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Monitoring the Circulation and Impact of SARS-Cov-2 Variants on Public Health During COVID-19 Pandemic: a Case Study in a South Brazil Population

Larissa Glugoski[1]
https://orcid.org/0000-0003-1631-0647

Laís Priscila Karas[1]
https://orcid.org/0000-0002-5585-7242

Viviane Nogaroto[2*]
https://orcid.org/0000-0001-5757-9648

Fernanda Couto Miléo[1]
https://orcid.org/0000-0002-0194-3014

Ana Luiza Augustinho[1]
https://orcid.org/0000-0002-3678-8309

Mackelly Simionatto[1]
https://orcid.org/0000-0002-5445-8696

Marcos Pileggi[2]
https://orcid.org/0000-0003-1633-8295

Bruno Ribeiro Cruz[1]
https://orcid.org/0000-0001-6940-0262

Giovani Marino Fávero[1]
https://orcid.org/0000-0002-1946-3262

Marcelo Ricardo Vicari[2]
https://orcid.org/0000-0003-3913-9889

[1]Universidade Estadual de Ponta Grossa, Laboratório Universitário de Análises Clínicas, Ponta Grossa, Paraná, Brasil; [2]Universidade Estadual de Ponta Grossa, Departamento de Biologia Estrutural, Molecular e Genética, Ponta Grossa, Paraná, Brasil.

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*Correspondence: vivianenogaroto@uepg.br; Tel.: +55-42-2102-8135 (V.N.).

HIGHLIGHTS

- Screening of the Gamma, Delta, and Omicron variants by RT-PCR technique.
- Genomic surveillance of COVID-19 in the municipality of Ponta Grossa.

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the responsible for the coronavirus disease 2019 (COVID-19) pandemic. Because of its high transmissibility, allied to the emergence of some variants of concern (VOC), like Alpha, Beta, Gamma, Delta, and Omicron, the detection of these strains must be rapid and efficient. Among the tests available, the reverse-transcriptase polymerase-chain reaction (RT-PCR) is considered the gold standard test for COVID-19 detection, and the use of specific primers and probes can discriminate the different COVID-19 variants. In this study, we screened 317 individuals tested positive for COVID-19 from the municipality of Ponta Grossa (Paraná State, Brazil), from April/2021 to February/2022, aiming to identify the Gamma, Delta, and Omicron variants, by RT-PCR, using specific probes. The Gamma variant was detected from April/2021 to September/2021. The Delta variant was subsequently detected from August/2021 to November/2021. The Omicron variant was the unique strain detected from December/2021 to February/2022 and, because of its high rate of transmissibility, it caused a considerable increase in the number of COVID-19 infections. Mass testing is considered an important strategy for COVID-19 control and, the correct detection of new strains, could improve the knowledge about the virus´ behavior, permitting the development of drugs and optimized vaccines.

Keywords: Coronavirus; Delta; Gamma; Omicron; RT-PCR; vaccination.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel Betacoronavirus [1,2] that has as characteristic its higher transmissibility, which led to the coronavirus disease 2019 (COVID-19) pandemic. The infectious agent is transmitted primarily through respiratory droplets, aerosol, and direct contact with contaminated surfaces, as well as fecal-oral transmission [3,4]. The symptoms of COVID-19 vary among the individuals, ranging from asymptomatic infection to severe respiratory failure and death [5].

SARS-CoV-2 are enveloped and positive-sense single-stranded RNA viruses (+ ssRNA) [6]. SARS-CoV-2 genome comprises 14 open reading frames (ORFs), which encodes nonstructural proteins (nsp), accessory proteins and four structural proteins (spike - S, envelope - E, membrane - M, and nucleocapsid - N) [7]. SARS-CoV-2 targets cells through the S protein, via receptor-binding domain (RBD), which binds to the human angiotensin-converting enzyme 2 (ACE2) receptor, causing conformational changes in the coronavirus and allowing its fusion to the host cell membrane, which requires the cleavage of S protein by cellular cathepsin L and the transmembrane protease serine 2 (TMPRSS2) [2,8,9], allowing the releasing of the viral RNA genome into the host cell. Then the nsp are synthesized to encode the viral replicase-transcriptase complex and catalyze replication of the viral RNA genome, and inhibition of the host's innate immune response [10,11].

