

# Genomic Profile of SARS-COV-2 Associated with COVID-19 Outbreaks in N'Djamena, Chad

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## Abstract

**Background:** SARS-CoV-2 has circulated worldwide with dramatic consequences. In Chad, we have no data reported of variants. The aim of this study was to identify the SARS-CoV-2 variants that circulated during the epidemic from 2020 to 2021. **Methods:** This is a cross-sectional, descriptive study carried out between 2020 and 2021. Samples from patients with suspected COVID-19 were tested in five laboratories in N'Djamena. One hundred quality samples of the positives were sequenced in Kinshasa using Oxford nanopore technologies minion and the Protocol Midnight SARS-CoV2. Data were processed using Excel version 16 software. **Results:** Of the 100 samples sequenced, 77 (77%) produced sequences, 23 (23%) did not. The genomic profiles were wild-type Wuhan and minor mutations (19A, 19B (A), 20A (B.1, B.2), 20B (AV.1), 20D (B.1.1.1 /C.36), 20C), variant of concern Alpha (20I), variant of concern Delta (21A/J), variant of interest Eta (21D), variant of concern Omicron (21K) and unclassified variant under surveillance (B.1.640). Of these variants, the maximums were detected in patients aged 26 - 35 with 30.26% and 25.26% in 36 - 45. However, 24.67% were in travelers and 75.32% in residents, 35.06% in those vaccinated against COVID-19 and 62.33% in non-vaccinates. The estimated case-fatality rate was 2.44% (107/4374). **Conclusion:** This work has provided preliminary data on COVID-19 and SARS-CoV-2 variants circulating during the 2020-2021 epidemics in Chad.

## Keywords

COVID-19, SARS-CoV-2, Genomic Profile, Variant, Chad

## 1. Introduction

COVID-19 has been characterized by several epidemiological waves and the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants from the ancestral strain in Wuhan, China [1]. This virus poses one of the greatest threats to mankind, due to its ability to evolve in the population and rapidly progress through variants [2] [3]. Variants have been classified, according to their degree of importance, as variants of high consequence (HCV), variants of concern (VOC), variants of interest (VOI) or variants under surveillance (VUM) [4] [5]. VOCs have been identified as the driving force behind viral circulation and dispersal, with higher rates in Northern Europe, Central America and sub-Saharan Africa [6] [7]. The emergence of variants with high pathogenicity and transmissibility has modified approaches to preventive measures, travel restrictions and the adoption of quarantine measures [8] [9]. In Africa, the spread of SARS-CoV-2 has been gradual and varied from country to country [10]. In Central Africa, the first case was confirmed in Gabon in March 2020 and around Chad, the first case of SARS-CoV-2 (COVID-19) infection was recorded in Cameroon on March 6, 2020, and from May 2, 2020, a total of 2069, cases and 61 deaths were recorded [6] [11]. In Chad, the first case was confirmed on 19 March 2020, in N'Djamena on a passenger arriving from Douala in Cameroon [12]. From 2020 to 2021, three epidemic peaks were recorded, with 6183 cases in the 23 provinces, and 184 deaths. Cases were concentrated in the capital, with 4373 cases and 107 deaths [13]. Data on variants were not available due to the absence of trained laboratory staff, lack of infrastructure and sequencing equipment. In addition, strategies for shipping samples to specialized laboratories outside the country were not implemented. The present study was undertaken to contribute to the knowledge base on the SARS-CoV-2 epidemic, and to provide preliminary data on some of the variants that circulated from residual samples from N'Djamena, Chad.

## 2. Materials and Methods

### 2.1. Framework of the Study, and Period

This was a cross-sectional, descriptive study carried out from March 19, 2020, to December 31, 2021, in N'Djamena. The COVID-19 testing centers were the laboratories of Laboratories of the National General Reference Hospital, Bon Samaritan Hospital, Military Hospital, Tuberculosis Program and Biosafety and Epidemics Laboratory.

### 2.2. Inclusion and Non-Inclusion Criteria

This study included male and female residents of N'Djamena or travelers who tested positive for COVID-19, and contacts of individuals who tested positive by PCR for SARS CoV-2. Negative test subjects were excluded from the study.

