Received: 16 November 2022 | Accepted: 30 December 2022

DOI: 10.1002/jmv.28481

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

The third vaccine dose significantly reduces susceptibility to the B.1.1.529 (Omicron) SARS-CoV-2 variant

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Funding information
Ministério da Ciência, Tecnologia e Inovação; Financiadora de Estudos e Projetos; Conselho Nacional de Desenvolvimento Científico e Tecnológico; Fundação de Amparo à Pesquisa do Estado de São Paulo; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Instituto Serrapilheira

Abstract

The main coronavirus disease 2019 (COVID-19) vaccine formulations used today are mainly based on the wild-type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoprotein as an antigen. However, new virus variants capable of escaping neutralization activity of serum antibodies elicited in vaccinated individuals have emerged. The Omicron (B.1.1.529) variant caused epidemics in regions of the world in which most of the population has been vaccinated. In this study, we aimed to understand what determines individual's susceptibility to Omicron in a scenario of extensive vaccination. For that purpose, we collected nasopharynx swab (n = 286) and blood samples (n = 239) from flu-like symptomatic patients, as well as their vaccination history against COVID-19. We computed the

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data regarding vaccine history, COVID-19 diagnosis, COVID-19 serology, and viral genome sequencing to evaluate their impact on the number of infections. As main results, we showed that vaccination in general did not reduce the number of individuals infected by Omicron, even with an increased immune response found among vaccinated, noninfected individuals. Nonetheless, we found that individuals who received the third vaccine dose showed significantly reduced susceptibility to Omicron infections. A relevant evidence that support this finding was the higher virus neutralization capacity of serum samples of most patients who received the third vaccine dose. In summary, this study shows that boosting immune responses after a third vaccine dose reduces susceptibility to COVID-19 caused by the Omicron variant. Results presented in this study are useful for future formulations of COVID-19 vaccination policies.

KEYWORDS
antibody, COVID-19, neutralization, vaccination policy, vaccine doses

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the Betacoronavirus genus of the *Coronaviridae* family.[1] The SARS-CoV-2 first emerged in Wuhan, China, in 2019,[2,3] and, to date, infected hundreds of millions of people, resulting in more than 6 million deaths.[4] Such high epidemiological impact led to the development of several vaccine formulations that were specially aimed to control severe forms of the disease worldwide.[5] However, the consecutive emergence of new genetic variants of SARS-CoV-2 have brought new questions and challenges regarding the use of these vaccines,[6] as the main vaccine formulations used up today are essentially based on the spike glycoprotein from the wild-type (WT) viral strain.[7,8]

The emergence of new variants allowed SARS-CoV-2 to circumvent the neutralization activity of serum antibodies elicited in vaccinated or infected individuals.[9–11] Although the overall vaccines efficacy in protecting individuals from developing severe COVID-19 has been clearly demonstrated,[12] little is known about their capacity of preventing infection with the B.1.1.529 (Omicron) variant. In fact, the Omicron variant, as well as its subvariants, caused outbreaks in regions with largely vaccinated populations.[13–17] Thus, it is important to understand what determines individuals' susceptibility to COVID-19 in the context of their vaccination history.

In this study, we aimed to understand what determined individual's susceptibility to COVID-19 in a Brazilian population exposed to an Omicron epidemics in early 2022 and mostly vaccinated with two doses. Results presented in this study are useful to better understand susceptibility to infection with the SARS-CoV-2 Omicron variant according to vaccine history.

2 | METHODS

2.1 | Samples and ethics

We saw up to 60 patients per week during a COVID-19 epidemic in early 2022, from January to March, in Barreiras, Bahia, Brazil. The population study consisted of 286 enrolled patients (109 males and 177 females, with ages ranging from 7 months to 84 years), from which we collected 286 nasopharynx swab and 239 blood samples from flu-like symptomatic patients, as well as information of their vaccination history against COVID-19. Swab samples were used in molecular diagnosis based on reverse transcription (RT) followed by quantitative polymerase chain reaction (RT-qPCR), viral genome sequencing, and viral genotyping based on RT-qPCR. Serum samples obtained from blood samples were subjected to analyses based on enzyme-linked immunosorbent assay (ELISA) and neutralization assays (which will be described below).

All the research complied with all relevant ethical and biosafety guidelines. Ethics approval was obtained from institutional ethics committee of the Federal University of Western Bahia (CAAE 40779420.6.0000.8060). All procedures and possible risks were explained to volunteers. Informed consent was obtained from all participants.

2.2 | ELISA assay

Serum samples were first analyzed using the EIE COVID-19 IgG N/S kit (Biomanguinhos), according to the manufacturer's instructions and as previously described.[18] The serum levels of antibodies specific to SARS-CoV-2 were defined according to optical density values. In brief, the ELISA assay with solid-phase bound nucleoprotein (N) and spike (S) recombinant antigens was carried out with volunteer's serum samples. Kit

controls and samples were added to wells after dilution (1:101) with kit diluent and incubated for 30 min, at 37°C. The plates were washed five times using kit washing buffer and incubated with previously 1:100 diluted kit conjugate for 30 min, at 37°C. After a new washing cycle, plates were developed by addition of kit developing solution into the wells. After 10 min, the reactions were stopped with 2 M H_2SO_4 and the optical density (OD) was measured at A450nm wavelength.

