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RESEARCH ARTICLE

Initial detection of SARS-CoV-2 Omicron BA.4 and BA.5 sub variants associated with the onset of the fifth wave of COVID-19 in Cameroon between December 2021 and June 2022: phylogenetic and whole genome analysis

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ABSTRACT

Background: Two sub variants (BA.4 and BA.5) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant are concerning as they are spreading rapidly worldwide; however, no published data concerning these variants are available in Cameroon. We report the early detection of these new sub variants that are associated with the onset of the fifth wave of coronavirus 2019 (COVID-19) in Cameroon.

Methods: Positive samples were selected for next-generation sequencing (NGS). BA.4 and BA.5 complete genome sequences underwent sequence data analysis, epidemiology analysis of COVID-19's resurgence and wave, recombination and pairwise matrix analysis, and phylogenetic analysis. We selected the first nine SARS-CoV-2 Omicron BA.4 and BA.5 sub variants detected in Cameroon using local whole genome sequencing for the NGS analysis.

Results: During the fifth wave of resurgence of COVID-19 cases in Cameroon, it was found that the Northwest and Littoral regions were the most affected areas, while the Center and Littoral regions recorded the highest number of new deaths. The study identified evidence of recombination between the BA.2 sub variant and BA.4 and BA.5 Cameroonian strains. This result highlights the dynamic nature of SARS-CoV-2 evolution. The BA.5 strain (entitled hCoV-19/Cameroon/23850/2022) showed the highest sequence similarity to the first reported genome of the Omicron strain with 497 mutations. Phylogenetic analysis revealed that these nine Omicron sub variants were grouped into a distinct and highly distant cluster separate from the first Omicron variant detected in Botswana and were intermixed with sequences from other countries (the United States, Denmark, Scotland, and England), thus implying multiple introductions of the BA.4 and BA.5 sub variants in Cameroon.

Conclusions: Omicron BA.4 and BA.5 sub-lineages are associated with the onset of the fifth wave of COVID-19 in Cameroon. In addition to providing early warning of COVID-19 resurgence, continuous local genome sequencing of emerging variants is essential for detecting variants of concern, thereby guiding the country's response. This study emphasizes the value of real-time surveillance.

Keywords: COVID-19; Omicron BA.4 and BA.5 sub-variants; fifth wave; whole genome analysis.

Introduction

Emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants has fundamentally shaped coronavirus 19 (COVID-19) epidemiology globally and is of particular significance in Africa. Cameroon reported its first COVID-19 cases on March 5, 2020[1] followed by four distinct pandemic waves that were associated with successive variants of concern (VOC).[2] By mid-2022, the Omicron variant and its sub-lineages achieved global dominance, representing 84% of sequences submitted to the Global Initiative for Sharing All Influenza Data (GISAID).[3]

The evolution of Omicron has been characterized by successive sub-lineages beginning with BA.1–3 during South Africa's fourth wave (November 2021–January 2022).[4] Subsequently, BA.4 and BA.5 sub-lineages emerged and drove South Africa's fifth wave (April–June 2022) as these sub-lineages demonstrated enhanced transmissibility.[5] These variants significantly impacted healthcare systems, particularly by causing an increase in hospitalizations among elderly populations.[6]

Genetic analysis revealed that BA.4 and BA.5 possess distinct molecular signatures as these two sub-lineages have 33 and 34 spike mutations, respectively.[7] These sub variants present a closer genetic relationship to BA.2 than BA.1 and harbor unique spike protein mutations (L452R and F486V) that enhance their immune evasion capabilities.[8] Recent studies have demonstrated that BA.5 shows an increased resistance to neutralizing antibodies compared to its predecessors.[9]

The World Health Organization (WHO) defines COVID-19 resurgence as a spike in new cases following minimal transmission for at least two weeks and establishes a framework with three critical phases: (1) resurgence response, (2) control, and (3) alert.[10] This framework guides regional preparedness and intervention strategies on a global level.

While recent surveillance in Cameroon (December 2022–March 2023) has indicated the emergence of newer variants, including XBB.1, BQ.1.1, and atypical BA.4.6/XBB.1 recombinants,[11] our crucial study focuses on the initial emergence and characterization of BA.4 and 5. This study provides essential baseline data for understanding the evolutionary trajectory of SARS-CoV-2 in Cameroon and contributes to a broader understanding of variant emergence patterns in Africa. Although WHO declared an end to COVID-19 as a global health emergency in May 2023,[12] understanding the evolutionary dynamics of SARS-CoV-2 variants remains critically important for several reasons: (1) it helps predict future viral behavior patterns, (2) informs preparedness strategies for potential resurgences and (3) provides valuable insights for managing emerging respiratory pathogens.[13] Furthermore, this historical analysis of variant emergence and spread patterns serves as a crucial reference point for developing improved surveillance systems and response mechanisms for addressing future pandemic threats.[14]

This study aimed to analyze the phylogenetic relationships between Cameroon's BA.4/5 sub-lineages and the original Omicron variant, evaluate their correlation with Cameroon's fifth COVID-19 wave, and compare whole genome sequences with international data to understand viral transmission dynamics.

