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Whole-genome analysis of influenza A(H1N1) pdm09 viruses in Cameroon (2019–2024) using nanopore sequencing

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Abstract

Background Since 2019, Cameroon has reported a high number of seasonal influenza cases caused by the A(H1N1) pdm09 subtype, which remained the predominant global strain as of 2024.

Methods To characterize the evolutionary dynamics of circulating A(H1N1)pdm09 viruses, whole-genome sequencing was conducted using Oxford Nanopore Technologies, with multiplexing native barcode expansion kits. DNA repair and end preparation were performed using the NEBNext Ultra II End-Repair/dA-tailing kit. Phylogenetic trees for HA genes segments were inferred using the maximum likelihood (ML) method implemented in IQ-TREE v3.0.1under the LG + F + G4 substitution model. Additionally, Mutation analysis was performed across all eight gene segments using MEGA with A/Wisconsin/67/2022 (A/H1N1pdm09) serving as the reference strain. Identified amino acid substitutions were annotated and their potential phenotypic effects were evaluated using FluSurver.

Results All Cameroonian A(H1N1)pdm09 strains from 2019 to 2024 belonged to subclade 6B.1A.5a.2a. Phylogenetic analysis revealed annual divergence from Northern Hemisphere vaccine strains, suggesting a mismatch with locally circulating variants. Several functionally relevant mutations were identified in the viral genes, including A3L, A214T, and F12V in HA; R159K and A267V in PA; A241E and T137A in M2; I42L and V7I in NS1; and I84V and I33V in PB1. Many of these mutations have been associated with increased virulence. In addition, amino acid substitutions were observed in the NA protein at V13I, S200N, L339S, S37T, V80M, and I163V, relative to the 2024 vaccine strain A/Wisconsin/67/2022.Overall, the number of amino acid mutations between circulating strains and the vaccine strain was notably high, indicating that local viruses may be evolving away from the vaccine strain selected for the 2023–2024 season.

Conclusions These findings underscore the ongoing genetic evolution of the influenza A(H1N1)pdm09 virus in Cameroon and highlight the importance of local genomic data into the selection of WHO vaccine candidate strains for the Northern Hemisphere.

Clinical trial number Not applicable.

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Keywords Influenza A(H1N1)pdm09, Genetic drift, Whole-genome sequencing, Vaccine mismatch, Antiviral resistance, Nanopore technology, Cameroon

Introduction

Seasonal influenza is an acute respiratory infectious disease caused by influenza viruses of type A (subtypes A(H1N1)pdm09 and A(H3N2)) or type B [1]. Each year, seasonal influenza affects approximately one billion individuals globally, with 3 to 5 million severe cases, resulting in 290,000 to 650,000 respiratory-related deaths [2–4]. In high-income countries, most influenza-associated fatalities occur among individuals aged ≥ 65 years, while nearly 99% of influenza-related deaths in children under five are concentrated in developing countries [5, 6]. Pandemic influenza A viruses (IAV) can lead to even more devastating outcomes, as demonstrated by the 1918 "Spanish flu," which resulted in the deaths of approximately one in every 30 individuals worldwide within 18 months [3]. The A(H1N1)pdm09 virus replaced the seasonal A(H1N1) virus and continued to circulate in subsequent years alongside the A(H3N2) and B influenza viruses worldwide, causing annual seasonal epidemics [7, 8].

The genome of the A(H1N1)pdm09 virus comprises eight negative-sense single-stranded RNA segments that encode eleven proteins, including haemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), polymerase proteins (PB1, PB2 and PA), matrix proteins (M1 and M2) and non-structural proteins (NS1 and NS2) [9, 10]. Influenza viruses are divided into three types (influenza A, B and C viruses) on the basis of the antigenic specificity of the nucleoprotein (NP) and the matrix protein (M) [11, 12]. Subtypes are further defined by the genetic diversity of surface glycoproteins: HA (18 subtypes) and NA (12 subtypes) [13].

Due to the absence of proofreading function in the viral RNA-dependent RNA polymerase, influenza viruses undergo frequent mutations (estimated at 2.3×10^{-5} per site per replication cycle) [14]. This, combined with host immune selection pressure, drives antigenic drift that can alter viral attachment to host cells and reduce immune recognition [15, 16]. Intra-subtype reassortment events also contribute to viral evolution, making genome-wide surveillance essential for early detection of emerging variants, particularly in highly recombinogenic pathogens like influenza.

Previous studies conducted in Cameroon characterized circulating influenza subtypes between 2014 and 2019 using Sanger sequencing; however, this approach only partially covered three of the virus's eight single-stranded negative-sense RNA segments [17]. Such partial genomic coverage limits the detection of reassortment events and adaptive mutations that are critical for effective influenza surveillance [18].

To overcome these limitations, next-generation sequencing (NGS) technologies such as MinION (Oxford Nanopore Technologies) have emerged as powerful tools for real-time, high-throughput sequencing and multiplex barcoding [19].