The serum samples were also separately evaluated against the N or S protein by an in-house ELISA test, as previously described.[19] Briefly, 96-well polystyrene COSTAR microplates (Corning Inc.) were coated with 200 ng of recombinant fragments encoding the whole WT SARS-CoV-2 N protein (N-ELISA) produced by *Escherichia coli*, or the receptor-binding domain (RBD) region produced by Expi293™ cells (RBD-ELISA) in a pH 9.6 carbonate/bicarbonate buffer. Blockage was performed through a 3 h incubation with phosphate-buffered saline (PBS) supplemented with lysine and mannitol, and followed by the addition of serum samples diluted 1:100 in sample solution containing Tris-NaCl buffer supplemented with casein and EDTA, for 60 min at 37°C. The wells were washed three times with PBS-TWEEN 0.05% solution and incubated with anti-human IgG conjugated to peroxidase (Sigma Aldrich™ Sigma) at 37°C for 60 min. After a final wash, the wells were stained with Tetramethylbenzidine (Aldrich™ Sigma). The reaction was stopped after 10 min by the addition of 100 µl of H_2SO_4 at 0.2 N. The OD reading was measured at 450 nm in a plate reader (Labsystems Multiscan, ThermoScientific).

2.3 | Cell culture and SARS-CoV-2 propagation

The African Green monkey kidney cells Vero E6 (ATCC® CRL-1586™) and Vero CCL-81 (ATCC® CCL-81) were maintained according to ATCC® recommendations. The Vero E6 cells monolayer was infected with each SARS-CoV-2 variant to propagate a viral stock. The SARS-CoV-2 strains used in this study were: (i) WT virus SARS-CoV-2 (Wuhan strain—WT) (GISAID: EPI_ISL_2499748), a kind gift of Dr. José Luiz Proença-Módena (University of Campinas—UNICAMP, Campinas, SP, Brazil), and (ii) Omicron variant (GISAID: EPI_ISL_6794907), a kind gift of Dr. Edson L. Durigon (University of São Paulo, USP, São Paulo-SP, Brazil). Afterwards, the SARS-CoV-2 viral stocks were subjected to titration (in $TCID_{50}$/ml), as previously described,[20] and used for viral neutralization tests. The experiments using the SARS-CoV-2 were carried out under the laboratory Biosafety level 3 (BLS3) facilities at the Federal University of São Paulo, in accordance with World Health Organization (WHO) recommendations.

2.4 | Cytopathic effect-based virus neutralization test (CPE-VNT) for SARS-CoV-2 WT and Omicron

The neutralizing antibody titers were quantified against SARS-CoV-2 as previously described.[21] Briefly, monolayers containing 5×10^4 Vero cells (ATCC CCL-81) in 96-well culture plates were exposed to 1×10^3 $TCID_{50}$/ml of SARS-CoV-2 (Wuhan strain—WT) (GISAID: EPI_ISL_2499748) or Omicron variant (GISAID: EPI_ISL_6794907) previously incubated with 1:20–1:1280 twofold diluted, heat-inactivated human serum samples, in a final volume of 150 µl. After a 3-day incubation, all wells were evaluated by optical microscopy for the presence of characteristic SARS-CoV-2 CPEs. The absence of CPEs in at least the 1:20 dilution sample was considered a positive result of neutralizing antibodies to SARS-CoV-2. All procedures related to CPE-VNT were performed in a BLS3 laboratory at the Federal University of Sao Paulo, according to the WHO recommendations.[22]

2.5 | RNA extraction and RT-qPCR

The nucleic acid extractions from nasopharyngeal samples were carried out with the Extracta Kit—Viral RNA and DNA (MVXA-P016FAST) (Loccus), using an Extracta32 instrument (Loccus), following the manufacturer's instructions. Laboratory diagnosis was based on RT followed by RT-qPCR assays using the INFA/INFB/SC2 kit (Bio-Manguinhos), following the manufacturer's instructions. In brief, it is a quadriplex assay that detects specific genomic regions of influenza virus A (INFA), influenza virus B (INFB), and SARS-CoV-2 (SC2), in addition to the internal control (CI). The endogenous CI is a human constitutive gene—RNAse P (RP). The Molecular Kit INFA/INFB/SC2—Bio-Manguinhos is intended for diagnosis and epidemiological surveillance. Thermocycling was carried out in a QuantStudio 5 instrument (Applied Biosystems) with a hold stage composed of a first step of 15 min at 50°C, followed by a second step of 2 min at 95°C. The PCR stage was composed of a first step of 20 s at 95°C, followed by a second step of 30 s at 61°C, repeated 40 times.

2.6 | Viral genotyping by RT-qPCR

Viral genetic variants were screened using the 4Plex SC2/VOC kit (Bio-Manguinhos), following the manufacturer's instructions. In brief, this assay has a quadriplex format (four-target detection) using TaqMan probes and that can detect the SARS-CoV-2 RNA through targeting amplification in the N gene, and simultaneously screening samples with suggestive profiles for the different variants of concern (VOCs) of deletions (Del) S106, G107, and F108 in the *ORF1a* gene (nsp6) and DelH69 and V70 in the Spike gene of the samples tested. This protocol combines the detection and initial screening of Alpha, Beta, Gamma, Delta, and Omicron VOCs. As an CI, the assay detects a region of the human constitutive gene, *RNAseP* (RP). Thermocycling was carried out in a QuantStudio 5 instrument (Applied Biosystems) and involved RT and inactivation steps composed of a 50°C for 2 min, 95°C for 10 min, followed by 40 amplification cycles of 95°C for 15 s and 60°C for 60 s.

