REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

BRIEF COMMUNICATION

http://doi.org/10.1590/S1678-9946202163058

Early detection of SARS-CoV-2 P.1 variant in Southern Brazil and reinfection of the same patient by P.2

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ABSTRACT

Multiple variants of the Severe Acute Respiratory Syndrome coronavirus 2 virus (SARS-CoV-2) have been constantly reported across the world. The B.1.1.28 lineage has been evolving in Brazil since February 2020 and originated the P.1 variant of concern (VOC), recently named as the Gamma variant by the newly WHO nomenclature proposal, and P.2 as a variant of interest (VOI). Here we describe an early case of P.1 primary infection in Southern Brazil in late November 2020, soon after the emergence of the variant in Manaus, Northern Brazil. The same male patient was reinfected by another B.1.1.28 variant, namely P.2, in March, 2021. The genomic analysis confirmed genetically significant differences between the two viruses recovered in both infections, the P.1 lineage in the first episode and P.2 in the reinfection. Due the very early detection of P.1, we have also investigated the circulation of P.1 in the same region by differential RT-qPCR, showing that this was an isolated case of P.1 at the time of detection, and this variant has disseminated and became prominent from late January to the end of March, 2021. SARS-CoV-2 recent reports of reinfection have raised critical questions on whether and how well a first infection protects against reinfection.

KEYWORDS: Covid-19. Reinfection. SARS-CoV-2. P.1 lineage. Brazil.

INTRODUCTION

The emergence of the novel SARS-CoV-2 lineages is currently a worldwide concern. The continuous rise of variants is probably related to an increased rate of virus transmission and evasion from the host's immune system. Lately, research groups have reported the emergence of multiple variants of concern (VOC) or of interest (VOI), such as P.1[1], P.2[2], N.9[3] and B.1.351[4] in specific Brazilian regions. P.1 (VOC), recently named as Gamma variant by the newly WHO nomenclature proposal[5] as well as P.2 (VOI) variants have evolved from the lineage B.1.1.28[1,2] and harbor E484K (P.1 and P.2) and N501Y (P.1) mutations, which are associated with increased transmissibility or immune evasion[6]. The persistence of protective immunity provided by the coronavirus disease 2019 (COVID-19) or vaccination is not yet well established and some cases of reinfection have been reported[7,8].

The B.1.1.28 SARS-CoV-2 lineage has been circulating in Brazil since February 2020; P.2 (alias for B.1.1.28.2) was first detected in Rio de Janeiro[2] harboring the mutation S:E484K, and P.1 (alias of B.1.1.28.1) was first detected in Japanese travelers returning from the Amazonas State and due to the presence of several

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Received: 18 June 2021

Accepted: 23 June 2021

important mutations in the receptor binding domain - RBD of the viral spike (K417T, E484K, and N501Y) is of particular concern in Brazil. The P.1 variant is widely spread in the country, being associated with local outbreaks of great magnitude due to an enhanced transmissibility. The P.1 lineage more likely appeared in Manaus, Northern Brazil[9] in mid-November, even though, recent studies have shown an earlier possible origin for a P.1 ancestral in mid-August 2020 and for the common P.1 variant in mid-October[10]. The first described cases attributed to P.1 in Southern Brazil so far are from the last week of January 2021.

In this study, we report a case of SARS-CoV-2 P.1 infection in a 39-year-old individual from Campo Bom city (latitude 29º40'44" South and at longitude 51º03'10" West), Rio Grande do Sul State the Southernmost State of Brazil, in late November 2020. The same patient acquired a second infection by another variant, the P.2 lineage, in March 2021. Due the very early detection of P.1, we also have investigated the circulation of P.1 in the same geographic region using a differential VOC RT-qPCR. The study was carried out from the time of the first infection (November 2020) to April 2021, since P.1 emerged in Northern Brazil in mid-November[11] and the first cases described in Southern Brazil occurred in late January 2021.

MATERIAL AND METHODS

RT-qPCR for SARS-CoV2 detection

Naso-oropharyngeal swab samples from the same patient (LMM38991 and LMM50731) were received at Laboratorio de Microbiologia Molecular of Universidade Feevale, Novo Hamburgo, Rio Grande do Sul State, Brazil, (November 30th, 2020 and March 11th, 2021) for SARS-CoV-2 detection. Total nucleic acid was extracted with the commercial MagMAX™ CORE Nucleic Acid Purification Kit (Applied biosystems™, Thermo Fisher Scientific, Waltham, MA, USA) kit, using the automated equipment KingFisher™ Duo Prime (Thermo Fisher Scientific™). RT-qPCR for SARS-CoV-2 cDNA detection targeting the viral E gene was performed according to the Charité Institute, Berlin, Germany, protocols[12] and was carried out using AgPath-ID One-Step RT-PCR Reagents (Thermo Fisher Scientific™).