An innate characteristic of this viral type, due to the structure of its ssRNA genome, is a high mutational rate and consequent generation of distinct new-high transmissible SARS-CoV-2 variants. According to the World Health Organization [12], some variants of concern (VOC) have already been identified: Alpha - B.1.1.7 (earliest documented in United Kingdom, September/2020), Beta - B.1.351 (first reported in South Africa, May/2020), Gamma - P.1 (earliest documented in Brazil, November/2020), Delta - B.1.617.2 (first detected in India, October/2020), and Omicron - B.1.1.529 and descendent lineages (first time in South Africa, November/2021). These variants exhibit specific mutations in the S gene, favoring the interaction of the viral particle with the cell surface receptors ACE2, resulting in a faster synthesis of new viral copies [13,14,15]. Therefore, genetic variants of the cellular components that allow the interaction of the viral particle with the host cell are considered the targets for investigation [16].

Considering that there are many variants with different behaviors, the detection of these lineages must be efficient. Various tests have been designed for rapid COVID-19 detection. The reverse-transcriptase polymerase-chain reaction (RT-PCR) is highly specific and is considered the gold standard test for COVID-19 detection [17] since it detects specific viral RNA. Several modifications of this assay have been developed by different laboratories for testing COVID-19 samples, such the use of different primer/probe sets, targeting diverse segments of the SARS-CoV-2 genome [18]. The most usual assay used for COVID-19 involves a primer for regions of the viral nucleocapsid gene (N1 and N2), but there are assays that target the virus's RNA-dependent RNA polymerase and its envelope genes [17]. RT-PCR assays are designed as a two-target system in which one primer universally detects numerous coronavirus strains (it is recommended that the E gene), and a second primer set exclusively detects SARS-CoV-2 (in general, the RdRp gene) [17].

Moreover, different clinical samples displayed different sensitivities for SARS-CoV-2 detection [19]. A nasopharyngeal swab collection is recommended for RT-PCR COVID-19 assays, since for most individuals during the initial infection period, a single nasopharyngeal swab may harbor up to one million SARS-CoV-2 viral particles, attesting that nucleic acid tests offer the most sensitive tool for SARS-CoV-2 detection [20].

Various detection assays have been performed, but diagnostic challenges persist, especially considering the newly emerged variant strains [21]. Single-nucleotide polymorphisms (SNPs) are the most frequent variants in the genome of the SARS-CoV-2 [22] and some reports recommend the diagnostic target selection and optimization based on nucleotide-based and gene-based mutation-frequency analysis [23].

Mass testing is an important strategy for COVID-19 control. For example, in South Korea, mass testing programs and isolation contributed to early infection control [24]. However, the identification of cases is considered a challenge in low-income and middle-income countries, like Brazil [25]. The objective of this study was to perform genomic surveillance of COVID-19 in the municipality of Ponta Grossa (Paraná State, Brazil), so we screened the variants Gamma, Delta, and Omicron by RT-PCR technique in patients diagnosed as positive for COVID-19, comprising the period April/2021 to February/2022.

MATERIAL AND METHODS

The procedures were in accordance with the Comissão Nacional de Ética em Pesquisa (CONEP ID 4.833.527) and hospital scientific committee from Hospital Universitário Regional dos Campos Gerais - Wallace Thadeu de Mello e Silva (HURCG - project number 172). The analyzes comprised the period of April/2021 to February/2022 and included patients with mild or moderate clinical symptoms, hospital professionals from HURCG, in the general population from the Campos Gerais region (Ponta Grossa and neighboring cities in Paraná State, Brazil), who were attended at the laboratory for COVID-19 diagnosis. The research participants signed the informed consent form. Patients in the intensive care unit (ICU) on HURCG were excluded from the research due to disease severity, particularly because these individuals had already outdated the active viral phase period at the internalization moment.