In this study, we applied the MinION platform to perform whole-genome sequencing of influenza A(H1N1) pdm09 viruses circulating in Cameroon between 2019 and 2024, with the objective of elucidating their local evolutionary dynamics, and mutation profiles that contribute to viral diversity and transmission potential.

Material and methods

Study population and case definition

The Centre Pasteur of Cameroon (CPC) has operated an influenza surveillance system since November 2007. Initially, all six sentinel collection sites were located in the Centre region. In January 2009, the system expanded to three additional regions, and the CPC was designated by the World Health Organization (WHO) as the National Influenza Reference Centre for Cameroon. Over time, the network of sentinel sites has progressively expanded to cover all ten administrative regions of the country. These sentinel sites, represented by referral hospitals and district medical centers, are illustrated on the map of Cameroon in Fig. 1 (see Additional file 1 for detailed site information). Based on infection severity, referral hospitals were designated to manage severe acute respiratory infection (SARI) cases, while district medical centers were assigned to monitor influenza-like illness (ILI) cases. Each week, between 20 and 40 throat and/or nasopharyngeal swabs were collected from suspected outpatient cases presenting with fever (>38 °C) and a cough or sore throat within seven days of symptom onset, as well as from hospitalized patients meeting the SARI case definition. All sampling procedures adhered to WHO-recommended standards for identifying ILI and SARI cases.

Sample collection and Influenza detection

In accordance with authorization No3971CEI-Udo/07/2023/M from the Ethical and Institutional Committee of the University of Douala, nasopharyngeal samples were collected using polyester swabs placed in 2 mL of virus transport medium, stored at 4 °C, and transported to the CPC under cold chain conditions. Upon arrival, samples were either processed immediately or stored at – 80 °C. RNA was extracted from 140 µL of each nasopharyngeal specimen using the QIamp Viral RNA kit (Qiagen®, Hilden, Germany) following the manufacturer's instructions. Detection and subtyping of

