**Coronavirus Pandemic**

**Genomic epidemiology of SARS-CoV-2 in Senegal in 2020-2021**

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**Abstract**

Introduction: In Senegal, molecular diagnosis was widely used for the detection and management of COVID-19 patients. However, genomic surveillance was very limited in the public sector. This study aimed to share the experience of a Senegalese public sector laboratory in response to the COVID-19 pandemic, and to describe the distribution of variants circulating in 2020 and 2021.

Methodology: From July 2020 to December 2021, SARS-CoV-2 qRT-PCR was performed on nasopharyngeal samples from travelers and symptomatic patients at the Bacteriology and Virology Laboratory (LBV) of the Aristide le Dantec University Teaching Hospital. Samples with a cycle threshold (Ct) ≤ 30 were selected for whole-genome sequencing (WGS) using the Nanopore technology. In-house scripts were developed to study the spatial and temporal distribution of SARS-CoV-2 variants in Senegal, using our sequences and those retrieved from the GISAID database.

Results: Of 8,207 patients or travelers screened for SARS-CoV-2, 970 (11.8%) were positive and 386 had a Ct ≤ 30. WGS was performed on 133 samples. Concomitantly with high-quality sequences deposited in the GISAID database covering nine cities in Senegal in 2020 and 2021 (n = 1,539), we observed a high circulation of the 20A (B.1, B.1.416 and B.1.620) and 20B (B.1.1.420) lineages in 2020, while most of the samples belonged to Delta variants (AY34 and AY.34.1, 22%) in 2021.

Conclusions: Despite its late involvement, COVID-19 diagnosis was routinely performed in LBV, but genomic characterization remained challenging. The genomic diversity of SARS-CoV-2 strains in Senegal reflected that observed worldwide during the first waves of the pandemic.

**Key words:** COVID-19; SARS-CoV-2; Senegal; genomic diversity; wave.


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**Introduction**

After its first description in Wuhan, China, in 2019 [1,2], the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly around the world, causing COVID-19 to become a public health emergency [3] and then classified as a global pandemic by the World Health Organization (WHO) due to the number of infections worldwide.

Health organizations estimate that the number of people infected with SARS-CoV-2 has exceeded 611 million and the number of deaths has reached 6.5 million worldwide as of September 2022 [4]. The African continent, which was the last to report COVID-19 cases, has been the least affected in terms of morbidity and mortality, but this is likely to be underestimated due to lack of diagnosis. As of September 2022, the number of positive cases was estimated at ~12.8 million and the number of recorded deaths was close to 300,000 [5].

Senegal, a West African country, also reported SARS-CoV-2 cases in early March 2020 and, like other countries, began responding to the pandemic by implementing rapid diagnosis of individuals and isolation of patients to control the virus and limit its spread. Leveraging the expertise of the HIV laboratory system, qRT-PCR testing was implemented in public health laboratories in addition to private research institutes.

The SARS-CoV-2 genome has evolved due to the replication process of RNA viruses [6], resulting in the
emergence of new variants that are slightly different from the original strain. To date, 13 variants have been described, including 5 variants of concern (VOCs) and 7 variants of interest that shaped the circulation of the COVID-19 pandemic [7]. Therefore, genomic surveillance of SARS-CoV-2 strains is essential to provide information on virus biology, transmission, virulence, infectivity, and dynamics of different viral populations [8].

Viral genomic surveillance has been very limited in most African countries due to lack of whole-genome sequencing (WGS) equipment and expertise. This is reflected globally, with only 1% of sequences coming from Africa [9,10]. In fact, of the 10 African countries that published the most sequences on GISAID in 2021, only 3 were from West Africa [11]. SARS-CoV-2 genome sequencing must be performed on at least 5% of the number of positive cases in a given area, as recommended by the WHO [12]. However, at the beginning of the pandemic, WGS equipment in Senegal was mostly available in the private sector.

Here, we share the experience of a Senegalese public sector laboratory in responding to the COVID-19 pandemic and describe the spatial and temporal circulation of SARS-CoV-2 variants in Senegal during 2020-2021.

Methodology

Ethical statement
The samples used in this study were obtained from patients and travelers included in the PANBIO COVID-19 Ag Test Evaluation Study, approved by the National Ethical Research Committee (Protocol SEN20/87 N°005/MSAS/CNERS/Sec of January 20, 2021) and authorized by the Ministry of Health (N°088/MSAS/DPRS/DR of January 25, 2021).

