

# Setting up a Platform of a Sequencing Platform with the MinION Mk1C for Tracking SARS-Cov-2 Variants in Cote d'Ivoire (AFROSCREEN Project)

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## Abstract

Genomic surveillance has proven to be a critical source of information for understanding and responding to the COVID-19 pandemic. In Africa, genomic surveillance was very limited, and few sequences are available on GISAID database. Many initiatives, including WHO and other international partners, have been working to build capacity, particularly in low-income countries, to implement genomic surveillance. The AFROSCREEN project is about structuring an African network to build the capacities of laboratories, share data and effectively monitor the circulation of viruses. The aim of this study is to show the contribution of the WHO and AFROSCREEN project in the implementation of genomic surveillance of SARS-CoV-2 in the Ivory Coast. The acquisition of a MinION Mk1C sequencer from Oxford nanopore technology and training in sequencing and sequence analysis have enabled 395 sequences to be obtained. Of the 395 samples sequenced, 241 (61.01%) sequences yielded high-quality genomic data, achieving  $\geq 95\%$  genome coverage with a read depth of 30X and greater. These 241 sequences have passed quality control and have been deposited in the Global initiative on sharing of all influenza data (GISAID). In conclusion, support from the AFROSCREEN Project and WHO has enabled us to set up a platform for genomic surveillance of SARS-CoV-2 in Côte d'Ivoire. Nanopore sequencing is an easy method to implement, even for laboratories with limited NGS experience.

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## Keywords

NGS, Bioinformatics, Diagnostics, SARS-CoV-2, Variants

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## 1. Introduction

The coronavirus disease 2019 (COVID-19) is a major public health concern with a very important impact on the stability of the world [1]. The causative agent of this emerging virus has been identified by metagenomic RNA sequencing and virus isolation from bronchoalveolar lavage fluid samples from patients with severe pneumonia [2]. It was provisionally named 2019 nCoV. The first cases of COVID-19 were reported in December 2019 in Wuhan, the capital of China's Hubei province [3]. The virus quickly spread to several countries and forced the WHO to declare a public health emergency of international concern on 30<sup>th</sup> January 2020 [4] and then to declare a pandemic on 11<sup>th</sup> March 2020 [5]. On this date, the world recorded 118,465 confirmed cases, and 4295 deaths in 116 countries. Very quickly, the virus spread reaching all continents with an exponential increase in the number of deaths [6]. Analysis of viral genome sequence data identified a large proportion of cases belonged to a new single phylogenetic cluster. The new variant is defined by multiple spike protein mutations (deletion 69 - 70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H) present as well as mutations in other genomic regions. It is known and expected that viruses constantly change through mutation, leading to the emergence of new variants [7]. The SARS-CoV-2 Spike protein plays a crucial role in the infectivity of the virus, facilitating its entry into human cells. Since the emergence of the virus, this protein has undergone multiple mutations, giving rise to various variants with specific characteristics [8]. In order to understand the evolution of the virus in the human population, the scientific community urgently generated and shared information about the SARS-CoV-2 virus, genomes and other data [9]. Genomic surveillance has proven to be a critical source of information for understanding and responding to the pandemic. By the end of 2020, over 200,000 sequences from the European region were available in the GISAID database (<https://www.gisaid.org/>). In Africa, genomic surveillance was very limited. During the same period, fewer than 5000 SARS CoV-2 sequences from Africa were available in the GISAID database [10]. The importance of improving viral genomic surveillance capacity worldwide is recognized. This is essential if scientists are to monitor the spread of viruses and individual mutations [11]. Collectively, this information is used to better understand how virus evolution can impact public health [12]. Many organizations, including WHO and other international partners [5], are working to build laboratory capacity, particularly in low-income countries, to implement genomic surveillance [13]. The African Centers for Disease Control and Prevention (Africa CDC) have played a central role in coordinating genomic surveillance of SARS-CoV-2 in Africa. They are working closely with initiatives, such as Africa