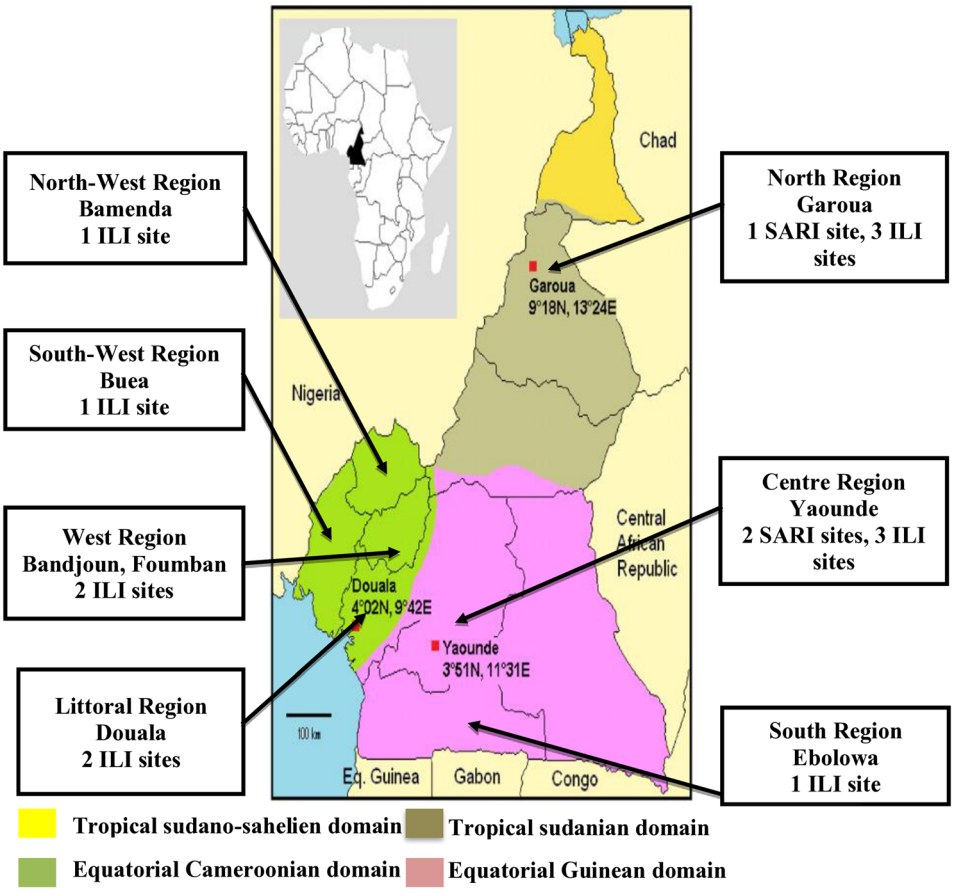

Fig. 1 Map showing the location of influenza sentinel sites across Cameroon

influenza A viruses were performed using the Superscript III Platinum One-Step Quantitative RT-PCR (qRT- PCR) System (Invitrogen, USA). All samples were tested using a multiplex kit targeting influenza A viruses. Positive samples were further subtyped to identify A(H1N1)pdm09. Amplifications were carried out on an ABI Prism® 7500 thermocycler (Applied Biosystem, Foster City, CA, USA). Each 25 µL reaction contained: 12.5 µL buffer (2X), 0.5 µL enzyme reverse transcriptase/Taq polymerase, 2 µL forward and 2 µL reverse primers (10 µM each), and 2 µL probe (2.5 µM), 1 µL water, and 5 µL of extracted RNA or control (negative and positive). All testing was completed within 24 hours. A total of 83 representative samples, collected from diverse geographic regions over a six-year period, were selected for sequencing. Only samples with Ct values < 25 were included: 2019 (n = 20), 2020 (n = 2), 2021 (n = 15), 2022 (n = 3), 2023 (n = 7), and 2024 (n = 36). Sequencing was performed using the Oxford Nanopore platform. After alignment with the reference strain A/Wisconsin/67/2022 using BioEdit, it was found that the samples from 2020 and 2022, as well as approximately 25% of those from 2019 and 2024, contained incomplete or unusable segments. In total, 62 out of 83 samples (74.7%) yielded complete influenza A(H1N1)pdm09 genomes. Consequently, nineteen samples were excluded from further analyses. The remaining 62 participants originated from all ten regions of Cameroon, with strong representation from the Centre region. The selected individuals ranged in age from 6 months to 75 years, with a median age of 37 years.

Library preparation and sequencing of A(H1N1)pdm09 Strain

The viral RNA segments of influenza A were simultaneously amplified using MB Tuni-12, MB Tuni-12.4 and MB Tuni-13 primers (Additional file 2), which targets highly conserved sequences located at the ends of each genome segment. The amplification conditions were summarized in Table 1. Amplified products were generated using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase. Purification was carried out using the AMPure XP Beads Nucleic Acid Purification kit (Beckman Coulter, Life Sciences, US). After purification, nucleic acid quantification was performed using the Qubit 4 dsDNA HS Assay kit (Thermo Fisher Scientific). Library preparation was conducted using the Oxford Nanopore Technologies (ONT) Ligation Sequencing kit (SQK-LSK109). Native barcode expansion kits

Table 1 Cycling conditions

Steps	Temperature (°C)	Time (mm:ss)
1	42	50: 00
2	50	10: 00
3	94	02: 00
4	94	00: 30
5	63	00: 30
6	48	03: 50
7	Repeat steps 4 to 6 for a total of 4 cycles.	
8	94	00:30
9	57	00: 30
10	68	03: 30**
	**** extend this step by 10 seconds/cycle**	
11	Repeat steps 8 to 10 for a total of 30	cycles
12	68	10: 00
13	4	hold

(EXP-NBD196) were used for multiplexing. DNA repair and end preparation were performed using the NEBNext Ultra II End-Repair/dA-tailing kit (New England Biolabs). To barcode the samples, 0.75 µl of initial cDNA was combined with the native barcodes and Blunt/TA Ligase Master Mix (New England Biolabs), and the reaction mixture was incubated at room temperature (15–25 °C) for 20 minutes. Samples were then pooled by combining 5 µL from each sample, resulting in a final volume of 390 µl. The DNA library was loaded onto a primed MinION Spot-On Flow Cell (R9.4). Sequencing was performed over 48 hours using FLO-MIN106 flow cells on MinION MK1b sequencing equipment, with high-accuracy base calling enabled via MinKNOW software (version 23.04.6, Oxford Nanopore Technologies). During sequencing, a QScore threshold of 8 was applied, due to low concentration observed in some samples within the pool.

Analysis of MinION data

Sequencing data were basecalled and demultiplexed using Guppy v6.5.7 (Oxford Nanopore Technologies). Raw reads were filtered according to strict quality criteria (Q score ≥ 10, minimum length ≥ 500 bp and maximum ≤ 3,000 bp) using Chopper v2.5.0, and adapter sequences were removed with Trimmomatic [20] and Porechop v0.2.4. Only genomes with a minimum coverage of 30 × and ≥ 90% of resolved positions were retained for analysis. Processed reads were mapped to influenza reference sequences using minimap2 v2.17 for consensus reconstruction and quasi-species analysis via IRMA-FLU MinION v1.0.3. Consensus sequences were generated using IRMA-FLU MinION with a minimum allele frequency threshold of 70% for base incorporation. Positions with coverage < 30 × or alleles below this threshold were masked as 'N'. Mutation detection and annotation were performed using FluSurver, which was used solely to identify amino acid substitutions relative to the selected reference sequences; no functional predictions were derived from this tool. Multiple sequence alignments were performed with MAFFT [21], and maximum-likelihood phylogenetic trees were inferred using IQ-TREE [22] with model selection implemented in ModelFinder [23]. Branch support was assessed using 1000 ultrafast bootstrap replicates. Phylogenetic trees were visualized using FigTree [24]. These steps ensure the reliability of sequences used for phylogenetic analyses.

