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Dynamics of SARS-CoV-2 variants characterized during different COVID-19 waves in Mali

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ABSTRACT

Background: The emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) variants may have contributed to prolonging the pandemic, and increasing morbidity and mortality related to coronavirus disease 2019 (COVID-19). This article describes the dynamics of circulating SARS-CoV-2 variants identified during the different COVID-19 waves in Mali between April and October 2021.
Methods: The respiratory SARS-CoV-2 complete spike (S) gene from positive samples was sequenced. Generated sequences were aligned by Variant Reporter v3.0 using the Wuhan-1 strain as the reference. Mutations were noted using the GISAID and Nextclade platforms.
Results: Of 16,797 nasopharyngeal swab samples tested, 6.0% (1008/16,797) tested positive for SARS-CoV-2 on quantitative reverse transcription polymerase chain reaction. Of these, 16.07% (162/1008) had a cycle threshold value ≤ 28 and were amplified and sequenced. The complete S gene sequence was recovered from 80 of 162 (49.8%) samples. Seven distinct variants were identified: Delta (62.5%), Alpha (1.2%), Beta (1.2%), Eta (30.0%), 20B (2.5%), 19B (1.2%) and 20A (1.2%).
Conclusions and perspectives: Several SARS-CoV-2 variants were present during the COVID-19 waves in Mali between April and October 2021. The continued emergence of new variants highlights the need to strengthen local real-time sequencing capacity and genomic surveillance for better and coordinated national responses to SARS-CoV-2.

Introduction

The unprecedented coronavirus disease 2019 (COVID-19) pandemic has lasted for more than 2 years worldwide due to many factors, including the rapid genetic evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The genetic evolution has given rise to

several variants, including variants of concern (VOCs), that may have contributed to prolonging the pandemic and increasing morbidity and mortality related to COVID-19. The first VOC (Alpha or B.1.1.7) was identified in October 2020, while the Omicron variant (B.1.1.529, including its sub-lineage BA.4/BA.5/BA.275) became dominant at the end of 2021 [6]. Several other variants emerged between the Alpha and Omi-

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Table 1
Primers used for reverse transcription polymerase chain reaction and Sanger sequencing experiments.

Primer set	Primer name	Sequence	Start	End	Size
Set 1	SARS-Spike-F1	GGGGTACTGCTGTTATGTCTTT	21478	22070	592
	SARS-Spike-R1	GGTCCATAAGAAAAGGCTGAGA			
Set 2	SARS-Spike-F2	CGAAGACCCAGTCCCTACTTAT	21947	22436	489
	SARS-Spike-R2	GAGGGTCAAGTGCACAGTCTA			
Set 3	SARS-Spike-F3	GACTCCTGGTGATTCTTCTTCA	22394	22858	464
	SARS-Spike-R3	CGCAGCCTGTAAAATCATCT			
Set 4	SARS-Spike-F4	CTCCAGGGCAAACTGGAAA	22852	23451	599
	SARS-Spike-R4	CACGCCAAGTAGGAGTAAGTTG			
Set 5	SARS-Spike-F5	GTTCTTTTGGTGGTGTCAGTGT	23385	23902	517
	SARS-Spike-R5	CTTCTTGGGTGTTTTTGTCTTGT			
Set 6	SARS-Spike-F6	GTTGCAATATGGCAGTTTTTGT	23872	24500	628
	SARS-Spike-R6	GCACTTCAGCCTCAACTTTGT			
Set 7	SARS-Spike-F7	GCTCCAATTTTGGTGGAA	24518	25086	568
	SARS-Spike-R7	GCGGTCAATTTCTTTTTGAATG			
Set 8	SARS-Spike-F8	CCTTTGCAACCTGAATTAGACTC	25038	25497	459
	SARS-Spike-R8	GAAAGGGAGTGAGGCTTGTAT			

F, forward; R, reverse.

cron variants. The emergence of these variants was the driving cause of different COVID-19 epidemic waves worldwide. Like the rest of the world, Africa has experienced and identified many different SARS-CoV-2 variants in addition to the VOCs [15].

Despite vaccination campaigns, many countries have experienced epidemic surges linked to variants that are highly transmissible in most cases. Multiple variants have been reported from Cote d'Ivoire [2], Liberia [14] and Senegal [1,10,11], among other countries [15], showing temporary cocirculation of variants.