Pathogen Genomics, to build sequencing and variant surveillance capacity on the continent. This collaboration aims to improve early detection of variants and inform public health decisions. In West Africa, several countries have set up genomic surveillance programs to monitor the evolution of SARS-CoV-2 and other pathogens. These initiatives include strengthening national laboratories, training specialized personnel and networking research centers to facilitate the sharing of data and resources. These collective efforts enable a better understanding of epidemic dynamics and a more effective response to emerging health threats. The AFROSCREEN project is a major initiative to strengthen genomic surveillance in Africa. Launched in 2021, it is funded by the French Development Agency (FDA) and coordinated by ANRS | Emerging Infectious Diseases, in partnership with Institut Pasteur and Institute of Research for Development (IRD). The AFROSCREEN project aims to establish a network across Africa to strengthen laboratory capacities, facilitate data sharing, and effectively monitor the spread of viruses. This initiative involves thirteen countries: Benin, Burkina Faso, Cameroon, Ghana, Guinea, Madagascar, Mali, Niger, the Central African Republic, the Democratic Republic of Congo, Senegal, Togo, and Côte d'Ivoire. In Côte d'Ivoire, the National Influenza Center, which also serves as the national reference laboratory for SARS-CoV-2, is one of the project's key beneficiaries. The purpose of this study is to highlight the role of the WHO and the AFROSCREEN project in advancing the genomic surveillance of SARS-CoV-2 in Côte d'Ivoire.

## **2. Material and Methods**

### **2.1. Acquiring and Handling of the MinIon Mk1C Sequencer, Oxford Nanopore Technologies (ONT)**

In the context of the COVID-19 pandemic, it was necessary to develop local capacity for the use of the SARS-CoV-2 genome within the reference laboratory of the Institut Pasteur de Côte d'Ivoire for the diagnosis of COVID-19. The overall objective was to identify SARS-CoV-2 variants and decipher the consequences of modifications to its Spike protein, using high-throughput sequencing with ONT. To this end, we participated in a two-week training workshop from 14 to 25 February 2022 in Accra (Noguchi Memorial Institute for Medical Research), entitled "Next Generation Sequencing for Genomic Surveillance". The course, organised in collaboration with the World Health Organisation (WHO), the West African Health Organisation (WAHO), AfroScreen and the African Centre for Disease Control (Africa CDC), was attended by ten (10) participants from eight (8) African countries: Benin, Cape Verde, Côte d'Ivoire, Guinea, Liberia, Mauritania, Sierra Leone and Togo. The aim of the training was to prioritize SARS-CoV-2 genomic surveillance activities in an efficient manner and to generate real-time data to guide responses to the SARS-CoV-2 pandemic. More specifically, the aim was to provide participants with practical training in genomic sequencing to improve their ability to understand the different sequencing techniques, particularly next-generation sequencing (NGS) techniques. Following this training, the laboratory

acquired two MinION Mk1C machines and reagents for sequencing SARS-CoV-2, as part of the AFROSCREEN project and WHO support for genomic surveillance. An international expert from the ONT has been invited to come to the SARS-CoV-2 diagnostic reference laboratory in Côte d'Ivoire from 26 to 28 October 2022 to configure the sequencers, install the bioinformatics analysis pipelines and train laboratory staff in the use of the MinION Mk1C, troubleshooting and sequence analysis.