Study participants
From July 2020 to December 2021, the Molecular Biology Unit of the Bacteriology and Virology Laboratory (LBV) of the Aristide Le Dantec Hospital (Dakar, Senegal) was involved in the diagnosis and follow-up of COVID-19 patients and in traveler screening. The remaining SARS-CoV-2 positive samples were then used for genomic surveillance. Non-consenting patients or patients with inconclusive diagnostic results were excluded from this study.

SARS-CoV-2 diagnosis
Nasopharyngeal swabs were inactivated at 56°C for 15 minutes, and viral RNA was extracted from the swabs using different platforms: m2000sp (Abbott Diagnostic, USA) and Amplix 12 (Biosynex, France) with Abbott mSample Preparation System DNA (Abbott Diagnostic, USA) and MagPurix Viral Nucleic Acid Extraction Kit (Zinexts Life Science, Taiwan), respectively, according to the manufacturer’s instructions. qRT-PCR was then performed with multiple kits targeting the RdRp, ORF1ab, E and/or N viral genes using either the Abbott RealTime SARS-CoV-2 Assay (Abbott Diagnostic, USA) or the DaAn Gene Co Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (Ltd of Sun Yat Sen University), Sansure Bio Tech Inc., or Lifedriver (Shanghai ZJ Bio-Tech Co., Ltd) according to the manufacturer’s recommendations.

Library preparation and genomic sequencing
Samples with a cycle threshold (Ct) value ≤ 30 were selected for SARS-CoV-2 genome sequencing. Sequencing was performed according to the Oxford Nanopore ARTIC protocol (nCoV-2019 sequencing protocol V3) as previously described [13,14]. Briefly, retrotranscription of RNA extracts was performed using the LunaScript™ RT SuperMix Kit (New England Biolabs). The obtained cDNA was then amplified using primer pools targeting different genes along the SARS-CoV-2 genome. The resulting gene pools were combined and quantified using the Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific, USA) prior to DNA end repair and barcoding of each sample. Following this step, the barcoded samples were pooled and then purified using AMPure XP magnetic beads (Beckman Coulter, USA) before adding adapters. Further purification was performed on the final library, followed by quantification before priming the flow cell to generate sequences on the MinION™ (Oxford Nanopore Technologies).

Generation of SARS-CoV-2 genome sequences
Prior to genome assembly, read quality control was assessed using NanoPlot [15]. Raw reads were then assembled using the ARTIC pipeline bioinformatics workflow [16,17]. Alignment metrics, consensus assessment and variant annotation were performed using SAMTools [18], BCFTools and SnpEFF [19], respectively. Genome sequences were submitted to the international GISAID database (https://www.gisaid.org).

Genetic diversity and mutation analysis
Phylogenetic analysis of the SARS-CoV-2 genomes was performed using the Nexstrain pipeline, which includes several quality control steps (such as...
alignment of sequences using Nextalign to identify gaps relative to the reference genome and running Pangolin to assign lineages.

To investigate the evolution of SARS-CoV-2 sequences over time in Senegal, in addition to the sequences we generated, we retrieved from the GISAID database (as of May 21, 2022) all sequences that met the following criteria: 1) a city in Senegal was associated with the sequence; 2) at least 95% of the SARS-CoV-2 genome sequence was covered; 3) a complete date of collection was provided; and 4) the date was comprised between January 1, 2020, and December 31, 2021. If the number of sequences covering a city was less than 15, the sequences were discarded. The final dataset consisted of 1,539 sequences covering nine cities in Senegal (Dakar, Diourbel, Fatick, Kaolack, Kedougou, Louga, Matam, Saint-Louis, and Thies) from February 28, 2020 to December 7, 2021. Custom R scripts were developed to study the genetic diversity of SARS-CoV-2 over time, by city, and by lineage [20].

**Results**

**Demographic and clinical characteristics of study participants**

From July 2020 to December 2021, 8,207 patients or travelers were screened for COVID-19 infection. The median age was 48 years (range: 28-50) and the sex ratio (M/F) was 1.96. A total of 970 (11.8%) patients were positive for SARS-CoV-2, including 768 symptomatic individuals. Of these positive cases, 386 samples had a Ct value of 30 or less. A total of 133 samples were processed for WGS (Figure 1). Of the 133 samples sequenced, only 12.8% covered at least 95% of the SARS-CoV-2 genome. The first sequences were dated July 28, 2020, and the last were dated September 6, 2021. Twenty-five sequences (18.8%) were from samples collected in 2020, and the remaining sequences (81.2%) were from 2021, including 66 sequences between July and September 2021.

