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Validation and implementation of TaqMAMA RT-PCR for SARS-CoV-2 variant surveillance: experience from a high-volume setting

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Abstract

Background The genomic surveillance of SARS-CoV-2 is challenging in high-volume, resource-limited settings. Faster and less expensive methods are required for the prompt detection of variants of interest. This study aimed to validate and implement the TaqMAMA RT-PCR method for the detection of SARS-CoV-2 variants.

Methods We developed the TaqMAMA RT-PCR method for SARS-CoV-2 variants. From the viral genomes obtained from the GISAID database, fluorescent amplification probes and oligonucleotides were designed to detect two specific mutations for each variant. The study consisted of an assay validation phase comparing the newly designed method to WGS in COVID-19-positive samples, followed by a large-scale implementation phase to calculate its performance.

Results During the assay validation phase, we included 232 samples for analysis using TaqMAMA and WGS. TaqMAMA identified 82.3% as positive, and had sensitivities of 82%, 100%, and 50%, specificities of 91%, 99%, and 100%, with PPVs of 99%, 75%, and 100%, and NPVs of 20%, 100%, and 100% for the Delta, Alpha, and Gamma variants, respectively. For the implementation phase, we included 1315 samples, TaqMAMA identified 68% positive samples, 97.5% as delta. The predicted performance using Bayesian statistics was 95%, 55%, and 0% for the positive, and 29%, 0%, and < 1% for the negative delta, alpha, and gamma variants, respectively.

Conclusions The diagnostic performance of TaqMAMA RT-PCR was acceptable for the detection of the most prevalent SARS-CoV-2 variants of interest. This method offers a cost and time-saving alternative for the genomic surveillance of SARS-CoV-2 in high-volume settings.

Keywords RT-PCR, TaqMAMA, SARS-CoV-2, Genomic surveillance, Variants of interest, Whole genome sequencing, Diagnostic tools, Clinical microbiology

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Introduction

Until June 2023, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for COVID-19, has infected over 670 million people and caused over 6.8 million deaths worldwide [1]. During the initial stages of the SARS-CoV-2 pandemic, there was limited genetic evolution, probably due to the lack of immunity against the novel virus. In addition, coronaviruses have lower mutation rates than other RNA viruses, such as HIV-1 or influenza, due to their genetic proofreading mechanisms [2, 3].

Even with these mechanisms in place, the mutation rates observed in coronaviruses vary between 10^{-5} and 10^{-3} substitutions per nucleotide site per cell infection [4]. Moreover, the widespread replication from the persistence of the pandemic over time has allowed natural selection to act upon favorable mutations to create numerous variants different from the original Wuhan strain [5].

The World Health Organization (WHO) has classified relevant SARS-CoV-2 variants as variant of interest (VOI) and variant of concern (VOC). A VOI is a variant with genetic changes known to impact the virus characteristics and has been identified to cause significant transmission. A VOC is associated with changes of global public health significance; these include an increase in transmissibility or virulence or a decrease in either the effectiveness of public health and social measures or the available diagnostics, vaccines, and therapeutics [6]. The VOCs are named Alpha, Beta, Gamma, Delta, and Omicron, all with different mutations within the receptor binding domain [7, 8].

Genomic surveillance has been crucial for promptly detecting mutations, monitoring virus evolution, assessing variant-vaccine strain similarities since the availability of SARS-CoV-2 vaccines, and monitoring the evolutionary trajectory of novel viral variants [9]. Whole Genome Sequencing (WGS), or at least the partial sequencing of the S-gene, has been acknowledged as the most reliable technique for comprehensively studying and characterizing potential emerging strains, as this tool can identify point mutations throughout the genome that may be otherwise missed by alternative methods such as traditional RT-PCR [10]. However, the elevated cost of WGS makes the implementation of this tool within low-resource settings troublesome. The expenses associated with setting up and maintaining a next-generation sequencing facility are high, spanning from $80,000 to $700,000 USD. Consequently, there has been a systematic underperformance of WGS for genome surveillance in developing countries, evidenced by a significant gap in genome data distribution between lower and middle-income countries compared to the rest of the world [9, 11].

The lack of WGS has highlighted the importance of developing efficient alternative methods for developing countries, where, historically, variant identification has been at least partially reliant on traditional diagnostic tools such as RT-PCR. Moreover, although conventional RT-PCR is considered the gold standard for the detection of SARS-CoV-2, it has been shown that its diagnostic performance can be affected by viral mutations such as those seen in variants [12].

In recent months, to circumvent these problems, emerging RT-PCR techniques have been developed [13–15]. However, researchers are still trying to find a feasible, accurate, and affordable option for its widespread implementation.

TaqMAMA (TaqMan mismatch amplification mutation assay) is an innovative approach that combines the quantitative strengths of TaqMan with the allele-specific PCR of MAMA. This method enables robust discrimination between alleles and has been utilized for targeted mutation detection. Initially, this analytical tool successfully identified mutations in human cell lines. Subsequently, its effectiveness has been demonstrated in detecting variants across various diseases [16, 17]. Although TaqMAMA has been acknowledged for its high accuracy and cost-effectiveness, its performance in identifying SARS-CoV-2 variants has yet to be evaluated.

In the present study, we aim to validate, for the first time, the TaqMAMA technique for SARS-CoV-2 variant identification. Utilizing patient data from the largest tertiary COVID-19 referral care center in Mexico, we aim to generate data for the potential implementation of this technique within resource-limited settings where WGS is not readily available.