Nasopharyngeal swab samples were collected in appropriated viral transport medium and the RT-PCR assays were performed at the Laboratório Universitário de Análises Clínicas (LUAC) from State University of Ponta Grossa. For RNA extraction, approximately 300 µL of viral medium were subjected to an automated magnetic extraction procedure (EC Xtract plate, Nova Biotecnologia) on a Nucleic Acid Purification System-32 - Spirall robot. Two distinct regions of SARS-CoV-2 genome were analyzed for COVID testing. cDNA synthesis and RT-PCR assays were performed using a master mix containing a set of primers and probes for SARS-CoV-2 detection (One-step RT-PCR assay kit, Nova Biotecnologia), according to manufacturer's instructions, on a thermocycler Mx3005P qPCR System (Stratagene). Samples presenting a cycle threshold (Ct) ≤ 30 on the COVID-19 diagnostic test were posteriorly selected for the VOCs approach. Samples with Ct values > 31 on the COVID-19 RT-PCR test did not full out the requirements for variant-specific mutation detection assays by RT-PCR and were removed from the study.

After filtering steps (be internalized in UCI, more severe disease, and Ct ≤ 30 in the COVID-19 RT-PCR diagnosis), the research sampled 317 individuals (141 male and 176 female) positive for COVID-19. RT-PCR assays for VOC detection consisted of GoTaq® 1-Step RT-qPCR System kit (Promega), in addition to mutation-specific probes (TaqMan™ SARS-CoV-2 Mutation Panel Assay, Thermo Fisher Scientific), aiming to analyze the allelic discrimination among the variants Gamma - E484K (ID: ANU7GMZ; Thermo Fisher Scientific), Delta - L452R and P681R (ID: CVAAAAD and ID: CVEPRY4, respectively; Thermo Fisher Scientific), and Omicron - K417N (ID: ANZTTXP; Thermo Fisher Scientific), according to manufacturer's instructions.

Individual information about COVID-19 typical symptoms, including fever, tiredness, headache, cough, and sore throat, among others, were reported by some patients at the time of the test. Additionally, the individuals reported data about their COVID-19 vaccination schedule at the moment of the test, and we considered as immunized who get the 2nd dose of the Pfizer, AstraZeneca, and CoronaVac COVID-19 vaccines, or the 1st dose of Jansen.

RESULTS

Among the sampled individuals that participated in the research (n = 317), only 218 described what COVID-19 symptoms they were experiencing at the moment of collection. All individuals assessed were Ponta Grossa inhabitants or residents in neighboring cities. Cough, headache, and fever were the most cited symptoms for patients carrying Gamma (54.31%, 59.48%, and 38.79%, respectively) and Delta variants (64.79%, 49.30%, and 54.93%, respectively) (Figure 1). For Omicron, the most common symptoms were cough (37.69%), sore throat (33.08%) and myalgia (33.08%), among others (Figure 1). The loss of smell and taste were common in patients with Gamma (33.62%) and Delta (33.80%) variants, but rare in individuals with Omicron variant (6.15%) (Figure 1). At the time of infection, 50 individuals reported that they had already had their COVID-19 vaccination schedule completed (i.e., at least two doses), which presented mild illness symptoms and did not need hospitalization.