Reference dataset construction, sequence alignment, and phylogenetic analysis

Raw sequencing data were processed and edited using BioEdit v7.2.5. Reference sequences corresponding to the 2023–2024 Northern Hemisphere A/H1N1pdm09 vaccine strain (A/Wisconsin/67/2022) were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/about/). For phylogenetic analyses, a comprehensive reference dataset was assembled from complete A/H1N1pdm09 genome sequences retrieved from the GenBank databases. Only sequences with complete coding regions, known collection dates, and high coverage (>95% completeness) were included. To ensure geographic and temporal representativeness, we selected reference strains from neighboring Central and West African countries, as well as globally representative WHO vaccine and reference strains collected between 2019 and 2024. Multiple sequence alignment was performed using MAFFT v6.864, based on the Multiple Alignment using Fast Fourier Transform algorithm. Phylogenetic trees for HA gene segments were inferred using the Maximum Likelihood (ML) method implemented in IQ-TREE v3.0.1. The HA phylogenetic tree was reconstructed from amino acid sequences under the LG + F + G4 substitution model, as determined by ModelFinder, which accounts for the amino acid replacement matrix (LG), empirical amino acid frequencies (+F), and rate heterogeneity across sites modeled with a gamma distribution with four categories (+G4). Branch support was evaluated with 1000 ultrafast bootstrap replicates, with values ≥ 70% considered statistically significant. The resulting phylogenetic trees were visualized using FigTree v1.4.4. Study sequences were compared with global reference strains and representative isolates from different geographic regions. All newly generated sequences have been deposited in the GenBank database, with accession numbers provided in Additional file 3.

Mutation analysis

Mutation analysis was performed across all eight gene segments using MEGA version11, with A/Wisconsin/67/2022 (A/H1N1pdm09) serving as the reference strain. Identified amino acid substitutions were

annotated, and their potential phenotypic effects were evaluated using FluSurver https://gisaid.org/database-features/flusurver-mutations-app/ (accessed on August 10, 2025).

Results

Description of influenza A(H1N1)pdm09 positivity rates from 2019 to 2024

Between 21 January 2019 and 31 December 2024, a total of 7453 samples were processed through the national influenza surveillance system in Cameroon. Of these, 727 tested positive for influenza A viruses, and 412 were confirmed as influenza A(H1N1)pdm09 subtype. Annual sample volumes and positivity rates varied across the surveillance period. In 2019, 1072 samples were collected (897 ILI and 175 SARI), with 324 (30.2%) testing positive for influenza A and 100 (30.9%) of those identified as subtype A(H1N1)pdm09. In 2020, 362 samples were processed (244 ILI and 118 SARI), of which 37 (10.2%) were positive for influenza A and 08 (21.6%) for A(H1N1) pdm09. In 2021, 443 samples were analyzed (377 ILI and 66 SARI), yielding 105 (23.7%) influenza A-positive cases, including 83 (79.0%) A(H1N1)pdm09. In 2022, 628 samples were processed (455 ILI and 173 SARI), with 91 (14.5%) testing positive for influenza A and 01 (1.1%) for A(H1N1)pdm09. In 2023, a total of 1,848 samples were collected (1442 ILI and 406 SARI), of which 358 (19.4%) were positive for influenza A and 73 (20.4%) for A(H1N1) pdm09. In 2024, 3,017 samples were processed (2316 ILI and 701 SARI), with 490 (16.2%) testing positive for influenza A and 157 (32.0%) for A(H1N1)pdm09. A summary of annual sample collections and influenza positivity rates is presented in Table 2. Distinct seasonal peaks were observed in influenza activity, typically occurring between October and March, with variable contributions of A(H1N1)pdm09 to these peaks, notably in early 2019, 2021, and 2024.

Subclade analysis by amino acid substitutions and phylogenetic tree analysis of HA genes

Between 2019 and 2024, all 62 A(H1N1)pdm09 strains from Cameroon fell into clade 6B.1A, with 99% of the 2023–2024 strains grouped within subclade 6B.1A.5a.2a. They evolved separately from the reference vaccine strain for the 2023–2024 influenza season (Fig. 2), based on WHO-defined subclade criteria. These strains exhibited key amino acid substitutions in the HA1 region at positions: A3L, D111N, A267D, A241E, E277D, H468N, Q71K, A241V, R159K, T89K, I550V, and N147K, relative to the 2024 A/Wisconsin/67/2022 vaccine strain. The number of amino acid substitutions between the circulating strains and the vaccine strain was notably higher, further supporting the genetic divergence of Cameroon strains from the recommended vaccine strain. To assess the genetic relationships of Cameroonian strains with viruses from other countries, we performed a BLAST search using the HA gene sequences of the 62 Cameroonian strains. The closest matches identified through BLAST were strains isolated in Algeria, Spain, Moscow, Venezuela, Ivory Coast, Kenya, Zambia, and Serbia (Fig. 2). Subsequent phylogenetic analysis confirmed that the A(H1N1)pdm09 viruses from Cameroon, classified as subclades 5a.2a and 5a.2a.1, are genetically close to subclade 5b.