Methods

Study design

We conducted an observational cross-sectional study to evaluate the diagnostic performance of TaqMAMA, a novel RT-PCR method, compared to WGS for detecting SARS-CoV-2 variants of interest. The study was conducted at a COVID-19 tertiary referral hospital in Mexico City, the National Institute of Respiratory Diseases (INER), from April 26, 2021, to January 16, 2022.

The study design included two phases to assess the diagnostic accuracy of the TaqMAMA RT-PCR. First, an assay validation phase, and second, a large-scale implementation phase.

Validation phase

For the validation phase, we included RT-PCR-confirmed SARS-CoV-2 nasopharyngeal or oropharyngeal exudate sample remnants, determined with the GeneFinder™ COVID-19 Plus Real*Amp* Commercial Kit (OSANG Healthcare Co., South Korea), with a WGS-confirmed

Table 1 Selected mutations for the detection of SARS-CoV-2 variants

WHO name	Pango lineage	Clade	Selected mutations of S gene	
Alpha	B.1.1.7	20I	A570D	P681H
Beta	B.1.351	20 H	D80A	A701V
Delta	B.1.617.2	21I	L452R	P681R
Gamma	P.1.14	20 J	H655Y	V1176F

variant from April 26, 2021, to June 30, 2021, via random sampling from the institute's patient database. We excluded SARS-CoV-2 negative samples and those stored under inadequate conditions (temperature >-70ºC).

The primary objective of this phase was to determine if the TaqMAMA RT-PCR method has an acceptable performance compared to WGS, defined as ≥ 80% sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the Alpha, Delta, and Gamma variants.

As a secondary outcome, we performed a phylogenetic analysis of SARS-CoV-2 using the Illumina DRAGEN COVID Lineage App (Illumina, San Diego, USA).

TaqMAMA RT-PCR

The viral genomes of each variant of interest were obtained from the GISAID database (www.gisaid.org). Sequences corresponding to the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617) variants were selected and downloaded (Table 1). Sequence alignment was performed using the Kalign server (EMBL-EBI, Hinxton, UK) and the Molecular Evolutionary Genetics Analysis (MEGA-X, University Park, USA) program, comparing each group of sequences with the original SARS-CoV-2 sequence (NCBI identification number: NC_045512.2). This initial step aimed to discriminate and identify the specific mutations inherent to each variant.

The preparation of fluorescent probes and oligonucleotides was carried out as follows. Initially, forward and reverse oligonucleotide primers were designed to flank the region with the nucleotide change compared to the wild-type sequence. The discrimination between each variant sequence and the wild-type sequence was performed using the method described by Li et al. called PCR "mismatch amplification mutation assay" (MAMA) with TaqMan technology or TaqMAMA PCR [16]. In this method, the forward oligonucleotide is hybridized to the same region in mutant and wild-type sequences. However, the last nucleotide at the 3' end will match only with the variant sequence, inhibiting the amplification of wild-type sequences or those with different mutations. For the design of primers, two specific mutations of the Spike (S) gene were selected for each variant. The mutations utilized for each variant are shown in Table 1, mutations were named after the amino acid change in the spike protein. A probe was designed for each variant, complementary to a specific region flanked by the oligonucleotides. Each probe was attached to a fluorescent molecule at the 5' end, with a corresponding quencher located 20 nucleotides away from the fluorophore. Sequences of primers and probes are described in Table 2.

A TaqMAMA RT-PCR assay consisted of a series of 8 independent reactions per each confirmed SARS-CoV-2 positive sample. Each reaction is composed of the mixture of a forward primer, a reverse primer and the corresponding probe to identify one point mutation. Detection of a variant was confirmed when it was observed amplification in both of the reactions corresponding to the mutations of the variant.

Table 2 Designed primers and probes for TaqMAMA RT-PCR detection of SARS-CoV-2 mutations

Variant/ Mutation	Forward primer 5´ → 3´	Reverse primer 5´ → 3´	Fluorescent probe 5´ → 3´	Amplicon size (bp)
Alpha A570D	CAACAATTTGGCAGAGACATTAA	GCATGAATAGCAACAGGGAC	[FAM]CACAGACACTTGAGATTCT[BHQ1] TGACATTACACCATGTTC[C3]	191
Alpha P681H	CTAGTTATCAGACTCAGACTAATTCTGA	TACTGATGTCTTGGTCATAGACAC	[HEX]TCAATCCATCATTGCCT[BHQ1] ACACTATGTCACTTG[C3]	194
Beta D80A	ACCAATGGTACTAAGAGGTTTCC	AGGGACTGGGTCTTCGAATC	[HEX]TTGCTTCCACTGAGAAGTC[BHQ1] TAACATAATAAGAGGCTG[C3]	134
Beta A701V	CCTACACTATGTCACTTGGTCT	CAACAGCTATTCCAGTTAAAGCAC	[FAM]CACAGAAATTCTACCAGTGT[BHQ1]CTATGACCAAGACATC[C3]	237
Delta L452R	TCTAAGGTTGGTGGTAATTATAATTACGG	TCTGTATGGTTGGTAACCAACAC	[FAM]GATATTTCAACTGAAATCTA[BHQ1] TCAGGCCGGTAGCAC[C3]	201
Delta P681R	AGTTATCAGACTCAGACTAATTCTGG	GATGTCTTGGTCATAGACACTGG	[HEX]CTACACTATGTCACTTGGT[BHQ1] GCAGAAAATTCAGTTGTTTA[C3]	188
Gamma H655Y	GCTGTTTAATAGGGGCTGACT	CAATGATGGATTGACTAGCTACAC	[HEX]GGTGCAGGTATATGCGCT[BHQ1] AGTTATCAGACTCAG[C3]	138
Gamma V1176F	CATCTCTGGCATTAATGCTTCCT	CATTACTATGGCAATCAAGCCAG	[FAM]TCGATCTCCAAGAACTT[BHQ1] GGAAAGTATGAGCAGTA[C3]	184

