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Research Article

First identification of ba.5.2.1 omicron sub-lineages in fully vaccinated patients in Cameroon

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Omicron is the most mutated SARS-CoV-2 variant; its high transmissibility and immune evasion ability have raised global concerns. We reported here the first identification of BA.5.2.1 Sub-lineage of Omicron variant in two fully vaccinated patients in Cameroon. We used DICOV-MOC/I MULTIPLEX RT-PCR and CovidSeq on Illumina ISeq100 respectively for the screening of SARS-CoV-2 mutations and whole-genome sequencing. We detected antibodies to Spike (SP) and Nucleocapsid (NC) proteins from ancestral and Omicron SARS-COV-2 strains using a multiplex Luminex-based assay on a MagPix platform. On the 15th of June 2022, two patients (Cases 1 and 2) presented at CREMER laboratory in Yaoundé for COVID-19 test. Nasopharyngeal Swab RT-PCR results were positive with Treshold Cycle of 16.7 and 19.3 for both the cases respectively. Mutations screening revealed the presence of K417N and L452 mutations for Case 1 and a single K417N mutation detected for Case 2. We obtained two full-length of SARS-CoV2 genome. Phylogenetic analysis showed that the two strains belonged to the BA.5.2.1 sublineage of Omicron. Serological analysis of Case 1 plasma collected 25 days after the onset of symptoms showed high levels of antibodies against SP and NC of ancestral and Omicron variants.

Key words: SARS-CoV2, Omicron, BA.5.2.1, Vaccination, Serology, Cameroon.

INTRODUCTION

The SARS-CoV-2 Omicron variant was first reported to the World Health Organization (WHO) from South Africa on November 24, 2021, (Callaway 2021). It was classified by the WHO as a Variant of Concern (VOC) on November 26, 2021. Omicron is the most mutated SARS-CoV-2 variant, and its high transmissibility and immune evasion ability have raised global concerns, (Araf et al., 2022). Owing to its enhanced transmissibility, it has rapidly replaced the Delta lineage as the dominant variant in several regions of the world, (Ferenčak et al., 2022). At the moment that manuscript was written, omicron has five sub-lineages designated BA.1, BA.2, BA.3, BA.4 and BA.5. BA.4 and BA.5 further diversified in sub-variants, some of which are able to significantly escape antibodies produced after vaccination or natural infection, (Dhawan et al., 2022). The persistence of the epidemic with successive waves caused by different variants has led to events such as genetic

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recombination between different variants and sometimes even between sublines of the same variant. This is the case of the recombinant XBB, which is the result of recombination between the sublines BA.2.10 and BA.2.75 (Focosi et al., 2022). This hybrid, which was first detected in August 2022, has caused much concern due to its increased infectivity and ability to evade the immune system, WHO (2023). These Omicron sub-lineages have the ability to reduce the affinity of neutralizing antibodies more significantly than any other variant, making existing vaccines less effective, (Tuekprakhon et al., 2022). Several studies have shown that Omicron variant and its sub-lineages escape vaccination, (Iketani et al., 2022) and are the leading cause of re-infection in people who had already been in contact with the Alpha, Beta and Delta variants that preceded it, (Bellusci et al., 2022). In Cameroon, a SARS-COV-2 genomic surveillance platform consisting of five genomic sequencing laboratories has been set up in April 2021 (Altmann et al., 2021). This platform aims to monitor in real time the appearance of new Variants of Concern (VOC), of Interest (VOI) or the Variant Under Monitoring (VUM) and to follow up in order to inform the health authorities and policies makers. It is in this context that we received nasopharyngeal Swabs samples from suspected SARS-COV-2 cases first for molecular diagnosis and then sequencing to identify what variant is circulating. We reported here the first identification of Omicron BA.5.2.1 sub-variants in two fully vaccinated individuals with a specific clinical presentation (Deng et al., 2023).

