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USING NEXT-GENERATION SEQUENCING (NGS) TO PREDICT AND MONITOR THE EVOLUTION OF SARS-COV-2 VARIANTS IN BENIN

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ABSTRACT

The purposes of this investigation were identifying, describing and characterizing SARS-CoV-2 variants circulating in Benin (Delta, Omicron, Beta and B.1.640), using next generation sequencing NGS. The phylogenetic tree show evidence of rapid expansion of Delta variant compared and apart from its introductions and prevalence, pointed out comprehensively some specific mutations, such as L452R and T478K that promote infectivity and immune escape. Vaccinated participants showed significantly higher levels of neutralizing antibodies compared to those who were unvaccinated, regardless of the variant causing the infection. However, individuals infected with the Delta variant and those who tested PCR-negative appeared to have lower protection levels compared to those infected with Omicron, Beta, and B.1.640. Our results highlight the critical role of combining genomic surveillance with immunological assessments to gain a deeper understanding of variant evolution, immune responses, and their impact on public health strategies. This study provides essential tools to guide vaccine selection, develop effective immunization strategies, and improve epidemic control measures. Moving forward, we plan to analyze a larger sample size to create a more precise genetic map of SARS-CoV-2. This will further enhance genomic surveillance efforts, particularly in resource-limited settings, and contribute to regional and global pandemic preparedness.

KEYWORDS: Next-Generation Sequencing (NGS), COVID-19, SARS-CoV-2, variants.

INTRODUCTION

On March 16, 2020, Benin reported its first case of SARS-CoV-2 infection. Since the start of the pandemic, the country has recorded a total of 28,036 confirmed COVID-19 cases. Of these, 27,847 patients have recovered, while 163 deaths have been reported. As of now, 26 active cases remain in the country [WHO, 2024]. Throughout the pandemic, Benin's healthcare system faced significant challenges, particularly in managing severe COVID-19 cases while maintaining essential health services. The government initiated several vaccination campaigns, but coverage remained limited due to supply issues, vaccine hesitancy, and inadequate infrastructure.

The pandemic exposed the structural vulnerabilities of Benin's healthcare system but also drove advancements in crisis management and the use of technology for faster, more coordinated responses. Given its geographical position and cross-border trade and human exchanges, especially with Nigeria, Benin quickly recognized the need to monitor the introduction of foreign variants. Identifying local mutations and understanding the dynamics of community transmission became essential for adapting public health policies. In West Africa, collaboration among countries to share scientific resources became a key strategy, further emphasizing the importance of genomic sequencing in Benin.
COVID-19 served as a catalyst for adopting genomic sequencing in Benin, not just to address an immediate crisis but also to strengthen long-term surveillance capabilities. However, post-pandemic, genomic monitoring in Benin remains reliant on standard PCR tests, which are unable to differentiate between variants, as well as on insufficient sampling and a lack of local genomic data. These shortcomings hinder the rapid identification of emerging variants and the implementation of tailored health policies. Next-generation sequencing (NGS) presents an opportunity to revolutionize variant surveillance through a more dynamic system. Approaches to sequencing SARS-CoV-2 include targeted and metagenomic methods, some of which require prior knowledge of the virus's genome while others do not (Quince, 2017; Bragg & Tyson, 2014).

Researches were conducted to study the prevalence and evolution of SARS-CoV-2 variants in Benin (Yadouleton et al., 2022). In this study, we aim to investigate the temporal and spatial evolution of SARS-CoV-2 variants in Benin and demonstrate the feasibility and effectiveness of using NGS for genetic surveillance.

METHODS

Population and Sampling

This study is a descriptive cross-sectional analysis conducted over six months, from September 2021 to February 2022. It focused on travelers entering and leaving Benin via air travel and individuals admitted to health surveillance centers for SARS-CoV-2 testing. The sample size *N* was calculated using Schwartz's formula:

$$N = (Z\text{^}2.\,p(1\text{-}p))/m\text{^}2$$

Z, the 95% confidence level, is 1.96; p, the estimated prevalence of the variant of concern (Delta) during the study period, is 2% (GISAID); m, the margin of error, is set at 5%.

Based on this, a minimum of 245 SARS-CoV-2-positive individuals were required for the study. Using the same formula and estimating a general infection prevalence of 7%, approximately 3,500 individuals needed to be tested to reach the required sample size. Ultimately, 14,725 individuals were tested for the virus. All individuals admitted to health surveillance centers for SARS-CoV-2 testing were eligible for inclusion in the study. Participants testing positive

and providing informed consent were included in the analysis of immune response. Those who declined consent, despite eligibility, were excluded.