Viral RNA extraction was carried out using the ExiPrep 96 Viral DNA/RNA kit (Catalog number: K-4614) and the Bioneer instrument (Catalog number: EP96L-BXF006) (Bioneer Inc, Oakland, USA). The synthesis of complementary DNA (cDNA) and qPCR amplification was performed in a single reaction mixture (Table 3) using the SuperScript™ III Platinum™ One-Step qRT-PCR kit, which includes the SuperScript™ III reverse transcriptase enzyme and Taq Platinum™ DNA polymerase (ThermoFisher Scientific, Waltham, USA). The amplification program used for the biological samples was performed using a reverse transcriptase enzyme by incubating at 50 °C for 15 min (1 cycle), 95 °C for 2 min (1 cycle), and 45 cycles of 95ºC for 15 s and 60 °C for 60 s.

Whole genome sequencing

Total RNA was extracted using the ExiPrep 96 Viral DNA/RNA kit and the Bioneer instrument. WGS was performed using the Illumina COVIDSeq Assay (Catalog number: 20051274) following the instructions provided by the manufacturer. Briefly, each RNA sample was incubated with DNA hexamers (oligonucleotides with a random 6-base sequence) aligned to different RNA regions. Once the hexamers were aligned to the RNA strands, first-strand cDNA synthesis was performed using a reverse transcriptase enzyme by incubating at 25 °C for 5 min, 50 °C for 10 min, and finally 80 °C for 5 min. Once synthesized, the cDNA was amplified via endpoint PCR, resulting in multiple copies.

The amplified DNA was fragmented into short sequences through "tagmentation," a process where specific adapters are attached to the ends of the short DNA fragments.

Subsequently, the fragmented product was cleaned to remove any remaining genetic material. The adapter-ligated fragments were used to perform an endpoint PCR, which had two main objectives: increasing the number of copies of the short fragments and attaching index adapters (used in the sequencing protocol) to these fragments. The PCR products were incubated with magnetic beads, specifically bound to DNA fragments containing the adapters, with an expected minimum length. This allowed the PCR products to be purified by washing the magnetic beads. Once clean genetic material was obtained, it was separated from the beads using an elution buffer. The purified product was quantified and normalized to a concentration of 4 nM.

Finally, samples were adjusted for sequencing to a final concentration of 75 pM. The adjusted samples were prepared according to the manufacturer's instructions and sequenced using the Illumina MiSeq instrument (Illumina, San Diego, USA). Once the sequencing was completed, the obtained data were analyzed using the Illumina DRAGEN COVIDSeq Test and DRAGEN COVID Lineage tools (Illumina, San Diego, USA). Read length was 151 with paired reads. All samples had an average coverage of 99.69% of the SARS-CoV-2 genome (range 98.52–99.78) and an average depth (± SD) of 3829.41 ± 703.28 (range 1340.0-4662.0).

Table 3 TaqMAMA RT-PCR reaction mixture components for the synthesis of complementary DNA and amplification

Component for TaqMAMA RT-PCR	Total reactant Volume (25 µL)
SuperScript™ III RT/Platinum™ Taq Mixture	0.5 µL
2X reaction mixture	12.5 µL
Forward Oligonucleotide (5 µM)	1 µL
Reverse Oligonucleotide (5 µM)	1 µL
Fluorescent Probe (5 µM)	0.5 µL
RNA	5 µL
H2O	4.5 µL

Implementation phase

We included all RT-PCR-confirmed SARS-CoV-2 samples for the implementation phase from July 1, 2021, to January 16, 2022. We excluded SARS-CoV-2 negative samples and those stored under inadequate conditions (temperature >-70°C). All the samples underwent TaqMAMA RT-PCR using the method described in the validation phase. The primary objective of this phase was to estimate the expected performance of the TaqMAMA RT-PCR method utilizing the validation results.

The validation phase of the study compared TaqMAMA RT-PCR with WGS. Therefore, as a secondary analysis, we also aimed to compare the sensitivity of the new method with an RT-PCR commercial kit. For this purpose, we compared the TaqMAMA RT-PCR cycle threshold (Ct) values with the Ct values obtained from the amplification of the gene N of positive samples with the GeneFinder™ COVID-19 Plus Real*Amp* Commercial Kit.

Statistical analysis

Means with standard deviations and medians with interquartile ranges are used for normally and non-normally distributed quantitative variables, respectively; frequencies and percentages are used for qualitative data.