**Genomic epidemiology of circulating variants in Senegal**

Given the limited number of sequences we generated, hundreds of complete SARS-CoV-2 genomes were retrieved from the GISAID database in 2020-2021, covering nine cities in Senegal (Figure 2a). Of the 1,539 sequences investigated, 70.8% were from Dakar, 7.2% from Fatick, 4.5% from Diourbel, and 4.3% from Thies. Other cities were represented by less than 3% of the sequences.

Overall, the Delta (AY34 and AY.34.1, 25%), 20A (B.1.416, 13%), 20B (B.1.1.420, 12%) and Eta (B.1.525, 9%) lineages represented the major viral lineages within the sequenced SARS-CoV-2 genomes in Senegal over the time period (Figure 2b).

In 2020, some differences in the frequency of SARS-CoV-2 lineages were observed between cities in Senegal (Figure 2c). In Dakar, more than half of the sequences (53.6%) corresponded to the 20A lineage (B.1, B.1.416 and B.1.620), followed by the 20B lineage (B.1.1 and B.1.1.420, 26.2%). In Kedougou, Matam or Thies, most of the sequenced genomes (> 53%) belonged to the 20B lineage (B1.416). Of note, Louga and Saint-Louis were associated with some SARS-CoV-2 lineages that were poorly represented in other cities (36.4% and 37.5%, respectively). Finally, in Diourbel, 73.8% of the sequences were associated with the 20A lineage.

In 2021, regardless of the city studied, most of the samples belonged to the Delta lineage (AY.34, AY.34.1 and B.1.617.2), which first spread in India from April 2021 (Figure 2d). The Alpha lineage (B.1.1.7) was rarely found, except in Saint-Louis (42.9%) and Dakar (15.5%). The 20B lineage (B.1.1.420) was observed only in Dakar (13.3%), Saint-Louis (40.0%) and Thies (100.0%). Dakar was associated with similar proportions of the 20B (B.1.1.318 and B.1.1.420, 17.8%), Delta (AY.34 and AY.34.1, 26%), Alpha (B.1.1.7, 15.5%) and Eta (B.1.525, 19.5%) lineages.

**Figure 1.** Flow chart of diagnosed patients and SARS-CoV-2 genome sequencing in Senegal.

![Flow chart](image-url)
Figure 2. Distribution of SARS-CoV-2 Pango lineages in Senegal.

(a) Map showing the number of sequences investigated across nine Senegalese cities in 2020 and 2021. 
(b) Distribution of SARS-CoV-2 Pango lineages in Senegal in 2020-2021. Each lineage is represented by a specific color. Percentages are indicated within the plot. The proportion of each lineage for each city in Senegal was calculated in (c) 2020 and (d) 2021.
**Temporal trends of SARS-CoV-2 variant detection and frequency**

Senegal was one of the first African countries to report SARS-CoV-2 cases in March 2020, with an average of 4.1% of the total positive cases in all of Africa (Figure 3a). Then, the prevalence of SARS-CoV-2 in Senegal remained low, with an average of 0.98% of the total cases within the continent. Different variants emerged and became dominant at different times (Figure 3b). The 20A lineage was highly prevalent during the first two months of the pandemic in Senegal, first with B.1 and then with B.1.416 until October 2020. Then the 20B lineage (B.1.1.420) emerged and replaced the 20A lineage. In early 2021, the Alpha variant (20I, i.e., B.1.1.7) spread rapidly throughout the world, including Senegal, but was quickly replaced by the Eta variant (21D, i.e., B.1.525) (Figure 3b and c). However, as in the rest of the world, the Delta variant (mainly AY.34 and AY34.1) started to increase in June 2021 and became the major and only circulating lineage by the end of 2021.

**Genetic diversity of SARS-CoV-2 variants and mutational analysis**

Among the lineages investigated in the present study (n = 17), the Delta lineages (AY.34, AY.34.1 and AY.34.1.1) were associated with the highest genetic diversity, followed by the Alpha (B.1.1.7), Eta (B.1.525) and 20B (B.1.1.318) lineages (Figure 4a). Overall, these lineages had between 30 and 50 mutations compared to the Wuhan-Hu-1 reference genome. Some other lineages, such as B.1, B.1.356 or B.1.416 (belonging to 20A or 20C lineages) had few mutations because they were mostly found at the beginning of the pandemic.

