Chapter

Perspective Chapter: Real-Time Genomic Surveillance for SARS-CoV-2 on Center Stage

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Abstract

The course of the COVID-19 pandemic depends not only on how the SARS-CoV-2 virus mutates but on the actions taken to respond to it. Important public health decisions can only be taken if we know viral dynamics, viral variants distribution, and whether new variants are emerging that may be more transmissible or/and more virulent, displaying evasion to vaccines or antiviral treatments. This situation has put the use of different approaches, such as molecular techniques and real-time genomic sequencing, to support public health decision-making on center stage. To achieve this, robust programs based on: (i) diagnostic capacity; (ii) high-throughput sequencing technologies; and (iii) high-performance bioinformatic resources, need to be established. This chapter focuses on how SARS-CoV-2 evolved since its discovery and it summarizes the scientific efforts to obtain genomic data as the virus spread throughout the globe.

Keywords: genomic surveillance, SARS-CoV-2, variant of concern, sequencing, PCR, genotyping, phylogenetic trees

1. Introduction

At the early stages of the COVID-19 pandemic, sequencing the full genome of SARS-CoV-2 was key to investigate the newly emerging outbreak of pneumonia in Wuhan, China. In addition, this fact provided evidence that it was being caused by a novel virus belonging to the family *Coronaviridae* [1–3]. The viral sequence became public very rapidly, permitting the scientific community to carry out analyses and pandemic preparedness to start promptly [4]. Back then, having available the full viral genomic sequence made possible the development of rapid and affordable molecular diagnostic tools to isolate infectious patients (symptomatic and asymptomatic). This was the first weapon to control the disease spread, given the lack of approved therapeutics and vaccines at that time [5, 6]. Later on, genomic surveillance of the virus played a center role in the prevention and control of the disease throughout the course of the pandemic [7]. In fact, it made it possible to study many different aspects of the disease, such as the transmission patterns of the virus, the time and from where the virus was introduced into a country, and local and superspreading events. Most notably, genomic surveillance was key to track virus evolution, evidencing the emergence of genomic variants worldwide. Those variants that are more transmissible or virulent, and/or can decrease the effectiveness of treatments, vaccines, and public health measures were defined, by the WHO - in consultation with the Technical Advisory Group on Virus Evolution - as variants of Concern (VOC) [8]. To date, five VOCs (named Alpha, Beta, Gamma, Delta, and Omicron) have emerged at different times and places as a result of viral evolution displaying different features compared to the first strain isolated from Wuhan. Among these, Alfa, Beta, Gamma, and Delta are now designated former VOCs as they appear to no longer circulate in the population. Omicron and its descendent sub-lineages are, at the time of writing this chapter, the only circulating VOCs [8]. The five VOCs have demonstrated to be able to act as 'game changers', reshaping infection dynamics and causing new waves of infections in many countries. Periodic genomic sequencing of viral samples kept the world informed in a global pandemic setting and facilitated proper public health measures to be made. Implementation of comprehensive real-time genomic surveillance programs is vital for monitoring, detecting, and characterizing new variants, helping sanitary authorities to better manage the crisis.

2. SARS-CoV-2 then

Whole-genome sequencing of specimens of an outbreak of pneumonia in Wuhan, China in December 2019, led to the discovery of a previously uncharacterized virus capable of infecting humans [2]. The first annotation of the complete 29,903 nucleotide-length genome of SARS-CoV-2 revealed it was a positive-sense, single-stranded RNA virus from the genus Betacoronavirus (β-CoVs). Comparative phylogenetic analysis shed light on the genomic organization of SARS-CoV proving that it shared key structural similarities with coronaviruses including SARS-CoV the causative agent of the severe acute respiratory syndrome outbreak in Asia in 2003. In addition, this novel virus, like many other members of the β-CoVs genus, had its evolutionary roots in viruses known to commonly infect bats [9, 10]. Yet, none of the SARS-CoV-2 related coronaviruses that can be found in public databases present more than 99% similarity to SARS-CoV-2 across the genome as a whole, suggesting that none of these viruses could be its direct ancestor. Efforts to find possible reservoirs and/or an intermediate host of the virus in wild animals' reservoirs, mostly bats and pangolins, had still not made clear the exact emergence event of the virus in the human population. The genome of SARS-CoV-2 has a rather mosaic pattern, to which different progenitors seem to contribute [11].