We validated the diagnostic performance of the TaqMAMA RT-PCR compared to the diagnostic standard, WGS. Based on this objective, we calculated the following variables: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and Cohen's kappa coefficient for agreement. For the implementation phase, we calculated each variant's pre-test probability (prevalence) during the study period. We employed Bayesian statistics to estimate the post-test

probability via Fagan's nomogram with the previously calculated LR + and LR- [18]. Positive and negative Ct Values were compared between groups using unpaired T-tests. TaqMAMA and commercial RT-PCR kit Ct values were compared with Spearman correlation.

Statistical analysis was conducted using SPSS 24.0 (IBM, Armonk, New York, USA) and R Statistical Software (v4.3.0; R Core Team 2023).

Ethical considerations

This study was approved by the Institutional Review Board of the National Institute of Respiratory Diseases (Comité de Ética en Investigación INER, study no. E06-22), which complies with the Helsinki Declaration. Informed consent was waived due to the study's observational nature and the national state of emergency.

Results

Validation phase

For the initial assay validation phase, we included a total of 232 nasopharyngeal or oropharyngeal exudate frozen samples confirmed positive for SARS-CoV-2 by RT-PCR obtained at our Institution from April 1, 2021, to June 30, 2021, from adult subjects. WGS was conducted in all samples and identified 224 (96.5%) as Delta, 6 (2.6%) as Alpha, and 2 (0.9%) as Gamma variants. Among the total samples, 193 (82.3%) were positive when analyzed through TaqMAMA RT-PCR. The Delta variant accounted for 184 (95.3%) cases, the Alpha variant for 8 (4.2%), and the Gamma variant for 1 (0.5%), as shown in Table 4. Agreement was 82% (kappa = 0.24), 99% (kappa = 0.85), and 99% (kappa = 0.66) for the Delta, Alpha, and Gamma variants, respectively. Neither WGS nor TaqMAMA RT-PCR detected Beta variants.

Compared to WGS, TaqMAMA RT-PCR demonstrated a sensitivity of 82%, specificity of 91%, PPV of 99%, NPV of 20%, LR + of 9.1, and LR- of 0.2 for the Delta variant. Regarding the Alpha variant, TaqMAMA RT-PCR effectively detected 100% of cases, with a specificity of 99%. However, two positive cases were detected corresponding to a different variant with the same mutations, resulting in a PPV of 75%. TaqMAMA RT-PCR had a sensitivity of 50% and specificity of 100% for the Gamma variant (Table 4).

Figure 1 presents a maximum-likelihood phylogenetic analysis of the detected SARS-CoV-2 viral mutations of each variant during the study period. As depicted, most of the observed clusters corresponded to different strains of the Delta variant, followed by four Alpha variant strains. For the Gamma variant, the analysis identified only one strain.

Implementation phase

An implementation phase was conducted by performing TaqMAMA RT-PCR in 1315 SARS-CoV-2-confirmed samples from July 1, 2021, to January 16, 2022. TaqMAMA RT-PCR detected a variant in 897/1315 (68.2%). From these, 97.4% (874/897), 2.2% (20/897), and 0.6% (6/897) samples were identified as Delta, Alpha, and Gamma variants, respectively.

In order to assess performance acceptability, post-test probabilities were calculated with a Fagan nomogram for each variant based on pre-test probabilities and likelihood ratios, as displayed in Fig. 2. Pre-test probability values were based on each variant's prevalence based on the total sample size ($n = 1315$).

For the Delta variant, the pre-test probability was 67%, LR + 9.1, and LR- 0.2, resulting in a positive post-test probability of 95% and a negative post-test probability of 29%. Regarding the Alpha variant, the pre-test probability was 1.5%, LR + 100, and LR- 0, resulting in a positive post-test probability of 55% and a negative post-test probability of 0%. For the Gamma variant, the pre-test probability was 0.45%, with an LR- of 0.5, resulting in a negative post-test probability of < 1%.

As a secondary analysis, we compared the Ct values from amplification of gene S using TaqMAMA RT-PCR assay with the Ct values from amplification of Gene N obtained with the Genefinder commercial RT-PCR SARS-CoV-2 detection kit. The correlation between the two tests was strong, with a coefficient (Spearman R) of 0.71 (Fig. 3).

Discussion

In our study, we tested TaqMAMA RT-PCR, an allele-specific PCR-based (ASPCR) method suitable for broad and cost-effective genotyping applications [16, 17]. Initially, we conducted a validation phase in which the

Table 4 Assay validation results

Variant	WGS positive tests *n* = 232 (100%)	TaqMAMA RT-PCR positive tests *n* = 193 (100%)	TaqMAMA RT-PCR Sensitivity (%)	TaqMAMA RT-PCR Specificity (%)	TaqMAMA RT-PCR PPV (%)	TaqMAMA RT-PCR NPV (%)	LR (+)	LR (-)
Delta	224 (96.5%)	184 (95.3%)	82	91	99	20	9.1	0.2
Alpha	6 (2.6%)	8 (4.2%)	100	99	75	100	100	0
Gamma	2 (0.9%)	1 (0.5%)	50	100	100	100	—	0.5

WGS whole genome sequencing, PPV positive predictive value, NPV negative predictive value, LR likelihood ratio

