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Genomic monitoring unveils a high prevalence of severe acute respiratory syndrome coronavirus 2 Omicron variant in vaccine breakthrough cases in Bahia, Brazil

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SUMMARY

OBJECTIVE: Genome sequencing has been proved to be an excellent tool to monitor the molecular epidemiology of the disease caused by severe acute respiratory syndrome coronavirus 2, i.e., coronavirus disease 2019. Some reports of infected, vaccinated individuals have aroused great interest because they are primarily being infected with circulating variants of concern. To investigate the cases of infected, vaccinated individuals in Salvador, Bahia, Brazil, we performed genomic monitoring to estimate the magnitude of the different variants of concern in these cases.

METHODS: Nasopharyngeal swabs from infected (symptomatic and asymptomatic), vaccinated or unvaccinated individuals (n=29), and quantitative reverse transcription polymerase chain reaction cycle threshold value (Ct values) of ≤30 were subjected to viral sequencing using nanopore technology.

RESULTS: Our analysis revealed that the Omicron variant was found in 99% of cases and the Delta variant was found in only one case. Infected, fully vaccinated patients have a favorable clinical prognosis; however, within the community, they become viral carriers with the aggravating factor of viral dissemination of variants of concern not neutralized by the currently available vaccines.

CONCLUSION: It is important to acknowledge the limitations of these vaccines and to develop new vaccines to emergent variants of concern, as is the case of influenza vaccine; going through new doses of the same coronavirus vaccines is "more of the same."

KEYWORDS: COVID-19. SARS-CoV-2. Genome. Vaccines.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), a disease that emerged in December 2019 in Wuhan, China, fueled worldwide efforts to develop vaccines to control the rapid spread of infection.

Currently, there are several vaccines against SARS-CoV-2 approved by the World Health Organization (WHO)[1]. However, the viral replication of this RNA virus has general characteristics common to other RNA viruses; the high mutation rate, principally in the spike (S) glycoprotein, allows it to generate new viral variants to improve its chances of survival in the host and escape to immune detection[2].

It has been shown that SARS-CoV-2 vaccines do not protect against viral infection, although they significantly reduce morbidity and mortality in infected patients. Moreover, the neutralizing antibodies persist for no more than 4 or 5 months, even with a full two-dose regimen[3]. These factors may contribute to the fact that vaccinated individuals (two doses), as well as those administered booster doses, may acquire the SARS-CoV-2 infection. Although the clinical situation of the vaccinated (two-dose regimen) and infected patients is associated with a significant reduction in COVID-19 symptoms and, protection against severe disease, they become a possible viral carrier and can trigger viral dissemination within the community[4,5].

Recent studies of infected, vaccinated individuals have generated notable interest because they show that these individuals can primarily be infected with the circulating variants of concern (VOCs), such as Omicron (B.1.529). Omicron variant is more aggressive, with greater transmissibility and infectivity than the Delta variant[6].

We conducted a genomic study to estimate the magnitude and range of SARS-CoV-2 VOCs in cases of infected (symptomatic and asymptomatic), fully vaccinated, or unvaccinated individuals.

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METHODS

Study design and participants

From December 2021 to January 2022, the Laboratory of Virology, Institute of Health Science, Federal University of Bahia, confirmed the SARS-CoV-2-positive quantitative reverse transcription polymerase chain reaction (RT-qPCR) in a total of 29 vaccinated or unvaccinated individuals. These individuals visited a public health unit to confirm whether they were infected with SARS-CoV-2 or not. According to their vaccination status, the individuals were classified into three groups: fully vaccinated (two-dose regimen), fully vaccinated with three doses, and unvaccinated. The fully vaccinated individuals reported being vaccinated with Coronavac (Butantan, Brazil) Moderna AstraZeneca (Oxford, UK), or Pfizer (Pfizer, USA), and only the elderly people (>60 years) reported having received the third dose of Moderna AstraZeneca or Pfizer. The unvaccinated individuals were defined as those who had not received any vaccine. Inclusion criteria were as follows:

(1) any gender or age with COVID-19 infection;
(2) without comorbidity;
(3) symptomatic or asymptomatic;
(4) not hospitalized, and
(5) vaccinated or unvaccinated.