## **2.2. Whole Genome Sequencing of SARS-CoV-2**

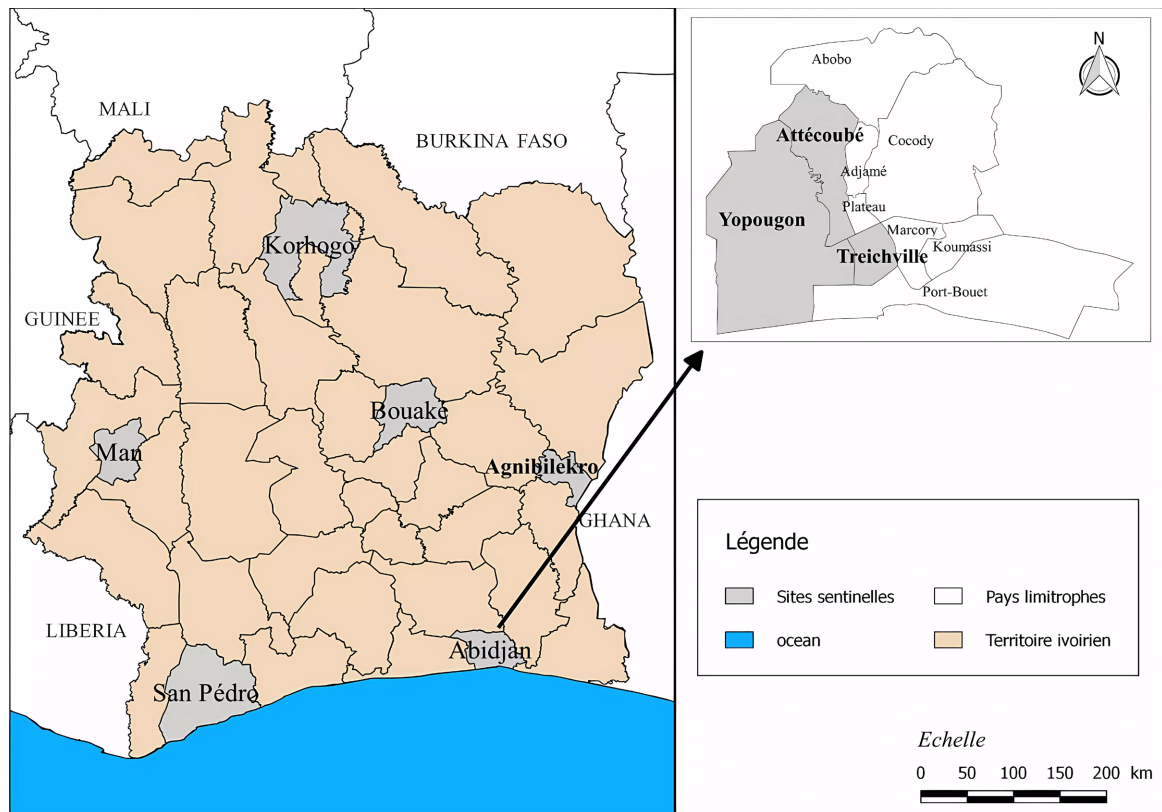
### **2.2.1. Sample Collection**

The samples were collected as part of the sentinel influenza surveillance network in Côte d'Ivoire. Since 2020, SARS-CoV-2 surveillance has been integrated into influenza surveillance. The influenza surveillance network comprises 10 sites across the country (**Figure 1**). Influenza sentinel surveillance network is a primary care surveillance network that has been in existence since the 2006-2007 season. In view of the COVID-19 pandemic and the co-circulation of influenza virus and SarsCoV-2, the World Health Organization (WHO) recommends the integration of SarsCoV-2 virus into influenza surveillance [14]. Starting with the 2022 season, the Ministry of Health has decided to revitalize this network in line with WHO recommendations. The National Institute of Public Hygiene (INHP) has been put in charge of central coordination in collaboration with the National Reference Laboratory for Influenza at the Institut Pasteur of Cote d'Ivoire (IPCI). The aim of this network, known as the Influenza/Covid-19 sentinel surveillance network, is to detect an epidemic situation at an early stage through weekly monitoring of Influenza-Like Illness (ILI) and Severe Acute Respiratory Infections (SARI), and to identify the strains responsible, in order to adapt health measures. In this study, samples were collected by nasopharyngeal swabs from patients who met the case definition of ILI and SARI as described in the protocol for integrated surveillance of influenza viruses and SARS-CoV-2. Indeed, ILI (Influenza-Like Illness) and SARI (Severe Acute Respiratory Infection) are two clinical syndromes used in public health to monitor and track respiratory infections, including influenza and other respiratory viruses such as COVID-19. On the other hand, the samples were collected as part of the national response to the COVID-19 pandemic, including the sampling sites and the infectious diseases department at Treichville University Hospital (SMIT). After each collection, samples are packed in coolers with cold accumulators and transported to the reference laboratory. Samples from sentinel influenza surveillance were sent in an epidemiological form containing the patient's biometric data, medical history, and clinical symptoms. Samples from the COVID-19 response system were sent with forms containing basic information about the patient or person requesting the test.

### **2.2.2. RNA Extraction**

RNA was extracted from 200 µL of nasopharyngeal secretion swab samples in a viral transport medium (VTM). Extraction was performed using the QIAamp Vi-

ral RNA Mini kit (QIAGEN®, Hilden, Germany), according to the manufacturer's instructions with RNA elution in a final volume of 80 µL of AVE buffer. Nuclease-free water was included in each extraction as a negative control. Two aliquots of each extracted RNA sample were made; one aliquot was used for RT-PCR, and the second was stored at  $-80^{\circ}\text{C}$  for further analysis.



