

Impact of a Mass Campaign on the Evolution of the Fourth Wave of the Covid-19 Pandemic in the Central African Republic

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Abstract

Objective: In the context of increasing cases despite vaccination campaigns, a survey was conducted in the Bangui population from January 17 to 26, 2022, to evaluate the strains of *Severe Acute Respiratory Infection Coronavirus 2 (SARS-Cov-2)* circulating in a healthy population. **Materials and methods:** This study was conducted by taking nasopharyngeal samples from randomly selected volunteers. Antigen detection was performed systematically, and RT-PCR was done on the positive samples. **Results and discussion:** We collected 2,554 samples. Thirty were found RT-PCR positive (1.2%) and sent for viral genome sequencing. Twenty-eight *SARS-Cov-2* strains belonged to the Omicron type, and only 2 to the Delta type. **Conclusion:** Thus, infections were uncommon in the tested population, but the presence of Omicron and Delta types raises concerns that vaccination may not be effective in fighting the virus, and newly designed vaccines should be implemented to better protect the population at risk of infection and reinfection by these variants.



Keywords

COVID-19, SARS-CoV-2 Variants

1. Introduction

With 767 million cases and 6.9 million deaths reported as of July 2, 2023 [1], COVID-19 is by far the most devastating pandemic recorded in the 21st century. In the advent of emerging variants that evade vaccination [2]-[4], diagnostic laboratories remain the cornerstone of the response. They play a crucial role in decision-making based on scientific evidence, ensuring routine diagnosis and performing essential epidemiological functions. Genome sequencing of the virus allows for the monitoring of the emergence and spread of variants [5]-[8].

The Central African Republic, which recorded its first case on March 14, 2020, has experienced six successive waves from March 2020 to January 2023. From the beginning of January 2022, like other neighboring countries, it was characterized by a predominance of the Delta and Omicron variants [9] [10].

It is in this context that the National Clinical Biology Laboratory was tasked with conducting a diagnostic survey to evaluate the impact on the population as well as to determine the circulating variants.

2. Methods

A cross-sectional study was carried out on strategic points defined by the Minister of Health and Population.

Seven trained and experienced individuals, equipped with motorized transport and consumables, collected samples and performed antigenic tests in situ and on an ad hoc basis. Randomly chosen volunteers from 36 planned sites were sampled from January 17 to 26, 2022. Clinical, epidemiological and socio-cultural information was anonymously recorded using a codified form, which included socio-demographic data, exposure factors and the results of antigenic tests and nucleic acid amplification.

The antigen tests (Standard Q COVID-19 Antigen®; Biosensor, Chungcheongbuk-Do, Republic of Korea,) were used following the manufacturer's instructions after initial evaluation at the LNBCSP. Quality controls were performed throughout the process.

RNA extraction was carried out automatically on a King Fisher Flex Extractor® using the MagMAX Viral/Pathogen II Nucleic Acid isolation kit®; Thermo Fisher Scientific (Waltham, MA, USA) in accordance with the manufacturer's procedures. After the reverse transcriptase step, PCR was carried out on the ABI FAST platforms (Thermo Fisher Scientific) or the CFX96® using the DAAN GENE PCR kits from QIAamp® (BioRad, Marne la Coquette, France).

The different test results were given to the patients, and referrals for therapeutic and, if necessary, psychological care were provided.

Positive RT-PCR samples with a Cycle Threshold (CT) value less than 30 were selected and sent (frozen) for sequencing to the National Institute of Biomedical Research (INRB) in Kinshasa, Democratic Republic of Congo.

2.1. Sequencing Technique

Positive samples with CT values < 30 were sent to be sequenced in the DRC by Illumina.

Libraries were prepared according to the Illumina COVID-19 ARTIC v3 V.5 library construction and sequencing protocol, using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs Inc., Ipswich, MA, USA). Libraries were quantified (Qubit DNA BR, Thermo Scientific, Waltham, MA, USA), normalized and pooled, and sequencing was performed using an IlluminaMiSeq 100 (Illumina, San Diego, CA, USA).