Exclusion criteria included individuals with SARS-CoV-2-negative RT-qPCR results.

Molecular detection of severe acute respiratory syndrome coronavirus 2

Detection of SARS-CoV-2 was done from nasopharyngeal and oropharyngeal swabs (n=29) pooled together at the time of sample collection. The samples were submitted to RNA extraction (Maxwell® RSC Viral Total Nucleic Acid Purification Kit, Promega, USA) and subsequent to RT-qPCR (GoTaq® Probe 1-Step qRT-PCR System, Promega, USA) assay following the CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)–PCR Diagnostic Panel[7] (Table 1). Samples with cycle threshold (Ct) values ≤39 were considered SARS-CoV-2-positive.

Ethics statement

This research was reviewed and approved by the Ethical Committee of the Federal University of Bahia (CAAE 30687320.9.0000.5662), and informed consent of all participants or their legal guardians have been obtained.

cDNA synthesis and whole-genome sequencing

Samples (n=29) were selected for sequencing based on the Ct value (≤30) and availability of epidemiological metadata (sex, age, residence in Salvador, symptoms, etc.) (Table 2). The preparation of SARS-CoV-2 genomic libraries was done using the nanopore sequencing technology[8]. The SuperScript IV Reverse Transcriptase kit (Invitrogen, USA) was initially used

Table 1. Overview of the primers and probes* used for severe acute respiratory syndrome coronavirus 2 detection by real-time polymerase chain reaction assay.

2019-nCoV_N1-F	GAC CCC AAA ATC AGC GAA AT
2019-nCoV_N1-R	TCT GGT TAC TGC CAG TTG AAT CTG
2019-nCoV_N1-P	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
2019-nCoV_N1-P	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ
2019-nCoV_N2-F	TTA CAA ACA TTG GCC GCA AA
2019-nCoV_N2-R	GCG CGA CAT TCC GAA GAA
2019-nCoV_N2-P	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
2019-nCoV_N2-P	FAM-ACA ATT TGC /ZEN/ CCC CAG CGC TTC AG-3IABkF

*CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)–qPCR Diagnostic Panel.

Table 2. Demographic and clinical characteristics in the study group of vaccinated or unvaccinated participants during severe acute respiratory syndrome coronavirus 2 vaccine outbreaks, Bahia, Brazil, between December 2021 and January 2022.

Participants	Variable	Number/total
	Groups (years)	
Age	6–19	5/29
	20–30	8/29
	31–45	7/29
	46–60	5/29
	> 61	4/29
Gender	F	15/29
	M	14/29
COVID-19 Immunization: (Oxford/AstraZeneca; Pfizer BioNTech, or Coronavac)	2 doses	18/25
	3 doses	6/25
No COVID-19 Immunization		4/29
Symptomatic (self-referred by vaccinated with two or three doses and unvaccinated individuals)	Fever	18/29
	Sore throat**	
	Cough**	
	Headache	
	Body pain	
Asymptomatic (vaccinated individuals)	2 doses or 3 doses	11/25

**Most referred by the participants.

for cDNA synthesis as per the manufacturer's instructions. The cDNA generated was subjected to multiplex PCR sequencing using the Q5 High Fidelity Hot-Start DNA Polymerase (New England Biolabs, UK) and a set of specific primers designed by the ARTIC Network for sequencing the complete SARS-CoV-2 genome (version 4)[9]. All experiments were performed in a biosafety level 2 cabinet. Amplicons were purified using 1´ AMPure XP Beads (Beckman Coulter, USA) and quantified on a Qubit 3.0 fluorimeter (Thermo Fisher Scientific, USA) using Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). DNA library preparation was performed using the Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies, UK) and the Native Barcoding Kit (EXP-NBD104 and EXP-NBD114, Oxford Nanopore Technologies, UK). Sequencing libraries were loaded into an R9.4 flow cell (Oxford Nanopore Technologies, UK). In each sequencing run, we used negative controls to prevent and check for possible contamination with <2% mean coverage.