**Figure 1.** Sentinel sites of the influenza surveillance network in the Ivory Coast.

### 2.2.3. Detection of SARS-CoV-2 in Nasopharyngeal Swabs by RT-qPCR

All samples from the Côte d'Ivoire influenza sentinel surveillance network were amplified using the CDC influenza SARS-CoV-2 (Flu SC2) multiplex Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) (CDC Influenza Virus Real-Time RT-PCR Influenza A/B Typing Panel (VER 2) (RUO) as per CDC protocol [15]. Samples collected as part of the national response to the COVID-19 pandemic, from sampling sites and the infectious diseases department of Treichville University Hospital (SMIT), were amplified using the STANDARD M nCoV Real-Time Detection SD BIOSENSOR kit (Ref. M-NCOV-01). For the study, 658 positive samples with CT value  $\leq 30$  were collected during the period from July 2022 to December 2023 for whole genome sequencing.

### 2.2.4. Library Preparation and Sequencing

The ONT library was prepared according to the ARTIC Midnight PCR tiling protocol for SARS-CoV-2 using the Rapid barcoding Kit (SQK-RBK110.96). The cDNA was prepared from viral RNA. A volume of 8 µl of each RNA sample was

transcribed with 2 µL of LunaScript RT SuperMix (LS RT) using a thermal cycler with the following program: 25°C for 2 min, 55°C for 10 min, 95°C for 1 min, and hold at 4°C. Preparation of the Master-Mix for cDNA Amplification was carried out using the Midnight RT-PCR Expansion kit (EXP-MRT001) containing separate primer pools used for overlapping mosaic PCR reactions covering the viral genome [16]. The PCR reaction mix for 96 samples contains 241 µL of nuclease-free water, 6 µL of Pool A or Pool B Midnight Primers, and 687 µL of Q5 HS Master Mix (Q5). The PCR amplification step was carried out under the following conditions: an initial denaturation step at 98°C for 30 s, followed by 35 cycles at 98°C for 15 s, 65°C for 5 min, and 4°C. After amplification, electrophoresis of the amplified products was performed, and only the 1200-bp amplicons were selected for further sequencing. Rapid barcodes were added to the 96-well barcode attachment plate by mixing 2.5 µL of nuclease free water, 5 µL of pooled PCR products (pools A and B) and 2.5 µL of barcodes from the Rapid Barcode Plate. The reaction was incubated in a thermocycler at 30°C for 2 minutes, then at 80°C for 2 minutes. The amplicons were then pooled, purified with an equal volume of AMPure XP magnetic beads (Beckman Coulter, Brea, California, USA) and quantified on a Qubit 4.0 spectrophotometer, using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, California, USA), followed by Library concentration normalization. For sequencing, the SQKRBK110.96 and EXP-MRT001 kit were used to prepare the Priming Mix and Library Mix, which were then loaded onto a Flow-cell inserted into the MinION Mk1C device (Oxford Nanopore Technologies, Oxford, UK) according to instructions. When the run is complete, the FastQ-pass file is retrieved for sequence analysis.

### 2.2.5. Nanopore Sequence Analysis

#### ARTIC-nCoV protocol

Sequence analysis was performed using the ARTIC-nCoV-bioinformatics protocol SOP-v1.1.3 and base calling was performed using Guppy v.4.2.2 (Oxford Nanopore Technologies) in high-precision mode (model dna\_r941\_450bps\_hac). ARTIC ONT sequencing data were demultiplexed using guppy\_barcode (v4.2.2) with the “require\_barcode\_both\_ends” option and a score of 60 at both ends. The analysis was performed using a copy of the ARTIC pipeline (v1.1.3) [2] to generate a consensus sequence for each sample in FASTA format. The pipeline comprises the following main steps: input reads were read-length filtered (ARTIC, 400-700) and mapped to the Wuhan-Hu-1 reference genome (accession MN908947.3) using minimap2 (version 2.17-r941). Adjusted reads were then used for variant calling with medaka (v 1.2.0). The final consensus was generated from a filtered VCF file and a position mask file with a coverage depth of less than 20. The final consensus file obtained was used on Nextclade and Pangolin for clade assignment.