Mali, a landlocked West African country, registered its first case on 25 March 2020 [5], and has faced several epidemic waves. As of 30 April 2022, Mali has recorded 30,833 cases and 732 deaths (Ministry of Health, www.sante.gov.ml). Even with a low burden of the disease compared with some other countries, the economic, social and health impacts on Mali have been serious and trying. The rapid introduction of 19B and the first variant, 20A, were reported by analysing 21 sequences collected during April 2020 [7]. However, genetic surveillance did not follow subsequent epidemic surges, and there is a need to understand the presence and burden of variants during the pandemic. This article describes the dynamics of circulating variants in relation to the COVID-19 waves that occurred in Mali. While whole-genome sequencing (WGS) platforms provide the most comprehensive data for genomic surveillance, Sanger sequencing offers an economical alternative for tracing SARS-CoV-2 evolution and identifying circulating variants [9]. Contiguous spike (S) gene sequences generated by eight overlapping sets of polymerase chain reaction (PCR) primers were analysed to characterize SARS-CoV-2 variants and assess their implications in COVID-19 waves.

Materials and methods

Study setting and clinical samples

This study was conducted at the University Clinical Research Center (UCRC) in Bamako between April and October 2021. UCRC is a joint initiative between the Government of Mali and the US National Institutes of Health (NIH) to facilitate clinical research and support Mali national disease surveillance systems through laboratory diagnostic capacity. It was the first laboratory designated to perform SARS-CoV-2 testing in Mali [3–5].

Specimens were obtained under public health surveillance and not as part of human subject research. Thus, submission to institutional ethics committees was not necessary, but good clinical and laboratory practices were strictly followed. Confidentiality with regards to all infor-

mation/data included in this manuscript was observed throughout the study by de-identifying the samples.

Sample collection and SARS-CoV-2 RT-qPCR screening

Demographics and clinical information were collected at the site of sample drawing, and analysed with the viral genomic information. Oro- and nasopharyngeal swab samples of patients, volunteers and some contacts of positive cases coming from health centres and referred to UCRC between April and October 2021 were tested using the national guidelines, which consist of testing symptomatic people and their household, social and professional contacts. Qiagen AVL lysis buffer was used for inactivation, and viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Screening by quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed using the Argene SARS-COV-2 R-gene Real-time PCR kit (bioMérieux, Marcy l'Etoile, France) run with ABI 7500 Fast Dx equipment (Thermo Fisher Scientific, Waltham, MA, USA). The Argene kit is designed to target two viral genes [nucleocapsid (N) and RNA-dependent RNA polymerase (RdRp)]; an internal control is introduced to samples during inactivation. Samples subjected to sequencing fulfilled the following criteria: (i) positive on RT-qPCR for SARS-CoV-2; and (ii) cycle threshold (Ct) value ≤28.

Primer design for S gene sequencing, RT-PCR amplification and Sanger sequencing

The entire S gene from nucleotides 21563–25384 of Wuhan-Hu-1 reference sequence (National Center for Biotechnology Information GenBank Accession No. MN908947.3) was targeted in eight overlapping amplicons, and used to design a panel of eight sets of primers (Table 1). The template RNA from SARS-CoV-2-positive samples was amplified in one-step RT-PCR (Qiagen) with the different primers (Table 1) using a MiniAmp Thermal Cycler instrument (Thermo Fisher Scientific). RT-PCR assays were performed using the following thermal cycling conditions: 50°C for 30 min (reverse transcription), followed by 95°C for 15 min (DNA polymerase activation and initial denaturation), and 35 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 60 s (extension). A final extension step was conducted at 72°C for 10 min before holding at 4°C. The PCR products were then analysed by electrophoresis using 1% agarose gel with 0.5X Tris acetate ethylenediamine tetra-acetic acid buffer, and stained with GelRed nucleic acid gel stain (Sigma Aldrich, St Louis, MO, USA). Amplification products

