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Genome diversity of SARS-CoV-2 lineages associated with vaccination breakthrough infections in Addis Ababa, Ethiopia

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Abstract

Background Extensive vaccination campaigns against COVID-19 have played a significant role in controlling virus spread and preventing severe illness. This study focused on breakthrough infections in vaccinated individuals, raising concerns about vaccine effectiveness against SARS-CoV-2 variant immune escape, with particular attention to lineage distribution among vaccinated and unvaccinated individuals.

Methods A case–control study was conducted from January to April 2023, sequencing 298 samples from participants who tested positive for COVID-19 via rapid diagnostic test (RDT) from 22 health facilities, including vaccinated and unvaccinated cases. Besides clinical and epidemiological data, nasopharyngeal swabs were obtained, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted to determine Cycle threshold (Ct) values, followed by whole genome sequencing of 298 samples fulfilling sequencing criteria to identify variants of concern and specific virus lineages.

Results Out of 298 samples sequenced, 281 fulfill quality for analysis with 44.8% (126) had received at least one COVID-19 vaccine dose, while 51.9% (146) were not vaccinated, and 3.2% (9) patients had no vaccination records. The analysis showed that all cases were of the Omicron variant, with the XBB.1.5 lineage being the most prevalent (38.4%), followed by FL.2 (9.3%) and XBB.1.9.1.2 (7.8%). The remaining 44.5% comprised a combination of 22 other lineages. The XBB.1.5 variant accounted for 51 (47.2%) cases among vaccinated individuals with at least one dose and 57 (52.8%) among unvaccinated, showing relatively similar prevalence across both groups. The viral load as indicated by the Ct value varied widely, with a significant appearance in the lower ranges (high viral load), suggesting active viral replication. Notably, 25% of samples exhibited high viral loads (Ct values 13–15), showing the high transmissibility of the XBB.1.5 lineage among both vaccinated and unvaccinated populations.

Conclusion The findings emphasize the need for continuous genomic surveillance and regular vaccine updates to address emerging SARS-CoV-2 variants, particularly the immune-evasive XBB lineage. The high prevalence of variants like XBB.1.5 in breakthrough infection underscores the importance of adaptive vaccination strategies

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and next-generation vaccines to maintain efficacy. Ongoing monitoring of variant dynamics is crucial for informed public health responses, strengthening pandemic preparedness and future outbreak prevention.

Keywords Breakthrough infection, Genomic diversity, Vaccination, Lineage, SARS-COV-2 variant

Introduction

The rapid global response to the COVID-19 pandemic led to the development and deployment of vaccines that have helped to mitigate the impact of the virus. Extensive vaccination campaigns have significantly reduced the spread of SARS-CoV-2 and prevented severe outcomes, such as hospitalizations and deaths [1]. These vaccines, including mRNA-based and viral vector vaccines, targeted the original strain of the virus, but the evolution of SARS-CoV-2 has led to the emergence of many variants, some of which have shown the capacity to evade immune responses generated by vaccination or previous infections [2, 3]. One of the most pressing concerns is the phenomenon of breakthrough infections, where vaccinated individuals contract COVID-19, often because of the immune evasion properties of these newer variants [4].

Breakthrough infections have raised questions about the long-term effectiveness of commercial COVID-19 vaccines over time, especially against the backdrop of evolving viral variants. Studies have reported mechanisms by which certain spike protein mutations in SARS-CoV-2 variants can lead to immune escape, resulting in breakthrough infections even among vaccinated populations [5, 6]. The study emphasizes the necessity for continuous monitoring of viral mutations to adapt vaccine strategies targeting new variants. Of particular interest are variants of concern (VOC), such as the Omicron variant, which has been linked to a higher rate of breakthrough infections due to its ability to evade vaccine-induced neutralizing antibodies [7]. In particular, the Omicron sub-lineages, such as XBB.1.5, have exhibited enhanced transmissibility and immune escape properties, prompting the need for ongoing monitoring of their spread among both vaccinated and unvaccinated populations [8, 9]. The research highlights the importance of genomic surveillance in tracking the dynamics of viral evolution and informing public health strategies to adapt vaccines to emerging variants [10].

Recent studies have shown that despite the high vaccination coverage in many regions, variants like XBB.1.5 continue to circulate widely, raising concerns about the efficacy of existing vaccines in the face of these mutations [11]. For example, research has shown that the XBB lineage has a higher capacity to evade both vaccine-induced and infection-acquired immunity, which may contribute to its prevalence even in vaccinated populations [12, 13]. As we continue to navigate the evolving landscape of the COVID-19 pandemic, continuous genomic surveillance and updated vaccine formulations are crucial in maintaining control over the virus's spread [14].

Ethiopia has faced significant challenges in controlling the spread of SARS-CoV-2, with multiple waves of infections driven by emerging variants. According to the report, Ethiopia has recorded over 500,000 confirmed COVID-19 cases and over 7,500 deaths, although the actual burden was likely higher because of limited testing and surveillance [15]. The country has undertaken extensive vaccination efforts, primarily relying on AstraZeneca, Johnson & Johnson, Sinopharm, and Pfizer vaccine types. By 2023, it was reported that Ethiopia had administered over 52 million vaccine doses, achieving approximately 50% full vaccination coverage among the eligible population [16]. However, breakthrough infections, particularly with immune-evasive Omicron subvariants, have raised concerns about waning immunity and the need for new strategies [17]. As a result, studies have emphasized the importance of continuous genomic surveillance and tailored vaccination policies to combat emerging variants in low-resource settings [18].

This study aimed to provide region-specific insights into vaccine effectiveness while highlighting the genomic diversity of SARS-CoV-2 variants and their impact within Ethiopia's diverse immunological landscape. By analyzing breakthrough infections, it informs public health decisions on vaccine rollout adjustments and future pandemic preparedness. Given Ethiopia's reliance on multiple vaccine types, the findings offer critical data to optimize vaccine selection and administration strategies for potential future variant-driven outbreaks.

Materials and methods

Study design and sample size

This study employed a case–control study design to assess the effectiveness of COVID-19 vaccination by analyzing the distribution of SARS-CoV-2 variants and lineages among vaccinated and unvaccinated individuals in Addis Ababa. Cases were defined as individuals with laboratory confirmed SARS-CoV-2 infection who had received at least one dose of a COVID-19 vaccine, breakthrough infections. Controls were individuals with confirmed SARS-CoV-2 infection who had not received any COVID-19 vaccination. This case–control study design is appropriate for this study because it allows to assess whether COVID-19 vaccination status (exposure)

is associated with differences in SARS-CoV-2 genomic profiles (outcome) among individuals with confirmed infection.

