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Contribution of Genomic Surveillance in the Detection and Monitoring of SARS-CoV-2 Variants during the 6 Pandemic Waves in the Central African Republic from 2020 to 2023

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Abstract

Objective: The COVID-19 pandemic has highlighted the need to strengthen diagnosis and genomic surveillance capacities. In 2021, Central African managed five waves of COVID-19 by integrating genomic surveillance into their health monitoring system. This study sought to report surveillance data from the National Laboratory of Clinical Biology and Public Health and describe the circulation of SARS-CoV-2 variants. **Materials and Methods:** This retrospective, descriptive observational study spans three years, from April 2020 to November 2023. It was conducted on a population of consenting volunteers from across the Central African Republic, who were tested using RT-PCR on nasopharyngeal samples. Data with sufficient information were obtained from the National Laboratory of Clinical Biology and Public Health (LNBCSP) databases. Sequencing was largely carried out at the National Institute of Biomedical Research (INRB) in Kinshasa until May 2023, and subsequently at the LNBCSP. **Results and Discussion:** Out of 97,864 RT-PCR tests performed, 9,764 were positive, resulting in a prevalence of 9.98%. The average age of the patients was 39.97 years ± 13.76, and the male-to-female sex ratio was 2.12. RT-PCR test positivity was significantly associated with age (p = 0.001), sex (p = 0.013) and clinical manifestations. Ten variants circulated during the five

recorded waves, with Omicron (B.1.1.529), Delta (B.1.617.2) variants being predominant. Notably, the B.1.620 and B.640 variants were prominent during the second wave. **Conclusion:** This retrospective study provides key insights into the COVID-19 pandemic in the CAR. It identifies risk factors and details the circulation of various SARS-CoV-2 variants. Enhancing national genomic surveillance capacities would enable the country to better respond to future pandemic challenges.

Keywords

SARS-CoV-2, Variants, Central African Republic, RT-PCR

1. Introduction

Coronaviruses are cold viruses that mainly affect birds and mammals, with bats and rodents serving as the primary reservoirs [1] [2]. While circulating human coronaviruses typically cause seasonal colds, emerging coronaviruses can lead to severe acute respiratory syndromes [3]. Three of the eight known human coronaviruses are emerging viruses: Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronaviruses type 1 and 2 (SARS-CoV-1 and SARS-CoV-2) [1] [2].

Originating in Wuhan in December 2019 and declared a pandemic by the WHO on March 11, 2020, the COVID-19 pandemic has resulted in 772,138,818 confirmed cases and 6,985,964 as of December 6, 2023. It is undoubtedly one of the most significant pandemics since the Spanish flu of 1918 [4]-[6].

During the peak of the pandemic, inequalities in access to diagnostic technology and vaccines negatively impacted response strategies in countries with limited resources [4] [6]. However, with the launch of the continental plan to intensify genomic surveillance in Addis Ababa in November 2021, remarkable progress has been made in the detection and monitoring of SARS-CoV-2 variants in Africa [7].

The Central African Republic is located in the heart of Africa, bordered Chad to the North, the Republic of Congo and the Democratic Republic of Congo to the south, Sudan and South Sudan to the east, and Cameroon to the west [8] [9]. The country detected its first case of COVID-19 on March 14, 2020, in an immigrant patient, using RT-PCR. From March 2021, the country integrated the continental genomic surveillance strategy by initially sending samples for sequencing to the National Institute of Biomedical Research (INRB) of Kinshasa. National genomic surveillance was launched on March 23, 2023 at the National Laboratory of Clinical Biology and Public Health (LNBCSP) [6] [10].

The study was conducted to identify the relevant information to extract three years of laboratory surveillance and variant tracking, with the aim of better managing future pandemics. To address these crucial concerns, our primary goal was to study the epidemiological and virological profiles of patients testing positive for

COVID-19 at LNBCSP.

2. Material and Methods

This was a descriptive retrospective study based on laboratory data in the National Laboratory of Clinical Biology and Public Health in Bangui and spanned from April 2020 to May 2023. We included consenting patients who had taken an RT-PCR test during the study period.

2.1. Laboratory Analyses

2.1.1. Sample Collection

Sample collection primarily took place at the COVID-19 sampling unit of the LNBCSP and the COVID-19 Patient Screening and Management Center. Additionally, samples were collected at various sampling sites in Bangui and the Provinces. Samples from mass screening campaigns were also analyzed during this study.