2.7 | SARS-CoV-2 genome sequencing

SARS-CoV-2-positive nasopharynx swab samples ($n = 95$) were subjected to genome sequencing using next generation sequencing

on Oxford Nanopore's MinIon platform (Oxford Nanopore Technologies). Viral RNA was extracted as described above. The complementary DNA and PCR products were obtained using Midnight RT PCR Expansion (EXP-MRT001) (Oxford Nanopore Technologies), generating amplicons with ~1200 bp, overlapping the entire SARS-CoV-2 genome, following the manufacturer's instructions. Rapid Barcoding Kit 96 (SQK-RBK110.96) (Oxford Nanopore Technologies) was used to barcode a pool of multiple samples. The pools of barcoded samples were purified and 800 ng were used for library preparation and sequencing using the Oxford Nanopore MinION SpotON Flow Cells R9 version (Oxford Nanopore Technologies), following the instructions of the manufacturer. Sequencing was performed using the so-called rapid precision base in MinKNOW software according to protocol (Community-Protocol-PCR tiling of SARS-CoV-2 virus-rapid barcoding and Midnight RT PCR Expansion (SQK-RBK110.96 and EXP-MRT001) (nanoporetech.com). ARTIC Network's RAMPART (https://artic.network/ncov-2019) was used to monitor the sequencing run in real-time to estimate the depth of coverage (20×) across the genome for each barcode (https://artic.net/wall). Analysis and consensus generation were performed according to the pipeline proposed by the ARTIC Network using the Medaka protocol (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). New SARS-CoV-2 full genome sequences obtained here were submitted to the Pangolin web application,[23] version v4.1.3, and pangolin-data version v1.17, available at: https://pangolin.cog-uk.io/. All consensus genomes were deposited in the Global Initiative on Data Sharing Avian Influenza-EpiCoV (GISAID-EpiCoV) database (see Supplementary Information 1 for details).

2.8 | Statistical analyses

The numbers COVID-19 positive and COVID-19 negative among vaccinated and non-vaccinated individuals, as well as among those who received or not a vaccine booster (a third dose), were subjected to Fisher's analysis. To compare the means of two groups, we used the Mann–Whitney test. To compare more than two groups, we used analysis of variance followed by Tukey's multiple comparisons. In all cases, statistical significance was set as $p \leq 0.05$.

3 | RESULTS

3.1 | Study population

In this study, we followed a COVID-19 epidemic in early 2022, from January to March, in Barreiras, Bahia, Brazil. At the beginning of the study, 97.89%, 92.44%, and 46.69% of the city population had received 1, 2, or 3 doses of COVID-19 vaccines, respectively. As shown in Figure 1, from the 286 nasopharynx swab samples analyzed by RT-qPCR, 95 tested positive while 189 tested negative for the presence of SARS-CoV-2. Among the 95 positive patients, 84 were previously vaccinated (received at least 1 dose), whereas 11 were not. Moreover, from the 84 positive and vaccinated individuals, 8 received only 1 dose, 60 received 2 doses, and 16 received the third dose (booster dose) (see Figure 1). On the other hand, from the 191 individuals negative for SARS-CoV-2, 166 were vaccinated, whereas 16 were not. Among the 166 negative and vaccinated individuals, 7

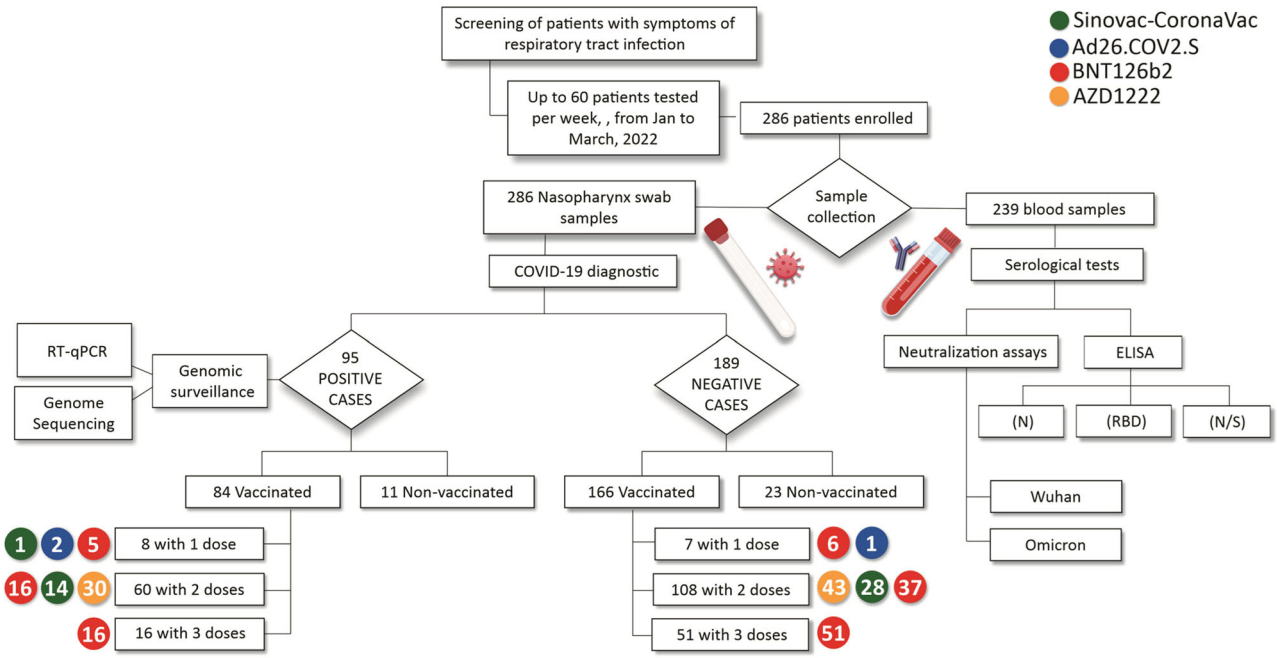


FIGURE 1 Study design. Up to 60 patients per week were subjected to laboratory tests for coronavirus disease 2019 (COVID-19) from January to March 2022, in Barreiras city, Brazil. They donated a nasopharyngeal swab and a blood sample. Nasopharynx swabs were used for reverse transcription quantitative polymerase chain reaction (RT-qPCR) and viral genome sequencing. Blood samples were used for serological assays. Patients were also interviewed regarding vaccine history and infection history.

received only 1 dose, 108 received 2 doses, and 51 received the third dose.