A target sample size was determined based on epidemiological sampling methods, considering assumptions of vaccine coverage in the population, expected vaccine effectiveness, and variant prevalence rates. assumed a vaccine effectiveness of 80% average for all vaccine types, an alpha of 0.05, 80% power. A case-to-control ratio of 1:1.2 was established to ensure adequate power for statistical comparisons. Participants were recruited from healthcare facilities across Addis Ababa and cases were identified based on positive RDT and RT-qPCR test results and documented vaccination history, while controls were selected from the same clinical settings to minimize selection bias. Cases and controls were randomly selected from individuals seeking care at health facilities, ensuring a representative sample. While matching was not strictly applied, demographic characteristics such as age, sex, and comorbidities were considered during analysis to minimize potential confounding factors.

The initial sample included 328 participants. However, 30 samples were excluded due to a low viral load Ct value, and 17 were removed after sequencing due to poor sequence quality. This resulted in a final dataset of 281 participants, consisting of 129 vaccinated cases and 152 unvaccinated controls, all meeting the criteria for the final analysis. By ensuring a systematic selection, this study aimed to provide robust insights into vaccine effectiveness and the lineage diversity of SARS-CoV-2 infections in Ethiopia.

Study period and setting

The study was conducted from January to April 2023 in Addis Ababa, Ethiopia, involving 22 public and private health facilities serving as central hub to COVID-19 testing and treatment efforts mandated by the Ministry of Health Ethiopia. This setting was chosen to ensure a representative sample of the urban population, where vaccination rates and the burden of COVID-19 have been significantly high. The study period was chosen to coincide with the peak circulation of the Omicron variant during the time.

Participants

Eligibility for participation required a positive RDT result, informed consent, and availability of comprehensive clinical and epidemiological data. The study included individuals aged 18 years and above, both male and female, who tested positive for COVID-19 through RDT during the designated study timeframe at those selected study sites. Both vaccinated and unvaccinated individuals were included to evaluate the occurrence and characteristics of breakthrough infections across various demographic factors, such as age, gender, and pre-existing health conditions. Selection bias was minimized by applying uniform inclusion criteria to all eligible positive test result individuals presenting to the participating healthcare facilities during the study period. Vaccinated individuals received at least one of the following vaccines: AstraZeneca, Johnson & Johnson, CanSino, Pfizer-BioNTech, Moderna, and Sputnik V, produced by different platforms (mRNA for Pfizer-BioNTech & Moderna, adenoviral vector for AstraZeneca, Johnson & Johnson, Sputnik V and inactivated virus for CanSino), verified by vaccination card and facility record logbook. Vaccinated individuals included in the study had received their vaccination at least 14 days before testing positive, allowing time for the expected protective immune response to develop. All participants provided informed consented, and data collectors emphasized that participation was completely voluntary, with the understanding that individuals could withdraw from the study at any point, even after providing consent.

Sample collection

Sample collection took place from January to April 2023, during a period of significant COVID-19 case surge in Ethiopia, primarily driven by the Omicron variant, and conducted in accordance with COVID-19 testing directives at selected health facilities. Those patients who tested positive with RDT were categorized into exposure (vaccinated) and control (unvaccinated) groups based on their vaccination data. Nasopharyngeal swabs were collected from all eligible participants, ensuring sufficient sample volume, and transported to the main laboratory in Viral Transport Medium (VTM) under cold chain conditions to maintain sample integrity.

RNA extraction and RT-qPCR test

To ensure optimal RNA extraction and real-time RT-qPCR testing for virus detection, a standardized protocol was followed based on the manufacturer's instructions, the MagaBio plus Virus DNA/RNA Purification Kit II, developed by BioFlux (Hangzhou Bioer Technology Co., Ltd., China). This protocol was implemented for RNA isolation, in line with the recommendations for COVID-19 testing. RNA extraction was carried out with a Bio-Rad Automated Extraction System (Bio-Rad Laboratories Inc., USA). This method maximizes the recovery of high-quality RNA by utilizing silica-based membrane technology, enabling efficient extraction of viral RNA.

Following RNA extraction, RT-qPCR was conducted using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Specific primers and probes targeting the SARS-CoV-2 genome, including

the N, E, and S genes, were used for amplification from the Shenzhen Gene and Bioengineering Company (Shenzhen, China), with the process monitored in real-time. The critical parameter measured during this process was the Ct value, which indicates the viral load in the samples. For genome sequencing, all samples with Ct values of 31 or lower were considered indicative of a significant viral load uniformly, appropriate for downstream analysis and suitable for whole-genome sequencing. Of the 328 samples tested via RT-qPCR, 298 samples met the pre-established Ct value criteria for sequencing. These samples were subsequently processed for whole-genome sequencing to further analyze the genetic variations of SARS-CoV-2 present in the collected specimens.

Library preparation and sequencing

Using SuperScript IV reverse transcriptase, cDNA synthesis and amplification were conducted on RNA extracted from 298 positive samples using the reverse transcription method with the Illumina COVIDSeq RUO Kits (Illumina Inc., USA). Initially, random hexamer was used to synthesize cDNA, effectively converting both coding and non-coding regions of the viral RNA genome into DNA. This process yielded a complete template for the subsequent amplification phase. Following this, PCR amplification of the cDNA was performed using multiplex ARTIC primer pools (ARTIC v4), targeting various regions of the SARS-CoV-2 virus genome, resulting in tiled fragments that ensured thorough coverage of the viral RNA. The next phase involved the creation of sequencing libraries. The amplified cDNA was fragmented to produce DNA segments of suitable sizes for sequencing. After fragmentation, size selection was performed to achieve uniform fragment lengths, enhancing sequencing quality. Adaptor ligation was then executed, where adaptors were attached to the cDNA fragments, enabling their binding to the Illumina sequencing flow cell. A subsequent PCR enrichment step was carried out to concentrate the library fragments with the correct adaptors, preparing them for sequencing. The prepared libraries underwent quantification using Qubit fluorometric assays to confirm optimal concentrations for sequencing. These libraries were subsequently loaded onto the Illumina MiSeq platform (Illumina Inc., USA), configured for paired-end 300-cycle sequencing, which allowed for sequencing from both ends of the DNA fragments, improving accuracy and coverage. This sequencing process generated high-quality data crucial for the comprehensive characterization and monitoring of the SARS-CoV-2 viral genome.