The swabs were immersed in virological transport media (MTV), hermetically closed with their caps, after breaking the stems of the swabs.

2.1.2. Sample Transportation

Samples, often collected and placed in double packaging, were transported to a biosafety level 2 plus container for inactivation. Prior to transportation, each sample was recorded and coded for subsequent identification. The samples were then transported to the technical room.

2.2. RT-PCR Test

The National Laboratory uses 3 PCR machines for conducting RT-PCR tests for SARS-CoV-2. These are the GeneXpert® system from Cefeid Diagnostics (Maurens Scopont, France), the ABI FAST from Applied Biosystems (Waltham, MA, United States), and the CFX96 from Biorad® (Marcy l'Etoile, France).

2.3. Real-Time RT-PCR Technique

Viral RNA was extracted from nasopharyngeal and oropharyngeal samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A real-time PCR targeting the ORF_1ab and N genes of SARS-CoV-2 was conducted using the SANSURE Biotech Kit (Changsha, People's Republic of China) according to the manufacturer's protocol on the 7500 Fast Dx Real-Time PCR (Applied Biosystems, Waltham, MA, USA).

2.4. GeneXpert® Technique

This fully automated RT-PCR technique involved analyzing 300 µl of liquefied sample contained in a specific cartridge. The test was considered negative if no fluorescence was detected after 40 cycles. Internal and external controls included in the cartridge validated the procedure.

2.5. Sequencing Sample Selection and Sequencing

Samples that tested positive with antigen-RDT and RT-PCR testing (CT value < 30) were selected for sequencing. The samples were then sent to the INRB in Kinshasa, DRC, for whole genome sequencing using Next Generation Sequencing (NGS). However, as from April 2023, positive samples that met the selection criteria were sequenced locally.

2.6. NGS Sequencing

The libraries were prepared according were prepared following the Illumina COVID-19 ARTIC v3 V.5 protocol, using the ARTIC preparation kit NEBNext Ultra II DNA library (New England Biolabs Inc., Ipswich, MA, USA). ThE libraries were quantified using the Qubit DNA BR (Thermo Scientific, Waltham, MA, USA), normalize, pooled, and sequenced on an Illumina MiSeq 100 (Illumina, San Diego, CA, USA).

2.7. Assembly

The quality of raw reads was checked using the FastQC tool with default parmameters. Read quality was improved by trimming using the software fastp (v0.23.4). The cleaned reads were assembled by reference-guided mapping using the Wuhan-1 reference sequence (accession ID: MN908947) and bowtie2 with default settings. A consensus sequence for the SARS-CoV-2 genome from each sample was obtained using the samtools/bcftool pipeline and then refined with the Pilon software v1.19. The sequences were annotated by identifying open reading frames (ORFs) potentially encoding SARS-CoV-2 viral proteins, as annotated in the Uniprot database. Only ORFs with more than 50% coverage were retained due to low quality in some assembled regions.

2.8. Phylogenetic Analyses and Identification of Variants/Lineages

Alignments including low coverage CAR sequences generated for this study, CAR and African sequences extracted from GISAID, and reference sequences representing all major variants of concern (VoC) in the GISAID database were built using MAFFT v7. A Neighbor Joining (NJ) Phylogenetic Tree of CAR genomes was constructed using the MEGA X software, applying a p-distance model and the pairwise deletion option. A Maximum likelihood (ML) phylogenetic tree of African genomes was inferred using FastTree v2.1.1, which calculated the approximate ML trees for very large alignments by applying a generalized time-reversible (GTR) model of nucleotides evolution and CAT approximation for varying rates of evolution across sites. Local support values were calculated with the Shimodaira-Hasegawa test. Trees were visualized and analyzed using FigTree v.1.4.4. For variant identification and mutation annotation, sequences were analyzed using Nextclade.

2.9. Data Analysis

Data collection sheets provided by the national coordination of the fight against

COVID-19 were used to collect sociodemographic, clinical, and biological parameters, including virological data of patients. These data was then entered and analyzed using Epi Info software v7.2.3.0. The Chi-square test, Pearson's test, and Fisher's exact test were used for categorical variables, with a 95% confidence interval. Differences were considered statistically significant for a p-value less than 0.05.