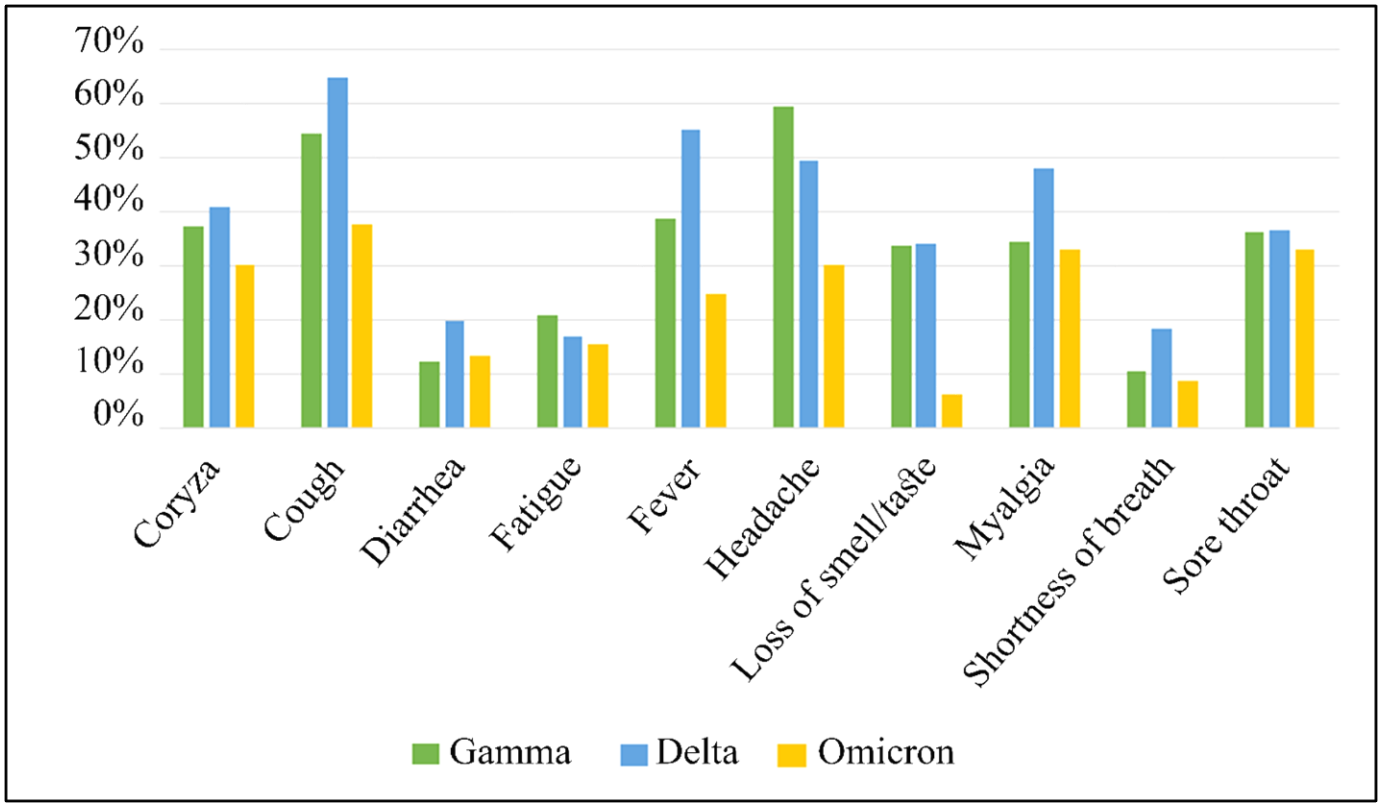

Figure 1. Typical COVID-19 symptoms presented by the sampled individuals, according to each variant (Gamma, Delta, and Omicron).

The Gamma variant was detected in 116 patients who assessed positive for COVID-19, from the months of April to September/2021, being a unique strain identified in the samples between the months of April and July (Figure 2). In June, it was detected the highest number of cases of this variant (43.96% of individuals positive for Gamma), which became much less frequent in September (only two cases).

The Delta variant was present in 71 patients and was first detected in August/2021 in the analyzed patients and was present until November (Figure 2). The incidence of this strain rapidly increased in August, when it reached a peak in the analyzed Delta cases (64.78%), being a unique variant detected on months of October and November.