### 2.3. Specimen Collection

NP and OP swabs were taken from individuals and placed in virus transport medium (VTM). To preserve confidentiality, samples were identified and separated from patient information. Samples were transported to laboratory in cold packs and stored at  $-70$  degrees pending processing.

### 2.4. Viral RNA Extraction

Viral ribonucleic acid (RNA) was extracted from 200  $\mu$ L of NPS universal transport medium using Qiagen's QIAamp<sup>®</sup> mini kit (QIAGEN, USA) in accordance with the manufacturer's instructions and temporarily stored at  $-20^{\circ}\text{C}$  pending reverse transcription [14]. All samples were inactivated and extracted, including the external control prior to extraction as a quality control for RNA extraction efficiency.

### 2.5. Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

SARS-CoV-2 was detected using reverse transcription polymerase chain reaction (RT-PCR), with the SARS-CoV-2 Maccura kit and the continuous enzyme in the manufacturer's kit (Biotechnology Co., Ltd, China). Primers and probes targeting the SARS-CoV-2 ORF1ab-Gene, E-Gene and N-Gene were used. Detection of the virus' ORF1ab-Gene, E-Gene and N-Gene targets was performed using MIC qPCR-Magnetic Induction Cycler (Bio Molecular Systems, Australia). Samples were considered positive if all three gene targets were amplified at a cycle threshold (Ct) of 37 or less. All samples with  $\text{Ct} \leq 37$  and were considered positive and stored in a freezer at  $-70^{\circ}\text{C}$ .

### 2.6. Whole-Genome Sequencing of SARS CoV-2

500-microliter aliquots of SARS-CoV-2 positive samples with a limit of detection (Ct)  $\leq 25$  were packaged and shipped to the Institut National de Recherches Biomédicales (INRB) in Kinshasa, DRC, for sequencing. Sequencing was performed on the MinION platform using the Midnight 1200 - Freed/Silander protocol [15]. Results were processed using Pangolin and Nextclade software [16].

### 2.7. Ethical Consideration

Authorization was obtained from the Department of Public Health and Prevention signed by Secretary General on February 23, 2021. Positive and negative PCR test results were returned to patients. The confidentiality of all patient results has been preserved.

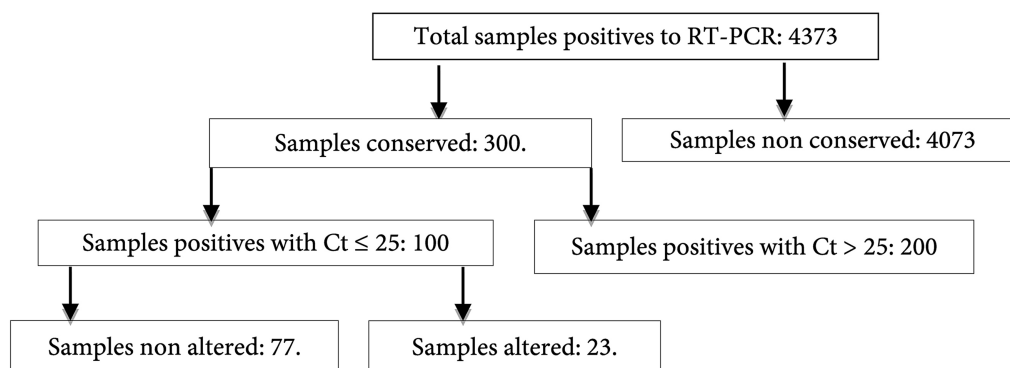
### 2.8. Data Analysis

Data were collected based on laboratory registers, epidemiological surveillance reports, clinical and socio-demographic data collection forms, and COVID-19 RT-PCR test results. The variables considered were sex, age, vaccination status, travel status and test result. Quantitative variables were collected: age, which was

divided into 10-year age brackets. The percentage of subjects in each age bracket was calculated and presented in table and figure form.