Methods

RATIONALE FOR METHODOLOGY

The methodological framework for this study was carefully selected to ensure comprehensive characterization of SARS-CoV-2 variants while maintaining analytical rigor.[15] We selected an integrated approach that combined molecular diagnostics, next-generation sequencing (NGS), and phylogenetic analysis for several key reasons: (1) real-time polymerase chain reaction (PCR) provided rapid initial screening with high sensitivity,[7] (2) NGS enables detailed genomic characterization with superior depth coverage,[16] and (3) phylogenetic analyses allowed robust evolutionary relationship determination.[17] This multi-faceted approach aligns with WHO recommended protocols for SARS-CoV-2 surveillance while concurrently enabling detailed variant tracking and characterization.[10]

STUDY DESIGN AND POPULATION

This retrospective observational study analyzed SARS-CoV-2-positive cases that had been recorded between December 2021 and June 2022. We chose this design to enable comprehensive genomic surveillance while minimizing selection bias.[18] Data were obtained from the National Public Health Laboratory and Centre Pasteur du Cameroon (NHL and CPC) databases, respectively, along with the national line listing.

SAMPLE COLLECTION AND PROCESSING

Following standardized national COVID-19 protocols,[19] nasopharyngeal and oropharyngeal specimens were collected from three strategically selected cohorts: (1) symptomatic individuals with acute respiratory infection manifestations, (2) high-risk contacts of confirmed cases, and (3) international travelers. This sampling strategy was designed to capture both community transmission and potential introduction of new variants.[9]

SARS-CoV-2 MOLECULAR DIAGNOSTICS

We selected the DAAN Gene extraction kit for RNA extraction due to its proven reliability and standardization capabilities.[20] Real-time amplification targeted the viral ORF1ab and N gene using TaqMan probes, which had been chosen for their high specificity and sensitivity.[21] The established threshold values (Ct < 30 for positive, Ct > 30 for negative) were based on validated protocols that ensured optimal diagnostic accuracy.[22]

NEXT-GENERATION SEQUENCING

The choice of the Illumina COVIDSeq protocol was based on its Food and Drug Administrations (FDA) approval status and demonstrated high-throughput capabilities.[23] This method generates 400bp amplicons through multiplexed PCR reactions and provides optimal coverage for variant detection. Library preparation procedures were standardized using IDT for Illumina PCR Unique Dual Indexes to minimize index hopping and ensure accurate demultiplexing.[24]

Sequence Analysis and Quality Control

The EDGE COVID-19 bioinformatics platform was selected for its comprehensive analysis capabilities and validated workflow.[25] Quality control measures included stringent parameters for trimming, alignment, and variant calling so as toensure reliable genomic data.[26] Lineage assignment utilized the latest versions of Pangolin COVID-19 software and NextClade for accurate variant classification.[27]

PHYLOGENETIC AND GENOMIC ANALYSIS

Maximum likelihood methods based on the Hasegawa–Kishino–Yano model were chosen for their robust statistical framework and capability for handling large datasets.[28] Tree visualization and recombination detection methods were selected based on their proven reliability in SARS-CoV-2 genomic analyses.[29]

Results

CHARACTERISTICS OF BA.4 AND BA.5 SUB-VARIANT STRAINS

In this study, we detected 381 COVID-19 positive cases that had been tested by reverse transcriptase polymerase chain reaction (RT-PCR). Of 381 positive cases, 318 (83.5%) full genomic sequences were obtained. The genome sequences range from 25 to 28.9 kb. Among the 318 complete sequences, the Omicron variant was predominant with 299 (94.0%) sequences: (1) 236 (78.9%) BA.1; (2) 26 (8.7%) BA.2; (3) 0 (0.0%) BA.3; (4) 3 (1.0%) BA.4; and (5) 6 (2.0%) BA.5. Other Omicron sub-lineages were also found: (1) 28 (9.4%) XG, (2) XP, and (3) other recombinants. Interestingly, this study present the first report of BA.4 and BA.5 sub variants in Cameroon. Regarding BA.4 and 5 sub-variant strains, the purpose of sampling was travel (six strains) and routine screening (three strains). Eight strains were isolated from Cameroonian patients and one strain from a Congolese patient, all of which were asymptomatic. Five strains were isolated from unvaccinated patients, whereas two strains were detected among vaccinated patients. The vaccination status of the two other cases was unknown (Table 2).

EPIDEMIOLOGICAL ANALYSIS AND LINK WITH RESURGENCE OF CASES

In terms of new cases, the study revealed that the Northwest (890 new cases) and Littoral (678 new cases) regions were the most affected areas during the last six weeks of the study period (from Epidemiological Week (EW) 28 through EW 33 in 2022). While the Central and Littoral regions presented the highest number of new deaths (four cases), the highest percentage of change in new cases over the last six weeks following the end of the study period was detected in the Northwest and East regions (1870% and 700%, respectively) as shown in Table 3.