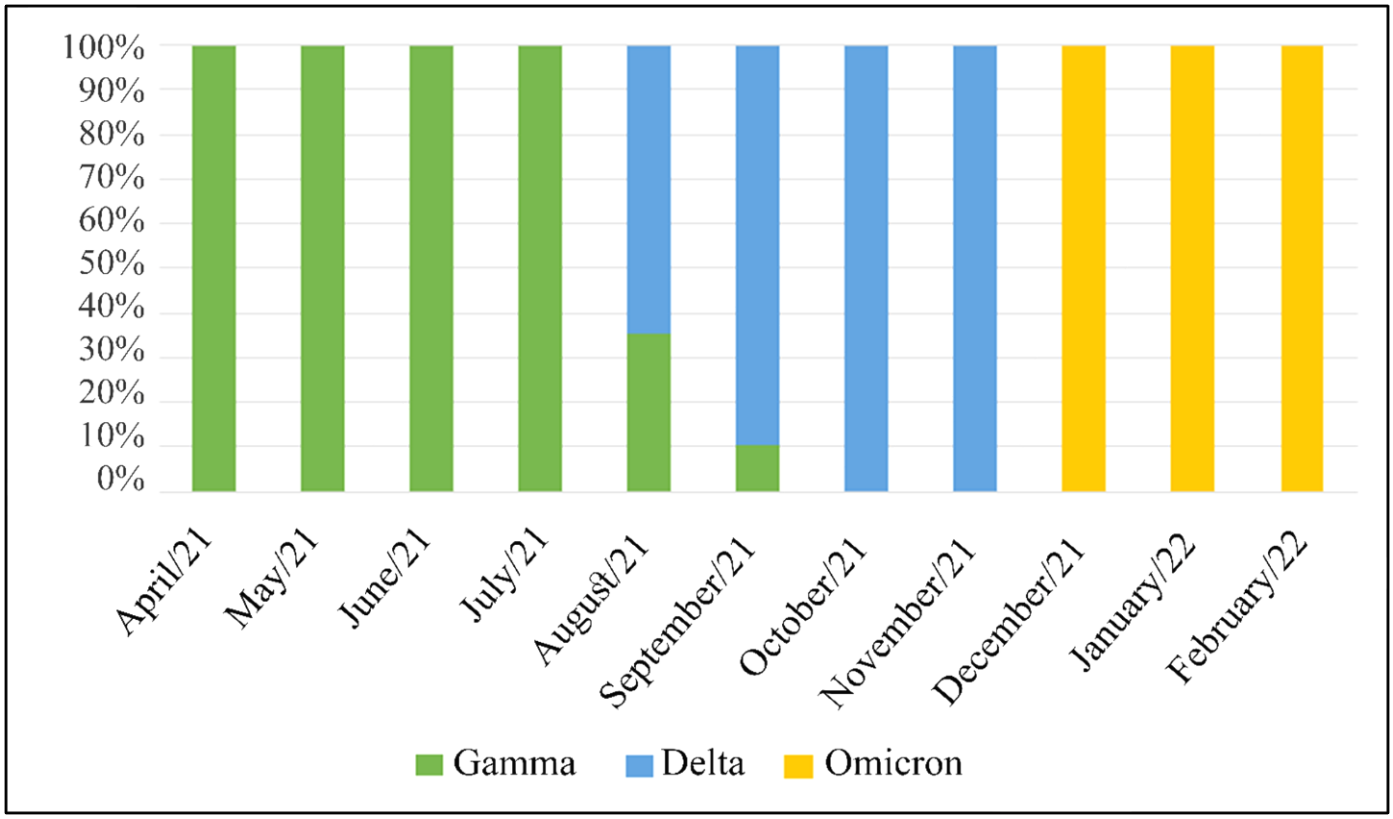

Figure 2. Prevalence of variants Gamma, Delta, and Omicron from April/2021 to February/2022.

The Omicron variant was detected in 130 patients from December/2021 to February/2022, being the unique strain presented in the samples analyzed in these months (Figure 2). According to our data, January was the most prevalent in the number of cases of omicron (84.61%).

DISCUSSION

The identification of variants by RT-PCR in patients positive for COVID-19 allowed the screening of mutations present in Gamma, Delta, and Omicron variants of SARS-CoV-2 in the Campos Gerais region and the circulation profile of these lineages throughout the analyzed period (April/2021 to February/2022). In a pandemic scenario, the knowledge of the genetic variation may improve the understanding of the pathogenesis, since some polymorphisms that impact disease susceptibility are considered important tools, since millions of people may be infected [16]. Additionally, this knowledge may improve the information for drug design and vaccine development [26].