Study Material

The biological material used in this study included two types of samples: Nasopharyngeal swabs for the detection and sequencing of viral RNA and venous blood samples from individuals who tested positive for SARS-CoV-2 to detect antibodies against the virus. Samples from travelers were collected at health surveillance centers in Cotonou (Congress Hall sanitary point and the airport travellers) and healthcare centers for admitted patients. Nasopharyngeal swabs were collected by inserting a swab into the subject's nostril, reaching the nasopharynx, and gently rotating it to collect cells. Positive samples, confirmed via RT-PCR, were aliquoted and stored at -80°C for subsequent RNA sequencing. Venous blood samples were collected in EDTA tubes by accessing the participant's vein and drawing blood shortly after applying a tourniquet.

Sample Preparation Protocols (RNA Extraction, Reverse Transcription, Amplification)

Sample preparation followed a three-step protocol PCR Amplification; (i) Two separate PCR reactions were performed for each SARS-CoV-2-positive sample. Two primer pools were used, Pool 1 contained 30 primers generating odd-numbered mosaic amplicons; Pool 2 contained 28 primers generating even-numbered mosaic amplicons, each approximately 1,200 base pairs in length. This mosaic design minimized overlap between amplicons, preventing them from annealing to one another and reducing PCR efficiency. (ii) Library Preparation and Pooling: After PCR, the two pools were combined for each sample. Libraries were barcoded, pooled into a single tube, and purified. (iii) Sequencing: The final sequencing step involved loading the prepared library onto a MinION sequencer. This device read the nucleotide sequences of the DNA fragments in the flow cell.

Sequencing Techniques

The study employed nanopore sequencing, a technology that enables rapid and efficient analysis of genetic material. The biological material analyzed consisted of viral RNA extracted from nasopharyngeal swabs of SARS-CoV-2-positive individuals. Two specific kits were used for sequencing: Rapid Barcoding Kit 96 (SQK-RBK110.96), Facilitated the identification of multiple samples; Midnight RT-PCR Extension (EXP-MRT001), Enhanced amplification of targeted viral RNA fragments.

Neutralizing Antibody Detection

The study also measured neutralizing antibodies against SARS-CoV-2 using the blocking ELISA method, a direct technique for detecting immune response. Plasma, obtained by centrifugation of blood collected in EDTA tubes, served as the biological material. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was employed for this analysis. This kit provided a standardized and reliable approach to evaluate participants' immune responses.

Bioinformatics Analysis of Results

Following the sequencing process, FASTQ files were exported and subsequently imported into the online platform https://edge-covid19.edgebioinformatics.org/ for the automatic identification of SARS-CoV-2 variants.

The bioinformatics analysis involved two key steps: the identification of critical mutations and the reconstruction of phylogenetic relationships to trace the evolutionary trajectory of the variants. A phylogenetic tree is constructed to

illustrate how sequences from Benin cluster relative to global sequences. This analysis is performed using the Nextstrain platform, which serves as a global repository for SARS-CoV-2 genomic data. Researchers worldwide contribute to sequencing and sharing SARS-CoV-2 genomic data, enabling the Nextstrain team to perform large-scale analyses at both global and continental levels. More specific, regionally focused analyses are often conducted by independent research groups.

In addition to studying SARS-CoV-2 evolution through these comprehensive analyses, the Nextstrain ecosystem includes a powerful tool called Nextclade. This tool allows for direct comparison of individual sequences with the SARS-CoV-2 reference genome, assigns sequences to specific clades, and positions them within the broader SARS-CoV-2 phylogenetic tree. By leveraging these bioinformatics tools, researchers gain deeper insights into the dynamics of viral evolution and the global spread of variants.

RESULTS

1. Sociodemographic Characteristics of the Study

To achieve the objectives of this study, we conducted extensive SARS-CoV-2 screening involving 14,725 participants. These participants were categorized based on sex, age, and origin. Age distribution was defined according to the human life cycle (birth, childhood, adolescence, adulthood, old age, and death). Our study sample specifically included adolescents, adults, and elderly individuals. The majority of participants (54%) came from health centers in Cotonou, and females were slightly more represented at 52%. Participants' ages ranged from 18 to 82 years, with an average age of 37.42 ± 6.98 years. Adults made up the largest group, accounting for 49% of the sample, followed by adolescents (26%) and individuals aged 65 and older (25%).