The closest related bat-borne virus at the whole-genome level identified so far is RaTG13 (from R. affinis, China, 2013), sharing 96.2% identity with SARS-CoV-2 [3]. Despite its apparent higher percentage of similarity, the receptor binding domain (RBD) sequence of the Spike (S) protein of SARS-CoV-2 shows a significant divergence from the RaTG13 strain. RaTG13 lacks the four-residue (PRRA) insertions at the furin cleavage site on the S protein, essential for viral binding to human cell receptors and infection [12, 13]. Furthermore, the authors referenced in [13] demonstrated the binding affinity between the RaTG13 RBD and human angiotensin-converting enzyme 2 (hACE2) to be approximately 70-fold lower than that between the SARS-CoV-2 RBD and hACE2. Further phylogenetic analysis identified pangolin-derived coronaviruses clustering with RaTG13 and SARS-CoV-2 and sharing a higher amino acid similarity to the RBD of SARS-CoV-2 (97.4%) [14]. This analysis raised the possibility that SARS-CoV-2 might have originated from a recombination event of a virus similar to pangolin-CoV with one similar to RaTG13 [15, 16]. Other groups have identified coronaviruses sampled from bats that shared higher similarity to the RBD of SARS-CoV-2, as STT182 and STT200 sampled from Cambodia [17] and BANAL-52 and BANAL-103 sampled from Laos [18]. Of note, comparative sequence analysis of the viruses sampled in Laos showed that those viruses have an RBD with only one or two amino acidic mismatches at the 17 residues that interact with the hACE2 receptor. Many more groups continued collecting genomic sequence data of coronaviruses and sampling animal reservoirs to better understand the exact spillover event and emergence process of SARS-CoV-2. These studies are of high importance due to the latent threat of the emergence and re-emergence of infectious diseases from animal origins.

3. Understanding SARS-CoV-2 long game: mechanisms for viral evolution and variant emergence

SARS-CoV-2 has acquired many mutations over the course of the pandemic, resulting in altered viral replication and transmission. One of the mechanisms that can explain the generation of new genomic variants is that, SARS-CoV-2 as well as all CoVs, rely on an error prone RNA-dependent RNA polymerase (RdRp) to replicate within the host's cells to produce more viral particles [19]. The errors that occurred during replication, however, are corrected by nsp14-ExoN, a 3′-to-5′ proofreading exoribonuclease that acts on both ssRNA and dsRNA, which helps the virus to reduce its error rate 100–1000-fold compared to other RNA viruses [20]. This repair mechanism however is not flawless, resulting in the retention of some of the mutations during replication. The error rate for SARS-CoV-2 replication has been estimated to be in the range of 10^{-4}–10^{-3} nucleotide substitutions per site per year, which means the viral genome can acquire approximately two mutations per month [21]. Although most acquired mutations are synonymous - producing a change in the ARN sequence but not the amino acid composition- some are non-synonymous, which allows the virus to acquire a different amino acid composition. When these mutations help the virus to reproduce and transmit better in the host, either by improving the virus intrinsic fitness, the interaction with key host cell components, or by permitting the virus to escape from the host immune system, they exert a positive selection force resulting in the appearance of new variants [22].

Another mechanism that has been proposed to drive SARS-CoV-2 variants' emergence is recombination [23–26]. For positive sense-RNA viruses, recombination can occur within a cell that was coinfected with more than one genomic species via a process known as 'strand switching' mediated by the viral RdRp, producing chimeric subgenomic RNA and proteins [27, 28]. Recombination has been commonly observed in β-CoVs, most notably in SARS-CoV and MERS-CoV [27, 29–31]. Unlike the mutations acquired due to errors occurring via the replication process, recombination allows the virus to acquire larger genome sections more quickly and causes dramatic changes in the SARS-CoV-2 phenotype [32, 33]. These recombination events have been identified throughout the genome, but most of them have been detected in the ORF1a and N-terminus regions of the S protein [25]. Mutations in the S protein have been of more attention as it plays a critical role in viral infection and immunity, although mutations in other genes can play a role in viral replication and fitness.