Data analysis

The raw sequencing data underwent a multi-step bioinformatics pipeline to ensure high-quality analysis. Initially, sequencing reads were assessed for quality using FastQC, which provided detailed metrics on per-base sequence quality, GC content, and adapter contamination. Low-quality reads ($Q < 20$) and adapter sequences were trimmed using Trimmomatic, ensuring the retention of good-quality sequence reads for downstream processing. After quality filtering, 281 high-quality samples were obtained. To facilitate genome assembly, the filtered reads were processed using SPAdes, a widely used assembler for viral genomes, which generated contiguous sequences (contigs). The assembled sequences were aligned to the SARS-CoV-2 reference genome using Minimap2, ensuring accurate genome reconstruction and identification of mutations. Lineage classification was performed using Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN), based on the SARS-CoV-2 classification updates from GISAID. The Nextclade tool was used for additional quality checks and clade assignment, ensuring consistency in lineage classification. Multiple Sequence Alignment (MSA) was performed using MAFFT, and the aligned sequences were used to generate a phylogenetic tree using the Neighbor-Joining algorithm in MEGA 11, with 1,000 bootstrap replicates to assess the robustness of the clustering. Omicron was designated as the root lineage to illustrate divergence patterns among sub-lineages and a phylogenetic tree was constructed to visualize the evolutionary trajectories of the identified lineages.

For statistical analysis, the processed sequencing data, along with metadata was imported into SPSS version 25. Descriptive statistics were used to summarize lineage frequency and distribution. Inferential statistical analysis was conducted using the Pearson Chi-Square test to assess associations between lineage distribution and demographic factors, while the Kruskal–Wallis test was applied for comparisons of viral loads across different lineages. This analytical approach provided critical epidemiological insights, aiding in understanding the impact of viral evolution on vaccine effectiveness and transmission dynamics.

Results

The data include 281 sequences analyzed (Table 1), with the age distribution, 90 patients (32.0%) in the 18–29 years age group, 69 participants (24.6%) in the 30–39 years, 48 participants (17.1%) in the 40–49 years, 28 participants (10.0%) in the 50–59 years, and 46 (16.4%) in the 60 + years age group.

Table 1 Age and lineage frequency distribution

Age Group			Lineage		
	Frequency	**Percentage**		**Frequency**	**Percentage**
18–29 years	90	32.0%	BA.2.75.3.4.1 1.1.1.18	24	8.5%
30–39 years	69	24.6%	BA.5	12	4.3%
40–49 years	48	17.1%	XBB.1	22	7.8%
50–59 years	28	10.0%	XBB.1.5	108	38.4%
60 + years	46	16.4%	XBB.1.9.1.2	26	9.3%
Total	**281**	**100%**	XBB.1.9.1.5	17	6.0%
			Others	72	25.7%
			Total	**281**	**100%**

The examination of lineage frequency shows that the XBB.1.5 lineage was the most dominant, comprising 38.4% (108 patients) of the total sequence analyzed (Table 1). This finding highlights the significant prevalence of certain variants within the sampled group, with BA.2.75.3.4.1.1.1.1.18 (8.5%, 24 cases) and XBB.1.9.1.2 (9.3%, 26 samples) being notably prominent. The "Others" category, representing 25.7% (72 cases), includes 22 lineages such as BA.5, BQ.1, BA.1.1, BA.2, XBB.1.5.40, XBB.3, and FL.2.6, with the most frequently detected in seven patients and the least in one sample. The lineages such as BA.1.1, BA.4.1, and XBB.1.11.1.3 account for less than 1% of the total, showing their rarity. Thirty-three distinct lineages were identified, reflecting genetic diversity and the potential for new variants to emerge.

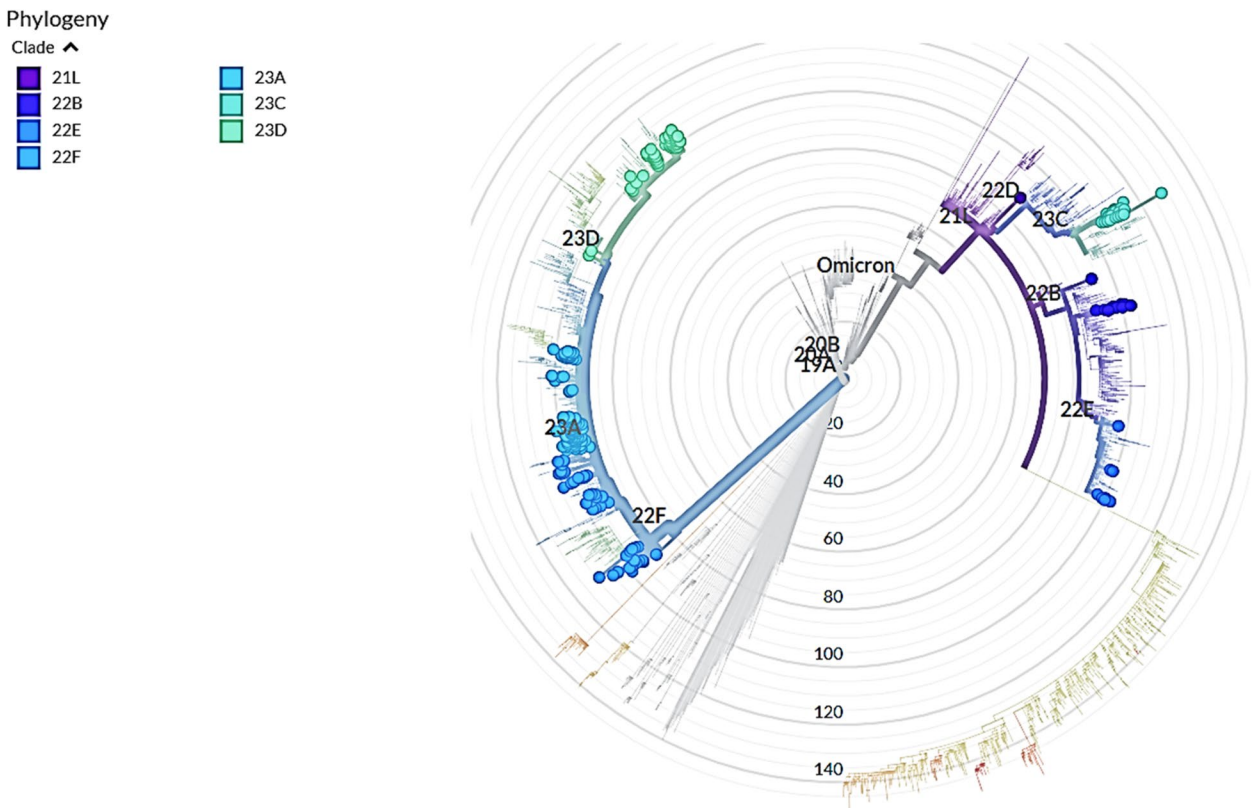

Fig. 1 Phylogenetic relationships and Nextstrain clade assignments of SARS-CoV-2 Omicron variant lineages and sub-lineages detected in Addis Ababa in 2023