were purified with ExoSAP-IT PCR product cleanup in accordance with the manufacturer's instructions (Thermo Fisher Scientific) to remove extra deoxyribose nucleotide triphosphate and primers. Purified samples were subjected to cycle sequencing performed using the Big Dye Terminator (BDT) v 3.1 kit (Thermo Fisher Scientific). The cycle mastermix included 3 μL of BDT V3.1 mastermix, 1 μL of 5X sequencing buffer V1.1, V3.1, 4 μL of molecular grade water and 1μL of primer (forward or reverse). Purified DNA (1–50 ng) template was added to the prepared mastermix. Samples were loaded into 96-well plates and run with the following conditions: one cycle at 96°C for 1 min and 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. The plate was centrifuged for 1 min before replacing the adhesive film with 96-well septa. The Big Dye XTerminator (BDX) purification kit (Thermo Fisher Scientific) was used to purify samples in the 96-well plate. In each well, a mix of 45 μL of SAM solution and 10 μL beads was added and vortexed for 30–45 min. After vortexing, the plate was centrifuged for 2 min and subjected to capillary electrophoresis using the protocol BDX_fastSeq_50_POP7_Z with BDT V3.1 on an ABI 3500 Genetic Analyzer sequencer (Thermo Fisher Scientific). The different fragments covering the entire S gene of the virus were sequenced, and the resulting sequences were aligned by Variant Reporter v3.0 using the Wuhan-Hu-1 strain as the reference.

Data analysis

The consensus sequences (FASTA files) were generated from assembly of the overlapping PCR products and their alignment to the Wuhan-Hu-1/2019 strain as the reference sequence. Quality control and coverage rate of the assembled S gene sequences was performed using the GISAID database. The Nextclade tool (Version 1.14.0) was used for SARS-CoV-2 S gene alignment, mutation calling, quality checks, phylogenetic placement and clade assignment. R-studio (Version 2021.09.0+351) and Graphpad Prism (Version 9.3.0) were used for demographic and clinical data analysis.

Results

In total, 16,797 oro- and nasopharyngeal swab samples were tested at UCRC between April and October 2021; of these, 6.0% (1008/16,797) tested positive for SARS-CoV-2 RNA using RT-qPCR. Of the positive cases, 16.07% (162/1008) had a Ct value ≤28 and were sequenced, resulting in 80 sequences covering the entire S gene (submitted under GenBank Accession Nos. ON909128-75, OP168680-99 and OP173206-17). These samples came from different health district centres in Bamako and the neighbouring areas. Patients attended these health district centres from different places, including Bamako city (77.5%). The majority of patients were male (51.25%), with a mean age of 38.79 years. One-third of cases were symptomatic (Table 2).

Seven distinct variants were identified, including three VOCs: Delta (62.5%), Beta (1.2%) and Alpha (1.2%); two variants of interest, Eta (30.0%) and 20B (2.5%); and two variants under monitoring, 19B (1.2%) and 20A (1.2%) (Table 3). In the samples, the Eta variant was predominant during the third wave of COVID-19 in Mali (March/May 2021); up to late 2021, this wave had caused the highest total COVID-19 case count until the Delta variant increased from June 2021 to October 2021 at the beginning of the fourth wave in Mali (Figure 1). In this study, during the sampling period, the Delta variant was linked to an increased number of cases (63%) (Figure 2A). The phylogenetic tree demonstrates the genetic differences among the identified lineages (Figure 2B), and indicates likely independent and distinct introductions of the virus into Mali.

Discussion

The ability of countries to cope with COVID-19 or subsequent pandemics is linked to their ability to detect infection rapidly, and to employ the appropriate preventive measures efficiently. At the start of the

Table 2

Sociodemographic characteristics and clinical status of patients.

Characteristics	n=80	%
Sex		
Female	34	42.50
Male	41	51.25
Missing	5	6.25
Age (mean, SD)	38.79	17.34
Residence		
Bamako (capital)	62	77.5
Outside Bamako	8	10.0
Missing	10	12.5
Collection site		
University hospitals	21	26.25
Health districts	43	53.75
Private clinic	1	1.25
Research laboratory	7	8.75
Missing	8	10.00
Clinical status		
Asymptomatic	25	31.3%
Symptomatic	26	32.5%
Missing	29	36.3%

SD, standard deviation.

Table 3

Nomenclature and prevalence of variants.