Table 2. Summary of genomic and epidemiologic characteristics of nine Omicron BA.5 and BA.4 sub variants detected in Cameroon.

N	Sample ID	Location	Sample Collection Date	Specimen Type	Purpose of Sampling	If travel, provenance/ destination	Nationality/ residence	Age (y)	Gender	Symptoms status	Vaccination status/vaccine-dose	qPCR Result Date	SARS-CoV-2 PCR Result	CT Value Gene N	CT Value ORF-1ab	Clade	Nextclade _pango
1	HCY--003411	Africa /Cameroon/ Littoral/Deido	06/06/2022	Nasopharyngeal and oropharyngeal swabs	Travel	Entry from Addis Ababa - Ethiopia	Cameroonian/ Cameroon	38	Male	Asymptomatic	Unvaccinated	06/06/2022	Positive	23,9	25,6	22B (Omicron)	BA.5
2	B-126495	Africa /Cameroon/ Littoral/Deido	07/06/2022	Nasopharyngeal and oropharyngeal swabs	Travel	Entry from Milan - Italy	Camerounian/ Cameroon	40	Male	Asymptomatic	Unvaccinated	07/06/2022	Positive	21,11	23,5	22B (Omicron)	BA.5
3	HCY--16401	Africa /Cameroon/ Littoral/Bangue	02/01/2022	Nasopharyngeal and oropharyngeal swabs	Travel	Entry from Lomé - Togo	Cameroonian/ Cameroon	56	Male	Asymptomatic	Unvaccinated	02/01/2022	Positive	22,5	25,6	22B (Omicron)	BA.5
4	HCY-49830	Africa /Cameroon /West/Mifi	09/01/2022	Nasopharyngeal and oropharyngeal swabs	Travel	Exit from Mifi - West Cameroon	Cameroonian/ Cameroon	38	Female	Asymptomatic	Unvaccinated	09/01/2022	Positive	30,8	25,9	22B (Omicron)	BA.5
5	332411	Africa /Cameroon/ South/Kribi	24/12/2021	Nasopharyngeal and oropharyngeal swabs	Travel	Exit from Kribi - South Cameroon	Italian/ Cameroon	62	Male	Asymptomatic	Vaccinated - AstraZeneca - 2 doses	24/12/2021	Positive	20,3	18,5	22B (Omicron)	BA.5
6	SUD-23850	Africa /Cameroon/ South/Kribi	12/06/2022	Nasopharyngeal and oropharyngeal swabs	Routine screening	Not applicable	Cameroonian/ Cameroon	36	Female	Unknown	Unknown	12/06/2022	Positive	17,5	22,3	22B (Omicron)	BA.5
7	0328873	Africa /Cameroon/ Center/Cité verte	20/04/2022	Nasopharyngeal and oropharyngeal swabs	Routine screening	Not applicable	Cameroonian/ Cameroon	31	Male	Unknown	Unknown	20/04/2022	Positive	32	35,2	22A (Omicron)	BA.4.1
8	B-349629	Africa /Cameroon/ Center/Cité verte	13/06/2022	Nasopharyngeal and oropharyngeal swabs	Routine screening	Not applicable	Cameroonian/ Cameroon	35	Female	Asymptomatic	Unvaccinated	13/06/2022	Positive	15,5	19,4	22A (Omicron)	BA.4.1
9	B-082189	Africa /Cameroon/ Center/Djoungolo	29/05/2022	Nasopharyngeal and oropharyngeal swabs	Travel	Exit from Yaoundé Cameroon to Kinshasa, DRC	Congolese (DRC)/DRC	42	Male	Asymptomatic	Vaccinated - J&J - 1 dose	29/05/2022	Positive	20,3	17,5	22A (Omicron)	BA.4.1

Table 3. Geographic distribution of new COVID-19 cases and deaths in Cameroon in the last 6 weeks after the study period (From EW28 - EW33 – 2022).

Region	Cases					Deaths				
Administrative region	Cumulative cases	New cases in the last 6 weeks (EW28-EW33)	New cases in the last 3 weeks (EW31-33)	New cases in the 3 previous weeks (EW31-33)	% Change in new cases in last 6 weeks ((D-E)/E) *100	Cumulative deaths	Total of new deaths in the last 6 weeks (EW28-EW33)	New deaths in the last 3 weeks (EW31-33)	New deaths in the 3 previous weeks (EW31-33)	% Change in deaths cases in last 6 weeks ((I-J)/J) *100
Adamawa	4 153	47	38	9	322%	58	0	0	0	0%
Centre	38 193	159	113	46	146%	517	4	3	1	200%
East	5 428	45	40	5	700%	84	0	0	0	0%
Far-North	2 738	2	2	0	>100%	64	0	0	0	0%
Littoral	34 565	678	533	145	268%	373	4	3	1	200%
North	2 168	3	3	0	>200%	43	0	0	0	0%
North-West	12 409	890	847	43	1870%	366	3	3	0	>200%
West	11 825	200	165	35	371%	269	0	0	0	0%
South	5 541	59	42	17	147%	73	0	0	0	0%
South-West	5 355	77	68	9	656%	88	0	0	0	0%
Total	122 375	2 160	1851	309	499%	1 935	11	9	2	350%