Antigenic site mutations in A(H1N1)pdm09 viruses

A comprehensive analysis of the 62 A(H1N1)pdm09 virus sequences revealed diverse amino acid substitutions across all eight gene segments. Mutations observed in the surface glycoproteins (HA and NA) are summarized in Table 3, while those in internal genes (M1, M2, NS1, NS2, NP, PA, PB1, PB2) are presented in Additional file 4. Only mutations present at a frequency of ≥ 20% are reported. In the HA segment, recurrent substitutions including A267D, D111N, E277D, and N147K were observed in nearly all sequences, suggesting conserved evolutionary changes among circulating strains. In the NA segment, mutations such as E433R, S200N, P458S, and I264T were frequently observed. Some of these substitutions have been previously linked to increased disease severity or antiviral resistance [25]. Table 3 provides a comparative overview of Cameroonian A(H1N1)pdm09 subclades and the contemporary vaccine strains, highlighting key HA and NA amino acid differences. Additional Table 4 details the recurrent mutations detected in the internal gene segments, emphasizing broader genomic variability beyond the surface glycoproteins. This organization allows readers to quickly visualize both surface

Table 2 Summary of yearly sample collection and influenza A and A(H1N1)pdm09 positivity rates, 2019–2024

Year	Total Sample	ILI cases	SARI Cases	Influenza A Positive (n, %)	A(H1N1)pdm09 Positive (n, %)	Main Peak Period
2019	1072	897	175	324 (30.2%)	100 (30.9%)	Jan–Mar 2019
2020	362	244	118	37 (10.2%)	8 (21.6%)	Late 2020
2021	443	377	66	105 (23.7%)	83 (79.0%)	Feb–Apr 2021
2022	628	455	173	91 (14.5%)	1 (1.1%)	Low activity
2023	1848	1442	406	358 (19.4%)	73 (20.4%)	Oct–Dec 2023
2024	3017	2316	701	490 (16.2%)	157 (32.0%)	Jan–Mar 2024
Total	7453			1405 (18.9%)	422 (30.0%)	