Differential RT-qPCR

A differential RT-qPCR for the P1 lineage[9] that detects the ORF 1b deletion (NSP6: S106 del, G107 del, F108 del) was performed. This deletion is a genetic signature of the VOCs P.1, B.1.1.7and B.1.351. The differential RT-qPCR was carried out using AgPath-ID One-Step RT-PCR Reagents (Thermo Fisher Scientific™); primers and probes were used following the same protocol as described by Naveca *et al*.[9]. A total of 302 samples was tested (Ct values for the E gene ranging between 10 and 20) randomly distributed over 22 weeks that started on November 2nd, 2020 until April 4th, 2021. New SARS-CoV-2 cases, hospitalizations and deaths in the neighboring regions were compared with the P.1 lineage study results. This study was approved by the National Committee of Research Ethics and the Institutional Ethical Review Board of the Universidade Feevale (protocol Nº 33202820.7.1001.5348), following Brazilian regulations and international ethical standards.

Viral Whole Genome Sequencing and Phylogenetic Analysis

RNA was extracted from naso-oropharyngeal swab samples and reverse transcription reaction was performed using the SuperScript IV reverse transcriptase kit (Thermo Fisher Scientific, Waltham, MA, USA). Preparation of the whole viral genome library was performed using the QIAseq SARS-CoV-2 Primer Panel paired for library enrichment and QIAseq FX DNA Library UDI Kit, according to the manufacturer instructions (QIAGEN, Hilden, Germany). Sequencing was implemented in an Illumina MiSeq platform using MiSeq Reagent Kit v3 (600-cycle) from Illumina Inc. (Foster city, CA, USA). All procedures were conducted in a laminar flow to minimize the risk of contamination. The FASTQ reads were imported to Geneious Prime, trimmed (BBDuk 37.25), and mapped against the reference sequence hCoV-19/Wuhan/WIV04/2019 (EPI_ISL_402124) available in the EpiCoV database from GISAID[13].

A total of 97 Brazilian SARS-CoV-2 complete genomes and the reference sequence (EPI_ISL_402124) (>29 kb) were retrieved from the GISAID database and aligned with the sequences generated herein. Sequence alignment was performed using the Clustal Omega software and the reference sequence from Wuhan was applied as the outgroup. The Maximum Likelihood phylogenetic analysis under the General Time Reversible model, allowing for a proportion of invariable sites and substitution rates were inferred empirically in IQ-TREE v2.1.2 web server[14] especially for maximum-likelihood (ML, applying 200 replicates and 1,000 bootstraps.

RESULTS

Case description and epidemiological findings

A 39-years-old male patient, presenting with comorbidities (chronic cardiovascular disease and diabetes

mellitus) reported two clinical episodes of COVID-19. The first one was on November 30th, 2020, and the second one on March 11th, 2021. During the first infection period, the patient's symptomatology was not reported. However, the patient claimed to have had contact with his brother, who had previously tested positive for SARS-CoV-2. He had also visited his SARS-CoV-2 infected father at the hospital in a room shared with other COVID-19 diagnosed patients and, despite the efforts, it was not possible to know if the patient has had previous contact with any traveler from Manaus, since the region is highly known for trade and tourism. In the second infectious episode, the patient experienced dyspnea, fatigue and respiratory distress and an oxygen saturation < 95% as a clinical sign. The second infection evolved with complications, and the patient was admitted to an Intensive Care Unit (ICU) and intubated due to a severe loss of pulmonary capacity. The patient unfortunately died on March 19th, 2021, 12 days after the onset of symptoms.

Diagnostic laboratory findings

Both diagnostic RT-qPCR assays were positive, presenting a Ct value of 30.07 (LMM38991) and 18.83 (LMM50731). The differential RT-qPCR retrospective study resulted in 226 negative samples and 122 positive samples for the P.1 variant. Except for the reinfected patient (who was not analyzed according to the CT value), the circulation of P.1 in Rio Grande do Sul State was evidenced in our sampling from January 27th, 2021 on (Figure 1A). Furthermore, the peak of P.1 lineage detections coincides with the peak of SARS-CoV-2 new cases (Figure 1B), hospitalizations (Figure 1C) and deaths (Figure 1D) in the neighboring region.