The vaccinated individuals enrolled in this study received the following vaccine formulations: CoronaVac (Sinovac), Ad26.COV2S (Janssen), AZD1222 (Oxford-AstraZeneca), and BNT162b2 (BioNTech and Pfizer). Most of the enrolled patients had been vaccinated with two doses of CoronaVac or AZD1222, whereas only three patients had been vaccinated with Ad26.COV2S. Moreover, all patients that had three vaccine doses received BNT162b2 at the third dose (see Figure 1).

3.2 | Impact of vaccination in the induction of serum specific antibody responses and infection by SARS-CoV-2

To perform a comprehensive characterization of our study population, we first evaluated whether the vaccination interfered in the number of infected or noninfect individuals. As shown in Figure 2A, even in a scenario in which most of the population had received at least one vaccine dose, the numbers of individuals infected by SARS-CoV-2 were not significantly reduced by vaccination ($p = 1.0$). However, among vaccinated individuals, those who were COVID-19 negative presented significantly higher serum levels of antiviral antibodies when compared with the COVID-19 positive individuals ($p = 0.0001$) (see Figure 2B,C). In contrast, serum levels of antiviral antibodies were shown to be statistically indistinguishable among nonvaccinated individuals ($p = 0.2922$) (see Figure 2D,E). Collectively, these results show that vaccination did not statistically reduced the number of individuals infected by SARS-CoV-2 in the epidemic we followed, even with an increased immune response found among vaccinated and not infected individuals.

3.3 | The third vaccine dose reduces susceptibility to the B.1.1.529 (Omicron) SARS-CoV-2 variant

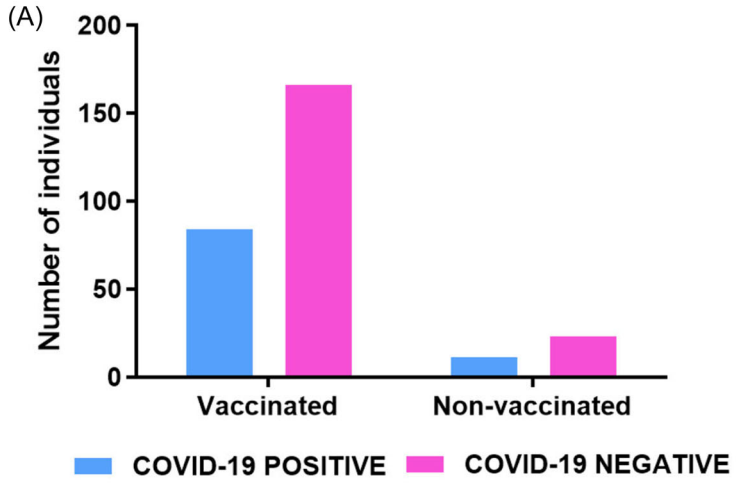
As shown in Table 1, the COVID-19 epidemic we followed in this study was mainly caused by the B.1.1.529 (Omicron) SARS-CoV-2 variant. All genome sequences that achieved enough quality to be deposited at GISAID-EpiCoV (58 samples) were classified as Omicron, which includes a myriad of subvariants (see Supplementary Information 1). In addition, all samples screened by RT-qPCR (77 samples) were suggestive of Alpha or Omicron variants. Notably, the number of patients infected by SARS-CoV-2 was significantly reduced in those who received the third vaccine dose (Figure 3A). In addition, patients who received the third vaccine dose presented significantly higher serum antiviral antibody levels (against S and N) than those who did not received the third vaccine dose (Figure 3B), giving strength to the hypothesis that higher levels of anti-SARS-CoV-2 antibodies can play a role in the infection control. These results show that the administration of the third vaccine dose significantly reduced susceptibility to COVID-19 in the epidemic caused by the Omicron SARS-CoV-2 reported in this study.

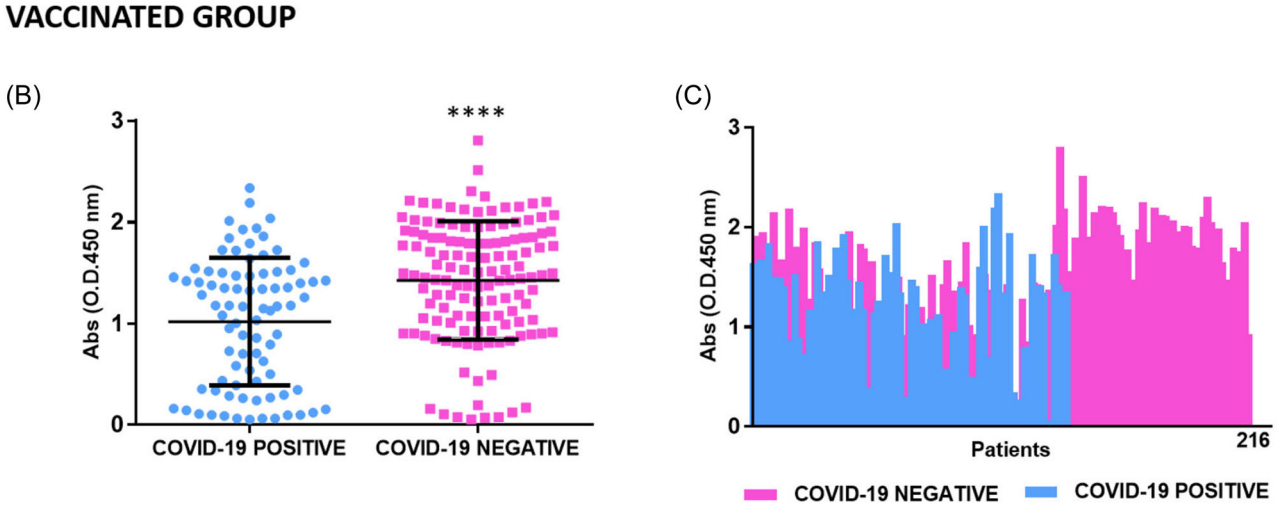
3.4 | Specific humoral immune response to viral structural targets and neutralization capacity