2.2. Bioinformatics Analysis

The raw FASTQ files were quality checked using FASTQC and MultiQC to generate a single quality report for all samples. To avoid introducing errors, vcf files were generated by filtering with read depth greater than 7 and mapping quality greater than 10 using bcftools. Only SNPs of high quality and with a depth of high site coverage were considered in the downstream analysis.

Our sample sequences and SARS-CoV-2 FASTA sequences are shared in the GISAID database. The readings from FASTQ samples were assembled using the Flye tool, and the resulting assembled contigs were joined using contigMerger to generate a single scaffold per sample. The per-sample scaffold was combined into a single multicast file that was used in the phylogenetic analysis. Multiple sequence alignment was performed using MAFFT version 7.310 and the phylogenetic tree was constructed using the maximum likelihood method in MEGA version 11. The resulting tree was manipulated using the Figtree package.

2.3. Data Analysis

Personal data were collected using survey sheets and entered into Epi-info version 3.3.7. Statistical analysis was performed using the chi-square test and the Odds Ratios.

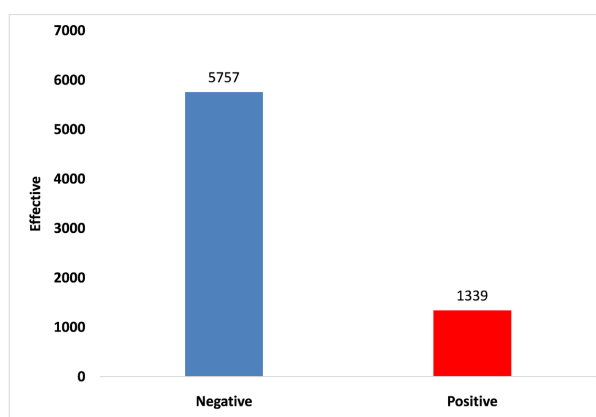
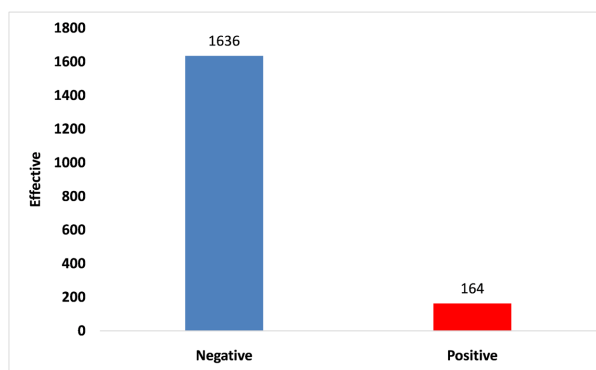
3. Results

The age of sampled individuals ranged from 1 to 66 years, with a mean age of 41 years. The sex ratio was 1.80 (M/F). Of the 2554 individuals sampled, 30 (1.2%) tested positive for antigen (**Table 1**).

Before the campaign (from the end of December 2021 to January 16, 2022), 7096 SARS-CoV-2 RT-PCR tests were carried out at the LNBCCP as routine diagnosis, of which 1339 (18.87%) were positive. After the campaign (from January 25 to 28, 2022), only 40 of the 613 SARS-CoV-2 RT-PCR tests carried out at the LNBCSP (6.53%) were positive compared to 573 (93.47%) that were negative (**Figure 1** and **Figure 2**).

Table 1. Demographic, epidemiological and biological characteristics of volunteers screened by antigenic tests.