When the peak incidence parameter was considered, none of the three scenarios (A-nationwide, B-Northwest region, and C-South region) exceeded the COVID-19 resurgence–response criterion of 30% greater than the previous peak (fourth wave). During the given period, the average number of cases during the peak nationwide decreased by 69% (723 versus 2,358 cases), whereas increases were observed in the Northwest and Littoral regions of 26% (379 versus 510 cases) and 23% (231 versus 188 cases), respectively. When considering a 7-day moving average, incident cases between EW 29 and EW 31–2022 significantly increased; on average, they rose by 109% nationwide, 375% in the Northwest region, and 60% in the Littoral region. At the national and regional levels, this increase was classified as a "resurgence alert" (Figure 1).

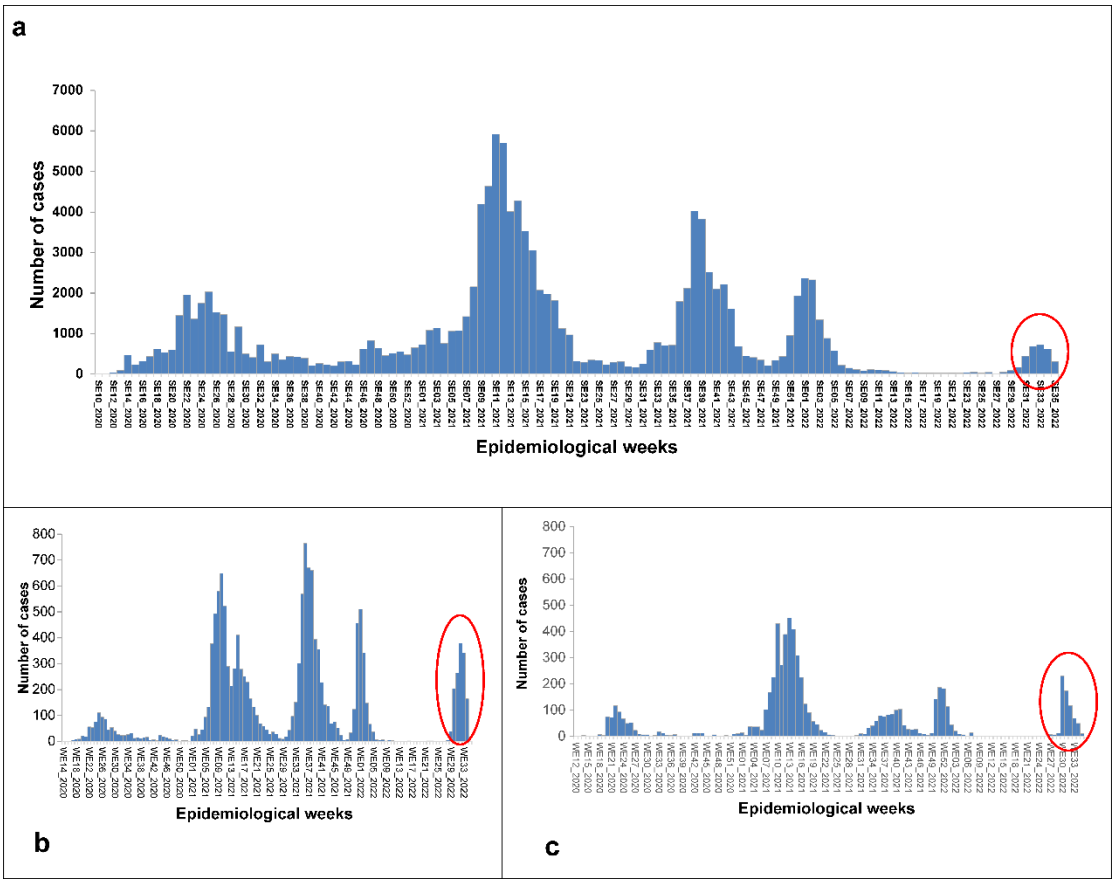

Figure 1. Comparison of epidemic curves of COVID-19 confirmed cases focusing on the 5th wave (red circled) of resurgence in Cameroon (A), and in the two most affected regions: North-West (B) and Littoral (C) regions.

Selected indicators for monitoring resurgence of COVID-19 cases in Cameroon from EW27 to EW33–2022

Following nearly 20 epidemiological weeks of low incidence (control phase), Cameroon has been on resurgence alert since EW31. An increasing trend in the number of new cases from EW28 through EW33 with an increase of 82.7% between EW 28 and 29, 70.5% between EW 29 and EW 30, 173.5% between EW 30 and 31, 54.6% between EW 31 and 32, and 5.5% between EW 32 and 33 has been detected. Most of the sequenced BA.4 and 5 sub-lineages originated from EW 23 and 30. The highest increases in new cases occurred in EW 28 (188.9%) and EW 31 (188.9% and 173.5%, respectively). Four health districts exceeded the resurgence threshold at EW33. These districts included the Deido Health Districts in the Littoral region, Bali and Fundong in the Northwest region, and Kribi in the South region. The Kumbo East and Kumbo West health districts in the Northwest region were on alert (Table 4).