The phylogenetic analysis illustrates the evolutionary relationships among Omicron-derived SARS-CoV-2 lineages (Fig. 1). In Nextstrain clade terminology, 21L corresponds to Omicron BA.2, while 22B corresponds to Omicron BA.5. Clade 22E represents Omicron BQ.1, a descendant of BA.5. Meanwhile, 22 F corresponds to Omicron XBB, which is a recombinant lineage derived from BA.2.10.1 and BA.2.75. Clade 23 A corresponds to Omicron XBB.1.5, an important descendant of XBB. Additionally, 23 C corresponds to Omicron XBB.1.9.1, and 23D corresponds to Omicron XBB.1.16. They all belong to the Omicron variant, Pango lineage B.1.1.529 and its descendants**.** In this sequence, the XBB.1.5 lineage appeared as the most prevalent variant and was found to be distributed across multiple branches, indicating its ongoing diversification and evolutionary adaptation. Other frequently detected lineages included XBB.1.9.1, XBB.1.17.1, and XBB.1.28, all clustering within the broader XBB-related clade. Notably, CH.1.1.18, a descendant of BA.2.75, formed a distinct branch, emphasizing its divergence from other co-circulating lineages. Earlier circulating variants such as BA.5 and its derivatives, including BQ.1 and BQ.1.1, were also observed but formed separate branches, consistent with their emergence in earlier epidemic waves before being largely displaced by XBB-derived lineages. The FL.2 and FL.5 variants clustered within the XBB sub-lineages, further supporting their evolution from XBB ancestors. The widespread distribution and branching of XBB.1.5 and related variants reflect their genetic variability and continued expansion in the population.

The distribution of virus lineages by sex is shown in Fig. 2. The total number of COVID-19 cases was higher in females (168 cases) than in males (113 cases). For the BA.5 lineage, there were 8 cases in females (66.7%) and 4 (33.3%) in males. The CH.1.1.18 lineage had 16 cases in females and 8 in males. The FL.2 lineage was almost evenly distributed, with 14 cases in females and 12 in males. The FL.5 lineage had 11 cases in females and 6 in males. The XBB.1 lineage showed 16 cases in females and 6 in males, while the dominant XBB.1.5 lineage had 63 cases in females and 45 in males. The total number of breakthrough cases was higher in females (168 cases) compared to males (113 cases), a pattern observed across most lineages, particularly for XBB.1.5. A chi-square test revealed a p-value of 0.069, which exceeds the significance threshold of 0.05, indicating no statistically significant association between virus variants and sex.

The data presents the distribution of first and second dose COVID-19 vaccinations across sex, comparing female and male vaccination rates (Table 2). For the first dose, 74 females (59.2%) and 51 males (45.1%) received one of the vaccines. In contrast, a larger number of patients (88 females, 70.7% and 58 males, 51.8%) had not

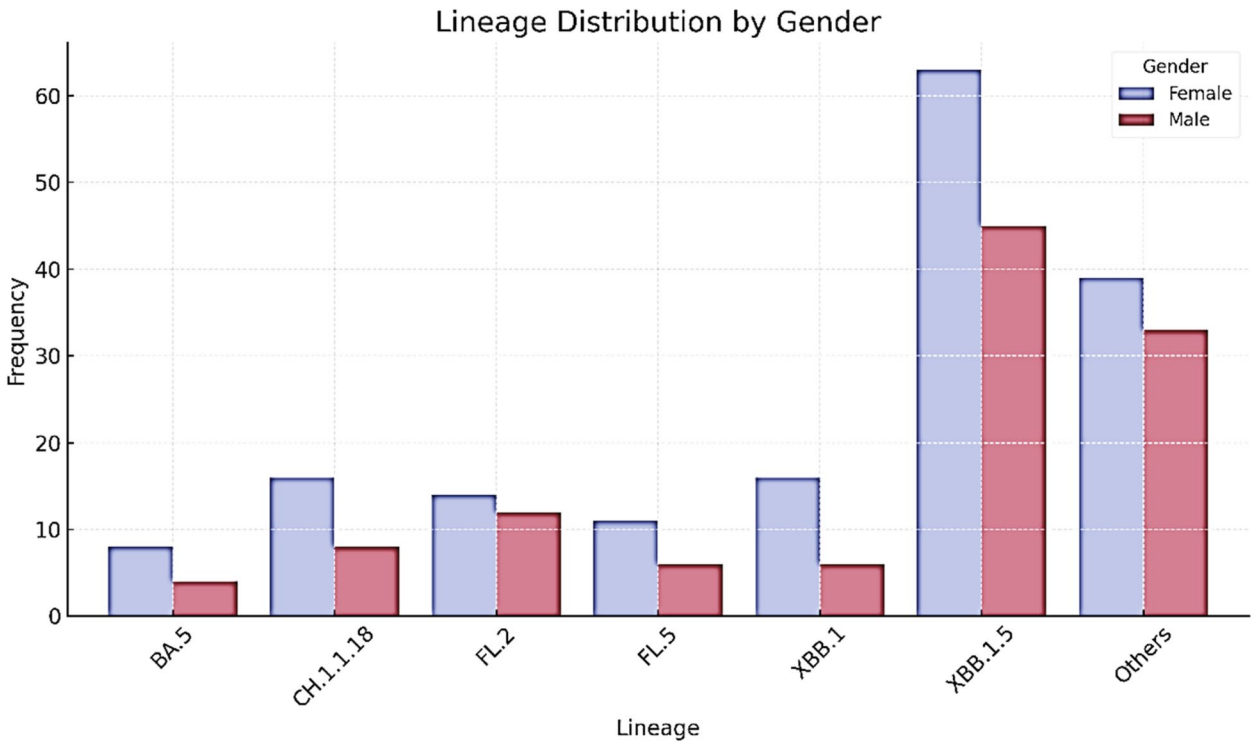

Fig. 2 Lineage distribution by sex

Table 2 Distribution of vaccination status by sex

		First dose vaccination			Second dose vaccination		
		Not known	No	Yes	No	Yes	Total
Sex	**Female**	6	88	74	136	31	168
	Male	4	58	51	81	31	113
	Total	10	146	125	217	62	281
p-value		*p = 0.000*			*p = 0.393*		