Settings	Results		Total	p-value 0.05
	Positive	Négative		
Mean age (years) ± DS	38.77 ± 17.28	40.69 ± 11.99	40.66 ± 12.07	
Median age (years)	40.00	40.00	40.00	
Age per category (years)				
1 - 10 years	3 (10.71%)	25 (89.29%)	28	<0.001
11 - 20 years	1 (1.08%)	92 (98.92%)	93	
21 - 30 years	5 (1.45%)	340 (98.55%)	345	
31 - 40 years	7 (0.78%)	892 (99.22%)	899	
40 - 50 years	9 (1.31%)	680 (98.69%)	689	
>50 years	5 (1.00%)	995 (99.00%)	100	
TOTAL	30 (1.17%)	2524 (98.83%)	2554	
Gender				
Male	20 (1.22%)	1623 (98.78%)	1643	>0.850
Female	10 (1.10%)	901 (98.90%)	911	
Sexe ratio M/F = 1.80				
Total	30 (1.17%)	2524 (98.83%)	2554	

**Figure 1.** Positivity of RT-PCR tests in the weeks preceding the campaign.**Figure 2.** Results of RT-PCR tests carried out during and after said campaign.

We selected 53 positive samples with CT values less than 30 for sequencing by Illumina® at the INRB in Kinshasa. Analysis of the sequences generated by Pangolin® revealed two major variants (Figure 3). In the study population, the Omicron variant predominated (92%), followed by the Delta variant (8%). This observation extends to a few positives from the general population diagnosed by routine RT-PCR.

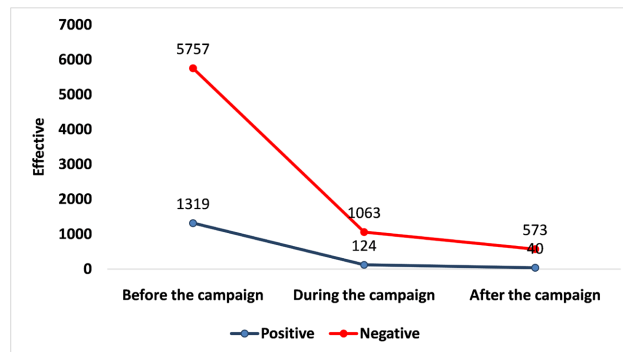


Figure 3. Impact of the campaign on RT-PCR test positivity.

4. Discussion

This sampling and diagnostic campaign was limited to Bangui and its surrounding areas. It does not provide any information about the circulation of SARS-CoV-2 and its variants in other parts of the country.

The antigen test used during the campaign (COVID-19 Ag, BIOSENSOR-Chungcheongbuk-Do, Republic of Korea) was evaluated locally before being deployed. Although the manufacturers reported its sensitivity and specificity as 96.52% and 99.68% respectively, our evaluation of quality control panels showed a sensitivity of 65.5% and a specificity of 95.6%. We acknowledge that the performance of the antigen test, compared to that of RT-PCR (considered as the reference test) can vary significantly depending on the CT values [7] [8] [11] [12] [13]. In our study, most of the antigen test positives had CT values lower than 25, indicating a high abundance of viral proteins in the samples, including nucleocapsid proteins targeted by the monoclonal antibodies in the test [7] [8] [13].

Age was associated with test positivity, with the age group of 1 - 10 years being the most affected (10.71%). Increased contamination rates in children and adolescents over the waves have been reported in studies from the Global North [10] [14]. This age group was not initially targeted by vaccination campaigns until the end of the second wave. Initially, natural cross-immunity directed against other circulating coronaviruses protected them against the early strains. However, with the selection pressure induced by vaccination coverage in those over 15, young children and adolescents have become significant reservoirs and sources of contamination for the elderly. Serious conditions, such as Kawasaki syndrome and severe acute respiratory syndromes, began to be reported in young patients, leading to the gradual expansion of the vaccination campaign to include minors

[15]. This study was conducted at a time when anti-COVID-19 vaccination had not yet been extended to under 17 years of age in the Central African Republic.

Unlike previous studies conducted in the Central African Republic [16] [17] and globally, this study does not report statistically significant differences in contamination risk factors between men and women. Although men are generally more exposed than women due to hormonal and occupational factors [15], vaccination may provide additional protection that reduces these disparities. However, we acknowledge that infections continue to occur, influenced by the genetic evolution of SARS-CoV-2 variants.