MATERIALS AND METHODS

Study site and sample collection

On June 15, 2022, two patients aged 59 and 60 were admitted for a SARS-CoV2 PCR test to the Center for Research on Emerging and Re-Emerging Diseases (CREMER) in Yaoundé, with symptoms similar to those of COVID-19. The first patient (Case 1) was a 60-year-old female patient who complained of sore throat, headache, myalgia and was short of breath since June 12, 2022 which marks the onset of symptoms. She reported having received two doses of the Sinopharm vaccine on April 21st and May, 17th, 2021 and a Johnson & Johnson vaccine dose in November 2021. The 2nd patient (Case 2) was a 59-year-old female whose onset of symptoms was on June 13th, 2022. She suffered from cough, fatigue, sore throat, headache, myalgia, chills, diarrhea and shortness of breathing. She took a single dose of Johnson & Johnson COVID-19 vaccine on August 14, 2021 and had no history of SARS-COV-2 infection. We took nasopharyngeal samples from the two patients and performed SARS CoV 2 Rapid Diagnostic Tests (TDR Antigen Panbio Abbott) at the CREMER Virology Laboratory. Both case 1 and 2 RDT were positive. The investigation team was dispatched to cases 1 and 2 respective family's houses for case contact tracing. Overall, 6 nasopharyngeal swabs (cases 1 and 2 and four family members) were collected. The four family members were aged 15, 56, 57 and 82 years old. The antigenic Rapid Diagnostic Test of the four members of cases 1&2 were negative. However all the swabs collected were further tested in the laboratory for RT-qPCR confirmation.

Nucleic acid extraction and diagnosis by RT-qPCR

Viral RNA extraction was performed using DaAn Gene's RNA/DNA Purification Kit (Guangzhou, China). Amplification and detection were done with a QUANSTUDIO 5 Thermocycler using the "Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)".

Mutation screening RT-PCR

We used DICOV-MOC/I MULTIPLEX RT-PCR kit (Montpellier, France) for the screening of SARS-CoV-2 mutations. DI SARS-CoV-2 MOC/I Multiplex is a qualitative multiplex system that allows, for each sample, the simultaneous amplification of target RNAs in a single reaction. This kit is a multiplex RT-PCR which specifically amplifies two target sequences in the gene coding for the nucleocapsid (N) protein and 8 target sequences in the spike (S) protein of the SARS-CoV-2 coronavirus. This kit is composed of two pools of Mixes (Mix 1 and Mix 2) corresponding respectively to ARMFD-COV-1 and ARMFD-COV-2 which are a Freeze-Dried Master Mix containing the Reverse Transcriptase, Taq polymerase, primers, hydrolysis probes, nucleotides and intercalant fluorescent for the amplification and detection of wild-type SARS-CoV-2 genome and MOC and MOI (L452R/Q, E484K/Q/N501/K417N,and P681H/R).

These two lyophilized pellets should be reconstituted separately in 825 µl resuspension buffer (included). Melting curves are used and the interpretation of the results is done through the patented DISoft™ software, using artificial intelligence available at: https://idsofthrm.di4diag.commande.

Whole-Genome Sequencing (WGS), bioinformatics and phylogenetic analysis

Full genome sequencing on the Illumina Platform (iSeq100) was performed on the 04 samples confirmed positive by RT-qPCR. The Illumina COVID Seq Assay kit was used following the manufacturer's protocol availabe at: https://biomedic.com.vn/en/san-pham/illumina-covidseq-assay-96-samples/ for cDNA synthesis and amplification as well as library preparation. The assembly of reads and the assignment of variants and/or lineages were carried out by the GeVarLi Pipeline (GeVarLi: GEnome assembly, VARiant calling and LIneage assignment) available at: https://forge.ird.fr/transvihmi/GeVarLi).

Mutation calling and lineage assignment were carried out using NextClade v.154 (https://clades.nextstrain.org/) and Pangolin Lineage Assigner v3.1.16 (https://pangolin.cog-uk.io/). The genome-based phylogenetic tree was constructed using IQ-TREE v1.6.12, (Nguyen et al., 2015). The generated phylogenetic trees were noted with FigTree v1.4.4 available at: http://tree.bio.ed.ac.uk/software/figtree/. To understand the introduction of this sub-lineage of Omicron in Cameroon, we used the information from the epidemiological investigation and the phylogenetic analysis of the SARS-COV-2 sequences obtained. On the epidemiological level, Cases 1 and 2 reported to have been in contact with foreigners during a family gathering from June 9, the day of their arrival in Cameroon. These foreigners came from France, Belgium, the United States of America (Houston, TX and Baltimore, MD and Senegal, West Africa). On the phylogenetic level, we downloaded the sequences of the BA.5.2.1 sub-lineage deposited in GISAID from France, Belgium, USA (TX and MD) and all of Africa. These sequences corresponded to samples taken between June 01 and 09, 2022. We constructed a phylogenetic tree with 212 sequences including 210 sequences downloaded from

GISAID and our 02 Cases. The 210 sequences from GISAID included the reference sequences of Wuhan (WIV04), 06 Alpha variant sequences, a sequence of Beta variant, a sequence of Mu variant, 02 sequences of Wild type and 199 sequences of different sub-lineages of Omicron variant.