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**Figure 3.** Evolution of SARS-CoV-2 main lineages in Senegal.

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**a)** Number of SARS-CoV-2 positive patients and related deaths in Africa and in Senegal between January 2020 and December 2021. The ratio between Senegal and Africa was calculated. **b)** Trends in the prevalence of the main variants circulating in Senegal between 2020 and 2021. Lineages are represented by different colors. **c)** Emergence and spread of variants of concern (VOCs) in Senegal in 2021. Each viral lineage is associated with a color.
Figure 4. Frequency of SARS-CoV-2 mutations for each viral lineage.

(a) Total number of nucleotide substitutions for each viral lineage. Box boundaries represent the first and third quartiles and the length of whiskers corresponds to 1.5 times the interquartile range. Each lineage is associated with a color.

(b) Frequencies of amino acid substitutions for each SARS-CoV-2 protein. Mutations are sorted and colored per protein.

(c) Frequency of Spike amino acid mutations in the main SARS-CoV-2 variants circulating in Senegal. A blank case indicates no mutation. The redder the color, the higher the frequency of the mutation.
The most frequent substitution in all samples was the Spike D614G mutation (93.1%), followed by ORF1b P314L (87.6%) (Figure 4b). The frequency of mutations then decreased dramatically, reaching 42.3% for the I82T mutation in the M gene, and 37.3% for the Q677H mutation in Spike. Focusing on mutations with a frequency of at least 3%, we observed that the Spike gene was associated with the highest number of mutations (n = 23), followed by ORF1a (n = 18) and N (n = 16). Other genes had from 1 to 8 mutations compared to the Wuhan-Hu-1 reference sequence.

Some variants with different evolutionary lineages had similar amino acid substitutions (Figure 4c). Focusing on the Spike gene, we observed that all lineages almost systematically had the D614G mutation, except for the first lineages that spread in the world, i.e., A.21 and A.27 (belonging to the 19B lineage). Another example is the E484K mutation, which was observed in our dataset in the 20A (B.1.619 and B.1.620), 20B (B.1.1.318), and Eta (B.1.525) variants. In contrast, some mutations were found to be variant-specific, such as the T19R mutation that was detected only in the Delta lineages (AY.34, AY.34.1, AY.34.1.1 and B.1.617.2); or the Q52R mutation, which is specific to the Eta variant (B.1.525) (Figure 4c). The mutations found in the lineages 20A and 20B were generally found at low frequencies (< 10%), with the exception of L18F (65.5%) and N440K (56.8%) in 20B (B.1.1.420), and D614G (> 99%) in both 20A and 20B. Finally, we observed that some mutations were found at high frequencies at the beginning of the pandemic, disappeared, and then reappeared in other lineages a few months later (Figure 4c). This was the case for the L452R mutation (57.1%), which was initially found in the 19B lineage, then disappeared from circulating Alpha, Eta and other variants, and then reappeared in all Delta variants (with frequencies ranging from 77.8% to 100.0%). Taken together, these results revealed the high dynamics of SARS-CoV-2 genome evolution in Senegal.

Discussion

In Senegal, epidemiological surveillance started late and was mainly carried out by two private laboratories, the Institut de Recherche en Santé de Surveillance EpidémioLogique et de Formation (IRESSEF) and the Institut Pasteur de Dakar (IPD). Based on our dataset, the mapping of the sequences shows that most of the surveillance was based in the Senegalese capital (Dakar), a situation that emphasizes the disproportionality of the technical facilities in the country, accentuated by the population density in this locality. In the public sector, to our knowledge, only one laboratory had experience in sequencing, the LBV of the Aristide le Dantec Hospital, which had to be strengthened with next-generation sequencing (NGS) techniques in partnership with IRESSEF and the Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine (MRC Gambia | LSHTM).

Regarding case detection, the LBV contributed to 1.4% (970/68,412) of the cases of COVID-19 identified in the country from the declaration of the first case of COVID-19 to September 2021, and a total of 8,207 (0.9%) of diagnostic tests performed among the 829,291 tests performed during the same period. The observed positivity rate (12%) is undoubtedly related to the high proportion of symptomatic patients (79%) who were followed up at the outbreak treatment center at the Aristide Le Dantec Hospital. The overall positivity rate in the country varied between 3 and 4% during the same period [21].