4. Tracking SARS-CoV-2 evolution worldwide

Tracking the evolution of SARS-CoV-2 was made possible by the state-of-the-art molecular and bioinformatic tools that were rapidly adapted for an efficient data-driven response against the COVID-19 pandemic. Although these tools are now used widely, they did not exist a couple of decades ago. SARS-CoV-2 is the first virus to which its evolution has been monitored and assessed in real time since its discovery, leading to the accumulation of an unprecedented volume of data - more than 11 million SARS-CoV-2 genomes have been sequenced up to the time of writing this chapter [34].

4.1 Whole-genome sequencing

Next-generation sequencing has been the major molecular tool to identify and study viral genomic variants. Its widespread application turned out to be possible thanks to the extensive collaborative efforts of the scientific community for developing standardized sequencing protocols and making public the bioinformatics workflows for consensus genome assembly. The main method used for the identification of SARS-CoV-2 variants relied on sequencing the whole genome of the virus. For this purpose, several sequencing technologies have been employed, including Illumina, Oxford Nanopore, Pacbio, Ion Torrent, BGI, Sanger and Qiagen; where the first two remain the most used platforms. The protocol for sequencing SARS-CoV-2 by these two technologies employs a tailed amplicon sequencing approach. The protocol for nanopore sequencing of tiled PCR-generated amplicon pools was developed by the ARTIC Network [35] in 2017 for sequencing Ebola, Zika, and Chikungunya viral genomes [36]. It has been adapted throughout the course of the pandemic in order to convert into a rapid and cost-effective method to acquire high-quality genomic data at a great scale [37]. This method proved to be helpful for obtaining whole viral genomes from clinical samples with limited viral genomic material promptly, as it is the only technology capable of sequencing in real-time long-read nucleic acid sequences. Other approaches like sequence capture methods, that enrich libraries by using sequence capture with a respiratory virus panel containing probes against SARS-CoV-2, have also been used particularly as a tool for recovering more data for low input samples [38].

Having the information on the entire viral genome not only permits us to identify viral variants, but it is also essential to perform phylogenetic analysis to study viral evolution. It has helped scientists to answer several questions, for example, understanding whether an outbreak was caused by imported viruses or by community transmission, through tracking when and where new mutations are introduced in a geographical region [39]. This information was particularly valuable at the beginning of the pandemic when there were no treatments available, as it provides evidence of high-risk transmission routes prompting enhanced public health control measures. Additionally, phylogenetics was also used to monitor the effectiveness of global travel restrictions and lockdowns. Later, with the appearance of the variants of concern harboring distinctive mutations, having their whole-genome sequence provided valued data for understanding their emergence and distribution across the globe and also to investigate the risk associated with specific mutations.

4.2 RT-qPCR

Another molecular tool that turned out to be extremely valuable to investigate the prevalence of emerging viral variants is multiplex real-time RT-qPCR [40]. This assay has been employed by different groups to identify genomic fingerprints associated with emerging VOCs. VOCs specific mutations, such as amino acid S deletion 69–70 (del69-70) found first in Alpha and later in Omicron BA.1, S deletion 241 in VOC Delta, and ORF1a nucleotide deletion 3675–3677 in VOC Gamma and Beta, generate a negative or a significantly weaker positive result in the PCR when the target probe is designed to align with the deletion. This failure of the amplification target caused by the specific mutation in a gene is known as gene target failure or gene dropout [41–43]. Choosing this method works perfectly for monitoring the introduction of a variant in a population when another one is already circulating at high prevalence, as it becomes evident very quickly when the two variants display a different PCR gene dropout pattern [44]. For example, this method was used to evidence the introduction of the VOC Gamma into Uruguay, as this VOC has the ORF1ab deletion which displaced the other lineages with no ORF1ab deletion circulating before Gamma introduction [45]. The use of this method is more convenient in resource-limited settings compared to sequencing, as the latter is time-consuming, costly, and requires extensive data processing [46]. RT-qPCR-based variant analysis of SARS-CoV-2 is rapid, low-cost, and does not require bioinformatics expertise. Another TaqMan-based RT-qPCR that was widely used to estimate VOC prevalence was an assay that can detect specific amino acid substitutions or single nucleotide polymorphisms (SNPs) present in VOCs, particularly in the S gene [47]. Ascertaining SNPs allowed certain countries to estimate the prevalence of the variants carrying specific mutations in a population.