To confirm whether only high anti-SARS-CoV-2 antibody levels or the active immunization with the third vaccine dose are required to reduce infection levels, we compared the serum antibody levels among the study population. The Figure 4A shows that individuals who received the third vaccine dose developed higher serum levels of specific antibodies to the SARS-CoV-2 spike protein RBD than patients who did not receive the third vaccine dose. However, serum levels of specific antibodies to the nucleoprotein (N) were statistically indistinguishable among those patients who received or not the third vaccine dose. Aiming to better understand why individuals who received the third vaccine dose were less susceptible to infection, we carried out neutralization assays of all collected serum samples with the Wuhan and Omicron variants. As shown in Figure 4C, patients who received or not the third vaccine dose neutralized the Wuhan variant better than the Omicron variant. However, patients who received the third vaccine dose presented higher neutralization titers to the Wuhan virus strain in comparison with patients who did not receive the booster dose. As these results did not explain the lower susceptibility to COVID-19 seen in individuals who received the third vaccine dose, we analyzed the serological data of patients who received the third vaccine dose separately. We then grouped patients who received the booster and were infected and those who received the booster and were not infected. Patients who received the vaccine booster and were not infected presented higher serum anti-RBD (Figure 4D) and anti-N (Figure 4E) antibody levels than patients who received the third vaccine dose and were infected. Furthermore, they also showed higher neutralization titers to the Omicron virus variant, although their neutralization titers to the Wuhan virus strain have been statistically indistinguishable from patients who received the third vaccine dose and were infected. Even with an increased capacity in neutralizing Omicron, patients that received the booster dose and were not infected still had higher neutralization titers to the Wuhan strain (see Figure 4F). It is important to highlight that most of patients that received the third vaccine dose were not infected (51 COVID-19 negative vs. 16 COVID-19 positive; see Figure 1). Together, these results indicate that patients who received the third vaccine dose developed higher neutralization titers to SARS-CoV-2. In addition, most of these patients also developed higher neutralization titers to the Omicron virus variant. In summary, we showed that a boosted immune response induced by the third vaccine dose reduces the susceptibility to COVID-19, as schematically represented in Figure 5.

4 | DISCUSSION

In this study, we followed a COVID-19 epidemic caused by the Omicron SARS-CoV-2 variant in a scenario of extensive vaccination. We wanted to know what was determining individual's susceptibility to COVID-19 and noticed that a lower proportion of the population





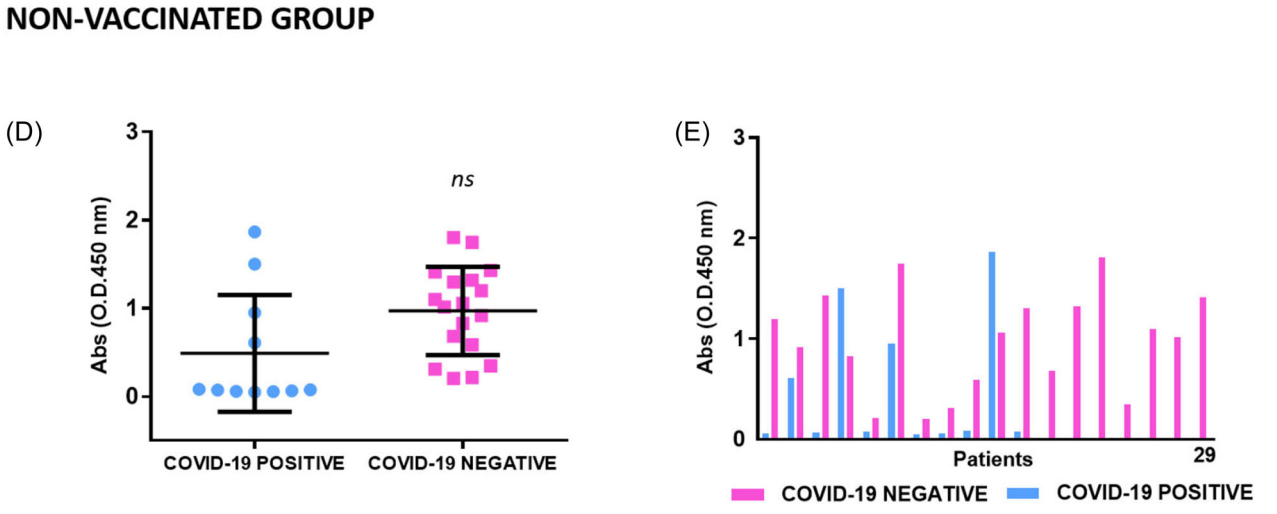


FIGURE 2 (See caption on next page)

TABLE 1 Genomic surveillance of SARS-CoV-2 positive samples detected in in Barreiras, Bahia state, Brazil, from January to March, 2022.

Analysis	Number of samples analyzed	Omicron[c]
Genome sequencing[a]	58	58
RT-qPCR[b]	91	77

Abbreviations: RT-qPCR, reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]SARS-CoV-2-positive samples ($n = 95$) were subjected to genome sequencing. All sequenced samples that achieved enough quality were deposited at GISAID-EpiCoV ($n = 58$).

[b]RT-qPCR capable to detect mutations that are indicative of Alpha or Omicron SARS-CoV-2 lineages was performed.