Luminex serology

Five milliliter of venous blood was drawn in an EDTA treated tube from Case 1, 25 days after the onset of symptom. We detected plasma IgG antibodies to Spike and Nucleocapsid proteins from ancestral and Omicron SARS-COV-2 strains using a multiplex Luminex-based assay on a MagPix platform as described earlier by (Ayouba et al., 2020).

RESULTS

A total of six samples were taken on June 15, 2022, including the two cases at CREMER and the four contact cases among family members in their homes.

RT-qPCR Results

Out of the six nasopharyngeal samples, four were positive by RT-qPCR including Cases 1 and 2 which were positive to the antigenic RDT and two others that we named Case 3 and Case 4 among the four family members of cases 1 and 2 which were all negative to the antigenic test. The viral loads were high, i.e a CT (Cycle Treshold) of 16.7 and 19.3 respectively for Cases 1 and 2. The viral loads of Cases 3 and 4 were lower with CTs of 32.6 and 35.9 respectively. This could explain the fact that these Cases presented no symptoms and also the non-detection by the rapid tests.

Mutation screening

We next screened for SARS-CoV2 mutations in samples from the four samples positive by RT-qPCR. For Case 1, two mutations were detected (K417N and L452R) and a single mutation detected for Case 2 (K417N). The mutation constellations of the two Cases indicate the presence of Omicron variant but the sublineage was not identified. Cases 3 and 4 were at the limit of detection because of the high CTs (Table 1). This prompted us to carry out a complete genome sequencing of these.

Whole-genome sequencing (WGS) and bioinformatics

We obtained two full genome sequences of Case 1 (CENT324651) and Case 2 (CENT324652) with following characteristics. For Case 1, we obtained the Mean depth of 3174, the Standard deviation of 1503, the Coverage of 99.86% with the numbers of 796810 reads. For Case 2, we obtained the Mean depth of 1535, the Standard deviation of 782, the Coverage of 99.72% with the numbers of 318357 reads. The mutations identified in the Spike (S) gene are identical in the two sequences obtained. These mutations are: Δ69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. Phylogenetic analysis confirms that the two strains belonged to the BA.5.2.1 sublineage of Omicron (Figure1). This was the first identification of this sublineage in Cameroon, in fully vaccinated individuals. The two genomes were deposited and publicly available in the Global Initiative on Sharing All Influenza Data (GISAID) under the accession numbers EPI_ISL_16858758 and EPI_ISL_16858768 (Table 2).

DISCUSSION

COVID 19 disease caused by SARS-CoV-2 started in China in late 2019 and has spread to almost every Country in the world. In the course of its evolution, variants of concern (VOC) have appeared that sometimes escape the different vaccines that have been developed by (Almann et al., 2021). Some of these emerging VOC have acquired mutations in several genes, primarily in the Spike that increased viral transmissibility and/or promote escape from the host immune response. However, tools including mutation screening RT-PCR and genomic sequencing have been developed to identify circulating SARS-CoV-2 variants of concern early, (Tao et al., 2021). Screening for SARS-CoV-2 mutations by RT-PCR performed on nasopharyngeal samples is a rapid and inexpensive strategy to characterize known SARS-CoV-2 mutations as part of a surveillance program to control the virus (Haim-Boukobza et al., 2021). Mutation screening by RT-PCR has been implemented in Cameroon since October 2021 as a component of the National SARS-CoV-2 Genomic Surveillance Strategy. Its implementation in routine laboratories was simple compared to whole genome sequencing and has been widely deployed.

Table 1. Mutation screening using DICOV MOC/I SARS-COV-2 KIT.

			ARM-COV-1			**ARM-COV-2**	
	RDT result	RT-qPCR (Ct)	K417N	P681H/R	N501Y	L452R	E484K/Q
Case 1	POS.	16.7	Detected	ND	ND	Detected	ND
Case 2	POS.	19.3	Detected	ND	ND	ND	ND
Case 3	NEG.	32.6	ND	ND	ND	ND	ND
Case 4	NEG.	35.9	ND	ND	ND	ND	ND
			Detected: Presence of mutation		ND: Mutation not detected		