### 3. Results

Of the 4373 RT PCR-positive SARS-CoV-2, 100 samples were taken and sequenced. Seventy-seven (77) samples produced sequences and 23 were altered and could not be sequenced, giving a success rate of 73% and a failure rate of 23% (Figure 1).



**Figure 1.** Selecting the samples for sequencing.

#### 3.1. Profile of Patients Surveyed

**Table 1** shows that of the 77 samples sequenced, 51 (66.23%) were males and 26 (33.76%) females, for a male/female ratio of 1.96. Patient ages ranged from 5 to over 65 years, with a maximum between 26 - 35 and 36 - 45 years. According to vaccination status, 48 (62.33%) samples were from unvaccinated individuals, 27 (35.06%) from vaccinated individuals and 2 samples had no information on vaccination status. A total of 58 (75.32%) were residents and 19 (24.67%) travelers.

**Table 1.** Distribution of sequenced samples by sex, age, vaccination status and residence.

<i>Variables</i>	<i>Number</i>	<i>Rate</i>
<i>Sex</i>	n	%
<i>male</i>	51	66.23%
<i>female</i>	26	33.76%
<i>Groups of age</i>		
[5 - 15]	3	3.89%
[16 - 25]	7	9.09%
[26 - 35]	22	28.57%
[36 - 45]	18	23.37%
[46 - 55]	11	14.28%
[56 - 66]	6	7.79%
[66 and +]	4	5.19%
<i>No information</i>	4	5.19%

## Continued

<i>Vaccination status of patients</i>		
<i>Vaccine</i>	27	35.06%
<i>No vaccine</i>	48	62.33%
<i>No information</i>	2	2.59%
<i>Patient origin</i>		
<i>Travelers</i>	19	24.67%
<i>Residents</i>	58	75.32%

### 3.2. Variant and Clade Profile

Sequencing of the 77 samples detected five variants. Three (3) wild-type strains 15 minor mutations, 3 variant Alpha, 38 variant Delta, 11 variant Omicron, 3 Eta and 4 unclassified B.1.160 variants. Bioinformatics analysis of the sequences yielded eleven grouped clades of lineages and sub-lineages. Wild Wuhan strain and minor mutations (19A, 19B (A), 20A (B.1, B.2), 20B (AV.1), 20D (B.1.1.1 /C.36), 20C), variant of concern Alpha (20I), variant of concern Delta (21A/J), variant of interest Eta (21D), variant of concern Omicron (21K) and unclassified variant under surveillance (B.1.640).

### 3.3. Variant According to Epidemic Period

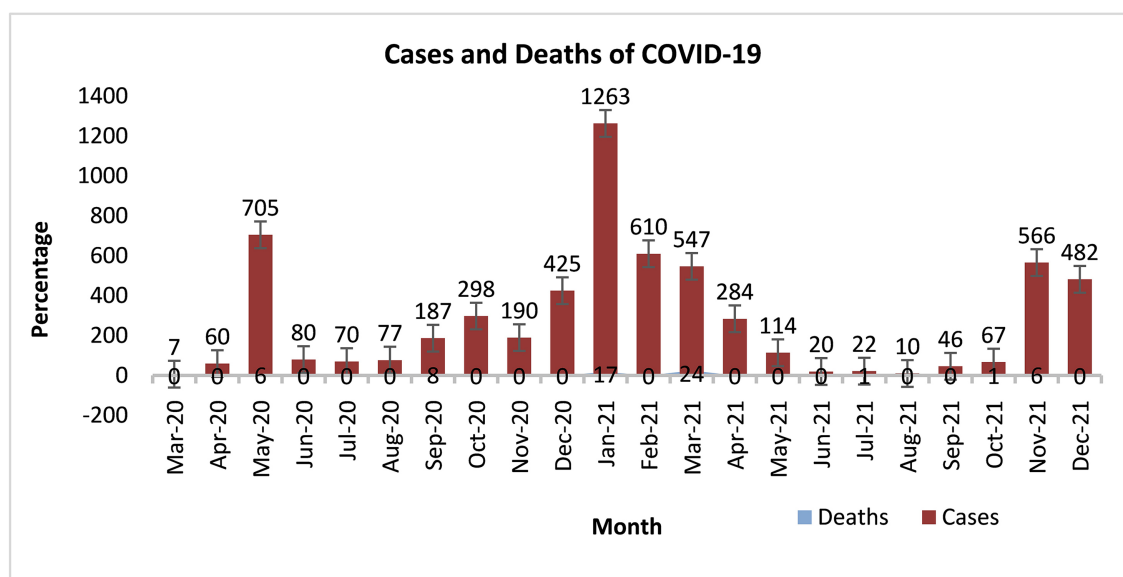
**Table 2** shows the period of appearance of the viruses. The wild strain at the start of the epidemic, followed by the Alpha variant in the last quarter of 2020. The alpha variant was associated with the Eta variant and B.1.640 in the first half of 2021. The Delta variant with B.1.640 from July to November 2021. And the Omicron variant in December 2021 was associated with the Delta variant.