Among the 14,725 individuals included in this study, 91.8% tested negative on the PCR test, while the remaining 1,207 were positive. This corresponds to a SARS-CoV-2 infection prevalence of 8.2% in our study sample. Of the 1,207 individuals infected with SARS-CoV-2, 59% were men, and the majority (57%) came from health centers in Cotonou. The rest of the infected participants comprised 11% incoming travelers and 32% outgoing travelers. The infected participants were aged between 18 and 75 years, with an average age of 38.25 ± 6.85 years, reflecting that the majority of infections occurred among adults.

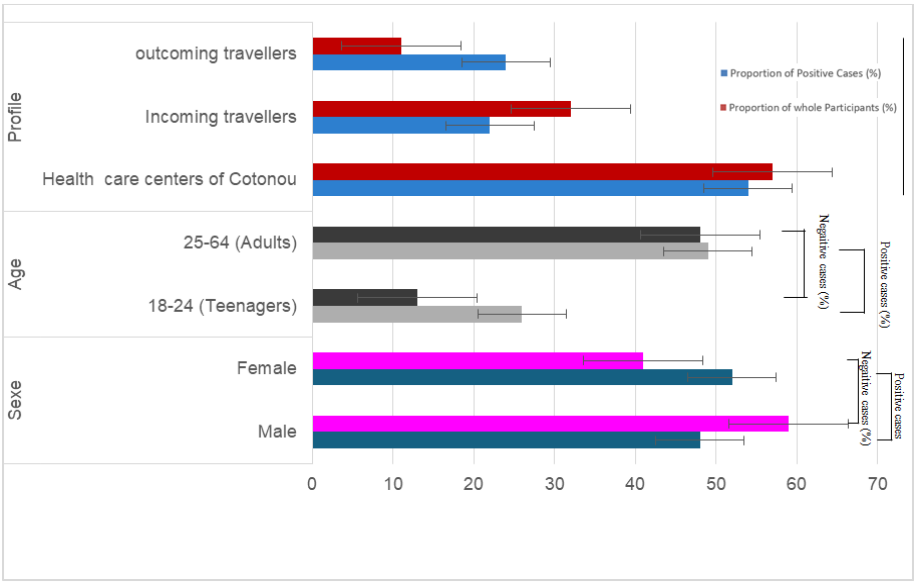

Figure 1: Sociodemographic Characteristics of Study Participants.

2. Temporal Evolution of SARS-CoV-2 Infection Cases in the Study Sample

The number of individuals tested ranged from 623 to 1,231 per week, with an average of 818 people screened weekly. Positive infection cases varied between 48 and 92, with an average of 67 positive cases per week. The highest positivity rate was observed during the 4th week of screening, while the lowest rate occurred in the 3rd week.

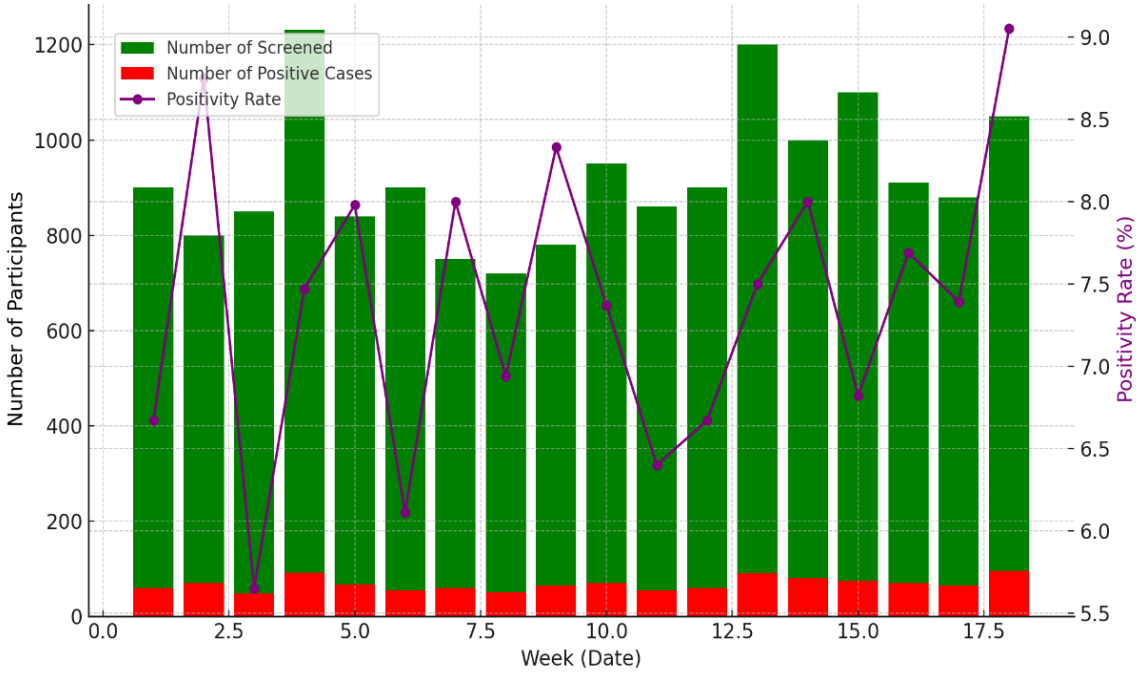

Figure 2: Temporal evolution of SARS-CoV-2 Infection cases.