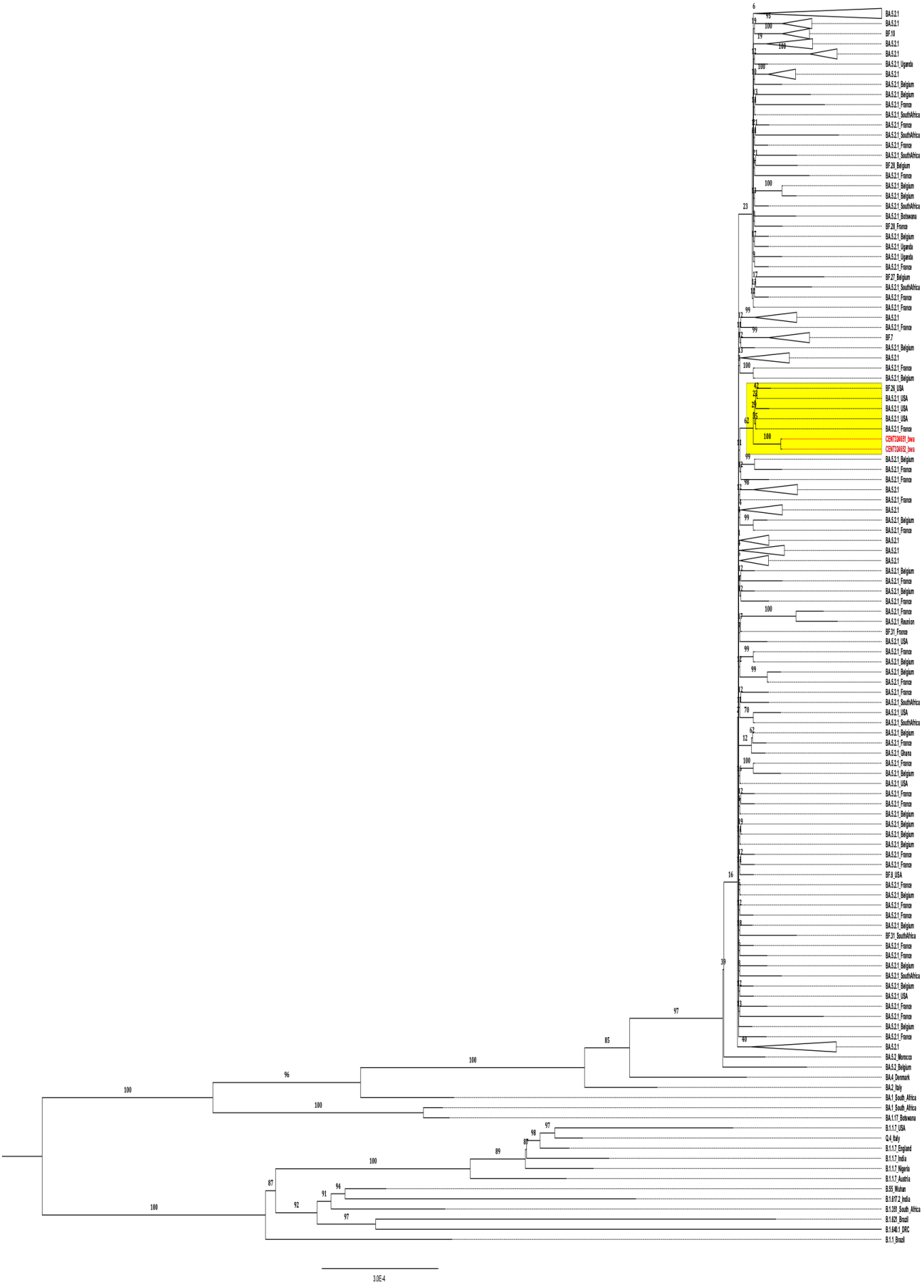

Figure 1. Phylogenetic tree of 212 SARS-COV2 full genomes. Note: A maximum likelihood tree was inferred using iQ tree with 1000 fast bootstraps on an alignment of 212 full genomes of SARS-COV2. The tree shows the clustering of the two index sequences, in red, with sequences from USA and France highlighted in yellow. Numbers on branch nodes indicated values of bootstrap supports.

Table 2. Serological analysis on Case 1 plasma collected 25 days after the onset of symptoms[&].

	Spike (MFI S/N ratio)[*]		Nucleoprotein (MFI S/N ratio)[*]	
	Ancestra	Omicron	Ancestral	Omicron
Case 1_1/2	16.69	8.85	34.15	32.26
Case 1_2/2	17.03	9.04	34.52	32.17

Note: [*]: Values indicate the ratio of sample signal and the cut-off value for each antigen. The reaction is positive if the ratio is ≥1.