**Table 2.** Chronology of variants by quarter for the 21 Months.

<i>Period</i>	<i>March-Oct 2020</i>	<i>Oct-Dec 2020</i>	<i>Jan-March 2021</i>	<i>April-June 2021</i>	<i>Jul-Sept 2021</i>	<i>Oct-Dec 2021</i>
<i>Strain and variants</i>	<i>Wuhan</i>	<i>Alpha Wuhan</i>	<i>Alpha Eta B.1640 (No classed)</i>	<i>Delta AlphaB.1640 (No classed)</i>	<i>Delta AlphaB.1640 (No classed)</i>	<i>Omicron Delta Alpha Wuhan</i>

### 3.4. Variants and Deaths in the Peaks

**Figure 2** shows three epidemiological outbreaks from March 2020 to December 2021. The wild strain, Alpha and Delta-Omicron variants are implicated in the epidemiological peaks, with a maximum of 705 cases in March 2020, 1263, in January 2021 and 596 in November 2021, associated with deaths and case-fatality rates. At the start of the epidemic, 60 deaths out of 705 positives (8.51%) were linked to the Wuhan strain; at the second peak, 17 deaths out of 1263 positives (1.34%) were linked to the Alpha variant; and at the third peak, 6 deaths out of 596 positives (1%) were linked to the Delta-Omicron combination.



**Figure 2.** Variants detected during the three epidemiological waves of COVID-19 and the corresponding lethality of the wave.

#### 4. Discussion

Our study had a reasonable sample size, with over 70% of samples sequenced. However, around 20% of the samples analyzed were failures. These failures could be linked to the fact that samples were not shipped within the two days initially planned for shipment from N'Djamena to Kinshasa but took 4 days to Addis Ababa. This long transit time led to a break in the cold chain, which would have led to the degradation of the nuclear materials, resulting in the deterioration of 23 samples.

The study revealed SARS CoV-2 variants circulating in N'Djamena, with higher proposals in males than females. The maximum number of people exposed were aged between 26 - 35 and 36 - 45 years, and particularly among people not vaccinated against COVID-19 and residents of N'Djamena. In view of these results, the trend in the sex ratio in favor of men has been reported in studies carried out in Iran and Algeria [17]. The age ranges observed in this study corroborate the results of Maiga in Mali in 2021 [18]. These results could be explained by the fact that they constitute the most active group of people, and therefore more exposed to the risk of contamination. Women are generally not very mobile in Chad, largely in the informal sector [19]. Sequences of SARS-CoV-2 variants were found in both vaccinated and unvaccinated samples. A similar situation was reported in a study carried out in Tunisia, which revealed fifteen cases of COVID-19 infection in healthcare personnel correctly vaccinated against SARS-CoV-2 who had experienced two episodes of infection [20]. These data confirm that vaccination cannot prevent reinfection, but it can mitigate its severity. Moreover, these variants can escape the immune system, reducing the efficacy of vaccines [21]. SARS-CoV-2 variants have been identified in travelers as well as in the community. This observation could be explained by

an exogenous and endogenous origin of the variants [22]. However, once the pathogen has been introduced, community spread takes over and develops significantly [23]. Reports of variants of concern (VOCs) in various parts of the world, notably the UK, South Africa, and Brazil [23] [24].