[c]SARS-CoV-2 whole-genome sequences obtained in this study were submitted to lineages assigner Pangolin web application, as described in the Methods section. Samples subjected to RT-qPCR were classified according to the presence of the deletions (Del) Del69, Del70, DelS106, DelG107, and DelF108 on the Spike protein sequence. In this last case, the presence of mutations indicates the variants of concern Alpha or Omicron.

had received the third vaccine dose, whereas most of the population had received one or two doses. Our data showed that the number of infected individuals was only reduced in the group that received three vaccine doses, which indicates that the susceptibility of individuals to the prevailing circulating virus variant was indeed diminished after the third vaccine dose. We also showed that such reduced susceptibility was based on a boosted immune response, with higher levels of serum antiviral antibodies, including those capable of neutralizing the virus. Although vaccines administered in the study population had been based on the wild-type SARS-CoV-2 strain (Wuhan WT), administration of the third vaccine dose induced increased neutralization capacity against both Wuhan and Omicron variants in most of the tested individuals.

This study shows the importance of vaccine policies aiming to boost the immune response of individuals against SARS-CoV-2. At present, there is a discussion regarding the need for updating vaccine formulations, especially due to the fast-evolving SARS-CoV-2 variants.[7,24] However, our results show that simply boosting people, at least for the third dose, significantly reduces susceptibility to COVID-19. It is necessary to investigate if a similar effect will be seen

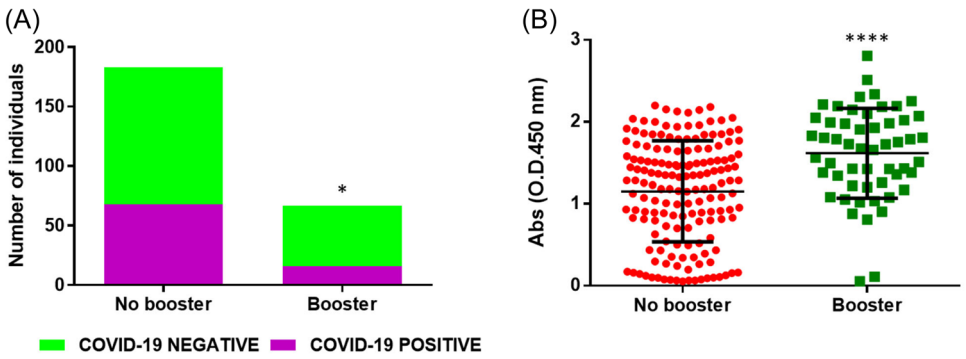


FIGURE 3 (A) Numbers of individuals infected or not by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) among those who received or not the third vaccine dose (booster), were subjected to Fisher's analysis. The numbers of coronavirus disease 2019 (COVID-19)-infected patients were significantly reduced with the vaccine booster ($p = 0.05$). (B) Serum levels of antiviral antibodies were shown to be significantly increased in samples from patients who received the third vaccine dose, in comparison with those who did not receive the vaccine booster with third dose after a Mann–Whitney test ($p = 0.0001$). Statistical significance was set as $p \le 0.05$. $^{*}p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$; $^{****}p \le 0.0001$.

FIGURE 2 Vaccine efficacy and preliminary serology in the study population. (A) Numbers of individuals infected or not infected by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) among vaccinated and nonvaccinated individuals were subjected to Fisher's analysis. The numbers of infected individuals were not significantly reduced by vaccine ($p = 1.0$). (B) Serum levels of specific antiviral antibodies (represented as optical densities (OD) from coronavirus disease 2019 (COVID-19)-positive and -negative patients after immunization were obtained by enzyme-linked immunosorbent assay (ELISA) and subjected to Mann–Whitney test. A significant increase of specific antibody levels was showed in the negative group (not infected) ($p = 0.0001$). (C) Histogram showing vaccinated patients serum levels of antiviral antibodies. Serum levels of antiviral antibodies are visually higher in samples of COVID-19-negative patients. (D) Serum levels of antiviral antibodies from COVID-19-positive and COVID-19-negative patients without immunization were subjected to Mann–Whitney test. The antibody serum levels in the compared groups were shown to be statistically indistinguishable ($p = 0.2922$). (E) Histogram showing nonvaccinated patients serum levels of antiviral antibodies. The antibody serum levels in the compared groups are visually indistinguishable. Statistical significance was set as $p \le 0.05$. $^{*}p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$; $^{****}p \le 0.0001$. Mean ± SD are presented in this figure.