The Gamma variant (P1) was a unique variant detected in our analyzes from April/2021. First detected in Manaus - Amazonas, Brazil (and named as “Brazilian variant”), this variant accumulated ten mutations in the S gene, including E484K and N501K [15]. Additionally, it may cause reinfection in individuals who have already had COVID-19 [15], as reported by few individuals that participated in our research (data not shown). During Gamma prevalence, Brazil experienced a period of vaccination starting, mainly with priority public (i.e., old, health workers, and immunosuppressed people). UCI occupation and lethality were extremely high, mainly among unvaccinated people. However, our study sampled subjects with mild and moderate symptoms of COVID-19, most of whom had already had one dose of the vaccine. The beginning of Gamma variant reduction from the middle of August, as well as its rapid decline in the subsequent month, is a result of the emergence of the Delta variant.

In our analysis, the Delta variant was first detected in August/2021, being prevalent at the end of this month, as well as on September/2021 and presented as an exclusive strain on October/2021. This variant presents 13 mutations that result in amino acid changes. The L452R and E484Q mutations, located at the S gene, have been routinely used to reference this strain, and the latter could be involved in the immune escape of the virus in those patients infected with SARS-CoV-2 [27]. Additionally, the Delta variant origin generated an alarm in public health workers due to its increase in transmissibility. The mutation P681R present in the Delta variant falls within an intensely studied region of the S protein, called the furin cleavage site [28,29]. The Delta was considered more contagious than previous variants based on the furin cleavage site changes [28,29]. The emergence of the Delta in Brazil did not impact the lethality of the population, as seen in the

Gamma variant. According to Adamoski and coauthors [30], considering the Paraná State, this fact is probably due to the advance of vaccination or by the close wave of Gamma infections, which was still ongoing when the first Delta cases were confirmed in the state.

From December/2021, Delta variant was replaced by Omicron, which was exclusively reported as unique variant in our analyses, and its rapid increase, resulted from its high rate of transmissibility, became evident in subsequent months. Although Omicron has caused a considerable increase in the number of COVID-19 infections, the number of deaths remained at low levels, because to both the reduced lethality of the Omicron variant and the increased population immunity due to vaccination and previous infection [30]. In Paraná State, about 70% of the population had the complete vaccination for SARS-CoV-2 during this period [30].

Among the sampled individuals, just one old patient progressed to a severe case and death. In fact, our findings indicated that many sampled individuals from November onwards, who had mild symptoms, already had their complete vaccination schedule (considering who get the 2nd dose of the Pfizer, AstraZeneca and CoronaVac COVID-19 vaccines, or the 1st dose of Jansen), or at least the 1st dose of any COVID-19 vaccine (data not shown). Additionally, the low intrinsic pathogenicity of the Omicron variant, allied to high seroprevalence of the population, caused the higher incidence of COVID-19 cases in the state, but with the lowest lethality rate of the entire pandemic [30].

The update of primer and probe sequences is crucial for COVID-19 new variant detection. Sequencing of the viral genome helps identify new variants of coronavirus, monitor mutations, and subtype classification and is less valuable in diagnosis because of its capacity and excessive cost [20]. The data presented allowed proving the high sensitivity of the probes/primers in the detection of different types of COVID-19 variants, in the case of this study, the Gamma, Delta, and Omicron variants. Therefore, the use of a specific combination of primers and probes enables the detection of new strains faster and more accurate using RT-PCR. The qualitative detection of SARS-CoV-2 antigens by RT-PCR is a crucial tool for public health epidemiological surveillance in screening the emergence of new SARS-CoV-2 variants, allowing some measures to be taken with the aim of minimizing the risks of new waves of infection.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet. 2003;361:1319-25.
2. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270-3.
3. Yu ITS, Li Y, Wong TW, Tam W, Chan AT, Lee JHW, et al. Evidence of airborne transmission of the severe acute respiratory syndrome virus. N Engl J Med. 2004;350:1731-9.
4. Otter JA, Donskey C, Yezli S, Douthwaite S, Goldenberg SD, Weber DJ. Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. J Hosp Infect. 2016;92:235-50.
5. He F, Deng Y, Li W. Coronavirus disease 2019 (COVID-19): What we know? J Med Virol. 2020;92:719-25.
6. Gorbalenya AE, Baker SC, Baric RS, Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5:536-44.
7. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol. 2009;7:439-50.
8. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271-80.
9. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host & Microbe. 2020;27:325-8.
10. Thiel V, Ivanov KA, Putics Á, Hertzig T, Schelle B, Bayer S, et al. Mechanisms and enzymes involved in SARS coronavirus genome expression. J Gen Virol. 2003;84:2305-15.
11. Gildenhuys S. Expanding our understanding of the role polyprotein conformation plays in the coronavirus life cycle. Biochem J. 2020;477:1479-82.
12. WHO Tracking SARS-CoV-2 Variants. [Internet]. [updated 2022 Jul 20]. Available from: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants

13. Daniloski Z, Jordan TX, Ilmain JK, Guo X, Bhabha G, tenOever BR, et al. The Spike D614G mutation increases SARS-CoV-2 infection of multiple human cell types. Elife. 2021;10:e65365.
14. Chaillon A, Smith DM. Phylogenetic analyses of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) B.1.1.7 lineage suggest a single origin followed by multiple exportation events versus convergent evolution. Clin Infec Dis. 2021;73:2314-7.
15. Sabino EC, Buss LF, Carvalho MPS, Prete CA Jr, Crispim MAE, Fraiji NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet. 2021;397:452-5.
16. Figueiredo DLA, Ximenez JPB, Seiva FRF, Panis C, Bezerra RDS, Ferrasa A, et al. COVID-19: The question of genetic diversity and therapeutic intervention approaches. Genet Mol Biol. 2022;44:e20200452.
17. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25:2000045.
18. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. Nat Microbiol. 2020;5:1299-305.
19. Sule WF, Oluwayelu DO. Real-time RT-PCR for COVID-19 diagnosis: challenges and prospects. Pan Afr Med J. 2020;35:121.
20. Zhou Y, Zhang L, Xie YH, Wu J. Advancements in detection of SARS-CoV-2 infection for confronting COVID-19 pandemics. Lab Invest. 2022;102:4-13.
21. Thomas E, Delabat S, Carattini YL, Andrews DM. SARS-CoV-2 and variant diagnostic testing approaches in the United States. Viruses. 2021;13:2492.
22. Yin C. Genotyping coronavirus SARS-CoV-2: Methods and implications. Genomics. 2020;112:3588-96.
23. Wang R, Hozumi Y, Yin C, Wei GW. Mutations on COVID-19 diagnostic targets. Genomics. 2020;112:5204-13.
24. Kim YJ, Sung H, Ki CS, Hur M. COVID-19 testing in South Korea: current status and the need for faster diagnostics. Ann Lab Med. 2020;40:349-50.
25. Turci MA, Holliday JB, Oliveira NCVC. A vigilância epidemiológica diante do Sars-Cov-2: desafios para o SUS e a atenção primária à saúde. Aps Em Revista. 2020;2:44-55.
26. Sironi M, Hasnain SE, Rosenthal B, Phan T, Luciani F, Shaw MA, et al. SARS-CoV-2 and COVID-19: A genetic, epidemiological, and evolutionary perspective. Infect Genet Evol. 2020;84:104384.
27. Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe. 2021;29:463-76.
28. Zhang J, Xiao T, Cai Y, Lavine CL, Peng H, Zhu H, et al. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science. 2021;374:1353-60.
29. Liu Y, Liu J, Johnson BA, Xia H, Ku Z, Schindewolf C, et al. Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. Cell Rep. 2022;39:110829.
30. Adamoski D, Baura VA, Rodrigues AC, Royer CA, Aoki MN, Tschá MK, et al. SARS-CoV-2 Delta and Omicron variants surge in Curitiba, Southern Brazil, and its impact on overall COVID-19 lethality. Viruses. 2022;14:809.