#### Quality control

The COG-UK consortium defined a consensus sequence as passing COG-UK quality control if more than 50% of the genome was covered by confident calls or if there was at least 1 contiguous sequence of more than 10,000 bases and no evi-



dence of contamination. This is considered the minimum amount of data to be phylogenetically useful [3]. A confident call was defined as having a minimum coverage depth of 10X for Illumina data and 20X coverage depth for Nanopore data [3]. If coverage fell below these thresholds, bases were masked by Ns. Low-quality variants were also masked with Ns. The QC threshold for inclusion in GISAID was higher, requiring over 90% of the genome to be covered by confident calls with no evidence of contamination.

2.2.6. Phylogenetic Analysis

A phylogeny was generated from all the consensus genomes of the ARTIC-ONT data obtained. A multiple FASTA alignment was created by aligning all samples to the reference genome MN908947.3 with MAFFT v7.470 [5]. Sequences were filtered to remove unnecessary aligned characters, as well as those containing more than 30% spaces, using BMGE [4] and save the remaining aligned characters in a FASTA file. A maximum likelihood tree was estimated with IQTREE2 (v2.0.4) [6] with an optimal evolutionary model (default option) with final branch collapse close to zero in polytomy, and the tree was visualized with Fig tree [17]. The pep-ilin GATK was used to search for variants and as a point of comparison for SNPs.

2.3. Results

2.3.1. Testing of Patient Sample

From July 2022 to December 2023, 658 positive samples (2022 = 427 and 2023 = 231) were collected. The age of infected patients ranged from 1 month to 82 years. There were significantly more males (543/658) than females (115/658) who tested positive for SARS-CoV-2. (Table 1). Molecular tests were performed at the influenza reference laboratory of the Institut Pasteur de Côte d’Ivoire.

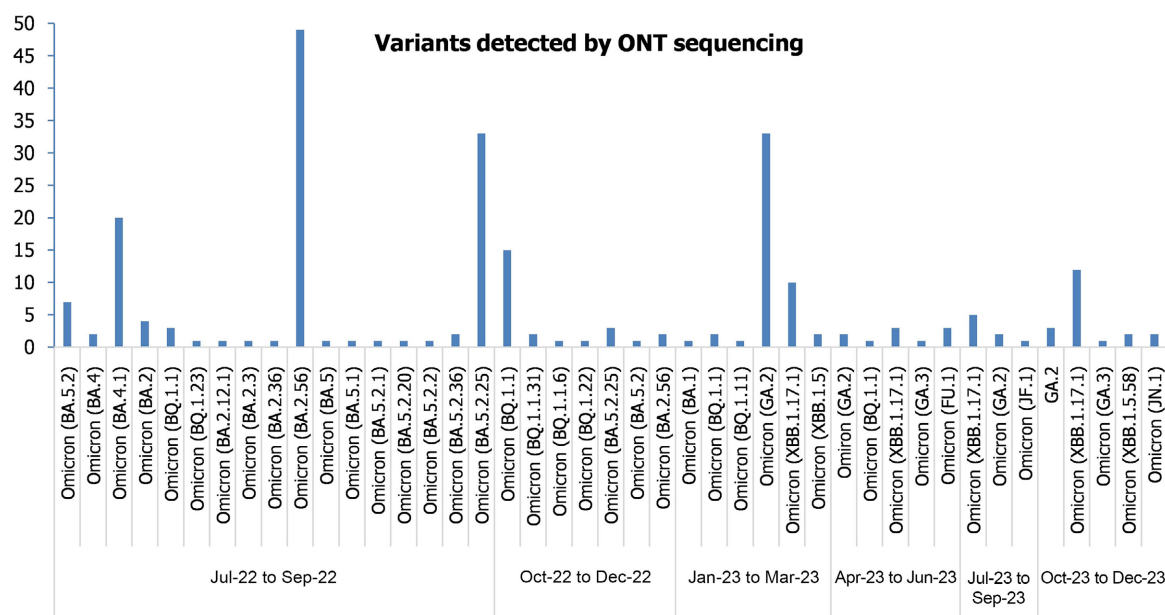
Table 1. Breakdown of samples.

