2%, n=19) (Table 2, Fig 1).

Table 1: Frequency distribution of the study participants in Burkina Faso (August 2021-September 2022) according to sociodemographic characteristics

Characteristics	Frequency	Percentage	p value
Sex			
Female	42	31.3	<0.001
Male	92	68.7	
Age group (in years)			
<30	42	31.6	0.007
30-50	60	45.1	
≥ 50	31	23.3	
Missing *	1	-	
Site			
Bagassi	20	14.9	<0.001
Ouagadougou	114	85.1	
Status			
Contact Case	14	12.3	<0.001
Suspected case	22	19.3	
Control case	23	20.2	
Screening case	4	3.5	
Traveler	51	44.7	
Missing*	20	-	
Symptoms			
Symptomatic	101	75.4	<0.001
Asymptomatic	33	24.6	

*Missing values were not included in calculations, but only presented as numbers

Table 2: SARS-CoV-2 lineages and variants detected

Lineage	Nextstrain clade	Number	Percentage
AY.122	21J (Delta)	4	3.0
AY.133	21I (Delta)	16	12.0
	21L Delta	3	2.3
AY.20 (B.1.617.2)	21J (Delta)	1	0.8
AY.34	21J (Delta)	1	0.8
AY.41	21J (Delta)	1	0.8
AY.88	21J (Delta) AY88	1	0.8
B.1.617	20A	1	0.8
B.1.617.12	21J (Delta)	1	0.8
B.1.617.2	20A	1	0.8
	21A (Delta)	2	1.5
	21I (Delta)	6	4.5
	21J (Delta)	17	12.8
	21M (Omicron)	1	0.8
B.1.617.2 (Delta)	21A (Delta)	1	0.8
	21I (Delta)	1	0.8
	21L (Delta)	2	1.5
	22 B (Omicron)	1	0.8
B.1.617.3	21J (Delta)	1	0.8
B.1.617.4	21J (Delta)	1	0.8
B.1.617.5	21J (Delta)	1	0.8
B.1.617.6	21J (Delta)	1	0.8
B.1.617.7	21J (Delta)	1	0.8
B.1.617.8	21J (Delta)	1	0.8
BA.1.1	21K (Omicron)	2	1.8
BA.1.1.1	21K (Omicron)	1	0.8
BA.1.13	21K (Omicron)	23	17.3
BA.1.13\r\n	21K (Omicron)	4	3.0
BA.1.B	21K (Omicron)	1	0.8
BA.2	21L (Omicron)	3	2.3
BA.2.12.1	22C (Omicron)	1	0.8
BA.2.3	21L (Omicron)	1	0.8
BA.4	22A (Omicron)	1	0.8
BA.4.1.2 (Omicron)	22A (Omicron)	1	0.8
BA.5.1.3	21L (Omicron)	1	0.8
BA.5.2 (Omicron)	22 B (Omicron)	4	3.0
BA1.13	21K (Omicron)	2	1.5
BE.1.1.1 (Omicron)	22 B (Omicron)	1	0.8
Omicron	21L (Omicron)	1	0.8
Omicron BA2	21L (Omicron)	3	2.3
Omicron BA2.12.1	22C (Omicron)	1	0.8
Omicron recombinant	Recombinant	1	0.8
Unassigned	20A	3	2.3
	20B	3	2.3
	21I (Delta)	3	2.3
	21K (Omicron)	1	0.8
	21L (Delta)	2	1.5
	21M (Omicron)	2	1.5
Total		133	100.0

Phylogenetic analysis:

Lineages were assigned to the sequenced genomes according to the Nextstrain SARS-CoV-2 clades. The phylogenetic tree shows a separation of the Omicron branch evolving with different clades (21K, 21L, 22M) and the Delta branch with its clades (21I, 21J) (Fig 1 A). Different variants were circulating during the study period as shown in Fig 1B.

The figure shows three distinct periods

of variant circulation. The first period runs from the start of the study to January 2022, when the Delta variant (20A, 20B, 21A, 21I, 21J) circulated predominantly. The second period runs from January 2022 to mid-August 2022, when Omicron (22A, 22B, 22C, 21M, 21L) circulated extensively. The final period, from mid-August 2022 to the end of the study, saw Delta, Omicron and Recombinant co-circulate.