Since January 2021, the diffusion of the most propagated SARS-CoV-2 variants in Cameroon (lineage B.1.1.7, B.1.351, B.1.617.2, B.1.1.519) were urgently screened, needing a surveillance with an RT-PCR screening assay. In this work, we used the DICOV SARS-COV-2 kit for mutation screening. Only two mutations were detected (K417N and L452). Automated analysis with Disoft Software using artificial intelligence showed it was is an Omicron variant. However, several sub-lineages of Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, B.E, BQ) circulate with different clinical, vaccine and diagnostic implications, (Wolter et al., 2022). We thus complemented this screening PCR with whole genome sequencing to differentiate the different sub-lineages. This highlights the limitations of the screening strategy based on the detection of few known mutations as well as its potential limitations in the race against rapidly evolving viruses such as SARS-CoV2. Whole genome sequencing confirmed the Omicron variant in these samples and accurately identified it as the Omicron sublineage BA.5.2.1 with numerous mutations. In the spike, 28 mutations were noted and one Δ69-70 deletion. NTD deletions at positions 69 and 70 have been identified in the sequences of many sub-lineages of Omicron variant BA.1, BA.4 and BA.5 and are associated with increased virus replication (Kemp et al., 2022). These deletions are not involved in the decreased sensitivity to neutralizing mAbs or plasma in convalescent individuals, (McCallum et al., 2021). Some of these mutations (G142D, G339D, S371F, D405N, K417N/T, N440K) are involved in the decrease of their sensitivity to monoclonal antibodies. The G142D mutations in NTD are frequently found in Delta and Omicron variants and interfere with the neutralization of many NTD-binding mAbs.The G339D and S371F mutations are present in the RBD core of the Omicron variant. The D405N mutation is present in the Omicron sublineages BA.2, BA.4 and BA.5 and results in decreased sensitivity to ETE and CAS. The K417N/T mutations are linked to the ACE2 binding site and are commonly reported in Beta (K417N), Gamma (K417T) and Omicron (K417N) variants. Finally, the N440K mutation is present in RBD and is increasingly common in the Omicron variant and several other sublineages, (Wang et al., 2021). To understand the immunological implication of these mutations, serological analysis using Luminex technology on a sample from case 1 taken 25 days after the onset of symptoms was performed. The anti-Spike and anti-Nucleocapsid IgG antibody titers raised against both the ancestral virus and the Omicron variant can be explained according to two hypotheses. Firstly, an infection with the wild type virus induced a first infection and subsequent re-infection with the Omicron sub-variant BA.5.2.1. The second explanation is that this first immunity was acquired after vaccination with the anti-Spike antigens of the wild SARS-COV-2 strain and subsequent infection with the Omicron sub-variant BA.5.2.1. The Omicron BA.5.2.1 variant sublineages isolated in this study with numerous immune escape mutations are of on-going concern. The Omicron Variant of Concern (VoC) and its sublineages have the largest number of amino acid alterations in its Spike protein to date. They can evade neutralizing antibodies, which can lead to re-infection in convalescent and vaccinated individuals, especially those who have not boosted their vaccination regimen. Sequencing has enabled the world to characterize the emergence of new variants of SARS-CoV-2 early and to efficiently develop strategies to limit its spread (https://www.who.int/publications-detail-redirect/9789240018440).

CONCLUSIONS

We showed here that the Omicron sublineage BA.5.2 was present in Cameroon in two vaccinated patients as early as June 2022. These Omicron variant lineages have vaccine escape mutations and induced severe conditions in the two patients. Real-time genomic monitoring is of paramount importance for early detection of emerging variants.

AUTHOR CONTRIBUTIONS

"Conceptualization, A.A. and E.D.; methodology, M.F.M. and C.G.; software, N.F.N.; validation, A.A., M.P. and C.K.; formal analysis, M.F.M., C.G., N.F.N., N.L., M.A.A. and G.R.A.; investigation, M.F.M., C.G., N.F.N.,N.L., M.A.A. and G.R.A..; resources, A.A., E.D., M.F.M., M.P., E.G., M.A.A., N.L., C.K and C.G.; data curation, M.F.M., C.G., E.G.N.F.N.,N.L.; writing—original draft preparation, M.F.M.; writing—review and editing, A.A., E.D., M.F.M., M.P., E.G., M.A.A., N.L., M.T., O.B., G.R.A.,W.B., N.F.N., C.K and C.G.; visualization, A.A., M.F.M., C.G.; supervision, E.D., A.A., M.P., M.T., W.B., O.B and C.K. project administration, A.A., E.G; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript."

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INSTITUTIONAL REVIEW BOARD STATEMENT

"Ethical review and approval were waived as these studies were conducted as part of the SARS-COV-2 genomic surveillance. This Genomic Surveillance Platform created in March 2021 by the Minister of Public Health of Cameroon includes five laboratories including CREMER (Centre de Recherche sur les Maladies Emer-gentes et Re-Emergentes).

INFORMED CONSENT STATEMENT

"Informed consent was obtained from all subjects involved in the study."

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest."

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