Genome sequencing and bioinformatics analysis

Two high-quality SARS-CoV-2 whole-genome sequences, named LMM38991/2020 and LMM50731/2021

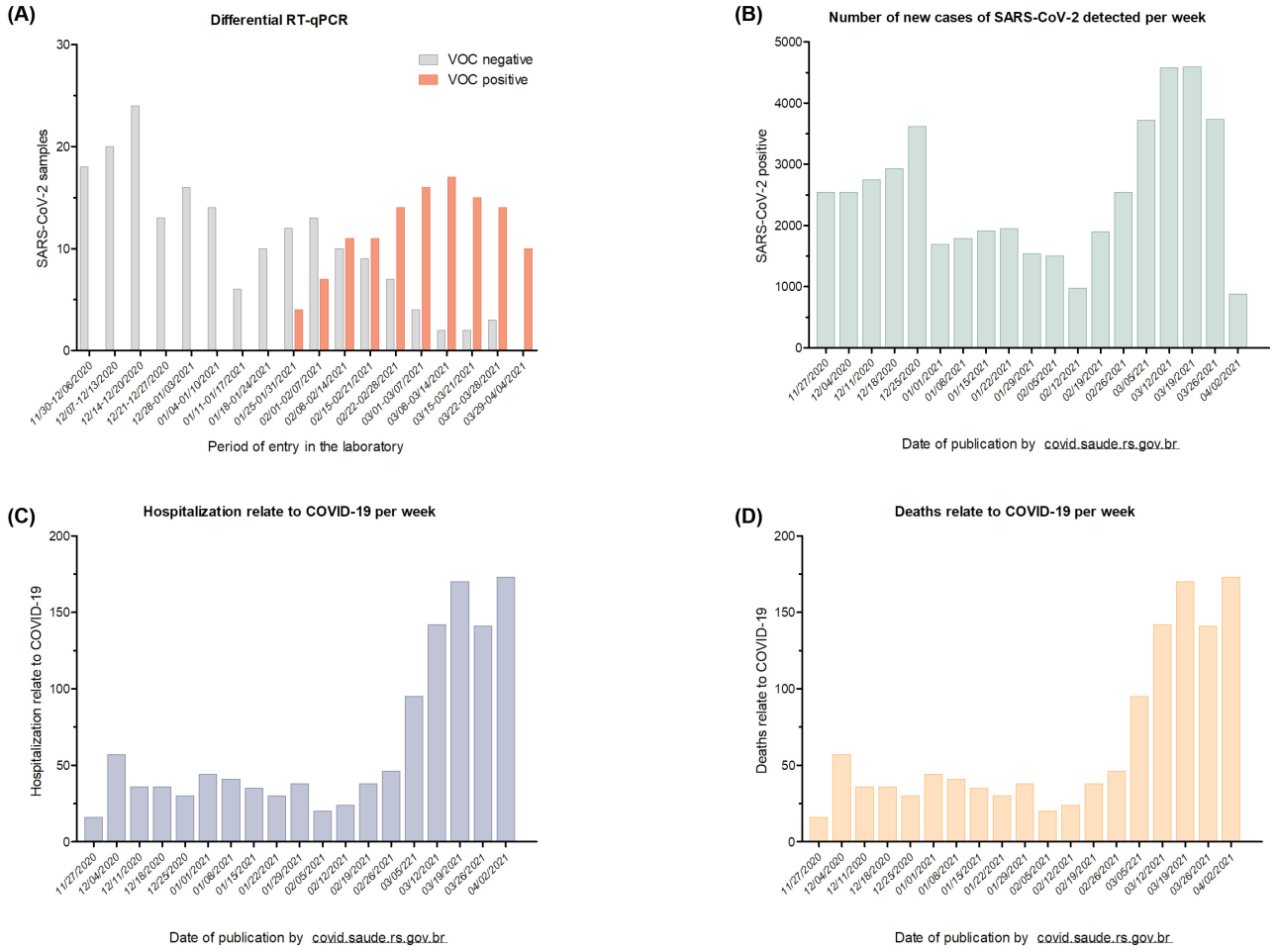

Figure 1 - Emergence of SARS-CoV-2 P.1 lineage and general situation of COVID-19: (A) Retrospective analysis based on differential RT-qPCR: The gray bars represent the negative results predominating during the first epidemiological weeks. Afterwards, positive samples (represented by pink bars) replaced the parental lineages; (B) COVID-19 new cases; (C) COVID-19 hospitalizations and (D) COVID-19 deaths.