Generation of consensus sequences from nanopore

The fast5 files generated during sequencing were basecalled under the high-accuracy model performed using Guppy v.6.0.1 (Oxford Nanopore Technologies, UK). The basecalled fastQ files with a minimum Q score of 7 were selected for subsequent trim adaptor and demultiplex processes performed using Guppy v.6.0.1. Furthermore, the fastQ files were submitted to the ARTIC Network's field bioinformatics pipeline v.1.2.1[9]. Briefly, a length filtering was performed to remove additional chimeras using artic guppyplex. The assembly was performed by Minimap2 v.2.17[10] using GenBank accession no. MN908947.3 as genome reference. The primer sequences were trimmed using align_trim.py, the assembly was then polished, and the variant calling was performed using Medaka v.1.0.3 (Oxford Nanopore Technologies, UK) and evaluated using LongShot v.0.4.1[11]. The consensus sequences were then masked with "N" at regions with coverage depth <20, and the variant candidates were incorporated into the consensus genome using BCFtools v.1.10.2[12]. The alignment statistics were calculated using SAMtools v.1.10 (using htslib 1.10.2)[12], exonerate v.2.4.0 (using glib version v.2.68.0),[13] and Seqtk v.1.3-r106[14]. This entire workflow is available at https://github.com/khourious/vgapONT.

Phylogenetic inference

Lineage assignment was performed using the Pangolin lineage classification software tool[15]. The newly identified isolates were compared with a diverse pool of genome sequences (n=3,441) sampled worldwide collected up to October 28, 2021. All sequences were aligned using the ViralMSA tool v.1.1.20[10,16], and phylogenetic analysis using the maximum likelihood approach was done using IQ-TREE2 v.2.2.0[17]. TreeTime v.0.8.5[18] was used to transform this ML tree topology into a dated tree using a constant mean rate of 8.0×10^{-4} nucleotide substitutions per site per year after the exclusion of outlier sequences.

Data availability statement

Newly generated SARS-CoV-2 sequences have been deposited in GISAID under the following accession numbers: EPI_ISL_9265729, EPI_ISL_9265745, EPI_ISL_9265746, EPI_ISL_9265743, EPI_ISL_9265744, EPI_ISL_9265749, EPI_ISL_9265728, EPI_ISL_9265747, EPI_ISL_9265748, EPI_ISL_9265730, EPI_ISL_9265752, EPI_ISL_9265731, EPI_ISL_9265753, EPI_ISL_9265750, EPI_ISL_9265751, EPI_ISL_9265734, EPI_ISL_9265756, EPI_ISL_9265735, EPI_ISL_9265732, EPI_ISL_9265754, EPI_ISL_9265733, EPI_ISL_9265755, EPI_ISL_9265738, EPI_ISL_9265739, EPI_ISL_9265736, EPI_ISL_9265737, EPI_ISL_9265741, EPI_ISL_9265742, EPI_ISL_9265740.

RESULTS

In this study, vaccinated individuals (25/29) were immunized with a complete two-dose regimen; elderly people >60 years (6/25) were immunized with three doses, and 7- to 13-year-old children (4/29) were unvaccinated (Table 2). The symptoms self-referred by participants were mild (fever, cough, sore throat, or headache) in individuals in the vaccinated and unvaccinated groups. Vaccinated individuals self-reported to be asymptomatic (11/25). In all cases, there was no need for hospitalization.