Variant	WHO label	Clade	Pango lineage	Frequency (%)
VUM	19B	19B	19B	1 (1.2)
	20A	20A	B.1.620	1 (1.2)
VOI	20B	20B	B.1.1.318	2 (2.4)
	Eta	21D (Eta)	B.1.525	24 (30)
VOC	Alpha	20I (Alpha, V1)	B.1.1.7	1 (1.2)
	Beta	20H (Beta, V2)	B.1.351	1 (1.2)
	Delta	21A (Delta)	B.1.617.2	6 (7.5)
		21I (Delta)	B.1.617.2 Subclade	12 (15)
		21J (Delta)	B.1.617.2 Subclade	32 (40)

WHO, World Health Organization; VOC, variant of concern; VOI, variant of interest; VUM, variant under monitoring.

COVID-19 pandemic, four laboratories in Mali had the capacity to diagnose SARS-CoV-2, and they continue to play an important role in the management of COVID-19 for the entire country. The national laboratory network is very important in a limited-resource setting for resource mutualization, coordinated diagnostic activities [3] and integrated national strategies [4], including patient management. It is also crucial to bring diagnostic resources closer to the most remote places, as well-equipped infrastructure in the country tends to be located in Bamako, the capital city. Through a network, all sites have access to laboratory services, although the time to results is still proportional to the distance from the place where the samples were collected.

Beyond diagnosis of SARS-CoV-2, understanding viral genomics is also relevant. The first variants described were detected quite early in Mali [7]. It is therefore necessary to strengthen the surveillance of variants in order to have a better understanding of the dynamics of the pandemic in the country, and to refine treatment and prevention measures. Positive samples were selected from national COVID-19 surveillance samples for sequencing in order to determine the prevalence of SARS-CoV-2 variants in Mali. This study demonstrated the introduction and circulation of several distinct SARS-CoV-2 variants, including three VOCs: Delta, Beta and Alpha. A similar study from Liberia showed the presence of different SARS-CoV-2 variants, including the Alpha, Beta, Iota, Eta and Delta variants, with intermittent cocirculation of several variants [14]. In a study from Senegal, different waves were linked to different circulating variants [1], denoting the need to strengthen monitoring of genetic variation to quickly characterize the circulating virus and adapt health policies. The geographic position of Mali, which shares borders with seven different countries, facilitates the movement of peo-
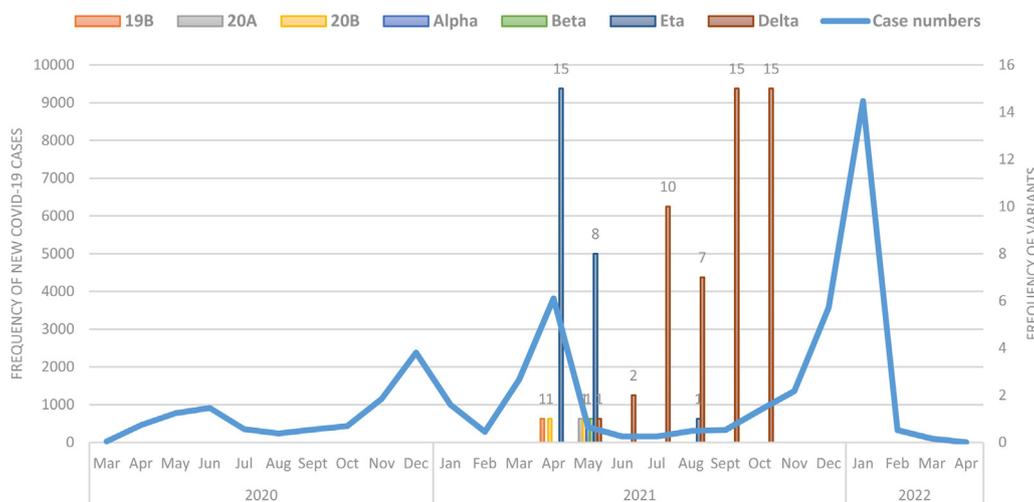
Figure 1. Variant frequency linked to different epidemic waves.
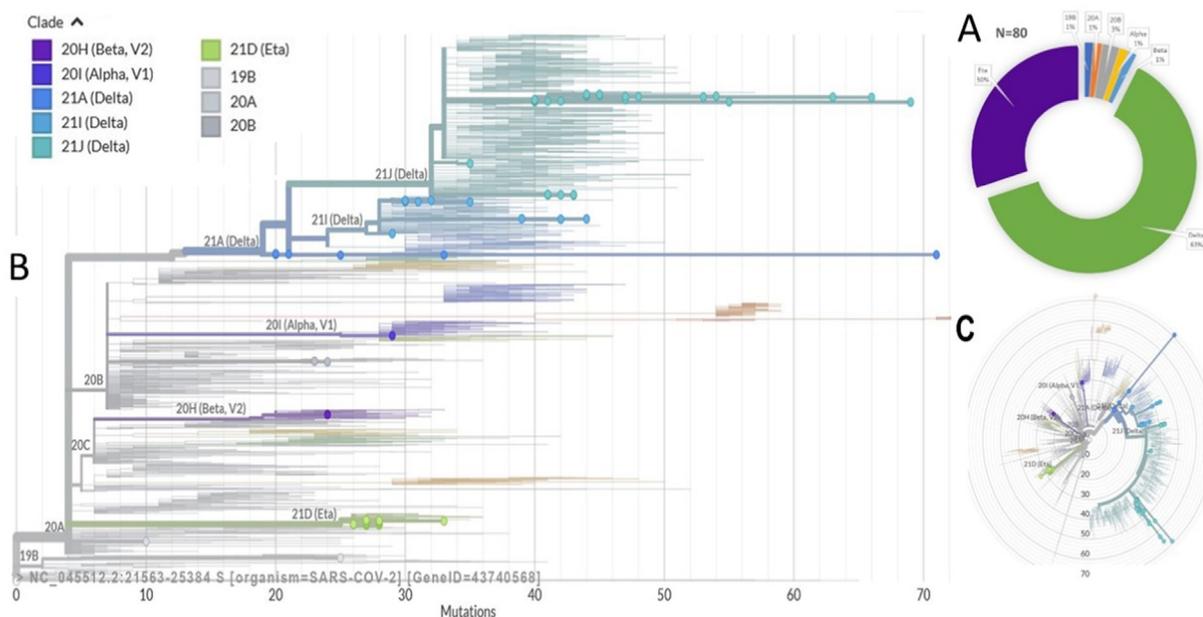