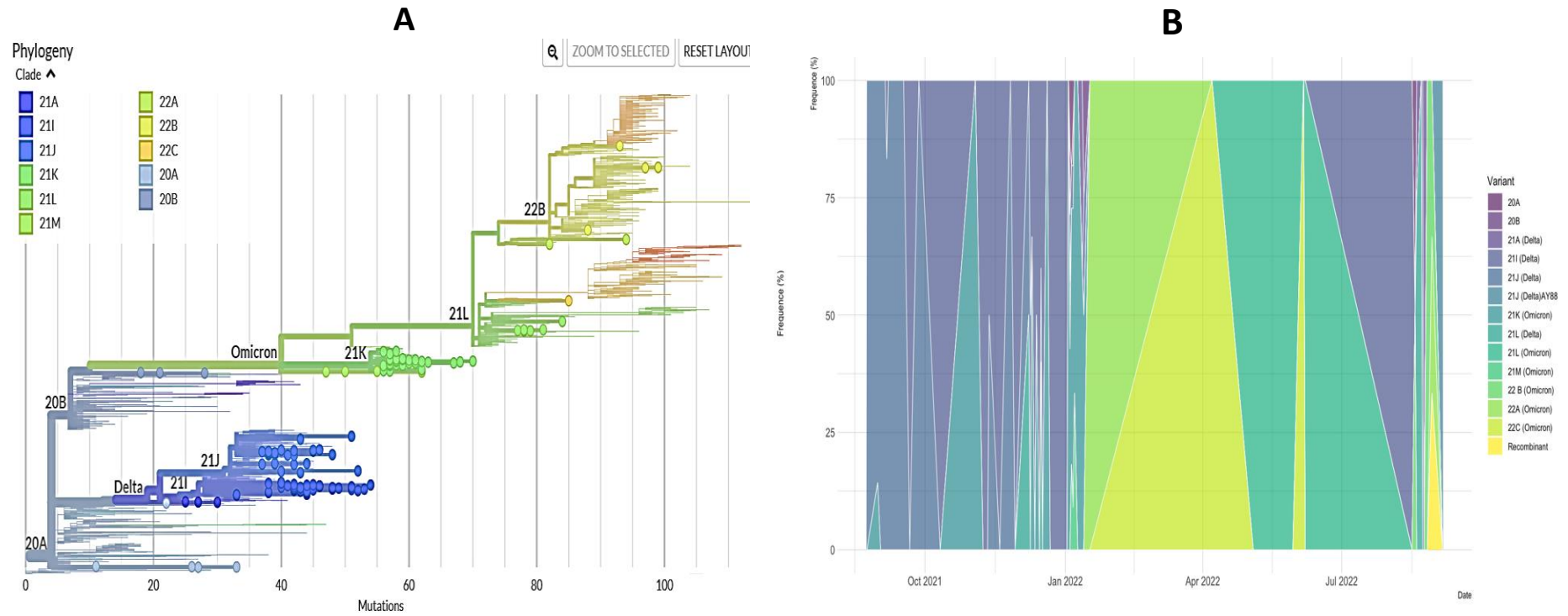
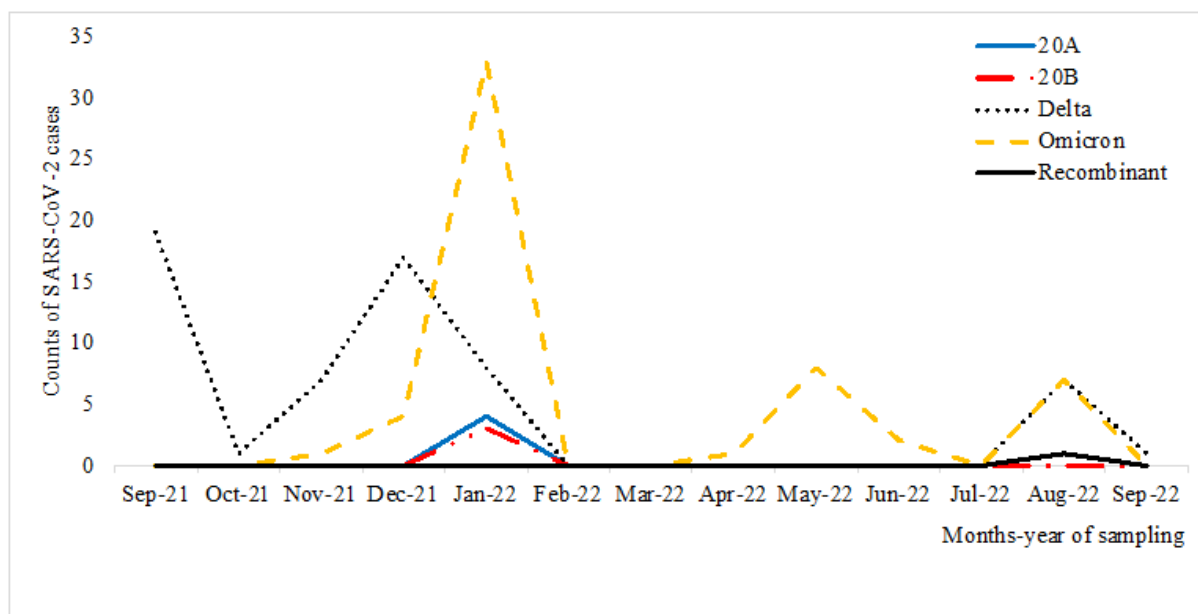


Fig 1. Phylogenetic analysis of SARS-CoV-2 genome in 134 sequences generated from high-risk populations in Burkina Faso. A phylogenetic tree generated by Nextstrain (A), and variants distribution during the study period (B).