Table 4. Resurgence of COVID-19 cases in Cameroon from EW27 to EW33, 2022

Characteristics	EW27	EW28	EW29	EW30	EW31	EW32	EW33
# new confirmed cases	18	52	95	162	443	685	723
% change in new cases in last 7 days	-58.1%	188.9%	82.7%	70.5%	173.5%	54.6%	5.5%
# new cases per week per million inhabitants	0.6	1.8	3.4	5.8	15.9	23.3	26.0
# tests performed	2,691	2,226	3,556	4,282	3379	4,774	2,816
Test positivity rate	0.7%	2.3%	2.7%	3.8%	13.1%	14.3%	25.7%
# tests performed per 10,000 habitants per week	0.9	0.8	1.3	1.5	1.2	1.7	1.0
# new deaths	0	0	1	1	0	2	6
# cases hospitalized	4	7	13	16	35	30	42
# hospitalized cases under oxygen (ICU)	0	1	1	1	4	7	13
Detection of Omicron sub variants (BA.4, BA.5)	Yes BA.4, BA.5			Yes BA.4, BA.5			
			Yes	Yes	Yes	Yes	Yes

Characteristics	EW27	EW28	EW29	EW30	EW31	EW32	EW33
In-country health districts in resurgence (N=197)			Bangue, Djoungolo	Bangue, Deido	Bangue, Logbaba, Ndu, Adamaoua urbain	Bangue, Bonassama, Edéa, Logbaba, Bali, Kumbo East	Deido, Bali, Kribi, Fundong
Neighboring countries in resurgence (N=6)	Yes Eq. Guinea	Yes Eq. Guinea	Yes Eq. Guinea	Yes Eq. Guinea			
Neighboring countries in alert (N=6)	Yes Gabon, Nigeria	Yes Gabon, Nigeria	Yes Gabon, Nigeria	Yes Gabon, Nigeria	Yes Eq. Guinea, Nigeria, Gabon	Yes Eq. Guinea, Nigeria	
Neighboring countries with >300% change in new cases during the week (N=6)	Yes CAR, Chad	Yes CAR, Chad	Yes CAR, Chad	Yes CAR, Chad	Yes Chad		

RECOMBINATION ANALYSIS

Recombination analysis using the Sc2rf pipeline showed evidence of recombination between the BA.2 sub-variant and eight Cameroonian strains (Figure 2).

PAIRWISE COMPARISON MATRIX

The pairwise comparison matrix shows that both BA.4 and BA.5 Cameroonian variants differ significantly from the first reported Omicron variant. The matrix indicates a range of mutations (from 497 to 21,527), thus distinguishing these sub variants from the original strain. The hCoV-19/Cameroon/23850/2022 strain revealed the highest sequence similarity (98.68%) to the reference strain with 497 mutations, while the hCoV-19/Cameroon/332411/2021 strain was the most divergent Omicron strain circulating in Cameroon (44.65%) with 21,527 mutations (Table 5).

Table 5. Pairwise comparative matrix of Cameroon Omicron with the first reported genome of Omicron Variant

Gisaid Sample ID	SIN	1	2	3	4	5	6	7	8	9	10	11
hCoV-19/Botswana/R40B59_BHP_3321001248/2021	1		9053	800	5309	769	523	497	21527	1784	2160	853
hCoV-19/Cameroon/003411/2022	2	76.53		8878	8263	8802	9121	9182	14172	7379	7054	8753
hCoV-19/Cameroon/0328873/2022	3	97.9	76.77		5519	230	363	347	21705	1524	2006	413
hCoV-19/Cameroon/126495/2022	4	85.57	70.87	85.41		5452	5606	5658	18062	4475	4071	5351
hCoV-19/Cameroon/16401/2022	5	97.99	76.85	98.68	85.46		405	383	21643	1488	1956	345
hCoV-19/Cameroon/349629/2022	6	98.6	76.79	98.92	85.63	98.8		146	21947	1781	2091	380
hCoV-19/Cameroon/23850/2022	7	98.68	76.83	99.02	85.69	98.95	99.6		22014	1857	2168	438
hCoV-19/Cameroon/332411/2021	8	44.65	42.95	44.57	42.48	44.61	44.6	44.61		20189	19878	21594
hCoV-19/Cameroon/082189/2022	9	94.89	76.75	95.27	84.82	95.22	95.24	95.23	44.58		1120	1441
hCoV-19/Cameroon/082216/2022	10	93.94	76.77	94.28	84.94	94.23	94.47	94.45	44.58	93.5		1869
hCoV-19/Cameroon/49830/2022	11	97.77	76.84	98.38	85.51	98.47	98.76	98.81	44.6	95.21	94.27	

The upper diagonal panel is the differences in nucleotide composition between the batch. The lower diagonal panel is percent identity. The matrix color ranges from red (high value) to green (low value).