| Year  | Number of Samples | Male | Female | Age (1 month to 5 years) | Age (6 to 15 years) | Age (16 to 25 years) | Age (25 to 50 years) | >50 years |
|-------|-------------------|------|--------|--------------------------|---------------------|----------------------|----------------------|-----------|
| 2022  | 427               | 360  | 67     | 36                       | 47                  | 95                   | 211                  | 38        |
| 2023  | 231               | 183  | 48     | 15                       | 39                  | 21                   | 131                  | 25        |
| Total | 658               | 534  | 115    | 51                       | 86                  | 116                  | 342                  | 63        |

2.3.2. Identification of SARS-CoV-2 Variants

During the study period, we locally collected 658 RT-qPCR positive samples for SARS-CoV-2. Using the MinION Mk1C instrument and the ARTIC SARS-CoV-2 network workflow, a total of 395 samples were sequenced and analysed. Of the 395 samples sequenced, 241 (61.01%) sequences yielded high-quality genomic data, achieving ≥95% genome coverage with a read depth of 30X and greater. These 241 sequences have passed quality control and have been deposited in the Global initiative on sharing of all influenza data (GISAID) (<https://www.gisaid.org/hcov19-variants/>). The results of this study revealed that in 2022, 4 Omicron lineages (BA.2, BA.4, BA.5, BQ), comprising twenty (20) sub-

lineages were detected with a predominance of BA.2.56 (33.11%), followed by BA.5.2.25 (23.37%), BA.4.1 (12.98%) and BQ.1.1 (11.68%). For the year 2023, 8 Omicron lineages (BA.1, BQ, GA.2, GA.3, FU.1, XBB.1, JF.1 and JN.1) comprising eleven (11) sub-lineages were identified with a predominance of GA.2 (45.97%) and XBB.1.17.1 (35.63%). The distribution of sub-lineages is shown in **Figure 2**. The proportion of lineages detected decreased over time. A peak in the detection of sub-lineages was observed from July to September 2022 (53.52%, 129/241), with a very low level observed in the third quarter of 2023 (3.31%, 8/241). Three lineages (BA.5, BA.4 and BA.2) were found during the first quarter of the study period, from July to September 2022. In the last quarter of the same year, 2 lines (BA.5 and BA.2) were detected. In 2023, 4 lineages were identified, namely BA.1, XBB.1, BA.5 and BA.1. A single lineage was present throughout the first quarter of 2023. (**Figure 2**)



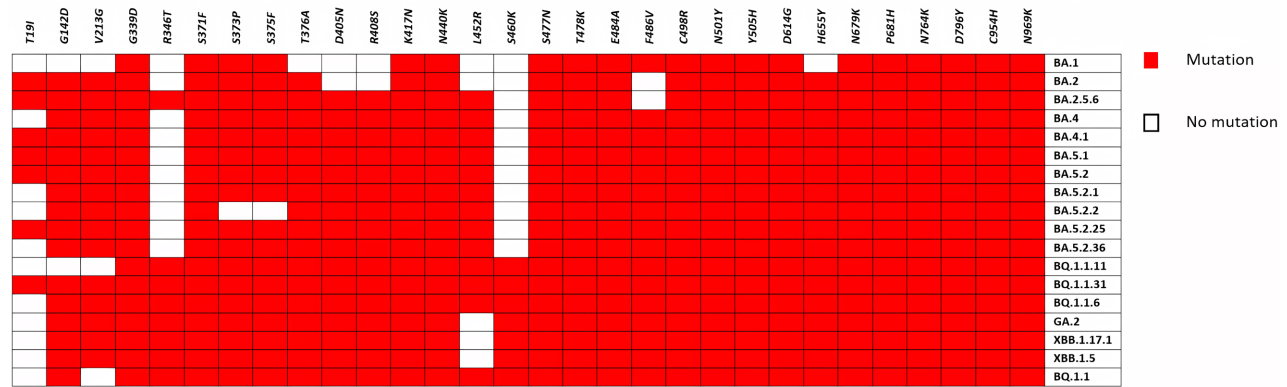
**Figure 2.** Periodic evolution of SARS-CoV-2 variants detected in samples.