Employing the methods mentioned above, genomics consortiums – networks of multidisciplinary workgroups with diverse expertise – were created in many countries around the world for real-time monitoring for SARS-CoV-2 genomic variants with public health implications. Following a sentinel strategy, samples that tested positive for SARS-CoV-2 are collected weekly from different regions within a country in order to adequately represent the geographic spread, to then send them for whole-genome sequencing to a laboratory that can perform molecular and bioinformatics analysis. As an example of this strategy, genomic surveillance of SARS-CoV-2 in Uruguay during the firsts months of 2021 was focused on a representative number of samples collected from all provinces of the country, as a need for detecting the imminent introduction of the VOC Gamma promptly, as this variant was circulating at great scale in neighbor countries (mostly Brazil and Argentina) by the time Uruguay was just starting to vaccinate its population. Phylogenetics analysis revealed that SARS-CoV-2 was introduced into Uruguay from multiple routes to then become the most prevalent variant in a matter of weeks, most likely causing the first wave of coronavirus in the country [45]. Later on, when Uruguay started to ease travel restrictions due to low positive cases and a high vaccination percentage, genomic surveillance started to focus mostly on the variants that could be imported by international travelers and their close contacts in the community. In this way, the introduction of the VOC Delta, Beta, and Alfa was detected in travelers, where only Delta surpassed the prevalence of Gamma that was previously circulating in great proportion.

4.3 Nomenclature schemas to explain SARS-CoV-2 phylogeny

Different standardized nomenclatures schemas have been developed by bioinformatic investigators in charge of tracking SARS-Cov-2 population dynamics to define and explain the divisions of the different viral genomes circulating globally.

There are currently mainly three schemas. Two are based on clade separation: the Nextstrain and GSAID [34, 48], where a clade is defined as a group of organisms that include a single ancestor in all its descendants. Both Nextstrain and GSAID aim to provide generic categorization of globally circulating diversity. The other schema, Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin) was developed to implement the dynamic nomenclature of SARS-CoV-2 lineages and to correspond to outbreaks [49]. Later on, the WHO recommended using letters of the Greek alphabet to designate viral variants in order to make the use of the nomenclature schemas easier for the general public without previous knowledge in bioinformatics and to ease the discussion about the topic [8].

5. Variants of concern

As of June 2022, there were five reported lineages defined as VOCs by the WHO [8]. Alpha, Beta, Gamma, and Delta were all first detected between late 2020 and January 2021. Omicron was the fifth emerging VOC, first reported in South Africa a year later in November 2021. Phylogenetic analysis of SARS-CoV-2 whole genomic sequences shows clearly that these variants did not emerge one from another but that they emerged from the ancestral SARS-CoV-2 genotype (Wuhan-Hu reference sequence) as shown in **Figure 1**.

VOCs have acquired many mutations independently by convergent evolution, which provided them with either enhanced transmissibility, higher severity of disease, or lower neutralization capacity by vaccine and/or sera from people who recovered from COVID-19. Most of these mutations are located at the subunit 1 (S1) of the spike protein, especially in the RBD and NTD domains. Interestingly, although VOCs are genetically divergent, they share many amino acid mutations at the S1 subunit as schematized in **Figure 2**, whereas they do not share mutations at the subunit 2 (S2). This phenomenon is hypothesized to have occurred most likely because the S1 subunit is the most immunodominant viral region and the one under a higher and constant selective pressure, as 90% of the neutralizing antibodies found in COVID-19 convalescent plasma were found to block the RBD [51].