The observed temporal trends highlight the importance of maintaining regular and targeted testing to monitor the progression of SARS-CoV-2 infections effectively. Periods of intensified screening, such as weeks 4, 13, and 14, reveal a positive correlation between the number of tests conducted and the detection of positive cases. Fluctuations in infection rates reflect potential influences like community transmission dynamics, testing campaigns, and population behaviors. Critical periods, such as **week 4**, stand out with the highest number of cases and positivity rates, warranting closer investigation into contributing factors (e.g., mass gatherings, easing of restrictions, or reduced adherence to preventive measures). Overall, these findings emphasize the need for proactive and adaptive strategies to control the spread of infections during peak transmission periods.

3. Identification of Circulating Variants in the Study Sample

SARS-CoV-2 cDNA was successfully amplified and sequenced in 420 samples out of the 1,207 positive cases. These 420 samples exhibited a high viral load, which is required for sequencing. Among them, 262 samples passed the quality control of the bioinformatics analysis, and the identified variants are as follows in figure 3.

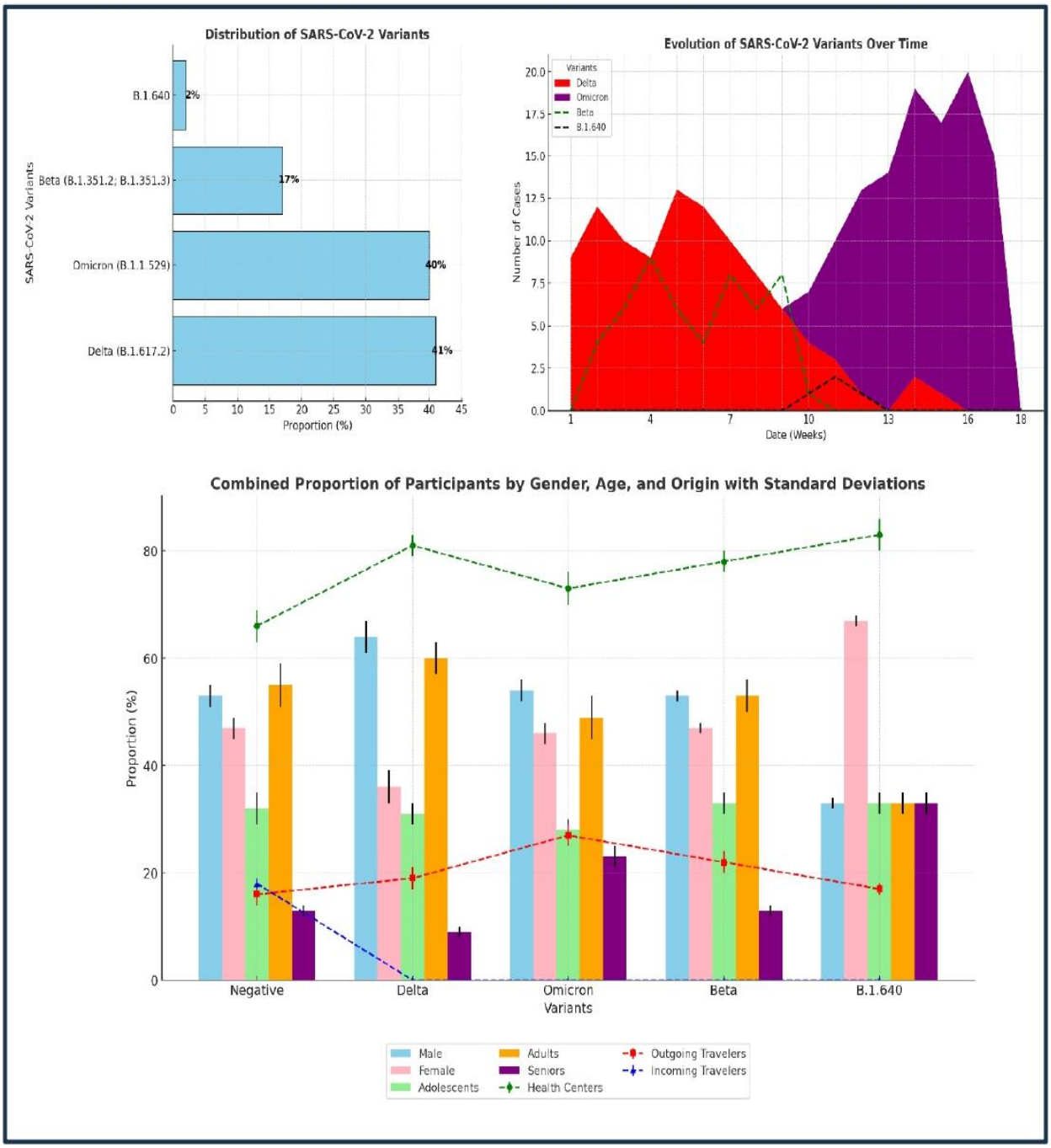

Figure 3: Identification of Circulating Variants in the Study Sample.

Out of the 262 samples that passed the quality control of the bioinformatics analysis, the WHO variants of concern — Delta, Omicron, and Beta — were identified at rates of 41%, 40%, and 17%, respectively. The variant under monitoring, B.1.640, was found in 2% of cases.

The early weeks of our screenings were marked by the Delta and Beta variants. The Delta variant persisted until the first week of December, with an average of approximately 8 infections per week, while the Beta variant lasted until the end of October, with an average of 5 infections per week. The first cases of Omicron variant infections emerged in the second week of November and continued until the end of our study, specifically the first week of January, with an

average of 13 infections per week. The B.1.640 variant, under monitoring, was short-lived during our study period, appearing only twice—in the first and third weeks of November—and once in the first week of December.

Except for participants affected by the B.1.640 variant, where 67% were female, the other groups were predominantly male, with proportions ranging from 53% to 64%. The participants in whom the variants were identified ranged in age from 19 to 72 years, with a mean age of 37.35 ± 2.35 years. Most variant groups were dominated by adults, except for the group of participants infected with the B.1.640 variant, which was evenly distributed across the three age categories. No incoming travelers were identified as being infected with any variant in our study. Most participants affected by the variants came from health centers in Cotonou, with proportions ranging from 73% to 83%. The remainder of the identified participants were outgoing travelers preparing to leave the country.