### 2.3.3. Mutation Analysis

As with all RNA viruses, SARS-CoV-2 exhibits a high degree of genetic variability. Mutations occur naturally during viral replication and variant strains can emerge. The Spike (S) protein was the target of the analyses carried out as part of this study, as it is the virus' gateway into the host cell. Several substitutions were detected within the lines identified in this study. In total, 31 mutations were observed compared with the spike protein of the Wuhan reference strain. 84% (26/31) of the mutations were present in all the variants detected. In the BA.2 line, two positions (460; 486) were not affected by mutations. Lineage BA.4 was not affected by mutations at positions 460 and 346. In line BA.5, in addition to the mutations mentioned above, mutations were observed at positions 460 and 346. Positions 19, 373 and 375, the BA.5 sublineages BA.5.2.2 (373; 375), provide a better characterisation



of the BA.5 lineage. Variant BQ.1 contains 100% of the mutations described. The BA.1 line showed 32% of the mutations. Compared with the other variants, only positions 346 and 460 were free of mutations for the BA.5.2.25 subline compared with the Wuhan reference strain. For the GA2, XBB.1.5 and XBB.1.17.1 sublines, positions 19 and 452 were mutation-free. Most of the other sublines had a large number of mutations in very few samples, resulting in relatively high genetic diversity (**Figure 3**).



**Figure 3.** Mutations identified in sequenced samples.

2.3.4. Phylogenetic Analysis



**Figure 4.** Phylogenetic analysis.

The sequencing data obtained as part of this study was used to create a phylogenetic tree representing the evolutionary relationships between the different viral strains. This tree shows how the different strains are related to each other and identifies clusters or groups of closely related strains. Phylogenetic analysis of the genomes shows similarities with certain lineages around the world that have the Wuhan variant as a common ancestor. However, as they diverge, they share more recent ancestors. We also found that all the variants detected in this study share a common ancestor with BA.1(Omicron). The COVID-19 pandemic started in mid-November 2019, but the tree shows very significant evolution in the last half of 2022, in contrast to 2023. (Figure 4)

### 3. Discussion

In the context of the COVID-19 pandemic, it was imperative to strengthen the capacity of laboratories in the African region to best identify the different variants of concern (VOCs) of SARS-CoV-2 [18]. The use of Oxford Nanopore technology was an alternative to the large sequencing platform due to its lower cost, performance and better adaptation to laboratories with modest infrastructures [19]. As a result, most African laboratories have used Oxford nanopore technology for SARS-CoV-2 sequencing [20]. It was important to set up this sequencing platform to identify SARS-CoV-2 variants in real time, and to avoid the long wait for results caused by sending samples to other laboratories outside the country. This study demonstrated the process of setting up genomic surveillance for SARS-CoV-2 at the Pasteur Institut of Côte d'Ivoire within the Epidemic Viruses Department. The laboratory's capacity for sequencing the SARS-CoV-2 genome has been strengthened with the acquisition of a MinION Mk1C and training in the use of pipelines for analysing SARS-CoV-2 sequences. With regard to the identification of the SARS-CoV-2 variant in Côte d'Ivoire from July 2022 to December 2023, the results show that 100% of the sequences obtained contained mutations belonging to the Omicron variant. Phylogenetic analysis of the sequences of the samples in the current study revealed a very close relationship between our Omicron variants and Omicron variants that have been sequenced in other countries in the sub-region. This same contact was established by [21] in their work. This could suggest a similar source of the Omicron variants circulating in these different regions. Indeed, the Omicron variant was first discovered in Botswana on 11 November 2022. According to a report entitled Tracking SARS-CoV-2 variants, published in 2022, the genomic sequence of this new SARS-CoV-2 lineage (B.1.1.529) was designated in the Global Initiative on Sharing All Influenza Data database. This variant was rapidly classified as the fifth variant of concern (VOC) and named Omicron by the World Health Organization (WHO). Less than four months after its first detection, the Omicron variant was detected in 188 countries. From 23 February 2022 to 24 March 2022, more than 99.7% of the sequences deposited on GISAID were from the Omicron variant [22]. From the B.1.1.529 sublineage, the significant evolution of the genomic regions of the Omicron variant has led to the