The RT-qPCR Ct values varied between 14.6 and 27.2, even in asymptomatic individuals (Figure 1). Of the vaccinated individuals (n=25), 24 confirmed the presence of Omicron variant and only one Delta variant. The unvaccinated individuals (n=4) confirmed to be infected by the Omicron variant. Our genomic

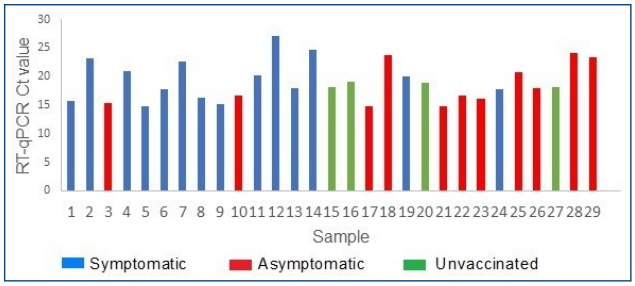

Figure 1. Distribution of RT-qPCR cycle threshold values in the clinical samples from vaccinated or unvaccinated individuals infected by severe acute respiratory syndrome coronavirus 2 variants. Cycle threshold values from vaccinated symptomatic individuals (blue), vaccinated asymptomatic individuals (red), and unvaccinated individuals (green). RT-qPCR: quantitative real-time polymerase chain reaction.

surveillance analysis revealed that in Bahia, three VOCs primarily dominated the epidemiology in the state. The Gamma variant (P.1), which was prominent during the second wave of the pandemic, persisted until August 2021, when it was replaced by the Delta variant (B.1.617.2) (Figure 2A), which in turn was replaced by the emerging Omicron variant in December 2021. The Alpha variant was also detected in Bahia at the end of December 2021, but it remained at a very low frequency (<1%). To explore the relationship between the sequenced genomes, we also constructed a phylogenetic tree with these VOCs and those from the other parts of the world. Our time-stamped phylogeny revealed that the Omicron isolates in Bahia are scattered throughout the phylogeny, suggesting that multiple independent introductions have occurred over time (Figure 2B).

DISCUSSION

Since the start of the COVID-19 pandemic in December 2019, efforts have been directed to the development of vaccines against SARS-CoV-2. Given the global situation, the WHO has authorized the use of several types of vaccines using mRNA technology, adenovirus vector vaccine, and inactivated viruses[1].

Recent reports confirm that the currently used vaccines do not protect against infection but do reduce the severity of cases. Indeed, countries that have vaccinated at least 50% of their population have managed to reduce the mortality rate. However, the current scenario shows the tendency of vaccinated individuals getting infected with emerging VOCs that cannot be neutralized by the immunity generated from these vaccines[2]. The new variants have emerged as a result of increased viral circulation, primarily in countries where vaccine coverage is low, which quickly spread worldwide. Examples of these variant leaks include Delta in India and Omicron in South Africa[19].

Our results reinforce the notion that virus migration generally follows national and international patterns of human mobility, facilitating the spread of emerging VOCs not only within countries but also globally. The identification of SARS-CoV-2 suspected cases through genome-wide sequencing in Bahia also revealed this, and as of 2020, co-circulation of three different VOCs, such as Gamma (P.1), Delta, and Omicron, appears to have dominated the epidemiological history in the state. The Alpha variant currently remains at a very low frequency (<1%) in the state, consistent with the effectiveness of SARS-CoV-2 vaccines against it while being less effective against new VOCs[5].

Initially, it was considered in the literature that reaching herd immunity (≥70%) would prevent the circulation of the virus in the population; this fact is now being questioned. The countries that have reached or surpassed this percentage (England, Israel, and Brazil) have high infection rates since the emergence of Omicron[20,21]. In Bahia, 74.9% of the vaccination coverage was recently achieved with two doses, but our work demonstrated breakthrough infection by Omicron with a high viral load even in double or triple vaccinated individuals. Moreover, asymptomatic vaccinated individuals can still spread the virus to other people.