Table 3 Virus lineage in association with fist dose vaccination

Lineages	Vaccination status			*p*-value
	Yes	No	Total	
BA.5	8	3	11	*p = 0.485*
BQ.1.1	3	6	9	
CH.1.1.18	10	14	24	
FL.2	14	11	25	
FL.5	4	13	17	
XBB	3	3	6	
XBB.1	13	9	22	
XBB.1.5	51	57	108	
Others	27	32	59	
Total	133	148	281	

Table 4 Virus lineage in association with second dose vaccination

Category		Second dose vaccination			*p-value*
		No	Yes	Total	
Virus lineage	BA.5	5	3	8	*p = 1.00*
	BQ.1.1	5	2	7	
	CH.1.1.18	10	1	11	
	FL.2	15	8	23	
	FL.5	13	2	15	
	XBB	11	1	12	
	XBB.1	3	0	3	
	XBB.1.28	1	0	1	
	XBB.1.5	69	17	86	
	Others	9	3	12	
Total		141	37	178	

received the first dose. Additionally, 10 individuals (6 females, 60% and 4 males, 40%) had an unknown vaccination status, as it was not recorded on their vaccination cards or accurately reported by the patients.

For the second dose, a larger proportion of patients had not completed their vaccination. A total of 207 individuals (136 females (65.7%) and 81 males (39.1%)) had not received the second dose. Only 62 individuals (30 females (48.4%) and 32 males (51.6%)) had received the second dose. Although slightly more females were vaccinated with the first dose compared to males, the data shows a significant drop in second-dose vaccination across both sexes. The analysis revealed statistical significance for sex distribution in relation to the first dose vaccination ($p = 0.000$), while no statistically significant association was found for the second dose vaccination ($p = 0.393$).

The Table 3 presents the distribution of SARS-CoV-2 lineages by vaccination status (Table 3). The XBB.1.5 lineage was the most frequent, with 108 total cases, divided nearly equally between vaccinated (51, 47.2%) and unvaccinated (57, 52.8%) individuals. The BQ.1.1 lineage was more common in unvaccinated individuals (6, 66.7%) than in vaccinated individuals (3, 33.3%), while the FL.5 lineage showed a higher prevalence in unvaccinated individuals (13, 76.5%) compared to vaccinated individuals (4, 23.5%). The FL.2 lineage was more prevalent among vaccinated individuals (14, 56%) than unvaccinated individuals (11, 44%), and the XBB.1 lineage also followed a similar trend, with 13 cases (59.1%) in vaccinated individuals and 9 cases (40.9%) in unvaccinated individuals. The chi-Square test yielded a p-value of 0.485, indicating no statistically significant association between vaccination status and the distribution of lineages.

The data reveals the distribution of SARS-CoV-2 lineages in relation to second-dose vaccination status (Table 4). The most prevalent lineage was XBB.1.5, with 86 total cases (69 unvaccinated and 17 vaccinated), followed by FL.2 (23 total cases, with 15 unvaccinated and 8 vaccinated). Other notable lineages include CH.1.1.18 (11 total cases, 10 unvaccinated and 1 vaccinated) and FL.5 (15 total cases, 13 unvaccinated and 2 vaccinated). For XBB, there were 12 total cases (11 unvaccinated and 1 vaccinated), while BQ.1.1 had 7 total cases (5 unvaccinated and 2 vaccinated). The XBB.1 and XBB.1.28 lineages had lower prevalence, with 3 and 1 total cases respectively, both of which were more common among unvaccinated individuals. The "Others" category totaled 12 cases, with 9 unvaccinated and 3 vaccinated. Although the second-dose vaccination shows a tendency for lower prevalence of certain variants, such as XBB.1.5 and FL.2.

The result of the Pearson chi-square test ($p = 1.00$) shows no statistically significant association between second dose vaccination and lineage prevalence.

The distribution of SARS-CoV-2 lineages varied across different vaccine types (Table 5). Among individuals who received AstraZeneca, the most frequently detected lineages were XBB.1.5 (9, 8.3%) and CH.1.1.18 (4, 16.7%). Those vaccinated with Johnson & Johnson (J&J) also had the highest occurrence of XBB.1.5 (9, 8.3%), followed by CH.1.1.18 (4, 16.7%) and FL.2 (3, 12.0%). Pfizer recipients had only a few cases, with XBB.1.5 (1, 0.9%) being the only detected lineage. CanSino vaccine recipients showed the presence of FL.2 (2, 8.0%) along with a small number of other lineages. Individuals categorized under Other Vaccines exhibited multiple lineages, with XBB.1.5 (10, 9.2%) being the most common, followed by XBB.1 (5, 22.7%) and FL.2 (3, 12.0%). Among the unvaccinated group, XBB.1.5 was the most frequently observed lineage, accounting for 9 cases (8.3%), followed by CH.1.1.18 (3, 12.5%) and FL.5 (1, 5.9%). Additionally, a small number of cases (6 in total) fell into the unknown vaccination category, with XBB.1.5 (3, 50.0%) and FL.2 (3, 50.0%) being the only detected lineages.