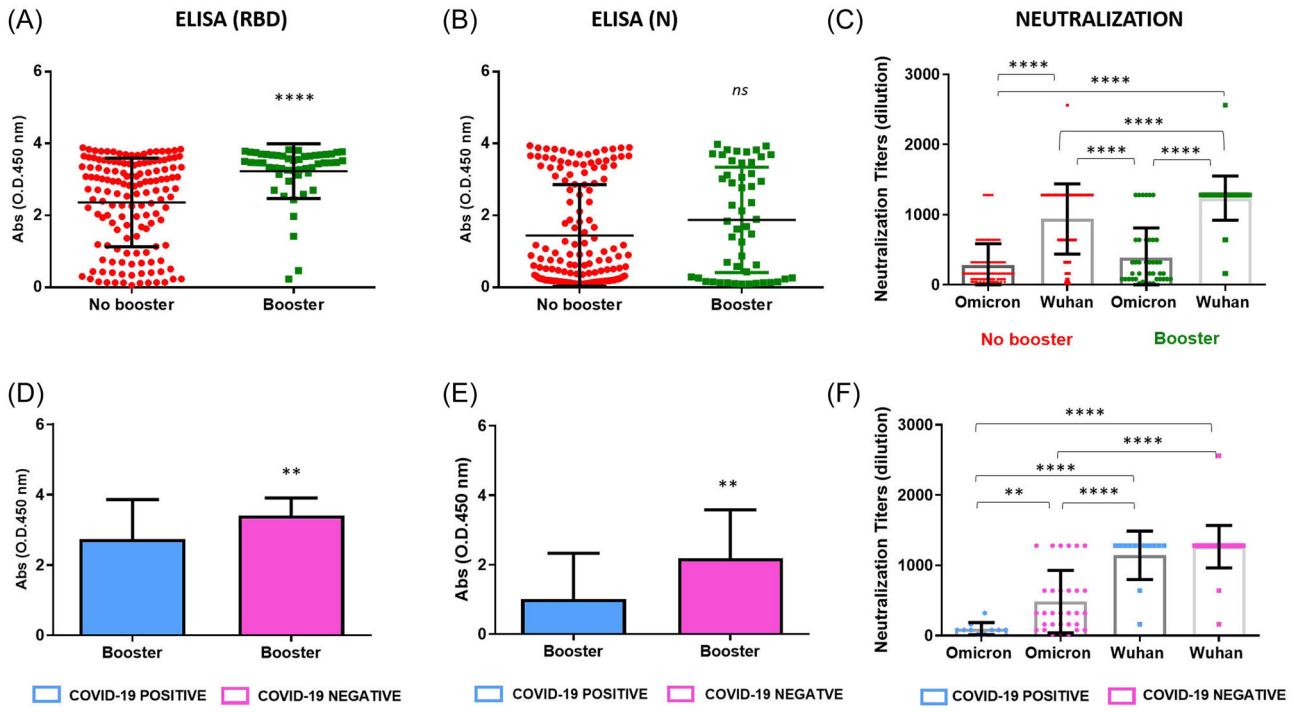


FIGURE 4 Serological profile of patients regarding vaccine booster. (A) Serum levels of antibodies specific to the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein were measured by ELISA. The levels of such antibodies were shown to be significantly increased in samples from patients who received the vaccine booster after a Mann-Whitney test ($p = 0.0001$). (B) Serum levels of antibodies specific to the N protein of SARS-CoV-2 from patients who received or not a third vaccine dose (booster) were shown to be statistically indistinguishable after a Mann–Whitney test. (C) Serum samples from patients who received or not the vaccine booster were subjected to neutralization assays using Wuhan and Omicron SARS-CoV-2 variants. Values of neutralization titers were subjected to an analysis of variance (ANOVA) followed by Tukey's multiple comparisons. Samples from patients who received the vaccine booster were grouped according to infection or absence of infection by SARS-CoV-2 (COVID-19 positive and COVID-19 negative). Serum levels of specific antibodies to RBD (D) and N protein (E) were shown to be significantly higher in noninfected patients after a Mann–Whitney test. (F) Serum samples from patients who received the vaccine booster and were or not infected by SARS-CoV-2 were subjected to neutralization assays using Wuhan and Omicron. Neutralization titers were subjected to an ANOVA followed by Tukey's multiple comparisons. Statistical significance was set as $p \leq 0.05$. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

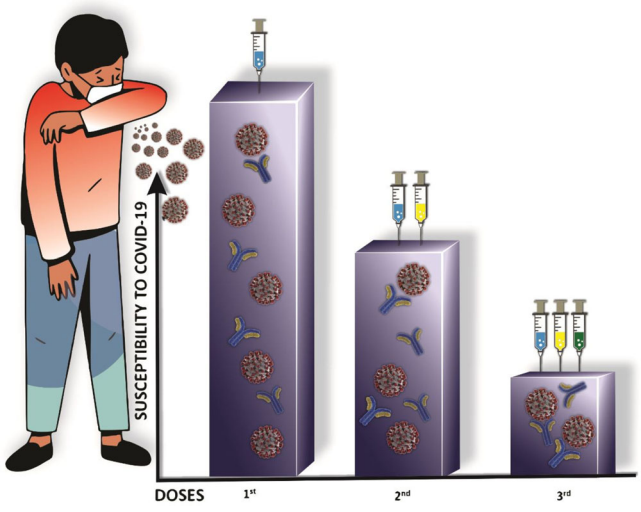


FIGURE 5 Summary of the main finding of this study. The susceptibility to coronavirus disease 2019 (COVID-19) decreases with the vaccine booster.

regarding administration of a fourth and subsequent vaccine doses. A vaccination policy based on booster shots could at least give time to update the current vaccine formulations. Moreover, the less susceptible to SARS-CoV-2 a given population is, the less probable a new virus variant will emerge, due to lower virus circulation, as new viral variants and VOCs emerge when the virus infects people and replicate to generate mutants.[9,25] Thus, our findings support present public health policies based on the continued administration of three, or more, vaccine doses both for prevention of the disease and its sequelae and the emergence of new SARS-CoV-2 variants capable to escape immunity induced by the present available vaccine formulations.

It is important to note that most of the individuals in our study population presented humoral immune response to SARS-CoV-2, including neutralization capacity. However, such a functional immune response was restricted to the wild type SARS-CoV-2 (Wuhan WT) strain in most of individuals. Only the third vaccine dose was capable of boosting the humoral immune

response to a level in which there was also a neutralization capacity against Omicron. A possible explanation for this phenomenon is that, after the booster dose, enhanced immune responses increase availability of antibodies targeting conserved epitopes required for virus neutralization activity, such as the most conserved epitopes of sarbecoviruses.[11] Indeed, our previous observations demonstrated that administration of the third vaccine dose significantly increases the humoral immunological status of most vaccinated individuals,[18] but we were surprised by demonstration that a boosted immune response could also prevent infection by the Omicron (and its subvariants) variant, largely known to be less susceptible to antibodies capable to neutralize other SARS-CoV-2 variants.