(EPI_ISL_1630158 and EPI_ISL_1629809) were recovered from the same patient, corresponding to the first infection and the reinfection, respectively. Consensus sequences LMM38991/2020 and LMM50731/2021 presented a mean coverage of 1,405x and 1,263x. The sequences were first classified using the Pangolin COVID-19 Lineage Assigner tool[15] indicating the presence of two discordant SARS-CoV-2 lineages: the P.1 lineage in the primo-infection (LMM38991/2020; Gisaid access EPI_ISL_ EPI_ISL_1630158) and the P.2 lineage (LMM50731/2021; Gisaid access EPI_ISL_1629809) in reinfection.

The phylogenetic analysis confirmed previous results. LMM38991/2020 was clearly clustered with the P.1 lineage, while LMM50731/2021 branched out into P.2 group (Figure 2). LMM38991/2020 and LMM50731/2021 displayed the typical P.1 and P.2 spike protein E484K mutations and INDELs. LMM38991/2020, presented all 11 typical P.1 amino acid non-synonymous changes in the S protein (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F) and LMM50731/2021 presented three previously reported amino acid changes in S protein (E484K, D614G, V1176F) of the P.2 lineage.

DISCUSSION AND CONCLUSIONS

This study described a COVID-19 primo-infection caused by a P.1 lineage followed by a reinfection by a P.2 lineage in a three-month interval. A male, 39-years-old patient with history of comorbidities, presented two clinical episodes of COVID-19. Few reinfection cases were described worldwide, especially with detection of different SARS-CoV-2 lineages[16]. In addition, there are apparently more cases reporting asymptomatic/mild disease during reinfection episodes[17], although some cases showed a more severe illness in the second episode[16,18] as the one described here. These evidences are reinforced by the apparent low viral quantification determined by RT-qPCR in the primo-infection.

Viral immune evasion or limited and transitory protective immunity might be the cause of this reinfection as observed in the last cases to date[7], especially emerging lineages reinfections (P.1 and P.2) that might also reflect the ability of S:K484 virus to escape from anti-SARS-CoV-2 neutralizing antibodies. Although P.1 and P.2 variants have been related to antibody evasion in patients previously immunized by non-mutated lineages[6] in the case described herein, the patient may have produced antibodies that included the S:484K site, but apparently these antibodies did not prevent a second infection by the P.2 lineage.