X-axis represents month-year of sampling; Y-axis represents the count of SARS-CoV-2 strains; colors correspond to each viral strain.

Fig2: SARS-COV-2 variant dynamics per month in Burkina Faso.

Dynamics of SARS-CoV-2 lineages over time:

The epidemiological pattern of SARS-CoV-2 as demonstrate continuously evolution over time. Between September and October 2021, the Delta variant predominantly circulated. This was subsequently followed by an overlapping circulation of both Delta and Omicron variants, with peaks incidences observed in December 2021 for Delta and January 2022 for the Omicron from February 2022, the Omicron variant was the main circulating strain with sporadic occurrence of other variants. Notably, a reemergence of the Delta variant was observed after several months of disappearance (Fig 2).

Discussion:

Our study provides a molecular characterization of SARS-CoV-2 strains circulating in Burkina Faso over the course of a year. Despite the implementation of preventive measures such as PCR testing and border controls during the pandemic, our findings reveal active circulation of Delta and Omicron variants, along with their respective lineages and sub-lineages. The high prevalence of the disease among travelers (44.7%) underscores their significant involvement in introduction of the disease into the country and spread of these variants. Specifically, given the status of Ouagadougou as the main entry point for travelers into Burkina Faso from the airport, majority of SARS-CoV-2 positive cases within this study were collated from and could explain the introduction of the various variants and lineages.

Our study period coincided with the global emergence of the second wave of SARS COV-2 notably marked by the emergence of

the Delta variant (21I) from late 2020 in India (16) and the Omicron variant (21K) from the end of 2021 in South Africa (17). This time-frame also aligns with the third and fourth waves of the pandemic in Africa, as previously documented (18). Our results indicate a predominance of the Delta variant (50.7%), with approximately 16 identified sub-lineages. The most frequent sub-lineage was B.1.617.2, accounting for 20.1% of cases. These findings are consistent with previous data from Ghana, which also exhibited the circulation of the delta variant B.1.617.2 and other sub-lineages from May to November 2021 (19). Similarly, variants such as Delta B.1.617.2 and AY.4 were also reported in Benin while the variants B.1.617.2 and 21A circulated in Guinea from May to July 2021. In addition, the sub-lineages B.1.617.2, AY.4, and AY.36 were reported in Nigeria with dominance of the AY.36 lineage (20–22). The Delta variant, known for its increased transmission, has been widely documented in other countries, with high prevalence in the United States (83%) and the United Kingdom (90%) during the same period(23).

Our data highlighted the emergence of the Omicron variant between October and November 2021, with a peak in January 2022, corresponding to the fourth wave of the pandemic in Africa (18). Similarly, this wave was also marked by the circulation of the Omicron variant also in Ghana (19) identified as lineages BA.1, BA.2, and BA.3. Interestingly, our study detected a circulation of the Omicron in Burkina Faso since October 2021, even before its official discovery in South Africa in November 2021 (21). With at least 32 mutations in the spike protein and others in the NSP12 and NSP14 regions, phylogenetic analysis showed

a distinct divergence of the Omicron branch from other variants (24). The peak observed of circulation recorded for the Omicron variant in January 2022 corresponded to its highest prevalence recorded during the same period in Bobo Dioulasso, the second largest city in Burkina Faso (25).

Both Omicron and Delta variants co-circulated during the same period in 2021, a pattern also observed in other regions. However, Omicron became the dominant strain from January 2022 due to its increased transmissibility, estimated to be 3.2 times greater than that of the Delta variant(21,26). Omicron is associated with asymptomatic or mild infections, which likely contributed to its rapid spread worldwide (21). Additionally, its ability to evade immunity, even in vaccinated or previously infected individuals, could have also facilitated its dissemination (21).

Conclusion:

Our study highlights the complex dynamics of SARS-CoV-2 variant circulation in Burkina Faso and provides new insights into its molecular epidemiology in the country. Our data exhibited the usefulness of genomic for monitoring of emerging variants. They also highlight the crucial need for continuous genomic surveillance of SARS-CoV-2 to adjust existing public health measures in response to the evolving pandemic.

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Contributions of authors:

The study was conceived by ZT; Data were collected by ZT and AC; Data analysis was done by ML and AG; The manuscript was drafted by AC and GN; ZT and MF made important contributions to the final manuscript. All authors read and approved the final manuscript submitted for publication.

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Conflicts of interest:

Authors declare no conflict of interest

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