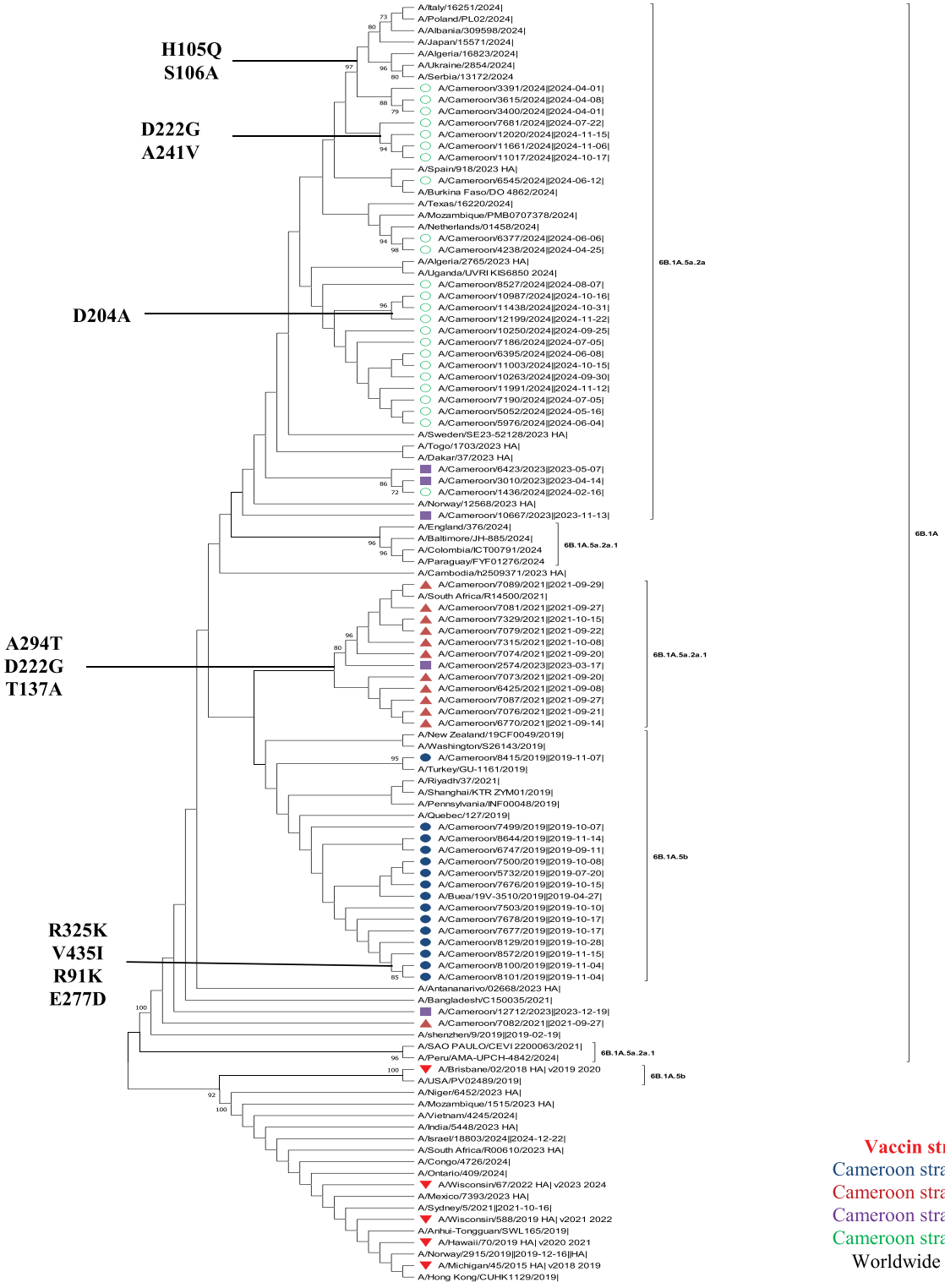

Fig. 2 Phylogeny of the HA1 gene of Influenza A(H1N1)pdm09 viruses detected in Cameroon (2019–2024). Cameroonian strains collected in 2019 are shown in blue, those from 2021 in pink, those from 2023 in purple, and those from 2024 in green, while vaccine reference strains are highlighted in dark red. HA1 amino acid substitutions are indicated in green, and bootstrap values > 70% are shown at the nodes

Table 3 HA and NA amino acid differences between Cameroonian A(H1N1)pdm09 subclades and vaccine strains (2019–2024)

Cameroon Subclade	Year(s) Detected	Key HA Mutations	Key NA Mutations	Vaccine Strain (Year)	Amino Acid Differences vs Vaccine
6B.1A.5a.2	2019–2021	D222G, T137A, N147K, S154P, R159K	V106I, H275Y, N200S	A/Wisconsin/67/2022	D222G, T137A, N147K, S154P, R159K, V106I, H275Y, N200S
6B.1A.5a.2a	2023	S220T, A214T, A241E, A267V, K173N	I223V, I264T, N284D	A/Wisconsin/67/2022	S220T, A214T, A241E, A267V, K173N, I223V, I264T, N284D
6B.1A.5a.2a.1	2024	T233A, A241V, D373E, S468N, Y506F	P458S, L329D, S366N	A/Victoria/4897/2022	T233A, A241V, D373E, S468N, Y506F, P458S, L329D, S366N

Table 4 Summary of detected mutations and their links to disease severity and antiviral resistance in A/H1N1pdm09 strains from Cameroon (2019–2024, N=62)

Mutation	Frequency(n/62)	Percentage (%)	Potential Impact
Group 1 : Mutations associated with increased disease severity			
D222G	31/62	0,5	Disease severity
Group 2 : Mutations associated with mild drug resistance			
V7I	10/62	16,1	mild drug resistance
V27I	14/62	22,5	mild drug resistance
I223V	07/62	11,2	mild drug resistance
I264T	05/62	08	mild drug resistance
P458S	11/62	17,7	mild drug resistance
Group 3 : Mutations associated with strong drug resistance			
H275Y	33/62	53,2	strong drug resistance
V106I	40/62	64,5	strong drug resistance
N284D	16/62	25,8	strong drug resistance

glycoprotein changes relevant to antigenicity and vaccine matching, as well as internal gene mutations that may contribute to viral fitness or antiviral resistance.

Discussions

This study aimed to characterize the genetic diversity and antiviral resistance profiles of influenza A(H1N1)pdm09 viruses circulating in Cameroon between 2019 and 2024, using whole-genome sequencing and phylogenetic analysis. Since September 2023, A(H1N1)pdm09 viruses have circulated globally and remained predominant across most regions, as reported by the World Health Organization [4]. Between 2019 and 2024, 7,453 influenza samples were analyzed, with A(H1N1)pdm09 remaining the predominant subtype, similar to several geographic regions worldwide [2]. The recent rise in clinical cases may be attributed both to reduced vaccine effectiveness [26, 27] and declining population immunity. In Cameroon, these trends coincide with the introduction of the influenza A vaccine into the Expanded Programme on Immunization (EPI) for high-risk groups, including children, pregnant women, the elderly, and individuals who are immunocompromised or have chronic conditions. Although vaccination coverage remains limited, it may have contributed partially to population immunity [28]. The stability of the dominant H1N1 clade (6B.1A.5a.2a and 6B.1A.5a.2a.1) suggests that the seasonal vaccine has a modest impact on viral evolution, with natural immunity playing a more significant role.