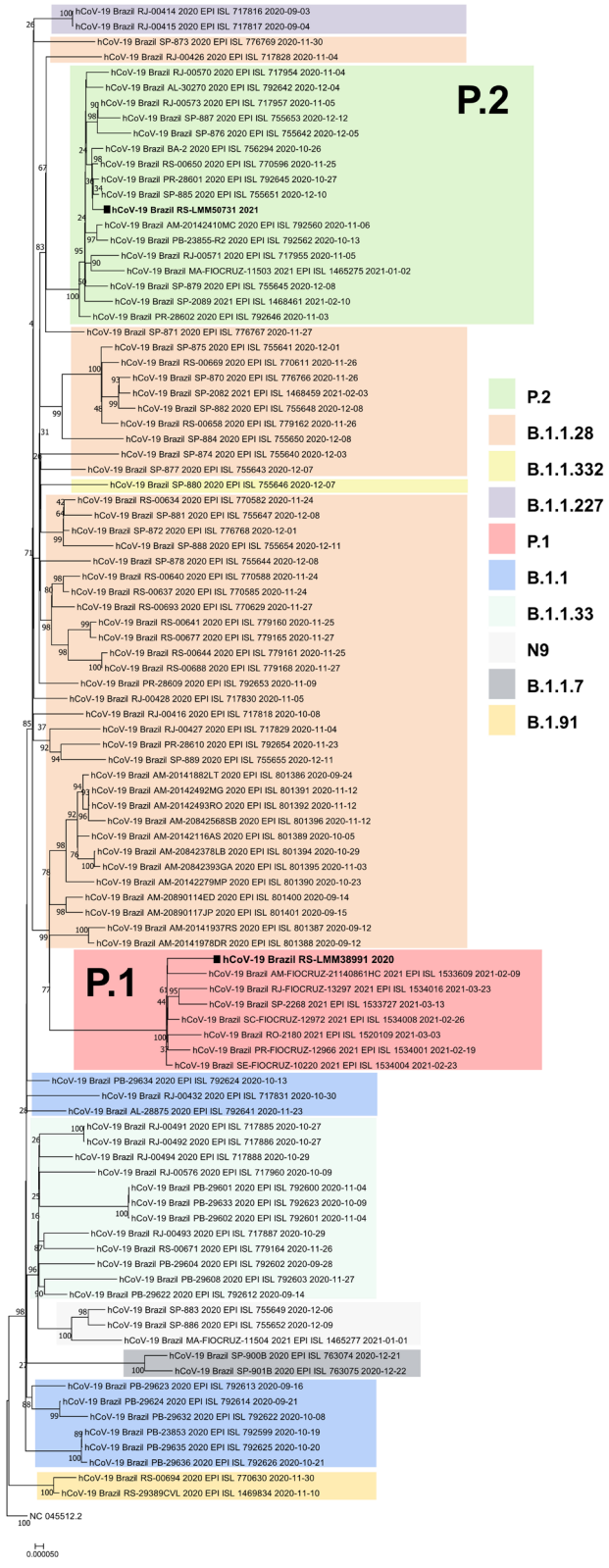

Figure 2 - Phylogenetic tree of SARS-CoV-2 complete genomes. The Maximum Likelihood phylogenetic analysis under the General Time Reversible allowing for a proportion of invariable sites, with empirical rates and substitution rates empirically inferred in the IQ-TREE (v2.1.2 web server applying 200 replicates and 1,000 bootstraps).

The P.1 lineage has most probably emerged in Brazil in middle November 2020 in Manaus, Northern region[9,11]. However, recent studies have shown an earlier possible origin for an ancestral P.1 in mid-August 2020 and for the nowadays common P.1 variant in mid-October[10]. Our findings showed that the P.1 lineage was present in Southern Brazil soon after, in late November. Later, other early detections of the P.1 lineage were described in other Brazilian States, such as Bahia, Salvador city[19].

According to our retrospective analysis, based on differential qRT-PCR, it is important to note that despite the case reported herein, the P.1 lineage did not spread in a first moment, as observed in other Brazilian cities and also in other countries. In Argentina, the first P.1 case was reported on February 8th, 2021, but the variant consistently spread between March and April, 2021[20]. Expressive number of positive P.1 cases were found only at the end of January in Southern Brazil, that replaced the parental lineages thereafter.

In summary, this study reported and characterized an early primary COVID-19 caused by the P.1 lineage followed by a reinfection episode, in Southern, Brazil some months later. The P.1 lineage is spreading rapidly across Brazil[21] and after its establishment, has been related to an exponential increment of transmissibility and hospitalization rates[11] as observed in our data. It is important to understand the new lineages origin, but since the patient made several personal contacts, including some with close family members that were also infected with SARS-CoV-2, there was a higher risk of infection. Despite our efforts, due the high number of infected contacts that our patient had prior to and during the first infection, it was impossible to establish the source of infection. Nevertheless, reinfection studies are essential to understand whether these are isolated or widely distributed cases[17]. These cases have attracted considerable attention since they indicate that SARS-CoV-2 infections do not uniformly confer protective immunity for infected individuals.

ACKNOWLEDGMENTS

This work is an initiative of *Rede Corona-ômica* BR MCTI/FINEP affiliated to *RedeVírus*/MCTI (FINEP = 01.20.0029.000462/20, CNPq = 404096/2020-4). See Supplementary Acknowledgments.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

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SUPPLEMENTARY ACKNOWLEDGMENTS

We gratefully acknowledge the following Authors from the Originating laboratories responsible for obtaining the specimens, as well as the Submitting laboratories where the genome data were generated and shared via GISAID, on which this research is based.

All Submitters of data may be contacted directly via www.gisaid.org. Authors are sorted alphabetically.