4. Neutralizing Antibody Protection Rates Against SARS-CoV-2 Variants

Plasma levels of neutralizing antibodies were measured in the 262 participants infected with the variants and in 38 participants who tested negative by PCR, bringing the total number of participants tested for neutralizing antibody levels to 300. The following graphs display these antibody levels according to the various groups that make up our study sample.

Only a quarter of the participants whose immune response was studied in this research had an antibody level below 30. In other words, 75% of these participants developed an anti-SARS-CoV-2 immune response. The neutralizing antibody levels among the 262 participants infected with variants in our study sample ranged from 0 to 98%, with an average rate of 78.71 ± 18.21%. The majority (86%) had neutralizing antibody levels greater than or equal to 30, whereas those who tested negative by PCR had neutralizing antibody levels ranging from 1 to 98%, with an average rate of 54.34 ± 28.01%. The majority (61%) of the latter group had neutralizing antibody levels below the threshold (30).

5. Proportion of Participants Infected by Variants and Their Immune Response Based on the Variant Responsible for Infection

All participants infected with the B.1.640 variant had neutralizing antibody levels greater than or equal to 30. Following this, the Omicron and Beta variants showed respective proportions of 87.5% and 86.67% of participants with antibody levels above the threshold. However, the Delta variant had the highest proportion of participants with antibody levels below the threshold (17%; fig4a).

There was no significant difference between the average neutralizing antibody levels of participants who tested PCR-negative and those infected with the Delta variant. Similarly, no significant difference was observed between participants infected with the Omicron, Beta, and B.1.640 variants. However, there was a significant difference between the average neutralizing antibody levels of these two groups of participants, with the latter group appearing to be better protected than the former (fig4b; fig.4c).

In all categories, the average antibody levels of vaccinated participants were higher than those of unvaccinated participants. A significant difference was observed between the neutralizing antibody levels of vaccinated and unvaccinated participants ($P<0.05$; fig.4b).