Finally, the decline in cases in 2020 can be attributed to reinforced COVID-19 public health measures (movement restrictions, widespread mask use, improved hygiene) as documented in several studies [29]. whereas the rebound in 2024 reflects the gradual relaxation of these measures, waning immunity after several years of low viral exposure, and the progressive introduction of seasonal influenza vaccination [30].

Genetically characterized haemagglutinin (HA) genes from Cameroon predominantly belonged to clade 6B.1A.5a.2, with further diversification into subclades 6B.1A.5a.2a and 6B.1A.5a.2a.1, defined by specific amino acid substitutions. This distribution mirrors findings from Australia, Indonesia, and New Zealand, where circulating A(H1N1)pdm09 strains during the same period also mainly belonged to clade 6B.1A.5a.2 and diversified into similar subclades, suggesting a comparable evolutionary pattern across these regions [31]. These observations highlight the ongoing diversification of Cameroonian A(H1N1)pdm09 strains, which were previously assigned to subclade 6B.1a in 2019, as reported by Monamele et al [32]. Our analysis further confirms the disappearance of clade 6C, which previously grouped some 2014 strain. Global comparisons showed similar evolutionary shifts, with recent viruses diverging from the WHO-recommended vaccine strain A/Wisconsin/67/2022 [33, 34], indicating limited protection by current formulations and reinforcing the need for annual vaccine updates [15, 16].

All eight genomic segments of the 62 sequences included in the final analysis were recovered with ≥ 90% coverage, as only genomes meeting the minimum coverage of 30 × and ≥ 90% resolved positions were retained. The D222G substitution in the HA1 subunit was detected in 50% of sequenced strains and has been associated with increased disease severity [35]. Analysis of the neuraminidase (NA) gene revealed resistance-linked mutations including H275Y,V106I and N284D [36–38], as well as novel substitutions I223V, I264T, P458S, which may confer mild resistance to neuraminidase inhibitors (NAIs). A complete overview of these mutations is provided in Table 4 ("Summary of Observed Mutations and Their Impact on Severity and Antiviral Resistance in A/H1N1pdm09 Strains from Cameroon (2019–2024, N = 62). Additional NA mutations associated with antiviral resistance such as N200S, I223V, and V241I have also been reported in previous studies [39, 40]. M2 protein substitutions (V7I, V27I) were identified in several strains, consistent with recent findings by Resende et al [41]. and earlier observations by Njouom *et al*., in classical A(H1N1) viruses prior to the 2009 influenza pandemic [42]. These mutations, along with the previously documented S31N substitution, indicate persistent natural resistance to adamantanes in local strains.

Despite potential introductions of H1N1 from international travelers (Algeria, Spain, Russia, Venezuela, Côte d'Ivoire, Kenya, Zambia, Serbia), the dominant clade remains unchanged, likely due to pre-existing population immunity and the lack of a selective advantage of imported strains. The persistence of this clade reflects partial population immunity, climatic and behavioral factors affecting transmission (temperature, humidity, healthcare-seeking behaviors), and the relatively slow antigenic evolution of this lineage.

This pattern indicates several likely independent introductions of the virus into Cameroon, rather than sustained circulation of a single lineage. The dataset included 138 reference sequences retrieved from GenBank, representing contemporaneous (2019–2023) isolates from Central, West, and East Africa, as well as a selection of global reference strains. Only complete or near-complete genomes with high coverage were retained to minimize artifacts due to missing data Moreover, several amino acid substitutions identified in the Cameroonian isolates mirror those detected in Sicily (Italy), Kenya, and China [31, 43–46], suggesting the occurrence of parallel evolutionary trajectories across distinct geographic regions.