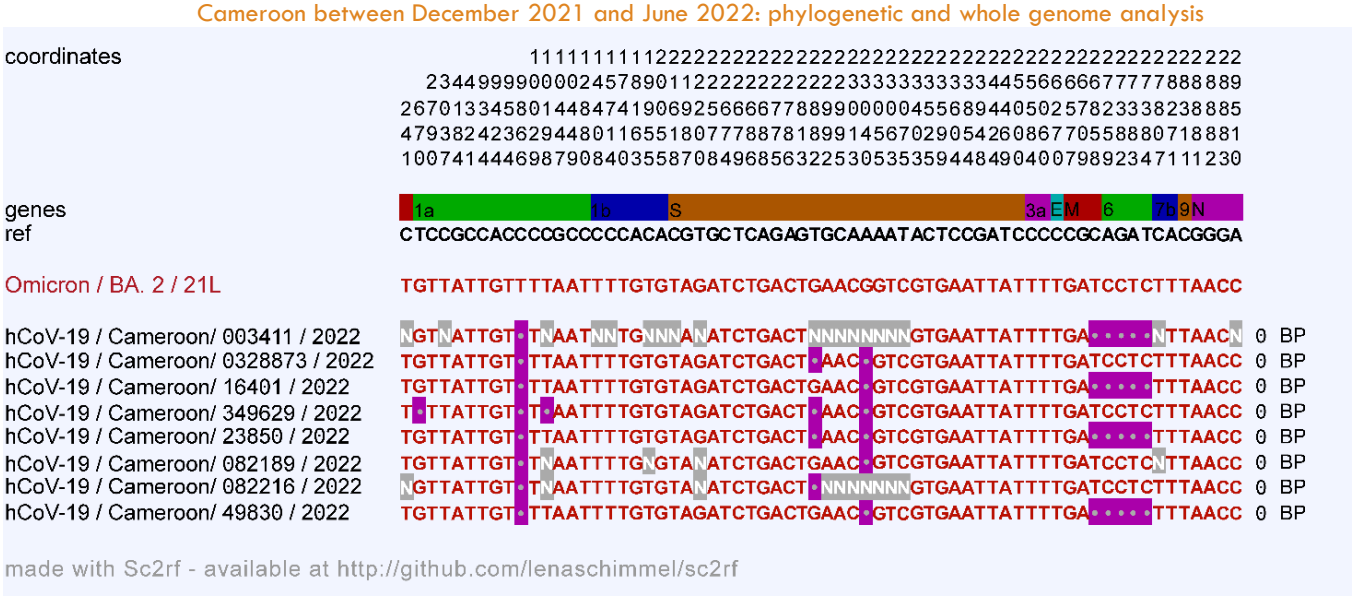

Figure 2. Sc2rf plot for SARS-CoV-2 recombination in Cameroonian strains.

PHYLOGENETIC ANALYSIS

The maximum likelihood (ML) phylogeny tree was obtained using the Molecular Evolutionary Genetics Analysis (MEGA) software and showed that the SARS-CoV-2 Cameroonian strains were phylogenetically clustered according to their geographical source. Moreover, Omicron BA.4 and 5 sub variants formed a distinct and highly distant cluster separate from the SARS-CoV-2 reference sequence MN908947.3 (Wuhan-Hu-1), as shown in Figure 3.