2.10. Operational Definition of Waves

The waves corresponded to periods of massive, exponential transmission that led to a sudden increase in severe cases, potentially overwhelming healthcare capacity and resulting in high mortality. In the laboratory, wave periods were characterized by a high rate of positivity, which gradually increased until it reached a peak in RT-PCR positivity, before subsequently decreasing.

3. Results

3.1. Sociodemographic Characteristics of Patients

Table 1 reports the sociodemographic characteristics of the patients and the correlations with RT-PCR test results, as well as evolving pandemic trends. The average age of the patients was 39.97 ± 13.90, with ages ranging from 6 months to 92 years. The M/F sex ratio was 2.16. RT-PCR test positivity varied by state clinic of patients, their gender and the pandemic evolution (Table 1).

Table 1. RT-PCR correlation and sociodemographic characteristics of patients.

Settings	Result				Total	p-value 0.05
	Positive	%	Negative	%		
Mean age (years ± DS)	38.84 ± 13.90		40.10 ± 13.74		39.97 ±13.76	
Median age (years)	38.00		40.00		40.00	
Age in category (ans)						
0 - 11 years old	2	0.85	232	99.1	234	0.001
1 - 20 years old	837	11.74	6295	88.26	7132	
21 - 40 years old	5003	11.15	39850	88.85	44853	
41 - 60 years old	3283	08.41	35761	91.59	39044	
61 - 80 years old	601	09.38	5806	90.62	6407	
81 years old and over et +	38	19,59	156	80.41	194	
Total	9764	9.98	88100	90.02	97864	
Gender						
Male	6574	09.69	61251	90.31	67825	4.481
Female	3190	10.62	26849	89.38	30039	
Sex Ratio M/F = 2.26						
TOTAL	9764	09.98	88100	90.02	97864	

Continued

Patients' condition						
Symptomatic	1511	16.82	7472	83.18	8983	0.001
Asymptomatic	8253	9.29	80628	90.71	88881	
Total	9764	9.98	88100	90.02	97864	
Year 2020						
Male	2223	19.91	8841	80.09	11164	
Female	802	18.91	3440	81.09	4242	0.079
Sex Ratio M/F = 2.63						
Total	3025	19.64	12381	80.36	15406	
Year 2021						
Male	2585	11.54	21352	88.46	24137	
Female	1671	14.46	9888	85.54	11559	0.001
Sex Ratio M/F = 2.07						
Total	4456	12.48	32240	87.52	35696	
Year 2022						
Male	1516	5.36	26743	94.64	28259	
Female	705	5.77	11520	94.23	12225	0.051
M/F Sex Ratio = 2.31						
Total	2221	5.49	38263	94.51	40484	
Year 2023						
Male	50	1.17	4215	98.83	4265	
Female	12	0.60	2001	99.40	2013	0.013
Sex Ratio M/F = 2.12						
Total	62	0.99	6216	99.01	6278	

P = Probability. For a value of $P < 0.05$, a difference is considered statistically significant.

The majority of recruits were male (69%). Women represented 31% of the study population (Figure 1).

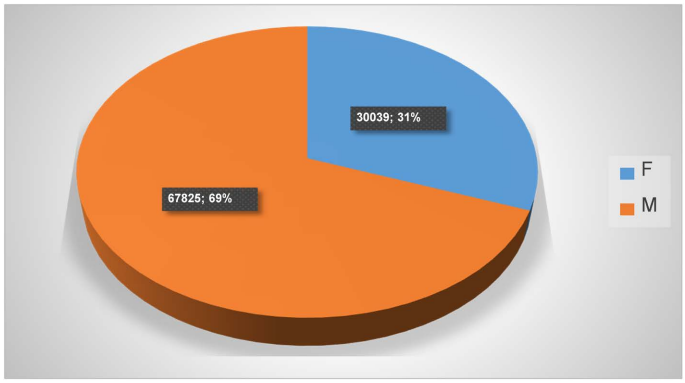

Figure 1. Distribution of patients by gender.

3.2. Pandemic Evolution and Positivity Rate of RT-PCR Tests

The number of RT-PCR tests carried out increased from 2020 to 2022 before decreasing in 2023.