As in the rest of the world, Senegal experienced several waves of COVID-19 characterized by different SARS-CoV-2 variants [22]. The Dakar region alone contains all the variants circulating during the pandemic. This is probably related to the geographical location of Dakar, which is not only an international crossroads but also the most densely populated region in Senegal with 22.9% (density of 7,200 inhabitants/km²) [23].

In the first wave of COVID-19, the co-circulation of the different variants was dominated by the B.1 and B.1.416 lineages. The latter lineage was widespread and was the most prevalent during the first wave. These lineages have also been described in other countries [24,25]. The emergence of these variants was accompanied by a low rate of infected patients and a limited number of deaths in the country. In response, health authorities implemented containment strategies, in particular curfews, while the establishment of outbreak treatment centers ensured rapid management and isolation of patients diagnosed as positive. In addition, systematic screening of contact cases was implemented to contain the virus. The measures put in place by the country allowed an effective response against the spread of the virus [26].

The second wave of infection was more virulent and infected a larger number of people. It was characterized by the emergence and spread of the lineage B.1.1.7, which was introduced in Senegal in December 2020 [27]. This variant is part of the VOC and was reported by the UK authorities on December 14, 2020. Lineage B.1.1.7 is characterized by a mutation in the Spike
protein (N501Y) that affects the conformation of the receptor binding domain [28]. Thirteen other mutations define the B.1.1.7 lineage, several of which are found in the Spike protein, including a deletion at positions 69 and 70 (del69-70) that is thought to increase transmissibility [29,30] and explains the increase in infected patients; and D614G which is one of the earliest mutations described during the evolution of SARS-CoV-2 and is thought to increase viral fitness [31] as well as infectivity [32].

The Eta variant, detected from March to July 2021, had significant mutations at a high frequency on the Spike protein (Q52R, A67V, T95I, E484K, D614N, Q677H, F888L). The Eta variant (also called B.1.525 lineage) coexisted with the Alpha variant during the second wave and was still detected during the third wave. This trend was observed throughout West Africa [33].

The Delta B.1.617.2 variant was the precursor of the third wave with a peak of infection 2 to 3 times higher than that of the second wave. The number of infected individuals reflects its high contagiousness, transmissibility, and infectivity, which have been described in numerous studies [34,35]. A high genetic diversity of the Delta variant has been described in several countries [36]. This diversity is the basis for the classification of the Delta variant into AY sublineages. Until December 2021, the Delta variant AY.34 predominated in Senegal, followed by the B.1.1.7 and B.1.617.2 lineages. At the same time, circulating lineages from AY.1 to AY.133 were identified worldwide, reflecting an accumulation of specific mutations in this variant [37]. During the same period in the United Kingdom, AY.4.2 alone represented 20% of circulating strains [38]. In addition to this lineage, the AY.43, AY.98 and AY.120 lineages were described in England from September 2020 to December 2021 [39].

Following the genetic diversity of variants in the West African region, the same trend was observed in Nigeria, in Oyo State, with a particular predominance of the Eta variant followed by the Alpha variant from January to April 2021, before the appearance of the Delta variant AY.36 [40]. Meanwhile, in Ghana, the B.1.1.318 lineage – which was detected in small proportions in Senegal – coexisted with the Eta variant and was predominant in the major cities. In addition, this variant, which had the third highest frequency of circulating variants in Ghana, was considered as a variant under surveillance in that country [41]. In South Africa, the second wave was dominated by the Beta variant [42], which was not detected in Senegal.

Conclusions

The COVID-19 epidemic in Senegal, as in other countries, was a unique opportunity to mobilize the health laboratory sector for molecular diagnosis, but also to start NGS techniques for strain surveillance in the private laboratory sector as well as in one public laboratory, e.g., the HIV reference laboratory. Here, we show that despite late involvement, the public sector was able to adapt its activities and routinely perform COVID-19 diagnosis and also start genomic characterization of strains. The results highlighted the great diversity of COVID-19 strains, but also the unevenness of genomic surveillance platforms at the national level. The Dakar region, which represents the Senegalese capital and international crossroads, was home to all circulating variants.

References


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