Viral sub-lineages from the ancestral Wuhan 19B strain to the variant of concern (Alpha, Delta, Omicron), the variant of interest (Eta) and the variant under surveillance (B.1.640) have been observed to circulate [25]. The presence of the Wuhan sequence and variants previously reported in other countries indicates a probable importation of the virus, because the first confirmed case of COVID-19 in N'Djamena was a passenger from Douala/Cameroon [10]. A similar result was obtained in Bamako, Mali [18]. In the same period, co-circulation of the Alpha and Eta variants was reported in Nigeria, a country bordering Chad [26]. In China, the wild Wuhan strain was sequenced as early as January 2020 [27]. Although samples had been collected, the first sequences of SARS-CoV-2 were not known until July 2021. Maiga's work in Mali in 2021 [18] reported only the Delta variant, whereas in our case the Alpha, Delta and Omicron variants were identified in our sequences. In addition, the Wuhan strain was present throughout the study period. As of October 7, 2021, 51 AU (African Union) member states reported the presence of variants of concern (VOC) Alpha (45 member states), Beta (39 member states), Delta (40 member states) and/or Gamma (2 member states). In addition, the beta variant circulated in Cameroon. The absence of certain variants could also be explained by the country's difficulties with diagnostic tools [28].

Our study shows that the country experienced three epidemic peaks between 2020 and 2023, characterized by early circulation of SARS CoV-2 variants of the Wuhan ancestral strain viral sublineage, followed by the Alpha variant in the last quarter of 2020, Alpha associated with Eta and B.1.640 in the first half of 2021. The Delta variant associated with B.1.640 from July to November 2021. And the Omicron variant in December 2021 was associated with the Delta variant. This gradual appearance of variants can be explained by the plasticity of the virus genome and its ability to produce variants rapidly. Numerous countries also experienced peaks, with similar situations recorded between January and February, with an average of 2544 daily cases. Burkina Faso, Nigeria, and Sierra Leone saw their peak in January 2021, with 36.3%, 42.2% and 55.1% of cases respectively. Ghana, Benin, Guinea Bissau, and Senegal reached their peak in February 2021. These countries accounted for 1.8%, 15.4%, 0.5% and 8.7% respectively of the total number of COVID-19 cases recorded in this second wave [29].

In this study, the ancestral Wuhan strain, Alpha, and Delta-Omicron variants were implicated in epidemiological peaks, with peaks in March 2020, January 2021, and November 2021, associated with deaths and high case-fatality rates (8.51%) during circulation of the Wuhan strain and a decrease to 1% with the other variants. This finding seems to differ from the literature, which attributes the high case fatality associated with the circulation of the SARS-CoV-2 variants

of concern, notably the Alpha, Beta, Gamma, and Omicron variants, which are highly transmissible and have a higher disease severity than the wild strain [30]. This very high case-fatality rate at the start of the pandemic may be linked to inadequate disease management capacity at the beginning of the pandemic, and the lack of appropriate treatment/therapeutic protocols in Chad.

## 5. Conclusion

Our study highlighted the circulation of SARS-CoV-2 and its variants in N'Djamena, Chad, as elsewhere in the world. These variants were at the origin of the outbreaks. Lethality was higher at the peak of the first wave than at the peaks of the last two waves. The variants were detected in both travelers and residents, as well as in immunized and non-immunized individuals. In view of the study's limitations, the results cannot be extrapolated to a national scale. It would be necessary to extend SARS-CoV-2 genomic surveillance to the entire country. Also, between the end of the study period on December 31, 2021, and the declaration of the end of the pandemic by the WHO in January 2023, the genomic profile of the virus is not known in Chad. Investigations in this direction could be envisaged.

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## Authors' Contributions

All authors contributed to the concept of this study. H.M., F.H.Y. and A.M.M. designed the study and wrote the initial manuscript. H.M and FHY analyzed data. F.H.Y and A.M.M. revised the manuscript and approved the final version. All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

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

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