emergence of several sublineages or sub-variants, referred to as recombinant BA.1, BA.2, BA.3, BA.4, BA.5 and BA.1/BA.2 [23]. A recent article published in the New England Journal of Medicine [24] explains how the BA.2.12.1, BA.4 and BA.5 sub-variants escape neutralising antibodies induced by vaccination and infection. This article reveals that the titres of neutralising antibodies against the BA.4 or BA.5 sub-variants and against the BA.2.12.1 sub-variant (to a lesser extent) were lower than the titres against the previous BA.1 and BA.2 sub-variants. These results establish a context for the increase in cases observed in 2022 by the BA.2, BA.4 and BA.5 variants in populations. In addition, the emergence of several omicron sublineages, such as BA5.2.25, has been the origin of waves of COVID-19 in Africa [25]. This suggests that Africa is still the epicentre of this variant, but diagnosis and knowledge of its evolution remain difficult. Lineages BA.4 and BA.5 emerged in South Africa in March 2022 and subsequently became dominant in South Africa and Portugal respectively. Our analysis showed that these lines were detected in the third quarter of 2022. This is partly explained by the mandatory antigen screening at Félix Houphouët Boigny International Airport. This trend could also be attributed to direct human interactions facilitated by cross-border movements, trade or conflicts in neighbouring countries. In addition, Omicron's transmission advantage appears to be largely attributable to immune escape, but also to the potential increase in intrinsic transmission capacity [26]. Although there is significant evidence of immune escape from transmission from natural or post-vaccination immunity, more data are needed to better understand the relative contribution of increased intrinsic transmissibility and immune escape in explaining transmission dynamics. There is evidence that the Omicron variant infects human bronchial tissue more rapidly and efficiently than the Delta variant [27]. Studies in other countries have shown that Omicron variants have dominated Delta variants. In particular, in China and South Africa, the Omicron BA.4 and BA.5 sublines were the most predominant compared to the BA.1 subline [20]. The XBB lineage, which appeared in Côte d'Ivoire during the first half of 2023, is one of the SARS-CoV-2 Omicron lineages with the best immune escape capacity, according to in vitro studies carried out on human serum samples [28]. In fact, this period of appearance of the XBB sub-lineage in Côte d'Ivoire is perfectly consistent with the first documented samples, which date from 9 January 2023 [25]. Interestingly, the XBB sublineage was only designated as a variant of interest (VOI) on 17 April 2023, based on its documented reports in 98 countries, and became dominant in most countries in the second half of June [29]. This is not characteristic of a dangerous variant, which usually spreads rapidly and affects a large population. XBB.1.5 evolved from XBB, which began circulating in the UK in September 2022. XBB had a mutation that allowed it to evade the body's immune defences. But this same quality also reduced its ability to infect human cells. According to [30], XBB.1.5 has a mutation called F486P, which restores its ability to bind to cells while continuing to evade immune defences. This allows it to spread more easily. These evolutionary changes have been like "springboards",

with the virus evolving to find new ways of bypassing the body's defence mechanisms.

## 4. Conclusion

In this study, we sequenced and analysed 395 SARS-CoV-2 samples from Côte d'Ivoire, collected between July 2022 and December 2023, using MinION Nanopore sequencing. Overall, the ARTIC protocol used generated the libraries required for successful sequencing. The MinION Oxford Nanopore technology enabled the identification of structural variations, which was one of the objectives of our study. Having established a local capacity to generate high-quality genomic sequences and to analyse them exhaustively in-house thanks to the training received via the Afroscreen project, we obtained a total of 241 good-quality sequences, which were deposited on the international GISAID platform. We have shown that Nanopore sequencing is an easy method to implement, even for laboratories with limited NGS experience. It is therefore a reliable alternative for rapid and effective surveillance. Monitoring the emergence and spread of new variants/sub-variants of SARS-CoV-2 is of crucial importance for the control of the COVID-19 pandemic, and only whole genome sequencing offers important information on the evolution of the virus.

## Acknowledgements

We would like to thank the coordinators of the AFROSCREEN project, particularly the Pasteur Network and the WHO, for the technical and financial support they gave us for carrying out this study.

## Importance

The AFROSCREEN project is about structuring an African network to build the capacities of laboratories, share data and effectively monitor the circulation of viruses. Thirteen countries—Benin, Burkina Faso, Cameroon, Ghana, Guinea, Madagascar, Mali, Niger, Central African Republic, Democratic Republic of Congo, Senegal, Togo and Côte d'Ivoire are involved in this project. In Cote d'Ivoire, the National Influenza Center, which houses the national reference laboratory for SARS-CoV-2 was one of the beneficiaries of the project. The aim of this study is to show the contribution of the WHO and AFROSCREEN project in the implementation of genomic surveillance of SARS-CoV-2 in the Ivory Coast.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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