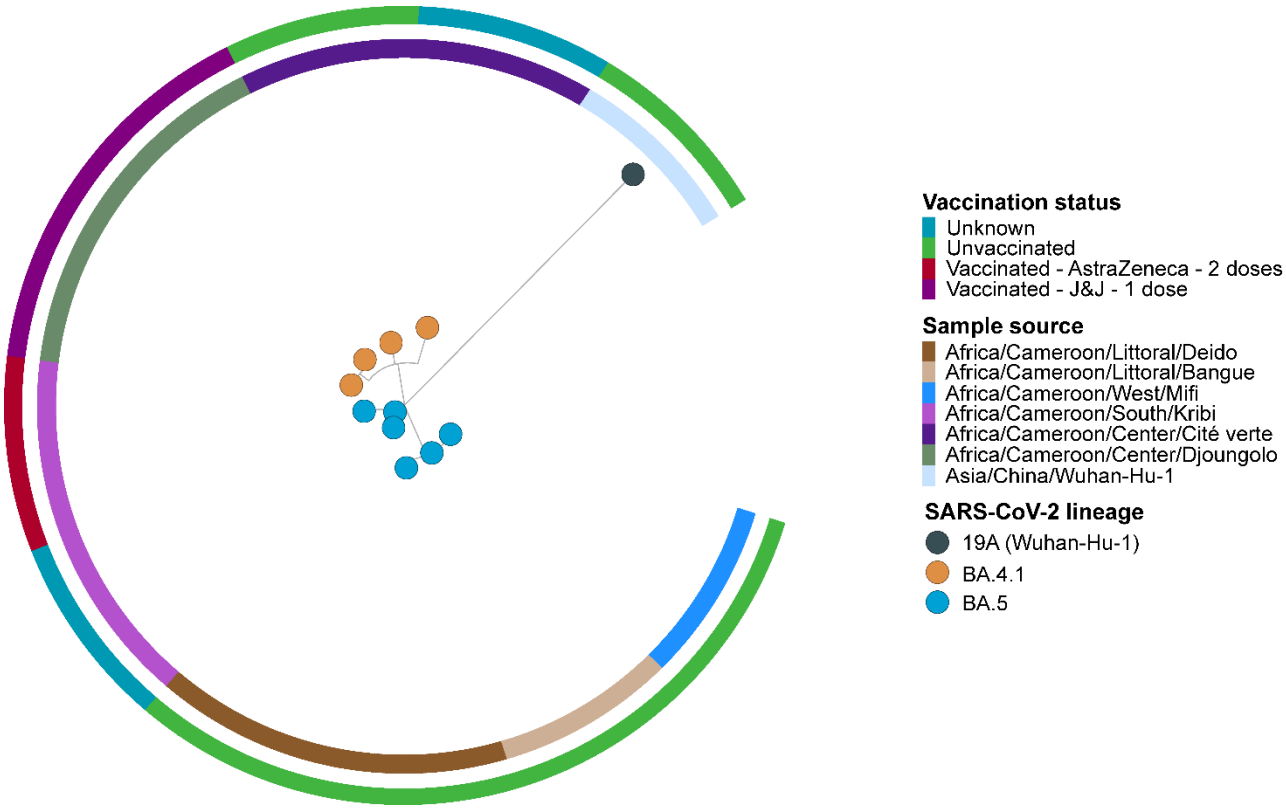

Figure 3. Phylogenetic tree of SARS-CoV-2 BA.4 and BA.5 lineages in Cameroon. The tip color indicates the SARS-CoV-2 lineages, while the inner and outer circles denote the sample source and vaccination status of patients, respectively. The SARS-CoV-2 reference sequence MN908947.3 (Wuhan-Hu-1) was added for comparison to show divergence.

In comparison to the first Omicron variant detected in Botswana, the BA.4 and 5 Cameroonian variants were clustered into two distinct clades: (1) Omicron clade 22A (BA.4 variant) that included hCoV-19_Cameroon_082216_2022, hCoV-19_Cameroon_082189_2022, hCoV-19_Cameroon_349629_2022, and hCoV-19_Cameroon_0328873_2022 and (2) Omicron clade 22B (BA.5 variant) that included hCoV-19_Cameroon_16401_2022, hCoV-19_Cameroon_49830_2022, hCoV-19_Cameroon_003411_2022, hCoV-19_Cameroon_126495_2022, and hCoV-19_Cameroon_23850_2022. Interestingly, the clade 22A (branch length: 0.0010) seemed to be more divergent than the clade 22B (branch length: 0.0008) from the Botswana strain (Figure 4).

Figure 4. Neighbor-joining (NJ) tree and ancestry of 9 BA.4 and BA.5 Cameroonian variants in comparison to the first Omicron variant detected in Botswana. Bootstrap values and branch lengths are indicated for all branches.

The clade 22A sequences were distributed in two main clusters: (1) strains from Cité-verte and (2) sequences from Djoungolo. The clade 22B sequences were also distributed in two main clusters: (1) a strain from the Littoral region and a strain from the South Cameroon band and (2) sequences mainly from the Littoral region. Interestingly, hCoV-19_Cameroon_49830_2022, which was epidemiologically linked to Mifi (West region), clustered with sequences from the Littoral region (Figure 4).

When comparing the nine BA.4 and BA.5 Cameroonian variants with the global reference dataset of Omicron variants using a customized NextStrain pipeline in GISAID, the study revealed that the nine sequences from Cameroon were intermixed with sequences from other countries (United States, Denmark, Scotland, England), which can also be seen in the broader phylogenetic analysis on GISAID. Indeed, the majority of Cameroonian samples were closely related to the United States Omicron samples. Moreover, the 22A Cameroonian Omicron clade revealed evolutionary disparity and high genetic diversity compared to the United States Omicron samples, thus suggesting that those pathogens had developed diverse mutations in the Cameroon population.

Discussion

Our comprehensive genomic and epidemiological analysis of BA.4 and 5 sub-lineages in Cameroon provides crucial insights into SARS-CoV-2 variant evolution and transmission patterns. The findings reveal significant implications for public health strategies while highlighting important considerations for future surveillance efforts.