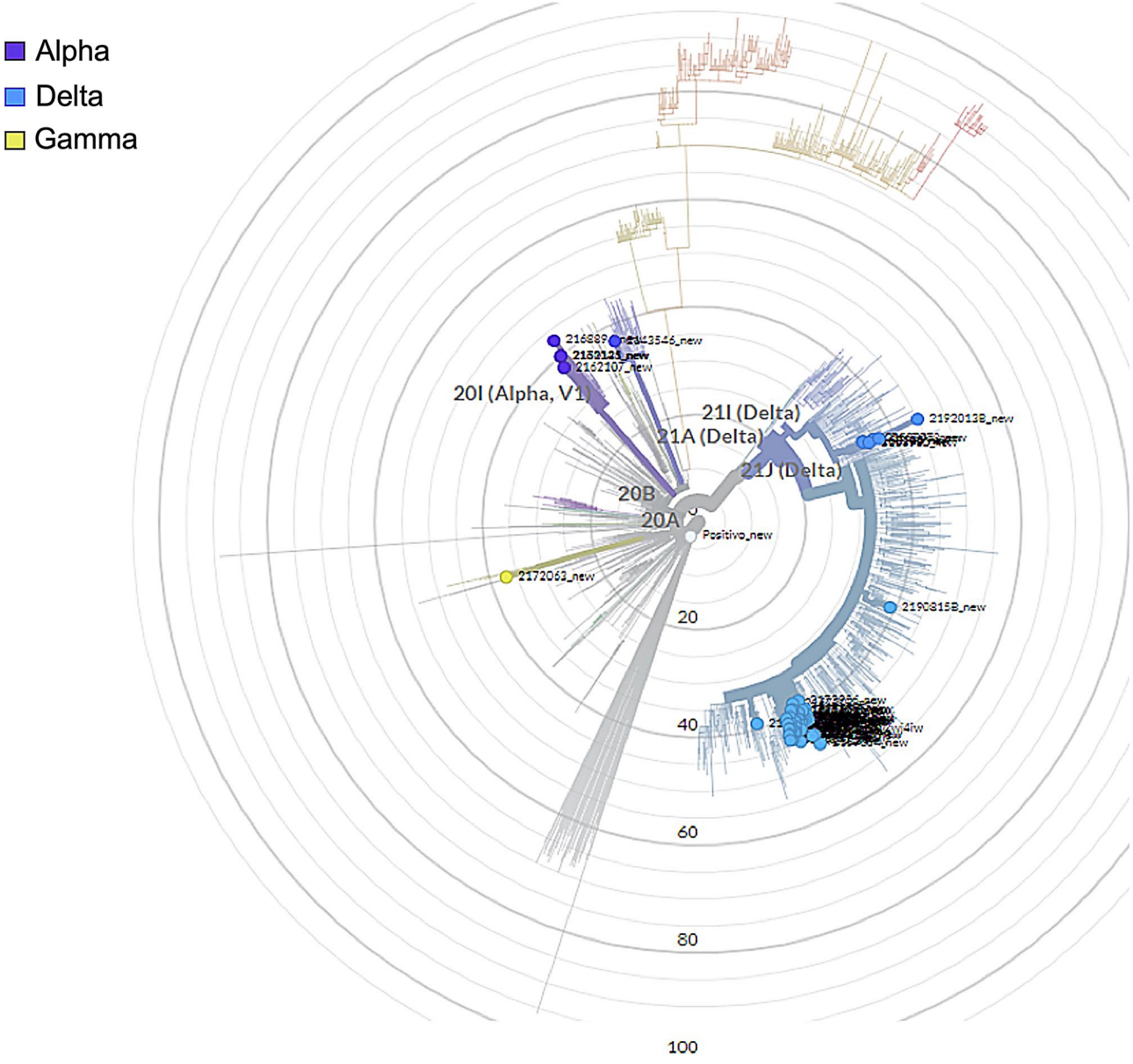

Fig. 1 Phylogenetic Tree of the Detected SARS-CoV-2 Variants. (**A**) The radial maximum-likelihood phylogenetic tree portrays the different SARS-CoV-2 strains detected during the study period from July 1, 2021, to January 16, 2022, when Delta was the most prevalent SARS-CoV-2 variant. Each dot represents a specific strain and is colored according to its clade. The blue, purple, and yellow dots correspond to the different delta, alpha, and gamma variant strains detected, respectively

TaqMAMA RT-PCR effectively detected a variant in 82% of cases compared to WGS, demonstrating high sensitivity and specificity, thus presenting an acceptable performance (≥ 80%) for Delta and Alpha variant detection.

To the best of our knowledge, this was the first study that analyzed the diagnostic performance of TaqMAMA RT-PCR and compare it to WGS in a high-volume center. However, previous studies have evaluated the performance of various RT-PCR methods for specific variant detection, reporting variable sensitivities and specificities ranging from 69 to 91.2% and 55 to 100%, respectively, correlating with our results, which showed a sensitivity of 50–100% and specificity of 91–100% depending on the analyzed variant [19, 20].

In addition, a study by La Rosa et al. tested a relatively similar in-house method using two short nested RT-PCR assays based on WGS, which yielded a 93% variant detection rate [21]. Another study, published by Hasan et al. in 2021, compared an RT-PCR genotyping strategy aiming to distinguish the Alpha variant from Beta/Gamma variants, suggesting that RT-PCR could be of value for epidemiological surveillance [22].