One limitation of our work is that the level of protective immunity of those who are currently vaccinated is not

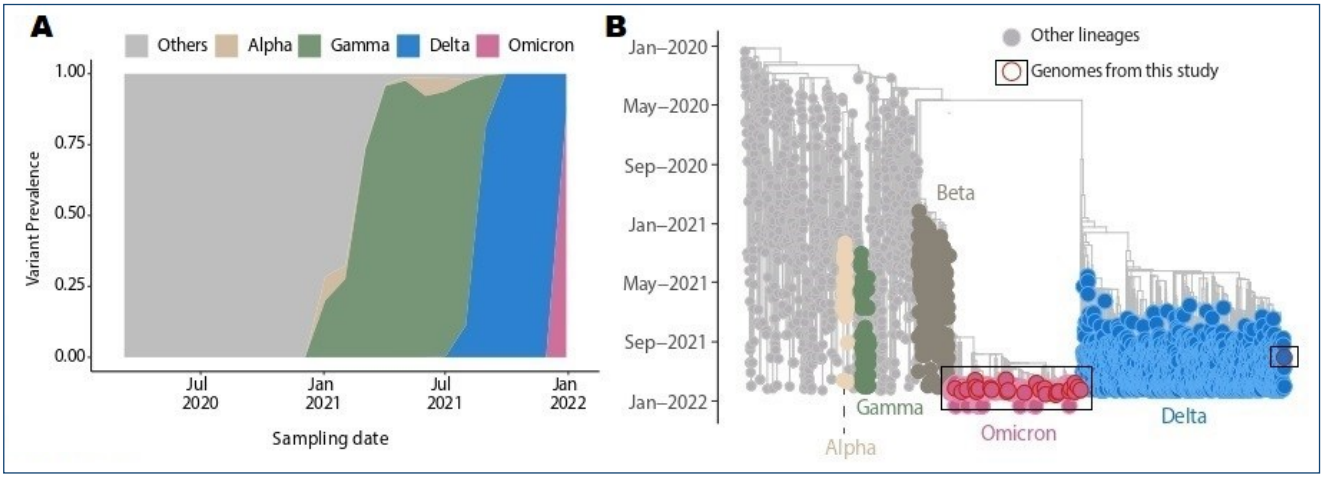

Figure 2. Genomic epidemiology of the severe acute respiratory syndrome coronavirus 2. Omicron variant in Salvador, Bahia, Northeast Brazil. (A) Dynamics of the severe acute respiratory syndrome coronavirus 2 epidemic in Bahia showing the progression in the proportion of circulating variants in the state over time, with the rapid replacement of the Delta by the Omicron variant. (B) Time-resolved maximum likelihood phylogenetic tree including the new Omicron and Delta isolates obtained in this study and n=4,249 representative severe acute respiratory syndrome coronavirus 2 genomes collected up to January 16, 2021. Alpha (brown), Beta (dark gray), Gamma (green), Delta (blue), and Omicron (red) variants of concern are highlighted in the tree. New Omicron (n=28) and Delta (n=1) genomes obtained in this study are highlighted with a black box in an each of these genomes. Genomes of the other lineages are shown in light gray.

known; however, even with three doses, there have been cases of viral infection. Dose reinforcements in countries such as England and Israel with a four-dose regimen raise doubts as to whether they will solve viral recrudescence for new VOCs in circulation[22,23].

It is important to acknowledge the limitations of these vaccines and urgently develop new vaccines to emergent VOCs, similar to the case of influenza vaccine; going through new doses of the same vaccines is "more of the same."

Measures to control the spread of the virus, such as the use of face masks, social distancing, and crowd avoidance, continue to be effective, but these need to be combined with new immunogens that prevent viral infection (an elementary concept for the approval of vaccines for widespread use).

AUTHORS' CONTRIBUTIONS

GSC: Conceptualization, Formal Analysis, Supervision, Writing – review & editing. **SIS:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing. **MG:** Formal Analysis, Methodology, Visualization, Writing – review & editing. **LM:** Formal Analysis, Validation. **HSH:** Methodology. **KVOMDA:** Methodology. **ACAB:** Methodology, Writing – review & editing.

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