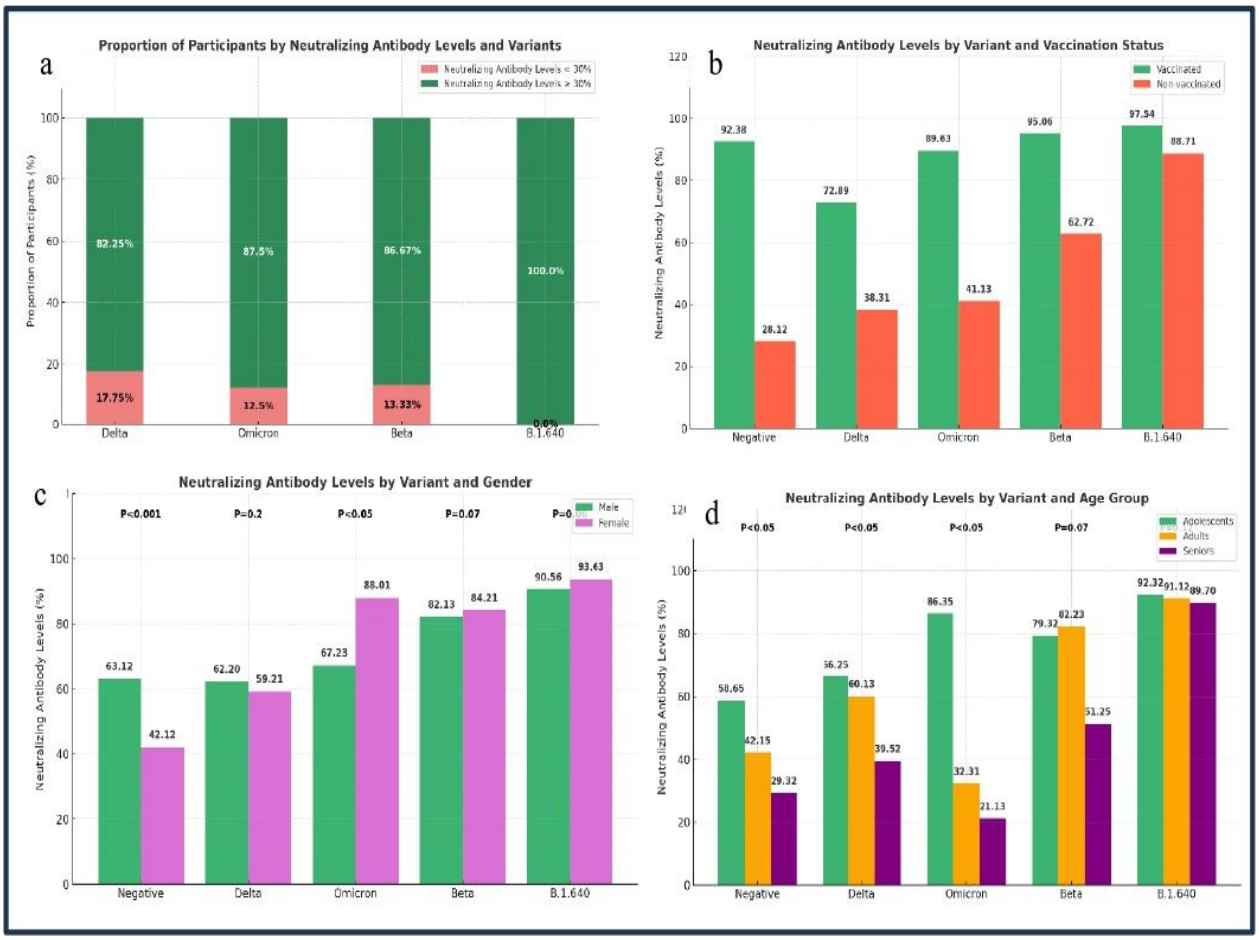

Figure 4: Comparison of Neutralizing Antibody Levels Based on Participant Characteristics and SARS-CoV-2 Variant.

6. Phylogenetic Tree

A phylogenetic tree illustrating how sequences from Benin cluster relative to global sequences is currently available on Nextstrain. Around the world, researchers are sequencing and sharing genomic data on SARS-CoV-2. The Nextstrain team analyzes this data on both global and continental scales. More specific analyses are often carried out by various research groups worldwide. This tool compiles publicly available SARS-CoV-2 analyses that use Nextstrain and originate from research groups across the globe. In addition to exploring SARS-CoV-2 evolution in completed analyses, the Nextclade tool allowed us to compare the sequences against the SARS-CoV-2 reference genome, assign them to clades, and see where they fit on the SARS-CoV-2 phylogenetic tree. The following figures present a phylogenetic analysis of SARS-CoV-2 in Benin.