The prevalence of SARS-CoV-2 infection was 24.43% in 2020, 14.26% in 2021, 5.8% in 2022 and 0.9% in 2023 (Figure 2 and Figure 3). Figure 3 shows an infection infection trend curve with a peak in the number of cases in 2021 and a decreasing trend from 2022.

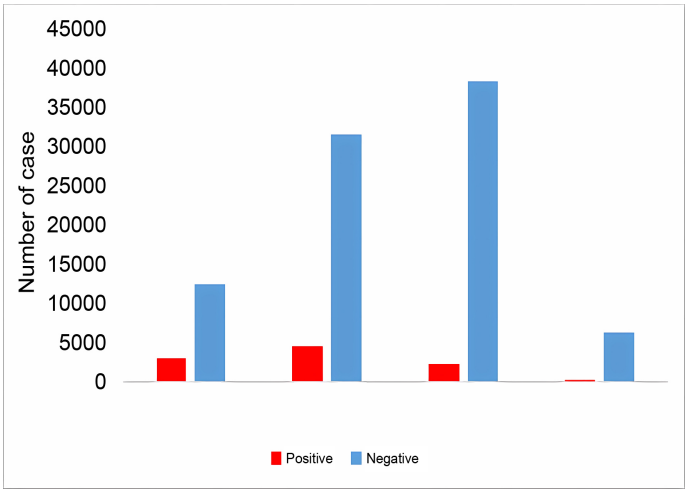

Figure 2. Results of patients who underwent the RT-PCR COVID-19 test at the LNBCSP, 2020 to 2023.

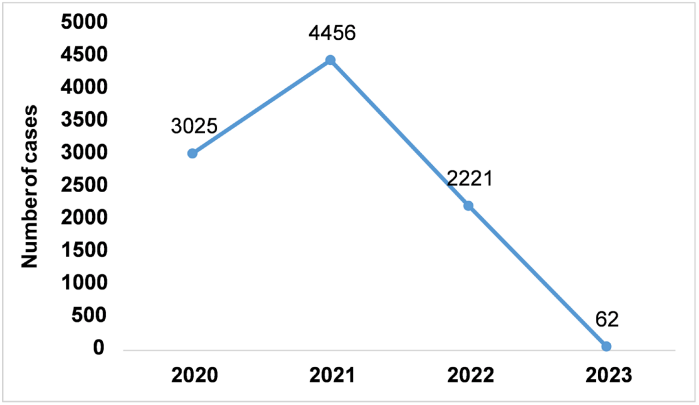

Figure 3. Evolution of COVID-19 RT-PCR test positivity over the years.

3.3. The Succession of Waves Recorded over the Years

Over 3 years of pandemic evolution, 6 waves have been recorded. The first wave occured between April and August 2020, followed by the second between March and June 2021. The third wave broke out between November 2021 and February 2022. The fourth and the fifth waves were recorded between May and July, and between September and November 2022, respectively. Finally, the sixth wave lasted from January to March 2023 (Figure 4).

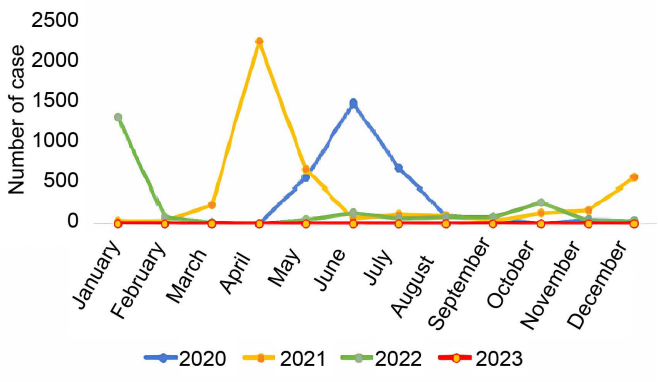

Figure 4. Evolution of SARS-CoV-2 infection in patient population tested at the LNBCSP from 2020 to 2023.

The observed mutations are grouped into the following clades: B.1.640.1, AY.33, AY.122, BA.1.1, BA.2, XBB.1, AY.4, B.1.1, B.1.617.2, BA. 5.1, BA.5.2.6, BE.1.1.1, BQ.1.1, BQ.1.1.1, BQ.1, B.1.620, AY.3, AY.12, BA.5, BA.5.2, BA. 4.1.8, XBB.1.17.1, BQ.1.2.2. A total of 23 different clades identified were and represented in a bar diagram. We noted a predominance of the B.1.620 variant, followed by other variants such as B. 1.640.1 and BQ.1.1.1 (**Figure 5**).