The box plot shows the distribution of Ct values across different SARS-CoV-2 lineages, where lower Ct values indicate higher viral loads. XBB.1.5 shows the widest range, suggesting diverse viral loads among cases (Fig. 3). The "Others" category also spans a broad range. Although their prevalence is lower, CH.1.1.18, FL.2, and XBB.1 show the lowest Ct value distributions, while FL.5 has a narrow range, indicating more consistent viral loads. XBB.1.28 has the lowest range (12 to 20), suggesting high viral replication with clinical outcome and transmissibility. The phylogenetic analysis showed that XBB.1.5 and its sub-lineages are highly diverse and spread across different branches, indicating their ongoing evolution and adaptation. This is particularly evident in breakthrough infections, where XBB.1.5 is the most prevalent lineage, accounting for 108 out of 281 cases, and is present in both vaccinated and unvaccinated individuals. Its widespread presence suggests that the variant has developed immune evasion capabilities, allowing it to infect people regardless of their vaccination status. This highlights XBB.1.5 as a key player in the current SARS-CoV-2 landscape, with its ability to evade immunity contributing to its success. It primarily appeared within the 13–15 and 16–20 Ct value ranges, implying a correlation with higher viral loads. This pattern may suggest enhanced infectivity/transmission potential relative to other lineages. Similarly, the CH.1.1.18 lineage exhibits a comparable trend, with 19 out of 24 cases falling within the lower Ct value range (13–20), showing higher viral loads. The FL.2 and FL.5 lineages, while slightly less frequent, also appear in the higher viral load categories, predominantly within the 13–20 Ct value ranges. Lineages such as XBB.1.28 and XBB.1, although less common, still align with the overall trend of being more prevalent in the higher viral load categories. Conversely, as viral load decreases (Ct values range 21–31), the occurrence of all lineages diminishes, with only a few cases identified in these ranges. This observation suggests that most infections are identified at higher viral loads, with the presence of the virus decreasing as Ct values rise. The chi-square test result shows that there was a statistically significant association between virus variants and Ct values ($p = 0.000$).

Discussion

This study examines the distribution of SARS-CoV-2 lineages in breakthrough infections among vaccinated individuals, comparing them to unvaccinated cases. The examination of SARS-CoV-2 lineages about factors such as vaccination status, age, sex, host related immune response and viral load offers valuable insights into the COVID-19 infection landscape. The findings from this

Table 5 Distribution of SARS-CoV-2 lineages by vaccine type

Lineage	AstraZeneca (n, %)	J&J (n, %)	Pfizer (n, %)	Cansino (n, %)	Other vaccines (n, %)	Unknown (n, %)	Total (n)
BA.5	2 (16.7%)	2 (16.7%)	0 (0.0%)	0 (0.0%)	3 (25.0%)	0 (0.0%)	7
BQ.1.1	3 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	4
CH.1.1.18	4 (16.7%)	4 (16.7%)	0 (0.0%)	0 (0.0%)	2 (8.3%)	3 (12.5%)	13
FL.2	3 (12.0%)	3 (12.0%)	0 (0.0%)	2 (8.0%)	3 (12.0%)	3 (12.0%)	14
FL.5	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	1 (5.9%)	3
XBB	2 (28.6%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	4
XBB.1	2 (9.1%)	1 (4.5%)	0 (0.0%)	0 (0.0%)	5 (22.7%)	2 (9.1%)	10
XBB.1.5	9 (8.3%)	9 (8.3%)	1 (0.9%)	0 (0.0%)	10 (9.2%)	9 (8.3%)	38
OTHERS	8 (24.2%)	3 (9.1%)	2 (6.1%)	3 (9.1%)	7 (21.2%)	2 (6.1%)	25
TOTAL	33 (11.6%)	24 (8.5%)	3 (1.1%)	5 (1.8%)	33 (11.6%)	6 (2.1%)	118