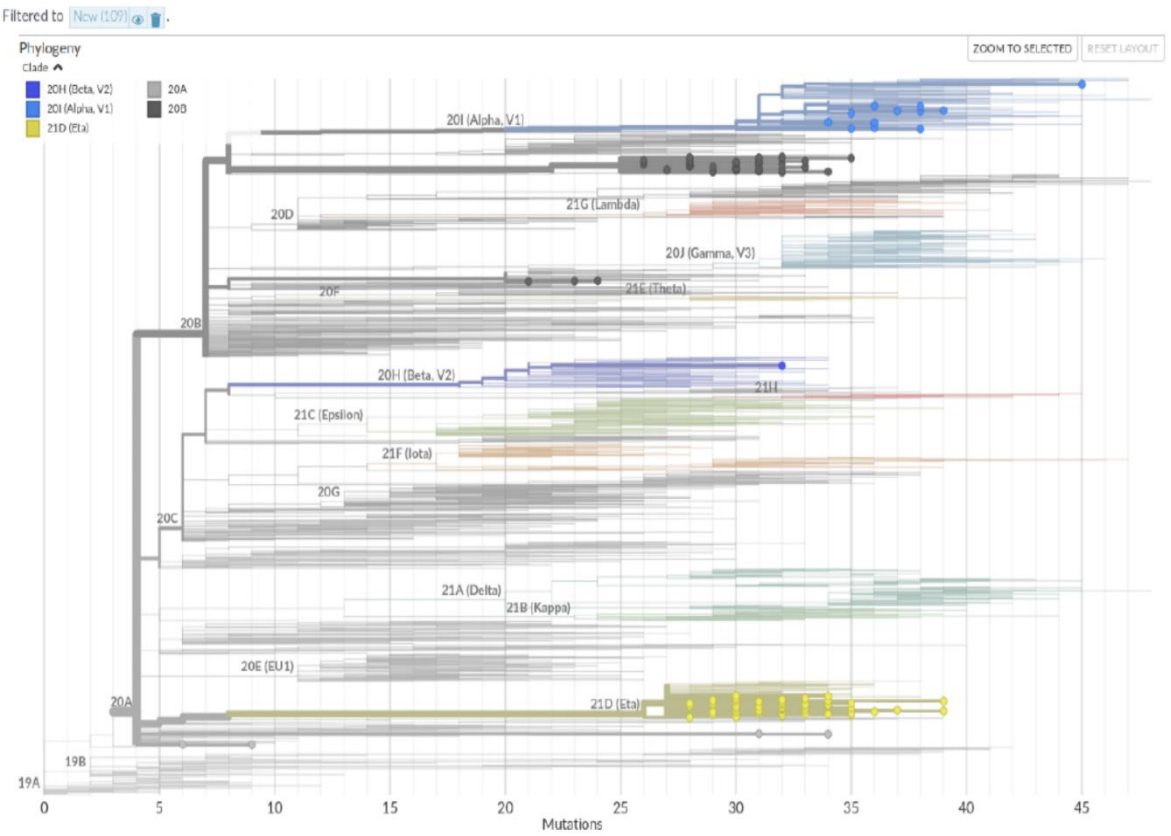

Figure 5: Phylogenetic tree of SARS CoV-2 clades.

DISCUSSION

Genomic sequencing allowed us to identify four variants responsible for the positive cases in our study sample: Delta, Omicron, Beta, and B.1.1.640. In response to these variants, 75% of the participants developed neutralizing antibody levels above 30%, indicating a positive immune response to SARS-CoV-2. Although most of our participants were unvaccinated, those who had been vaccinated produced significantly higher levels of neutralizing antibodies. According to a study conducted by Fiolet *et al.* (2022), COVID-19 vaccines are effective against the wild-type strain of the virus as well as variants of concern. These vaccines are described as safe and effective tools for preventing severe COVID-19 cases (Fiolet *et al*., 2022). Nevertheless, these three factors alone do not explain the high level of protection observed in our study sample. It is likely that most of our participants had previously been infected with SARS-CoV-2, allowing them to develop an effective immune response against future infections.