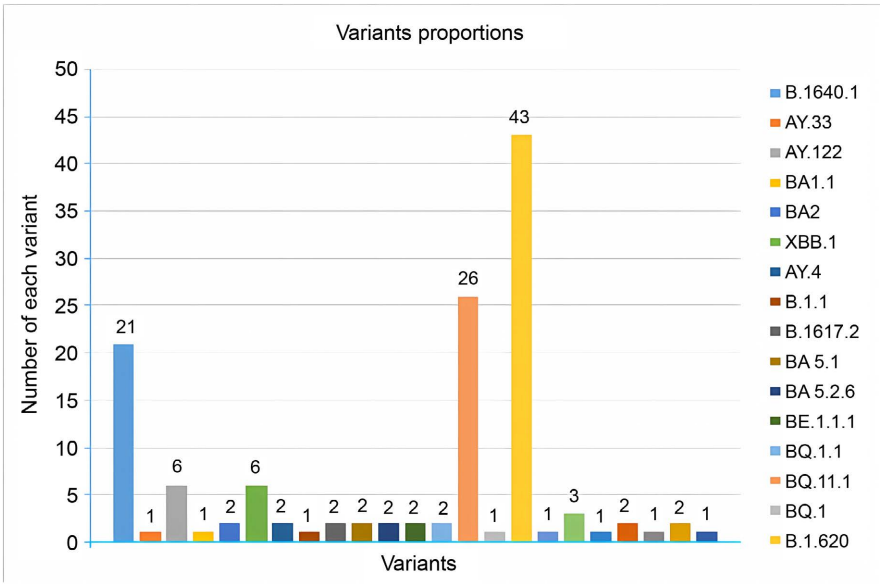

Figure 5. Landscape of variants detected from 2021 to 2023.

A total of 131 sequences with nucleotide coverage greater than or equal to 80% were included in this analysis, representing the period between 2021 and 2023 (**Figure 5**). From the sequences determined in our samples, we established a phylogenetic tree of SARS-COV-2 variants and sub-variants. It shows the evolution of mutations originating from infected hosts, depending on the proximity or distance between the strains of the SARS-CoV-2 virus resulting from the COVID-19 pandemic. **Figure 6** is the phylogenetic tree of the 23 representative clades: in red,

the Bootstrap value indicates the connections and distances between the mutations and the original sequence which circulated during our study period (Figure 6).

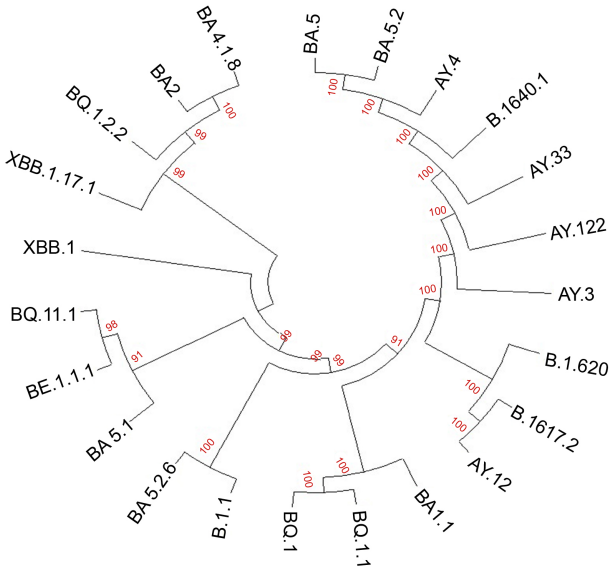

Figure 6. Phylogenetic tree of 23 variants, representative of each of the waves and years of their detection.

Figure 7 represents the phylodynamics of variants circulating in the Central African territory from South-East Asia and South-West Europe during this study period. This figure comes from the online software Nextstrain.