Importantly, the emergence of specific mutations and clade transitions can be contextualized within the observed epidemiological patterns. For example, the rise of subclades 6B.1A.5a.2a and 6B.1A.5a.2a.1 in 2023–2024 coincided with seasonal peaks in influenza A(H1N1) pdm09 cases in Cameroon, while earlier clade shifts corresponded temporally with the national vaccination campaigns targeting high-risk groups. These correlations highlight that genomic changes can shape the timing and intensity of influenza outbreaks and potentially affect vaccine performance. For public health decision-makers, our findings underscore the value of integrating routine Nanopore sequencing into the national influenza surveillance system. This approach would enable faster detection of emerging variants, timely adjustment of vaccination strategies, and more informed epidemic preparedness. This genetic relatedness may reflect Cameroon's strategic location in Central Africa and regional mobility patterns for trade, travel, and migration. Marked antigenic variability was detected in the HA gene of H1 influenza viruses, with half of the isolates carrying substitutions within the major head antigenic sites. Specifically, the mutations T137A, S154P, and R159K map to the 130-loop region of HA1 and are predominantly associated with the Sa antigenic site. However, some studies have suggested partial overlap between Sa and Sb, depending on alignment methods and strain context [47]Consistent findings were reported by Xu et al.[48], who identified 19 HA mutations in Wuhan strains, and by Su et al.[49], who described the fixation of substitutions conferring immune escape for instance, S220T within the Ca1/Ca2 sites and K180Q within the Sa site across multiple viral lineages. Moreover, the lack of 3′–5′ exonuclease proofreading activity in influenza viruses promotes frequent insertions and deletions, thereby accelerating antigenic drift and facilitating immune evasion [50].

Study limitations include restricted geographic representativeness and limited temporal coverage, particularly in 2020 and 2022, when samples were very limited or incomplete and thus excluded from the analysis. While Ct values mainly reflect sample quality, the use of a Ct < 25 threshold to ensure adequate sequencing success may have inadvertently excluded samples with lower viral loads, potentially reducing observed genetic diversity. Consequently, conclusions on year-to-year evolutionary dynamics should be interpreted cautiously, with comparisons primarily based on the seasons with sufficient data (2019, 2021, and 2024). Future work should expand spatiotemporal genomic surveillance to monitor transmission dynamics, antigenic drift, and vaccine strain relevance in Cameroon and the region.

Conclusion

The considerable genetic variation observed among A(H1N1)pdm09 strains circulating in Cameroon between 2019 and 2024 highlights ongoing viral evolution and the need for adaptive public health strategies. These findings underscore the importance of sustained genomic surveillance to inform vaccine strain selection, monitor antiviral

resistance, and strengthen pandemic preparedness in alignment with WHO recommendations.

Abbreviations

COVID-19	Coronavirus disease 2019
CPC	Centre pasteur cameroon
Ct	Threshold cycle
GISAID	Global initiative on sharing avian influenza data
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
WHO	World health organization
PB2	Polymerase basic protein 2
PB1	Polymerase basic protein 1
PA	Polymerase acidic protein
HA	Hemagglutinin
NP	Nucleoprotein
NA	Neuraminidase
M	Matrix segment
NS	Non-structural segment
ILI	Influenza-like illness
SARI	Severe acute respiratory Infection
DNA	Deoxyribonucleic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-12284-5.

Additional file 1: Temporal and regional distribution of sentinel sites

Additional file 2: Influenza A Virus Primers

Additional file 3: GenBank Isolate ID

Additional file 4: Internal gene mutations of Cameroonian A(H1N1)pdm09 subclades (2019–2024)

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Author contributions

R.N. designed the study. P.I.T., M.H.M.Y., A.M.N., L.L.M.E., M.R.N., R.M.N., J.L.M.N and D.T.T carried out the laboratory analysis and data curation. E.L., U.T., F.K.K and R.N. were involved in supervision and data validation. D.T.T., C.G.M., L.L.M.E., M.H.M.Y. and F.K.K. performed the data analysis. D.T.T. wrote the first draft of the manuscript. All authors revised and edited the manuscript. All authors are responsible for all aspects of the manuscript.

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Data availability

All genomic data generated and analyzed in this study are publicly accessible via the GenBank database. Researchers can access the data at https://www.ncbi.nlm.nih.gov/genbank/about/using the listed accession numbers: PX446230, PX446231, PX446382, PX446383, PX446384, PX446385, PX446386, PX446387, PX446388, PX446389, PX446390, PX446391, PX446392, PX446393, PX446394, PX446395, PX446396, PX446397, PX446398, PX446399, PX446400, PX446401, PX446402, PX446403, PX446404, PX446405, PX446406, PX446407, PX446408, PX446409, PX446410, PX446411, PX446412, PX446413, PX446414, PX446415, PX446416, PX446417, PX446418, PX446419, PX446420, PX446421, PX446422, PX446423, PX446424, PX446425, PX446426, PX446427, PX446428, PX446429, PX446430, PX446431, PX446432, PX446433, PX446434, PX446435, PX446436, PX446437, PX446438, PX446439, PX446440, PX446441 (Additional Table 3).

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics and Institutional Committee of the University of Douala (CEI-Udo) (Approval No. 3971CEI-Udo/07/2023/M) and was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki. Prior to enrollment, written informed consent was obtained from the parents of each child. All personal and identifiable information was anonymized to ensure the protection of participants' privacy. Informed consent was obtained from all participants, as well as from the legal guardians of those under 16 years of age, in accordance with Cameroonian regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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