Quantitatively, the neutralizing antibody levels of vaccinated participants were significantly higher than those of unvaccinated participants, regardless of the infecting variant. However, participants infected with the Delta variant and those who tested negative by PCR appeared less protected than those infected with the Omicron, Beta, and B.1.1.640 variants. Among this group, Omicron-infected individuals were the least protected, while B.1.1.640-infected individuals were the most protected. This difference in protection levels can be explained by specific mutations in the Spike protein of the Delta variant, particularly the L452R and T478K mutations, which impact infectivity and immune response targeting key antigenic regions of the receptor-binding protein. Studies have shown that the L452R mutation in the RBD (receptor-binding domain) increases the virus's ability to infect cells expressing the ACE2 receptor and the TMPRSS2 cofactor, while also enhancing the Spike protein's ability to evade immune response (Deng, 2021; Kumar *et*

al., 2021). This mutation may explain the surge in infections observed during our sampling period. The T478K mutation, in combination with L452R, enhances the stability and intra-chain interaction of the Spike protein, potentially altering its interaction with neutralizing antibodies (Kumar et al., 2021). The T478K mutation, also characteristic of the Omicron variant, results in the replacement of threonine with lysine, which increases the Spike protein's electrostatic potential, influencing its interaction with the cellular receptor. Combined with the K417N and N501Y mutations, the receptor's affinity for the Spike protein is further strengthened, which explains Omicron's high transmissibility and Beta's persistence during the study period (Leung et al., 2021).

The genomes of 14 viruses, including 5 main ones (20H, 20I, 21D, 20A, and 20B), are represented on figure 5 with their mutations (mutations relative to the ancestral strain). The mutations are depicted as colored circles, with each color representing a different mutation. In the actual SARS-CoV-2 virus sequences, the majority of mutations consist of substitutions, and the site provides information on their nature and location. The phylogenetic tree was constructed based on the characteristics of the mutations in these genomes (rather than their total number). The 5 main genomes share the mutation shown in gray, indicating that they inherited it from a common ancestor. These 14 viruses originate from the same clone, defined by the 20A genome.

In global ways, Ondoa *et al*. (2021) analyzed genomic data of SARS-CoV-2 from 33 African countries, providing a detailed view of the pandemic dynamics across the continent. Hoffmann *et al*. (2020) discussed NGS-based strategies to trace the origin and understand the evolution of infectious agents, with a focus on SARS-CoV-2. Bull *et al*. (2021) compared four NGS-based methods for sequencing the complete genome of SARS-CoV-2, evaluating their performance on clinical samples. Resende *et al*. (2021) compared Sanger sequencing and NGS for monitoring and detecting variants in Brazil, offering insights into the use of these technologies for epidemiological surveillance. Bojkova *et al*. (2020) characterized infectious SARS-CoV-2 isolates in primary human respiratory cells, identifying key features critical for viral replication. These studies illustrate the use of NGS to understand the evolution and spread of SARS-CoV-2, both in Africa and globally.

In Benin, the study of Yadouleton *et al.* (2022) highlights key similarities and differences with international studies. While global research focuses on analyzing the epidemiological dynamics, tracking the evolution of variants across multiple countries, and using robust bioinformatics tools for genomic sequencing, the Benin study targeted the emergence and rapid spread of the Delta variant over a specific period. International studies, such as those conducted in Brazil, revealed cycles of dominance of variants like Alpha, Delta, and Omicron, emphasizing key mutations in the Spike protein that influence transmissibility and immune escape. In contrast, the Benin study quickly identified Delta as the dominant variant within two months and demonstrated its correlation with rising cases through the detection of specific mutations. The methodological gap is notable: global research uses detailed phylogenetic reconstruction, while Benin applied phylogenetic alignment to trace the variant's origin and spread locally. Despite differences in sample size, scope, and sequencing infrastructure, both Benin and international studies emphasize the importance of next-generation sequencing (NGS) in monitoring variants of concern and identifying mutations influencing transmissibility and severity. Strengthening genomic surveillance in Benin by integrating international methodologies could enhance early variant detection, bridge existing infrastructure gaps, and allow the country to contribute more effectively to regional and global pandemic control efforts.

CONCLUSION

The SARS-CoV-2 pandemic is a major health event due to the global upheaval and profound uncertainty it has caused. This infectious disease has had deep repercussions on public health, the economy, industries, and transport systems worldwide. It represents the greatest health crisis since the Second World War. Since its emergence in December 2019 in Asia, the virus has spread to all continents except Antarctica. We aimed to clarify the transmission dynamics of the virus and trace its genetic spread in the Republic of Benin. At the end of this study, it is worth noting that, like other countries, Benin is not exempt from the consequences of emerging variants. After detailing the most recent genetic and phenotypic characteristics of the virus, we enhanced our understanding of immune responses and provided an overview of the genetic variants present locally.

This work equips us with decision-making tools to fight the epidemic on a daily basis, particularly in the selection of vaccines and the development of strategies for acquiring immunity. However, we hope to include a larger number of samples to establish a more precise genetic map.

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