Our study population was immunized with different vaccination regimens. Nevertheless, the effect of the third vaccine dose was seen independently of vaccine formulations patients received in the first and second doses. In fact, all individuals of our study received BTN126b2 in the third dose. A small proportion of these individuals did not develop the expected boosted immunity. In addition, most patients that received the third dose showed specific serum antibodies to the N protein. Moreover, boosted and COVID-19-negative patients presented higher serum levels of anti-N antibodies. Although these last-mentioned findings need clarifications, the main conclusion of this study is supported by robust statistical analyses.

4.1 | Limitations

The sample size used in this study was not representative of the city's population. We were not able to achieve the sample size planned in the beginning of the study using our sampling strategy, because the epidemic ended. We collected up to 60 samples per week, according to our laboratory analysis capacity. Thus, we used a convenience sample. In addition, we did not carried out neutralization assays considering Omicron sub-variants. We used only an Omicron virus strain. However, we highlight that we present clinical data showing that the third vaccine dose reduces susceptibility to Omicron, including a myriad of subvariants.

5 | CONCLUSION

In this study, we presented strong populational evidence that administration of the third vaccine dose reduces susceptibility to infection by the Omicron variant and, thus, development of COVID-19. Such protective effect represent an additional, and relevant, feature of vaccination, preclusion of new viral variants emergence. Thus, our data strongly support the conclusion that additional doses of the presently used anti-COVID-19 vaccines can relevantly contribute to the reduction of individuals' susceptibility to the Omicron variant, and its subvariants, and, consequently, reduces virus circulation among humans.

AUTHOR CONTRIBUTIONS

Jéssica P. Farias: Samples collection, ELISA data collection, patients' data collection, RT-qPCR data collection, data analysis, and writing. **Josilene R. Pinheiro**: Samples collection, patients' data collection, RT-qPCR data collection, genome sequencing data collection, and data analysis. **Robert Andreata-Santos**: ELISA data collection, VNT data collection, data analysis, and writing. **Mayanna M. C. Fogaça**: Samples collection, ELISA data collection, patients' data collection, RT-qPCR data collection, and data analysis. **Ruth D. da Silva Brito**: Samples collection, ELISA data collection, patients' data collection, RT-qPCR data collection, and data analysis. **Edgar F. da Cruz**: VNT data collection, data analysis, and writing. **Maria F. de Castro-Amarante**: VNT data collection, data analysis, and writing. **Samuel S. Pereira**: ELISA data collection, data analysis, and writing. **Shirley dos Santos Almeida**: VNT data collection and writing. **Ludimila M. Moreira**: Samples collection, patients' data collection, and RT-qPCR data collection. **Rafael da Conceição Simões**: Scientific support, data analysis, and writing. **Wilson B. Luiz**: Scientific support, data analysis, and writing. **Alexander Birbrair**: Financial support, scientific support, data analysis, and writing. **Aline Belmok**: Scientific support, data analysis, and writing. **Bergmann M. Ribeiro**: Scientific support, data analysis, and writing. **Juliana T. Maricato**: Scientific support, data analysis, and writing. **Carla T. Braconi**: Scientific support, data analysis, and writing. **Luís C. de Souza Ferreira**: Financial support, scientific support, data analysis, and writing. **Luiz M. R. Janini**: Financial support, scientific support, data analysis, and writing. **Jaime Henrique Amorim**: Study design, financial support, samples collection, patients' data collection, RT-qPCR data collection, data analysis, and writing.

ACKNOWLEDGMENTS

We are thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing PhD research fellowships to Josilene Ramos Pinheiro. We are also thankful for the support of the Epidemiological Surveillance Service of Barreiras city. This research was funded by Financiadora de Estudos e Projetos (FINEP), project 27968—FINEP/RTR/PRPq/REDE/COVID-19—Sub: 09 (Jaime Henrique Amorim); FINEP 29334-FINEP/UFMG/REITORIA/PRPQ/LABORATÓRIOS DE CAMPANHA FASE II-Sub: 06 (Jaime Henrique Amorim); FINEP 1227/21 Fase II—Corona-ômica BR MCTI Rede nacional de genomas, exoma e transcriptoma de COVID-19 (Jaime Henrique Amorim). This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): Postdoc scholarship 2021/05661-1 (Robert Andreata-Santos), Postdoc scholarship 2018/14459-9 (Maria F. de Castro-Amarante), Project No. 2020/08943-5 (Luiz M. R. Janini, Carla T. Braconi, Juliana T. Maricato), and Project No. 2020/05204-7 (Luís C. de Souza Ferreira). This work was supported by the National Council for Scientific and Technological Development (CNPq): Projects Numers 401506/2020-7 (Luís C. de Souza Ferreira), 405547/2021-8 (Jaime Henrique Amorim), and 303857/2021-8 (Jaime Henrique Amorim). This work was supported by Instituto Serrapilheira/Serra-1708-15285 (Aline Belmok, Alexander Birbrair). This work was also

supported by Postdoc scholarship No. 88887.372866/2019-00 (Edgar F. da Cruz) and PhD scholarship No. 88887.600175/2021-00 (Samuel S. Pereira) by the Coordination of Improvement of Higher Education Personnel (CAPES). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be provided under request.

ETHICS STATEMENT

Ethics approval was obtained from institutional review board (ethics committee) (CAAE 40779420.6.0000.8060) of the Universidade Federal do Oeste da Bahia. Samples were collected only after volunteers give written informed consents.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Farias JP, Pinheiro JR, Andreata-Santos R, et al. The third vaccine dose significantly reduces susceptibility to the B.1.1.529 (Omicron) SARS-CoV-2 variant. *J Med Virol*. 2023;95:e28481. doi:10.1002/jmv.28481