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EPI_ISL_801386, EPI_ISL_801387, EPI_ISL_801388, EPI_ISL_801389, EPI_ISL_801390, EPI_ISL_801391, EPI_ISL_801392, EPI_ISL_8013S4, EPI_ISL_801395, EPI_ISL_801396	Laboratonode Ecologia de Doencas Transmissiveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazonia	Laboratonode Ecologia de Doencas Transmissiveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazonia	Valdinete Nascimento, Victor Souza, Andre Corado, Fernanda Nascimento, George Silva, Agatha Costa, Debora Duarte, Luciana Gonçalves, Maria Julia Brandao, Michele Jesus, Felipe Naveca on behalf of the Fiocruz COVID-19 Genomic Surveillance Network
EPI_ISL_801400, EPI_ISL_801401	Laboratorio Central de Saúde Pública do Estado do Amazonas (LACEN-AM)		
EPI_ISL_1533609	Laboratonode Ecologia de Doencas Transmissiveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazonia	Laboratonode Ecologia de Doencas Transmissiveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazonia	Valdinete Nascimento, Victor Souza, Andre Corado, Fernanda Nascimento, George Silva, Agatha Costa, Debora Duarte, Karina Pessoa, Matilde Mejia, Luciana Gonçalves, Maria Julia Brandao, Michele Jesus, Felipe Naveca
EPI_ISL_1533727	Hospital Universitario da USP Sao Paulo	lnstituto Adolfo Lutz, lnterdiciplinary Procedures Center, Strategic Laboratory	Claudio Tavares Sacchi, Claudia Regina Gonçalves, Erica Valessa Ramos Gomes, Karoline Rodngues Campos, Cmo Vimcius Dias Lopes, Leonardo Jose Tadeu de Araujo
EPI_ISL_1534001	Laboratorio Central de Saude Publica do Estado do Parana (LACEN-PR)	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz, Institute, FIOCRUZ	Paola Resende, Luciana Appolinario, Fernando Motta, Anna Carolina Paixao, Ana Carolina Mendonca, Alice Sampaia Rocha, Renata Serrano Lopes, Mana do Carma Debur, Irina Nastassja Riediger, Marilda Siqueira on behalf of the Fiocruz COVID-19 Genomic surveillance Nelwork
EPI_ISL_1534004	Laborntorio Central de Saude Publica do Estado de Sergipe (LACEN-SE)	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz, Institute, FIOCRUZ	Paola Resende, Luciana Appolinario, Fernando Motta, Anna Carolina Paixao, Ana Carolina Mendonça, Alice Sampaia Rocha, Renata Serrano Lopes, Cliomar Alves dos Santos, Marilda Siqueira on behalf of the Fiocruz COVID-19 Genomic Surveillance Nelwork
EPI_ISL_1534008	Laboratorio Central de Saude Publica do Estado de Santa Catarina (LACEN-SC)	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz, Institute, FIOCRUZ	Paola Resende, Luciana Appolinario, Fernando Motta, Anna Carolina Paixao, Ana Carolina Mendonça, Alice Sampaia Rocha, Renata Serrano Lopes, Darcita Buerger Rovaris, Sandra Bianchini Fernandes, Marilda Siqueira on behalf of the Fiocruz COVID-19 Genomic Surveillance Nelwork
EPI_ISL_1534016	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute, FIOCRUZ	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute, FIOCRUZ	Paola Resende, Luciana Appolinario, Fernando Motta, Anna Carolina Paixao, Ana Carolina Mendonça, Alice Sampaia Rocha, Renata Serrano Lopes, Marilda Siqueira on behalf of the Fiocruz COVID-19 Genomic Surveillance Nelwork
EPI_ISL_1465275, EPI_ISL_1465277	Laboratono Central de Saude Publica do Estado do Maranhao (LACEN-MA)	Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute, FIOCRUZ	Paola Resende, Luciana Appolinarto, Fernando Motta, Anna Carolina Paixao, Ana Carolina Mendonça, Alice Sampaio Rocha, Renata Serrano Lopes, Lidio Gonçalves Lima Neto, Marilda Siqueira on behalf of the Fiocruz COVID-19 Genomic Surveillance Network
EPI_ISL_1468459	Secretaria Municipal de Saude de Valparaiso SP	lnstituto Adolfo Lutz, Interdiciplinary Procedures Center, Strategic Laboratory	Claudio Tavares Sacchi, Claudia Regina Goniçalves, Erica Valessa Ramos Gomes, Karoline Rodrigues Campos, Caio Vinicius Dias Lopes
EPI_ISL_1468461	Santa Casa de Aracatuba Hospital Sagrado Coracao de Jesus		
EPI_ISL_1469584	CENTRO MUNICIPAL DE SAUDE DE ROLANTE	Epiclin	Fernando Hayashi Sant'Anna, Ana Paula Muterle, Janira Prichula, Juliona Comertato, Carolina Comerlato, Eliana Mcircia Da Ros Wendland