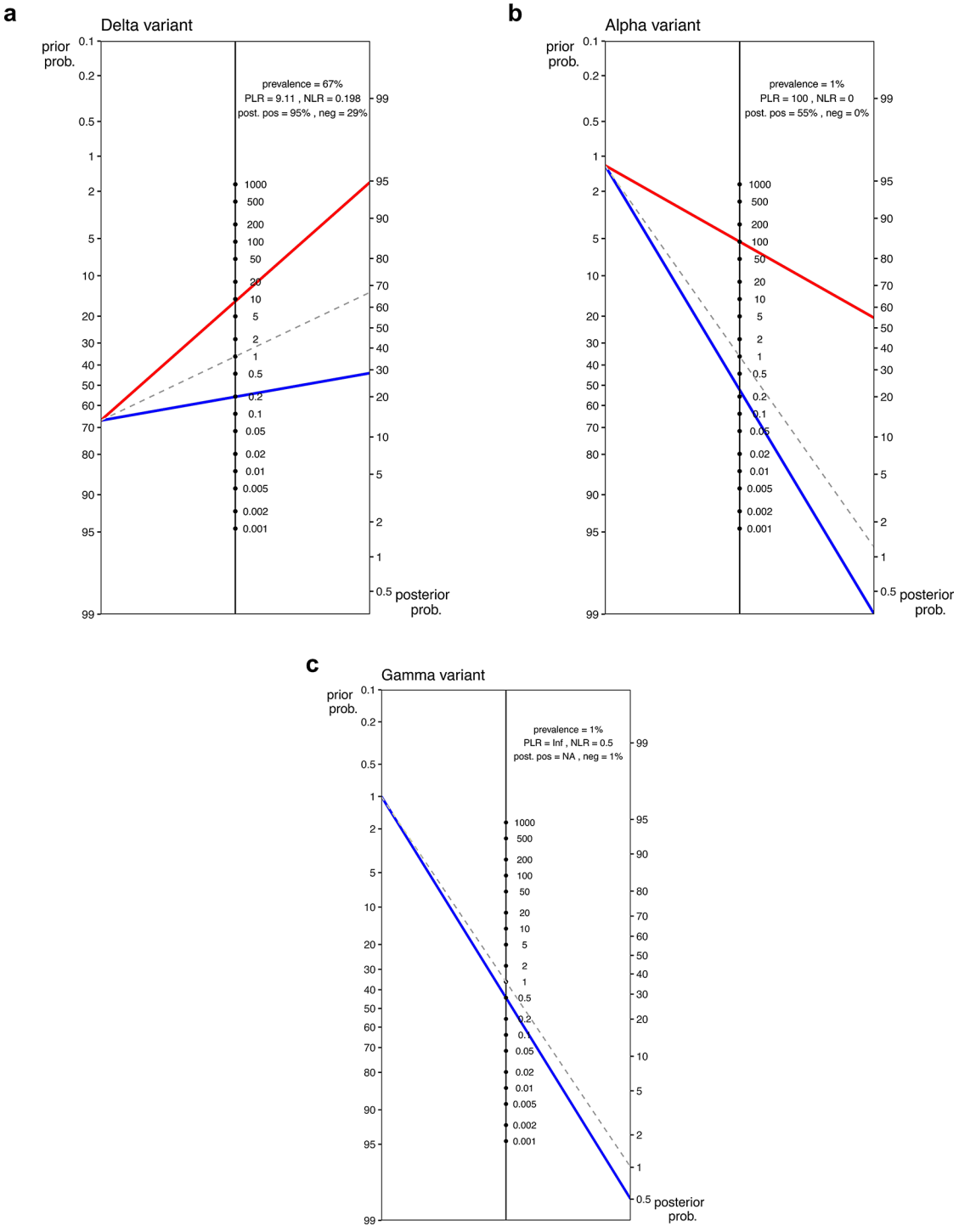

Fig. 2 Fagan nomograms for the diagnostic performance of TaqMAMA RT-PCR. The figure shows the diagnostic performance of TaqMAMA RT-PCR during the implementation phase for the (**a**) Delta, (**b**) Alpha, and (**c**) Gamma SARS-CoV-2 variants. The red and blue lines correspond to positive and negative tests, respectively. Abbreviations: Prob., probability; PLR, positive likelihood ratio; NLR, negative likelihood ratio; pos, positive; neg, negative; inf, infinite

Adding to its acceptable performance, TaqMAMA RT-PCR confers a significant advantage for variant detection compared to WGS due to its lower costs and faster results. Estimated to our implementation phase population, using TaqMAMA RT-PCR saved around USD 230,000, in addition to hundreds of hours of sample processing (Table 5). This innovative approach adds to the existing literature attempting to develop cost-effective RT-PCR methods [23].

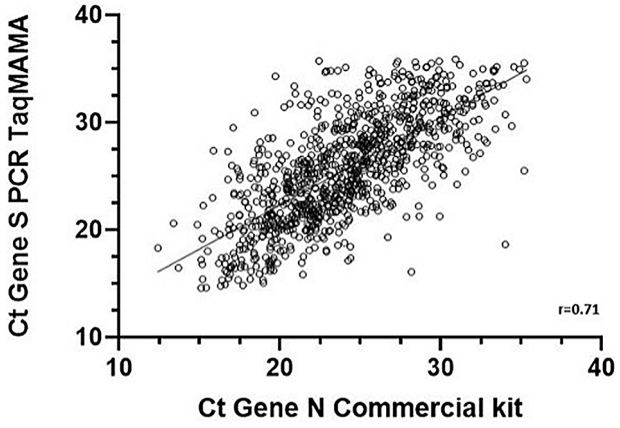

Fig. 3 Comparison between cycling threshold (Ct) values. The graph contrasts the Ct values obtained from the amplification of gene S with TaqMAMA RT-PCR and gene N with a commercial SARS-CoV-2 detection kit. Ct values lower than or equal to 35 from the samples analyzed in the implementation phase were used. Each circle represents the Ct value from the TaqMAMA RT-PCR and commercial kit from the same patient sample