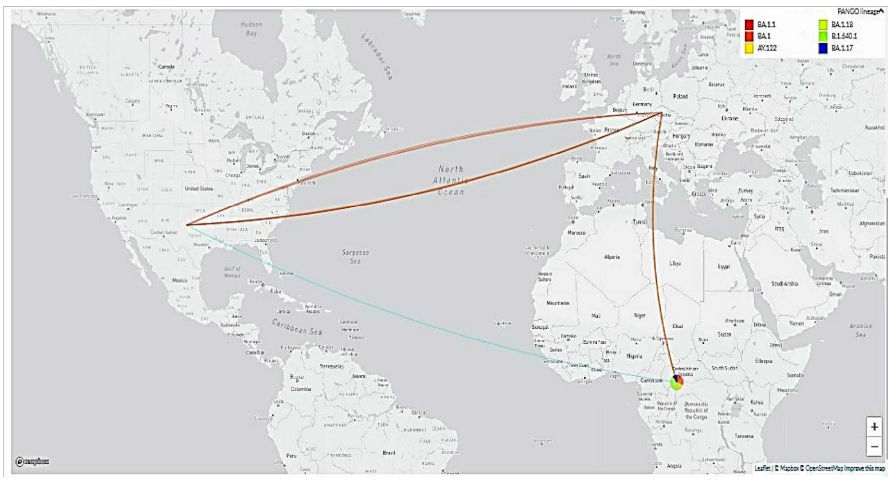

Figure 7. Origin and circulation of variants detected from 2020 to 2023 [11].

4. Discussion

The prevalence of SARS-CoV-2 infection was 9.9%, significantly higher in 2020 (24.43%) than in 2023 (0.9%). This trend is consistent with the findings of Lango-Yaya et al. who reported a prevalence of 24.71% in CAR in 2020 [12]. Similar

prevalence are reported in the sub-region around the same period [8]-[12]. It is important to note the evolution of the pandemic in the Central African Republic, marked by the period before the introduction of the vaccine and the vaccination campaign. The latter corresponded to a reduction in the number of new cases detected [6].

The increase in the number of RT-PCR tests coincides with the decrease in prevalence for two reasons. First, mass screenings include individuals without the disease who take precautions to maintain a negative RT-PCR test, especially for travel purposes [13]. Additionally, increasing screening capacity helps to rapidly identify trends and adjust the response to contain the pandemic by breaking the chain of transmission. Initially, the Central African Republic, facing a shortage of RT-PCR tests, had opted for the selective screening of symptomatic patients. While cost-effective, this strategy led to a rapid increase in contaminations shortly after the detection of the first case on March 14, 2020 [6]. However, with the support of partners including the African Union through the Africa CDC, the number of tests carried was significantly increased.

Individuals over 80 years and under 20 years were the most affected, followed by people aged 20 - 40 years, and this result was statistically significant. This younger age group corresponds to the active population, who frequently travel for professional, academic, or family reasons, thereby increasing their risk of contracting COVID-19. Studies carried out in CAR by Lango-Yaya, Manirakiza, and Rafaï support these findings [6] [9] [12]. Similar trends have been reported in the sub-region, indicating a high prevalence among the active population [14] [15].

However, the vulnerability of the elderly has already been reported by MASSAMBA in Senegal and WANG in China [16] [17]. Elderly individuals often produce insufficient neutralizing antibodies and have numerous comorbidities, which can lead to serious forms of COVID-19 and high mortality [18].

Positive RT-PCR results were significantly higher in the male population, especially before the introduction of the vaccine in CAR in May 2021 ($p = 0.001$). The male predisposition to being infected with SARS-CoV-2 has been reported in literature as being associated to hormonal reasons (androgenic) [16] [18]. However, with vaccination and immunity acquired after infection, trends have changed, alternating between the two sexes [19].

The presence of symptoms not only significantly increased the probability of positive RT-PCR tests but also that of antigenic tests used on the front line in countries with low RT-PCR testing capacities. In the CAR, for instance, there was a point in managing the pandemic where RT-PCR was reserved for symptomatic or severe cases of respiratory distress. Similar trends have been reported by Ntagereka in the DRC and by Tegally in a continental study [8] [21].

The CAR experienced 6 waves, with the most devastating being the second between March and June 2021. The devastation of the second wave was reported by Fokam and collaborators in Cameroon, Ntagereka in DRC and Manirakiza in CAR [9] [20] [21]. It is important to acknowledge that this phase of the pandemic

corresponded to an increasing trend of variants that could not adequately be controlled by initial response focused physical preventive measures.