Figure 2. (A) Percentage of clade among samples. (B, C) Phylogenetic tree of variants.

ple and the introduction of COVID-19, in line with the finding of different variants over the study period. An unexpected finding of this study was that the third wave was driven by the Eta variant and not the Delta variant, which may have had a delayed introduction into Mali compared with other areas of the world.

Limitations

This study was limited by the fact that the data presented are from convenience samples collected in Bamako and neighbouring areas, and referred to UCRC, one of the four centres in Mali certified to perform molecular diagnosis of SARS-CoV-2. Sampling was performed by different healthcare centres during the pandemic, and data recording during enrolment was not complete in all cases. Information on sex was missing for 6.25% of cases, age was missing for 17% of cases, residence was missing for 12.5% of cases, and clinical status was missing for 36% of cases (Table 2). The missing data did not impact the characterization of viral variants. Another limitation was the restriction to S gene sequencing. Sequencing strategies to characterize the genetic diversity of microorganisms are challenging in low-income countries, and next-generation

sequencing and WGS are out of reach in most instances. Thus, Sanger sequencing with S gene primers was used as an economical and logistical valid alternative in the absence of WGS capability. Until the emergence of the Delta variant, mutations did not significantly compromise available molecular diagnostic, antigenic and serological tools [11], which changed to some extent with the emergence of the Omicron variant [8, 12]. However, in-silico and empirical testing showed that the primers used in this study (Table 1) were not affected severely by these mutations, and although full S gene sequencing may not be obtained in all cases, the Omicron variant would not escape detection.