Table 5 Comparison between RT-PCR and whole genome sequencing for the detection of SARS-CoV-2 variants

	TaqMAMA RT-PCR	Whole Genome Sequencing
Test Description	Test for the qualitative detection of nucleic acids from the SARS-CoV-2 virus	Determination of the complete viral genome sequence
Sample types	Nasal, nasopharyngeal, saliva, bronchial aspirate bronchoalveolar lavage	Nasal, nasopharyngeal, saliva, bronchial aspirate bronchoalveolar lavage
Time per test	4 h	48 h
Infrastructural requirements	• Nucleic acid extraction area, with class II A2 biosafety cabinet • RNA extraction equipment and reagents • Primers, probes, and plasmid amplification in a Real Time PCR thermal cycler	• Nucleic acid extraction area, with class II A2 biosafety cabinet • Master mix preparation area with pre-PCR laminar flow cabinet • Area with workstations for addition of nucleic acids and PCR amplification • Area to work with amplicons to prepare libraries and post-PCR sequencing • Sequencer
Detected genes	Identification of two different mutations of gene S per variant	Whole viral genome
Result type	Qualitative	Qualitative
Cost per test*	$500 MXN (≈$27 USD)	$4000 MXN (≈$230 USD)

RT-PCR reverse transcriptase polymerase chain reaction; Ct cycle threshold; MXN Mexican Peso

*Costs in USD are based on the exchange rate for June 2023

During the implementation phase, the effectiveness of TaqMAMA RT-PCR in detecting variants was 68%. As an indicator of confidence in our diagnostic assay, we calculated post-test probabilities, yielding robust results for the Delta variant, which was deemed by the WHO as the predominant global COVID-19 variant in the summer of 2021 [24], accounting for up to 94% of total COVID-19 cases in Mexico during the study period [25]. Our study demonstrated a similar prevalence, identifying 96.5% of the variants as Delta.

The stage of the SARS-CoV-2 pandemic influenced the diagnostic performance of our method due to the modification of post-test parameters by the different variant prevalences, particularly given the high burden of Delta cases and the low proportion of Gamma and Alpha cases. Furthermore, some negative results could be attributed to emerging variants and not to poor TaqMAMA diagnostic performance. For instance, the TaqMAMA RT-PCR diagnostic performance for the Omicron variant could be further elucidated. As observed in Fig. 4, as of June 2023, Omicron subvariants were the most predominant SARS-CoV-2 strains. Although the validation phase of this study did not include data from Omicron amplification, it shows the potential application of the TaqMAMA PCR for the detection of the subvariants responsible for the new COVID-19 outbreaks.

In addition to temporality as the main limitation of our study, other factors that might have limited its scope include using WGS only during the validation phase and that the study was conducted in a single center.

The combination of acceptable sensitivity, high PPV, and low cost makes TaqMAMA RT-PCR a suitable test to provide epidemiologic surveillance at underserved regions, as validated in this large study, including more than 1500 samples. At a public health level, implementation of TaqMAMA RT-PCR could be a valuable strategy for rapid detection and tracking of SARS-CoV-2 variants, detection of new VOI and VOC, and monitoring variants per region, specific mutations, degrees of transmission, and patient outcomes, facilitating efforts such as the ones reported by Singh et al. and Ciubotariu et al. [26, 27]. As observed in our secondary analysis in the implementation phase, when comparing the Ct values of TaqMAMA with commercial RT-PCR SARS-CoV-2 detection kits, the results were similar, indicating that the designed RT-PCR protocol has a comparable sensitivity to the widely distributed kits. Moreover, the mutations detected with the developed RT-PCR technique were confirmed with a phylogenetic analysis using the complete viral genome sequence of the positive samples used in the validation phase.

These results reinforce that faster and cheaper variant detection techniques are key for the effective surveillance of the behavior of SARS-CoV-2 infections and the timely