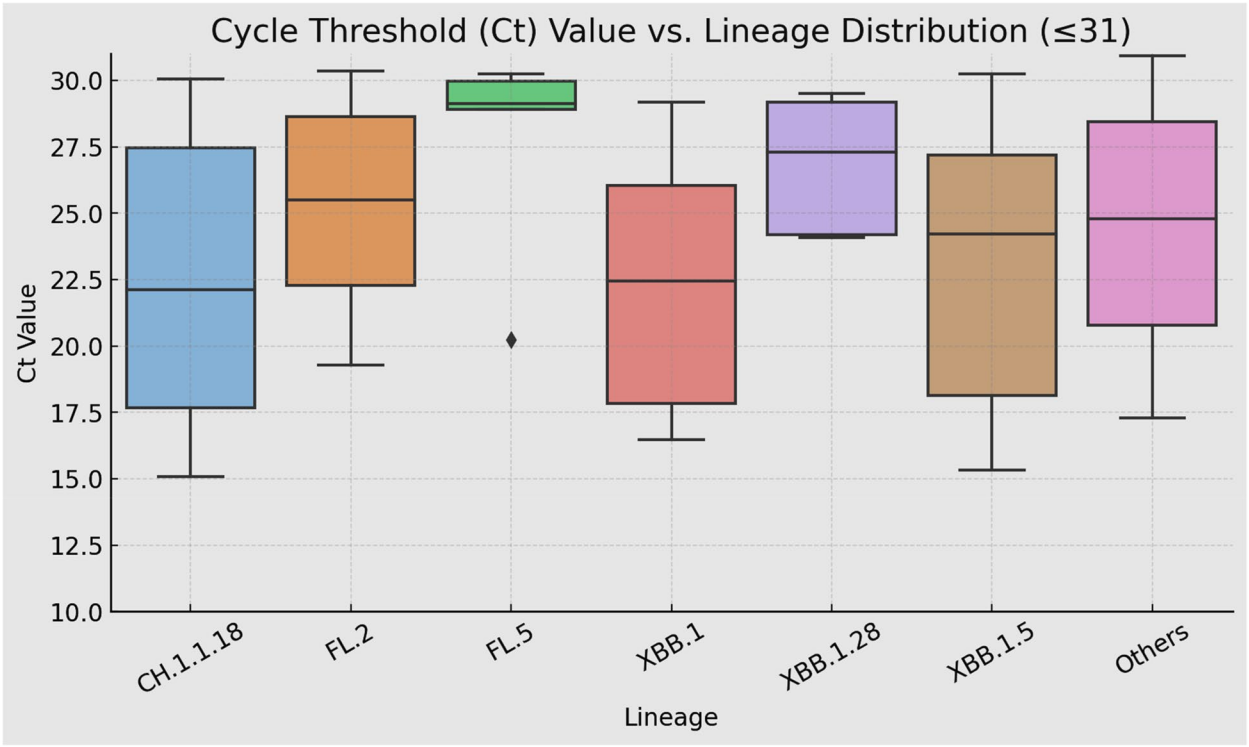

Fig. 3 Cycle threshold value in association with lineages

study revealed that the XBB.1.5 lineage was the most prevalent in Addis Ababa, accounting for 38.4%. This observation aligns with global patterns, where XBB variants, particularly XBB.1.5, are known for their increased transmissibility and ability to evade immune responses [19–21]. The predominance of XBB.1.5 is likely attributable to both sustained local transmission and multiple introductions via travelers, as evidenced by epidemiological trends and phylogenetic clustering patterns. Despite the high prevalence of the XBB.1.5 lineage, it was observed in both vaccinated and unvaccinated groups. Further investigation into the potential correlation between specific lineages, such as XBB.1.5, and breakthrough infections is needed. The lack of a statistically significant association between vaccination status and lineage distribution in this study ($p = 0.485$) suggests that while breakthrough infections occurred, other factors may be contributing to the prevalence of these variants, and further research is necessary to fully understand the impact of vaccination on the emergence of breakthrough infections. Moreover, other significant lineages, including XBB.1.9.1.2 (9.3%) and BA.2.75 (8.5%), demonstrate considerable presence, reinforcing reports of the virus's ongoing evolution and the emergence of variants that can bypass vaccine-induced immunity [22–24]. The study indicates a significant prevalence of the XBB lineage, with the XBB.1.5 subvariant emerging as a key factor in breakthrough infections due to its superior immune evasion properties. Studies have corroborated these findings, revealing that XBB.1.5 accounts for a substantial share of SARS-CoV-2 infections worldwide, highlighting the difficulties in managing these variants with current vaccines [20, 25]. For instance, XBB.1.5 was particularly dominant in various regions of North America and Europe, exhibiting a pronounced ability to evade immune responses, which is consistent with observations made in Addis Ababa [19].

The analysis reveals notable disparities in first and second-dose coverage about immunization. Of the 281 patients, 146 have not received vaccination, while 125 (74 females and 51 males) have at least received the first dose. This indicates that a sizable section of the population has not received any COVID-19 vaccinations. Furthermore, a significant disparity is seen in second-dose vaccination, as just 62 people (31 females and 31 males) have finished the two-dose course, whereas 207 have not. Since inadequate vaccination may make people more vulnerable to breakthrough infections, especially from a lineage like XBB.1.5 that has demonstrated resistance to vaccine-induced immunity, the lower percentage of second-dose vaccination draws attention to a serious problem. Additionally, the sex distribution shows that although there

are more women than men, both sexes display a similar rate of second-dose completion, with 31 individuals from each group. This suggests that barriers to completing the vaccination regimen are consistent across sexes. These gaps in vaccination coverage, coupled with the dominance of highly transmissible lineages, underscore the urgent need to address vaccine uptake and ensure full-dose vaccination to mitigate the spread of the SARS-CoV-2 virus and its variants. The study's findings resound with research conducted in various parts of the world. For instance, studies found significant gaps in vaccination coverage, which contributed to higher infection rates among unvaccinated individuals [23, 26], similar to the scenario observed in Addis Ababa. Furthermore, global studies on the Delta and Omicron variants have consistently shown that incomplete vaccination significantly raises the risk of breakthrough infections, emphasizing the importance of ensuring full vaccination regimens, particularly in the face of evolving variants [27, 28].

The XBB.1.5 lineage occurs almost equally among vaccinated (47 cases) and unvaccinated (55 cases) individuals, suggesting limited protection against this variant from vaccination. This observation is consistent with research showing that XBB.1.5 exhibits vaccine-escape capabilities, contributing to higher rates of breakthrough infections [6, 23, 27]. In contrast, the BQ.1.1 lineage is more prevalent among unvaccinated individuals, highlighting the potential benefit of vaccination in reducing the incidence of specific variants. Other lineages, such as FL.2 and XBB.1, appear more frequently among vaccinated individuals, showing continued susceptibility of these variants to vaccine-induced immunity. Additionally, a significant association ($p = 0.069$) was not observed between lineage and sex (Fig. 2), while infection rates were higher among females (59.6%) than males (40.1%). This finding is consistent with other studies that report sex-related differences in COVID-19 infection rates, potentially due to factors such as healthcare-seeking behavior, occupational exposure, or biological differences in immune response [28, 29]. The XBB.1.5 variant, in particular, shows a pronounced prevalence among females (63 cases) compared to males (45 cases), supporting the hypothesis of potential sex-related differences in susceptibility to infection. These results are consistent with another study which found a higher rate of infection among females in their study in the U.S., a trend that may be attributed to both biological factors and societal roles [28]. Similarly, the study in South Korea reported a higher prevalence of infection in females, highlighting the complex interplay of immune response and exposure risks in COVID-19 infection rates [29–31].

The results of the lineage distribution associated with second-dose vaccination offer key insights into how vaccination status impacts the circulation of specific SARS-CoV-2 variants. The data shows that while vaccination protects against the XBB.1.5 lineage, breakthrough infections still occur, with 25 cases among vaccinated individuals compared to 83 in unvaccinated individuals. This shows that vaccines significantly reduce infection risk but do not fully prevent XBB.1.5 infections, likely because of the variant's immune-evasive properties. The high prevalence of XBB.1.5 underscores the need for continued vaccine adaptation to enhance cross-protection and the potential role of booster doses in maintaining immunity against emerging variants. The FL.2 lineage showed a similar pattern to the XBB.1.5 variant, with a higher prevalence of infection in unvaccinated individuals (16 cases) compared to those who received the second dose of the vaccine (9 cases). The analysis of SARS-CoV-2 lineage distribution by vaccine type reveals that the XBB.1.5 lineage is most prevalent among the vaccinated group, and other variants such as BA.5 and BQ.1.1 are more common among AstraZeneca and Johnson & Johnson vaccine recipients. Furthermore, there is a notable variation in lineage distribution across different vaccine types, with other lineages like FL.2 and XBB appearing more frequently in AstraZeneca and CanSino. While vaccination, particularly with the second dose, reduces the risk of disease progress, it does not completely prevent infection, highlighting concerns about cross-protection against emerging variants and lineages. These findings suggest that vaccine adoption, while effective in protecting major variants like the original strain and early variants, may not offer full immunity against newer strains such as FL.2 and XBB.1.5. This underscores the need for continued vaccine adaptations and tailored approaches to ensure broader protection against evolving variants, alongside other preventive measures. Notably, the CH.1.1.18 lineage had 22 cases in the unvaccinated group versus just 2 in the vaccinated group, suggesting heightened vulnerability to this lineage among unvaccinated. This is in line with the findings which also showed that breakthrough infections were common with the Delta and Omicron variants, even in vaccinated individuals [27]. Their research found that while vaccines reduced the severity of disease, they did not fully prevent infection, particularly with immune-evading variants such as XBB.1.5. Reasonably, studies conducted by others support these findings, highlighting the prevalence of breakthrough infections with variants like Delta and Omicron among vaccinated individuals [11, 32]. These findings reveal that while vaccination significantly lowers the risk of severe disease, certain variants can still lead to substantial breakthrough cases, particularly among those who have not received a booster dose. This highlights that, while vaccination significantly reduces the