The ancestral Wuhan strain in China was gradually replaced by strains that acquired increased transmission and replication abilities. This was mainly due to mutations like D614G and N501Y, which enhance the spike proteins affinity for the ACE receptor. Moreover, mutations like P681H in the furin cleavage domain are thought to contribute to immune escape [21]. We detected 10 variants of interest and a few of concern. The B.1.1.7 or British variant was predominant during the second wave, while the B.1.620 and B.1.624 dominated the landscape in 2021 before being replaced by the Delta variant. Since the fourth wave, the Omicron variant appeared and has been the only variant circulating for two years [6] [10]. These findings are supported by studies conducted by Fokam in Cameroon, Vickos and Rafaï in Central African Republic [6] [10] [21]. Africa, which initially faced cases imported from at the start of the pandemic, eventually saw the emergence of variants of concern. wo of such variants were first detected on the continent due to an expansion of genomic surveillance: the beta variant, first discovered in a patient from Botswana, and the Omicron variant, with its first case detected in South Africa. The Central African region has been affected mainly by the B 1.620 and B1.640 variants [6] [10] [21]. The first was sequenced for the first time in a sample from the Central African Republic, while the second circulated there throughout 2021 [8]. The emergence of variants of concern emphasizes the need to strengthen national capacities in genomic surveillance to effectively adjust the response and alert the scientific community, as in the case of the B.1.620 variant pan-African cooperation [22] [23]. The national genomic surveillance initiative had a profound impact on pandemic management and outcomes in CAR by enabling the timely detection and tracking of SARS-CoV-2 variants, which in turn informed public health responses and policy decisions. Genomic surveillance has allowed for more precise epidemiological tracking, enabling health authorities to correlate variant prevalence with changes in disease severity and vaccine efficacy [24]. This data-driven approach has been crucial in adapting vaccination strategies and modifying public health measures to better control outbreaks, highlighting the critical role of genomic surveillance in enhancing the resilience and responsiveness of health systems during the COVID-19 pandemic in CAR.

5. Conclusion

This retrospective series, although limited to data from diagnostic test results of laboratory patients mainly from Bangui, provides significant insights into the management of the COVID-19 pandemic in the Central African Republic (CAR). The study highlights the critical role of genomic surveillance in identifying and tracking variants of concern, which has been instrumental in tailoring public health responses and vaccination strategies. To improve pandemic management, it is essential to address inequalities in access to genomic sequencing tools and bolster technical capacities across the region. Sub-regional collaboration should

be promoted to share resources and expertise. Additionally, efforts must continue to decentralize and expand sequencing capabilities beyond Bangui to better understand the genetic diversity of circulating variants. Sustained investment in genomic surveillance, vaccination, and public health infrastructure will be crucial in safeguarding public health and mitigating the impact of future pandemics.

Ethical Considerations

This study was approved by the Institutional Ethical Review Committee of the Ministry of Health and Population of the Central African Republic (CAR). Administrative authorization was obtained from the Minister of Health and Population of the CAR. Voluntary informed consent was obtained from the patients.

The study was carried out in strict compliance with the Declaration of Helsinki according to which no intervention likely to alter the dignity, integrity and right to privacy of participants will be implemented. We also received ethical clearance from the Ethics and Scientific Committee of the Faculty of Health Sciences of Bangui, CAR (N32/FACSS/CES.2020).

Human Ethics and Consent to Participate declarations: not applicable.

Consent for Publication

All authors read and approved the manuscript before publication.

Availability of Data and Materials

The data that support the findings of this study are available from Public Health Ministry of Central African Republic but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Public Health Minister.

Data on GISAID can however be consulted if necessary (https://www.epicov.org/epi3/frontend#5aa6aa).

Contribution of the Authors

Project design: CDR, ELY, MRDB, PS, MMS, BK, JBR, KGNF.
Laboratory techniques: CDR, ELY, SN.
Data analyses: AAOFTG, JAB, OS.
All authors acknowledge having read the manuscript.

Recruitment Consent of Publication

All patients recruited consent and acknowledged having voluntarily participated in the investigation carried out within the framework of public health.

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Conflicts of Interest

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

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