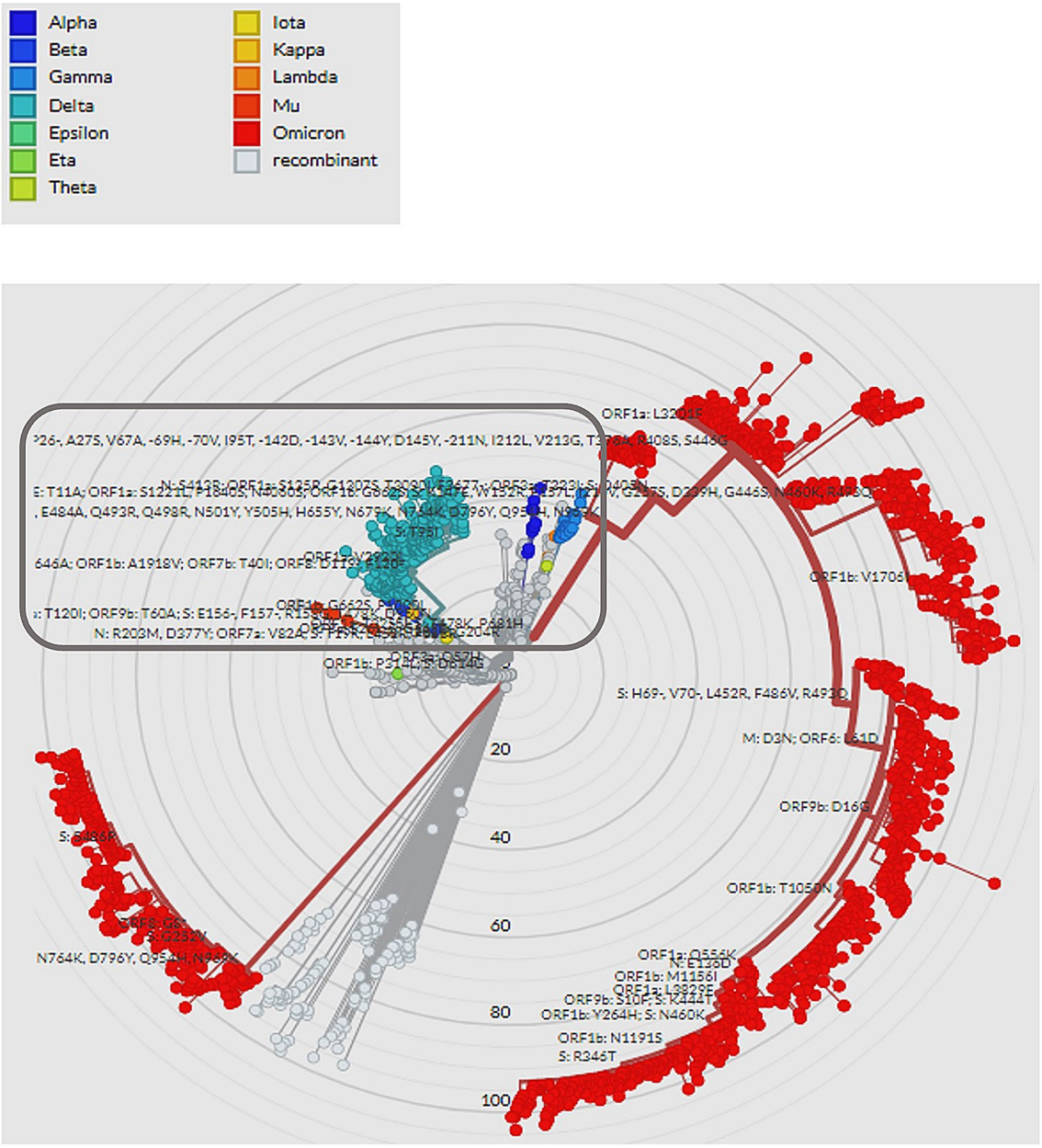

Fig. 4 Up-to-date phylogenetic tree. The graph displays the different SARS-CoV-2 strains using all the data analyzed in the implementation phase and including the branches of the published subvariants of Omicron as of June 2023. The gray square encloses the variants detected by TaqMAMA RT-PCR

determination of the outbreak-causing variants. A particular caveat of the TaqMAMA RT-PCR method is the need for constant updating and designing of primers to adapt to emerging variants. And it is important to note that if the TaqMAMA RT-PCR technique shows negative results, it could be an indicative of the appearance of a new variant, which emphasizes the importance of accompanying this PCR-based technique with WGS in such cases.

However, TaqMAMA RT-PCR would enhance testing of point mutations that are characteristic of a specific VOC in positive samples due to its low cost compared to other diagnostic tests, making it a reproducible and cost-saving alternative for SARS-CoV-2 genomic surveillance, and increasing access to healthcare.

Conclusion

The diagnostic performance of TaqMAMA RT-PCR was acceptable for the detection of the most prevalent SARS-CoV-2 variants of interest, as compared to WGS. Despite certain limitations, this method offers a cost and time-saving alternative for the genomic surveillance of SARS-CoV-2 in high-volume settings.

Abbreviations

WGS	Whole Genome Sequencing
WHO	World Health Organization
VOI	Variant of Interest
VOC	Variant of Concern
PCR	Polymerase Chain Reaction
cDNA	Complementary DNA
PPV	Positive Predictive Value
NPV	Negative Predictive Value

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10645-8.

Supplementary Material 1

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Author contributions
Conceptualization, EBV, JNAP; methodology, EBV and JNAP; software JNAP, HHCM; validation, DD, JNAP, and MAMS; formal analysis, EBV, DD, JNAP, and GCK; investigation ABP, AMN, AG, GBR, and GCK; resources, EBV; data curation, JNAP, DD, and EBV.; writing—original draft preparation, DD, AMN, AG, GBR, ABP, and GCK; writing—review and editing, JNAP, MADB, and MAGC; visualization, DD, MAGC, MADB, HHCM, and MAMS; supervision, EBV; project administration, EBV. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available in the BioProjects repository, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1037295, and in the BioSamples repository (see supplementary material for accession numbers). Further inquiries can be directed to the corresponding author.

Declarations

Ethical approval and consent to Participate
This study has been approved by the Institutional Review Board of the National Institute of Respiratory Diseases (Comité de Investigación y Ética en Investigación INER, study no. E06-22), which complies with the Helsinki Declaration. Informed consent was waived due to the study's observational nature and the national state of emergency.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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