Emergence and Transmission Dynamics dentification of BA.4 and 5 sub-lineages in Cameroon, which coincide with the country's fifth COVID-19 resurgence, reveals a complex transmission landscape. Our analysis identified two primary introduction routes: (1) international travel (44.4%) and (2) local transmission (55.6%), both of which align with patterns observed across Africa.[30] The predominance of BA.5 (32.5%) over BA.2 (20.51%) and BA.4 (11.54%) reflects global trends and suggests enhanced transmissibility of these variants.[31] This pattern of variant displacement provides valuable insights into viral fitness advantages and transmission dynamics.[32]

GENOMIC EVOLUTION AND VARIANT CHARACTERISTICS

A thorough genomic analysis revealed significant divergence among Cameroonian isolates, high contain distinct nucleotide signatures compared to the original Omicron variant. The observed recombination events between BA.2 and BA.4/5 variants, particularly the breakpoint between E and M genes, demonstrate ongoing viral evolution.[33] Phylogenetic analysis showed clear geographic clustering and multiple introduction events and contributes to our understanding of variant

dispersal patterns.[34] These findings align with current models of variant emergence and establishment across the African continent.[35]

PUBLIC HEALTH IMPLICATIONS AND RESPONSE STRATEGIES

Our findings present substantial implications for public health strategies. The multiple introduction routes that were identified necessitate enhanced border screening protocols and strengthened local transmission control measures.[36] The immune escape characteristics of BA.4/5 variants in combination with current vaccination coverage suggest potential vulnerabilities in population immunity.[37] These insights are crucial for guiding vaccination strategies and public health planning, particularly in regions with limited healthcare resources.

SURVEILLANCE SYSTEM RECOMMENDATIONS

The study emphasizes the critical need for enhanced monitoring systems in Cameroon and across Africa.[38] Our experience demonstrates that successful variant tracking requires integration of molecular and epidemiological data. While the WHO-AFRO network has been instrumental, our results indicate the necessity for expanded local sequencing capacity.[39] The current recommendation to sequence positive cases requires substantial infrastructure development and technical expertise and supports the establishment of regional sequencing hubs.[40]

STUDY LIMITATIONS AND FUTURE DIRECTIONS

Several important considerations warrant attention when interpreting our findings. The sample size of BA.4 and 5 sequences potentially affects the generalizability of phylogenetic analyses, though statistical validation supports the robustness of our conclusions.[41] The temporal distribution of sampling, while appropriate for initial variant detection, may not fully capture the dynamic nature of variant evolution. Additionally, incomplete clinical outcome data for some cases restricted our assessment of variant pathogenicity.[42]

FUTURE RESEARCH IMPLICATIONS

Moving forward, research efforts should focus on implementing systematic sampling strategies with broader geographic coverage and longer temporal spans for evolutionary analysis. Enhanced clinical data collection protocols will strengthen our understanding of variant dynamics and enable more robust phylogenetic analyses. The establishment of sustainable sequencing capacity in Africa remains crucial and requires continued international collaboration and resource allocation.[30] These improvements will significantly contribute to our understanding of SARS-CoV-2 variant dynamics in Central Africa while supporting the implementation of early warning systems and international coordination in variant monitoring.

Conclusions

Our comprehensive genomic and epidemiological investigation yielded three principal findings with significant implications for SARS-CoV-2 surveillance in Africa. First, we successfully identified and characterized BA.4 and 5 sub-lineages in Cameroon during the country's fifth COVID-19 wave and then revealed nine distinct sub-lineages with a clear dual transmission pattern (44.4% international travel, 55.6% local transmission). Second, the predominance of BA.5 (32.5%) over other variants, combined with unique genomic signatures and recombination events, provides compelling evidence for ongoing SARS-CoV-2 evolution within the African context.[43] Third, our integrated molecular-epidemiological approach established a robust framework for variant surveillance in resource-limited settings.

These findings have immediate practical implications. The necessity for sustained local genomic sequencing capacity is of utmost importance, followed by the importance of integrated surveillance systems combining molecular and epidemiological data. Additionally, the critical role of international collaboration in variant monitoring and the need for rapid response mechanisms to emerging variants has been clearly demonstrated through our findings.

Looking ahead, our research indicates several crucial developments needed in the field. The implementation of systematic sampling strategies with broader geographic coverage represents an essential next step. This step should be accompanied by the development of sustainable regional sequencing hubs and enhancement of cross-border surveillance networks. Strengthening of data-sharing platforms will further support these initiatives to ensure comprehensive variant monitoring across the continent.

Our results underscore the urgent need for sustained investment in African genomic surveillance infrastructure while providing a practical framework for future variant monitoring efforts. This work contributes to the global understanding of SARS-CoV-2 evolution and establishes a foundation for improved pandemic preparedness in Africa.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

This operational research was conducted with approval from Cameroon's Ministry of Public Health as part of the national COVID-19 surveillance program (N°368/NS/MINSANTE/SG/CCOUSP/CSO). This study was also approved by the Cameroon National Ethics Committee for Research in Human Health (N°2020/05/1224/CE/CNERSH/SP). This National Ethics Committee and the Ministry of Public Health waived the need for informed consent. The need for informed consent was waived by the *Comité National d'Ethique de la Recherche pour la Santé Humaine* [National Ethics Committee for Research in Human Health]. All methods were carried out in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION: Not applicable.

AVAILABILITY OF DATA AND MATERIALS: ALL data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS: The authors declare that they have no competing interests.

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