risk of SARS-CoV-2 infections, breakthrough cases still occur, particularly with evolving variants. This underscores the need for continuous genomic surveillance to monitor variant emergence and assess vaccine effectiveness. The results emphasize the importance of adaptive public health strategies, including booster doses and updated vaccines, to enhance cross-protection and maintain long-term immunity against emerging threats. Future studies should focus on longitudinal monitoring of vaccine-induced immunity, including the effectiveness and durability of booster doses against emerging SARS-CoV-2 variants.

Regarding viral load, a strong association ($p = 0.000$) was observed between lineages and Ct values. For instance, the XBB.1.5 lineage indicating a significant concentration of cases in the 13–20 Ct value range, suggesting higher viral loads and greater infectivity. Similar patterns were found in the CH.1.1.18 and FL.2 lineages, both of which also showed higher prevalence in the lower Ct value categories, showing their potential for enhanced transmission. Studies show that variants associated with lower Ct values corroborate this finding and are more infectious and have a higher potential for transmission [33–35]. These findings emphasize the importance of continuous monitoring of SARS-CoV-2 variant genome epidemiology and their public health implications, particularly considering ongoing viral evolution and the emergence of new variants. Study supports these observations and has a correlation between lower Ct values and higher viral loads in their study on Delta and Omicron variants [34]. Studies reported similar findings, highlighting that variant with lower Ct values, such as XBB.1.5, have a higher potential for transmission, which could contribute to their rapid spread [33, 36]. The exclusion of samples with high Ct values during sequencing may skew variant representation, especially if certain variants are associated with lower viral loads.

Conclusion

Vaccines continue to play a critical role in mitigating severe outcomes of COVID-19, but breakthrough infections have been observed among both vaccinated and unvaccinated individuals, with a higher prevalence of the XBB.1.5 lineage among the unvaccinated. Although vaccines provide substantial protection, they do not fully eliminate the risk of infection, particularly with the emergence of variants such as XBB.1.5, known for its increased transmissibility and immune evasion. This study also highlighted sex disparities, with females being more significantly affected, as well as age-related variations in susceptibility and infection severity. Additionally, a relationship between viral load and specific variants, particularly XBB.1.5, was noted, suggesting potential increases in transmission and infection severity. While the difference in viral load across variants like XBB.1.5, XBB.1, and CH.1.1.18 was not statistically significant, XBB.1.5 is important due to its higher prevalence and known immune-evasive properties, making it a significant variant in the ongoing pandemic. Given the continuous genetic evolution of SARS-CoV-2, ongoing genomic surveillance remains crucial for identifying emerging variants and adapting vaccination strategies. This research underscores the urgent need for sustained monitoring of SARS-CoV-2, particularly with variants that exhibit immune evasion. Studies should focus on the genetic traits of emerging variants, host factors influencing susceptibility, and the effectiveness of vaccines against new strains to guide public health interventions and vaccine development for future pandemics.

Abbreviations

AHRI	Armauer Hansen Research Institute
ARTIC	Adaptive Regional Tiling for Illumina COVIDSeq
BA	B lineage of SARS-CoV-2
BQ	B lineage of SARS-CoV-2
CanSino	CanSino Biologics COVID-19 Vaccine
cDNA	Complementary DNA
CH	C lineage of SARS-CoV-2
COVID-19	Coronavirus Disease 2019
Ct	Cycle threshold
EPHI	Ethiopian Public Health Institute
FL	F lineage of SARS-CoV-2
GC	Guanine-Cytosine
GISAID	Global Initiative on Sharing All Influenza Data
mRNA	Messenger Ribonucleic Acid
ML	Maximum Likelihood
Omicron	A variant of SARS-CoV-2
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SPAdes	St. Petersburg genome assembler
SPSS	Statistical Package for the Social Sciences
VOC	Variant of Concern
VTM	Viral Transport Medium
WHO	World Health Organization
XBB	X lineage of SARS-CoV-2

Supplementary Information

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Supplementary Material 1.

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Conflict of interest
We confirm that there are no conflicts of interest associated with this study. We have not disclosed any financial or personal relationships with individuals or organizations that could potentially influence or bias the findings of this study.

Clinical trial number
Not applicable.

Study limitation
The emphasis of the study on Addis Ababa, Ethiopia, could restrict its applicability to areas with differing demographics, health cares, or varying disease prevalence rates. Furthermore, since the study period was dominated by Omicron variant, it may not reflect the complete spectrum of other variants, potentially influencing the assessment of vaccine efficacy. Additionally, although genomic sequencing and viral load were analyzed, immune responses such as antibody titers and T-cell responses, which are essential for determining vaccine effectiveness, were not examined.

Authors' contributions
AMA conceptualization, investigation, supervision, draft writing and review; DM & AG, investigation, methodology, supervision, data curation & review; GTZ and AMG investigation, data curation and review; GBT, AA, AM, TB, TT, RA, FTW, TG, YT, BG, JBT, GTL, AA, SAB, BWL & JM investigation, data clearance and review; DN project supervision and paper review. DM & AG exclusively have equal contribution and will rank both second level authorship.

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Data availability
Data can be accessed from the GISAID data repository with accession number to datasets: EPI_SET_241010rh. Metadata and lineage list can also be found at: https://zenodo.org/records/13929701.

Declarations

Ethics approval and consent to participate
This study was approved by Ethiopian Public Health Institute Institutional Review Board (EPHI_IRB) Approval No. 415_2022. All participants have provided informed consent, and data collectors have made it clear that participation is entirely voluntary and the study was conducted in accordance with Declaration of Helsinki. Participants retain the right to withdraw from the study at any time without affecting their access to healthcare services.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests

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