Conclusions

The results show the presence of several SARS-CoV-2 variants associated with different COVID-19 waves in Mali. The second highest case count during the study period (peak during April 2021) was primarily driven by the Eta variant, whereas the beginning of the subsequent July/September wave was dominated by the Delta variant, representing delayed entry of the Delta variant into Mali compared with other countries. The ongoing dynamic waves of evolving SARS-CoV-2 vari-

ants underscore the need for timely molecular surveillance to direct and adapt preventive and therapeutic strategies. The importance of continued SARS-CoV-2 screening has been emphasized on a larger scale [13], and is also essential for Mali, a landlocked country connected with the outside world through trade and travel. This study highlights the importance of strengthening such diagnostic and surveillance capabilities at national level as well as regional level, not only in the context of the current COVID-19 pandemic but also in anticipation of future emerging and re-emerging infectious agents.

Declaration of Competing Interest

None declared.

References

[1] Ahouidi AD, Rodgers MA, Padane A, Leye N, Olivo A, Mbow M, et al. Emergence of novel combinations of SARS-CoV-2 spike receptor binding domain variants in Senegal. *Sci Rep* 2021;**11**:23644.
[2] Augustin Anoh E, Schubert G, Wayoro O, Pacôme M, Belarbi E, Sachse A, et al. SARS-CoV-2 variants of concern, variants of interest and lineage A.27 are on the rise in Côte d'Ivoire. *medRxiv* 2021 2021.05.06.21256282.
[3] Diarra B, Kone A, Guindo I, Bane S, Doumbia L, Timbine L, et al. A coordinated public health laboratory response to COVID-19 in Mali. *Front Trop Dis* 2022;**2**.
[4] Doumbia S, Sow Y, Diakite M, Lau CY. Coordinating the research response to COVID-19: Mali's approach. *Health Res Policy Syst* 2020;**18**:105.
[5] Dramé H, Diallo D, Coulibaly G, Kone M, Diallo F, Diarra H, et al. Contribution du laboratoire du centre universitaire de recherche clinique (ucrc) au diagnostic de sras-cov-2 au mali. *Mali Med* 2021;**XXXVI**:5.
[6] Gowrisankar A, Priyanka TMC, Banerjee S. Omicron: a mysterious variant of concern. *Eur Phys J Plus* 2022;**137**:100.
[7] Kouriba B, Durr A, Rehn A, Sangare AK, Traore BY, Bestehorn-Willmann MS, et al. First phylogenetic analysis of Malian SARS-CoV-2 sequences provides molecular insights into the genomic diversity of the Sahel region. *Viruses* 2020:12.
[8] Li A, Maier A, Carter M, Guan TH. Omicron and S-gene target failure cases in the highest COVID-19 case rate region in Canada – December 2021. *J Med Virol* 2022;**94**:1784–6.
[9] Lim HJ, Park MY, Jung HS, Kwon Y, Kim I, Kim DK, et al. Development of an efficient Sanger sequencing-based assay for detecting SARS-CoV-2 spike mutations. *PLoS One* 2021;**16**:e0260850.
[10] Padane A, Kanteh A, Leye N, Mboup A, Manneh J, Mbow M, et al. First detection of SARS-CoV-2 variant B.1.1.7 in Senegal. *New Microbes New Infect* 2021;**41**:100877.
[11] Rodgers MA, Olivo A, Harris BJ, Lark C, Luo X, Berg MG, et al. Detection of SARS–CoV-2 variants by Abbott molecular, antigen, and serological tests. *J Clin Virol* 2022;**147**:105080.
[12] Rodino KG, Peaper DR, Kelly BJ, Bushman F, Marques A, Adhikari H, et al. Partial ORF1ab gene target failure with Omicron BA.2.12.1. *medRxiv* 2022:e0060022.
[13] Schrörs B, Riesgo-Ferreiro P, Sorn P, Gudimella R, Bukur T, Rösler T, et al. Large-scale analysis of SARS-CoV-2 spike-glycoprotein mutants demonstrates the need for continuous screening of virus isolates. *PLoS One* 2021;**16**:e0249254.
[14] Shobayo B, Mishra M, Sameroff S, Petrosov A, Ng J, Gokden A, et al. SARS–CoV-2 sequence analysis during COVID-19 case surge, Liberia, 2021. *Emerg Infect Dis* 2021;**27**:3185–8.
[15] Wilkinson E, Giovanetti M, Tegally H, San JE, Lessells R, Cuadros D, et al. A year of genomic surveillance reveals how the SARS-CoV-2 pandemic unfolded in Africa. *Science* 2021;**374**:423–31.