The variants detected during our study were primarily the Omicron (92%) and Delta (8%) variants. The Delta or B.1.617.2 variant emerged in India in March 2021 [2]. The Central African Republic reported its first cases of a Delta variant outbreak between April and May 2021 just before the introduction of the anti-COVID-19 vaccines. This timing explains the severity of the second wave, which significantly impacted a non-immune population, unlike countries with high vaccination coverage where the impact in terms of serious cases and mortality was minimal.

The Omicron variant, originating from South Africa, was identified in November 2021 and quickly spread to Europe and other continents, becoming the dominant variant for more than 2 years. Currently, there are more than 500 sub-variants circulating, highlighting the need for continued health monitoring through genomic surveillance. This study reported a predominance of the Omicron variant, but the co-circulation of the Delta variant indicates its relatively recent introduction into the Central African territory.

This variant has severely tested the effectiveness of vaccines based on viral vectors like AstraZeneca®, showing that mRNA vaccines, with their greater adaptability, are the only ones to offer effective protection against contamination. However, all vaccines are recognized as effective against severe forms and against deaths due to COVID-19 [18] [19].

In countries with limited availability of mRNA vaccines, a mass campaign could make a significant difference [11]. This study demonstrated an impact on the evolution of a wave as the positivity rate of RT-PCR tests dropped from 18.87% to 6.53% in less than 10 days. This decline could have been more significant if there had not been a decrease in volunteer participation after the reduction in the number of infections. The few cases tested at the end of the campaign were either contact subjects or individuals who were already ill.

Continuous mutation of the virus can render vaccine protection ineffective over time. Therefore, screening and genomic surveillance should be coupled with booster doses of adapted vaccines (mRNA) to minimize the impact of outbreaks [9] [18] [20].

5. Conclusions

This campaign reports that the first wave was marked by very high contagiousness attributable to the high circulation of the Omicron variant. The mass cam-

campaign played an essential role in containing the said wave, confirming the success encountered everywhere by aggressive strategies against the pandemic.

This survey is of capital interest in following a population exposed to the risk of circulation of new variants already reported in neighboring countries. This real-time knowledge of variants makes it possible to assess vaccine effectiveness and better understand the risks of new waves occurring, given the great genetic variability of SARS-CoV-2 strains that can escape vaccine immunity.

There is an urgent need to strengthen the Central African Republic's capacities in molecular diagnostic tests and genomic surveillance technology to better monitor the evolution of variants, which are often deemed unpredictable.

Ethical and Administrative Procedures

This study which was part of the national response against Covid-19 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. 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. Voluntary informed consent was obtained from the patients. In addition, we received ethical clearance from the Ethics and Scientific Committee of the Faculty of Health Sciences (N32/FACSS/CES.2020).

Consent for Publication

All authors read and approved the manuscript before publication.

Availability of data and Materials

Given the sensitive nature of the data, access to the database is only possible with authorization from the Ministry of Health and Population. The same is true of the preliminary report of this mass campaign available in the archives of the Department of Health.

Data on GISAID can however be consulted if necessary.

Fund

The mass campaign benefited from Covid-19 Funds provided by the World Bank via the government. The tests were provided by WHO, the recipient of these Logistics Funds, and by Africa CDC and FIND.

Contribution of the Authors

We certify that all authors contributed to the production of the article, whether in technique, coordination and documentary review.

CDR, ELY, PS, SP, JEKK, EJK, RMS, BK, MRDB, ALF: project design and laboratory techniques.

CDR, NK, LSH, OS, WSN, FXMK, LB, GG, JDDL, BK, AS, PS: data analysis and interpretation of results and coordination of screening activities.

CDR, NK, LSH, WSN, FXMK, GG, JDDL, BK, MMS, MRDB, AS, CDMK, NRN: Elaboration of the manuscript and documentary review.

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Conflicts of Interest

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

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