5.1 Alpha

The VOC Alpha – defined as Pango lineage B.1.1.7 and Nextstrain clade 20I/V1– was the first VOC, identified by the COVID-19 Genomics UK Consortium (CoG-UK). It was first sampled in late September of 2020 [52] from a rapid rise in cases in the southeast of England during a national lockdown and caused a second wave of infections in December 2020 [44, 53]. It has 17 mutations located at the S, N, ORF1ab, and ORF8 genes and exhibited around a 50% increase in transmissibility over previously circulating lineages [44, 54]. The several amino acid mutations carried in S protein were proven to be of epidemiological concern as they play a crucial role in the infectivity and pathogenicity of the virus. The N501Y substitution (also found in

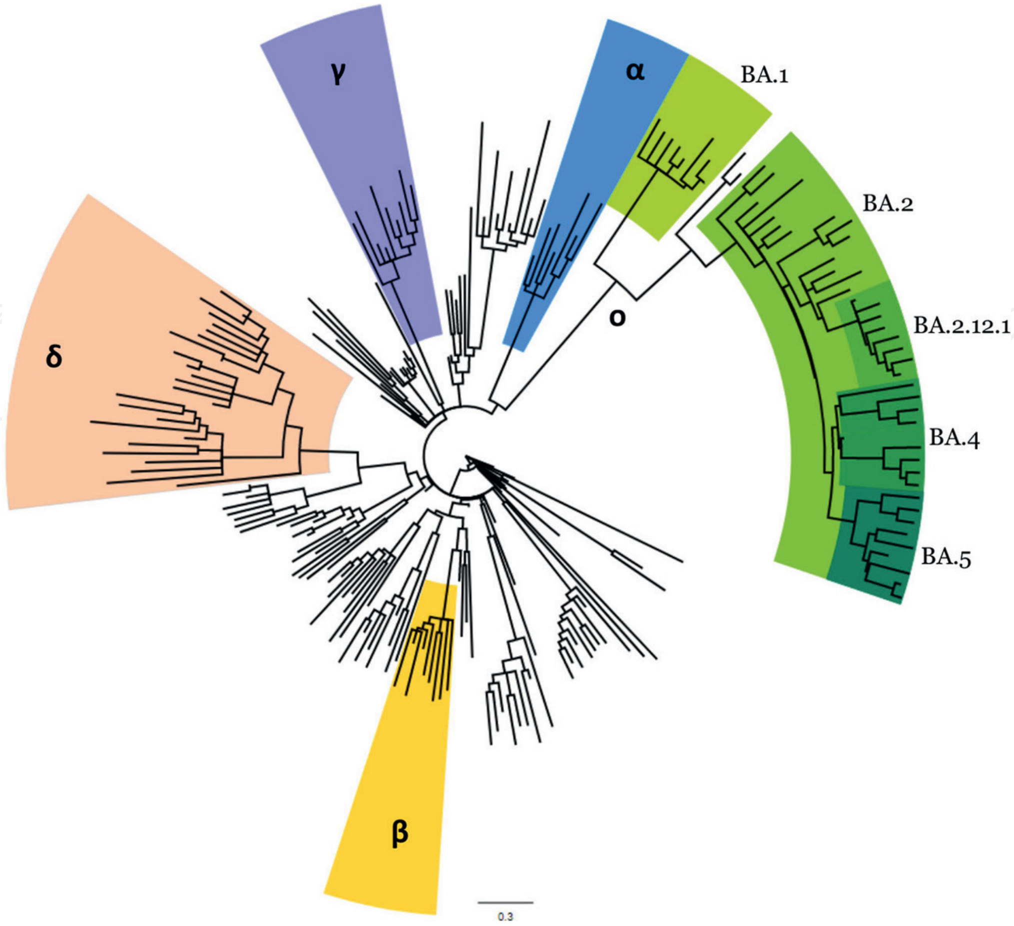


Figure 1.
Phylogenetic tree showing divergent SARS-CoV-2 VOCs emerging from the ancestral Wuhan Hu-1 strain sequence. Data was downloaded from Nextstrain [48] under a CC BY 4.0 license and corresponds to a subsampling of reference genomic sequences from GISAID [34]. The phylogenetic tree was edited with FigTree v1.4.4 [50].

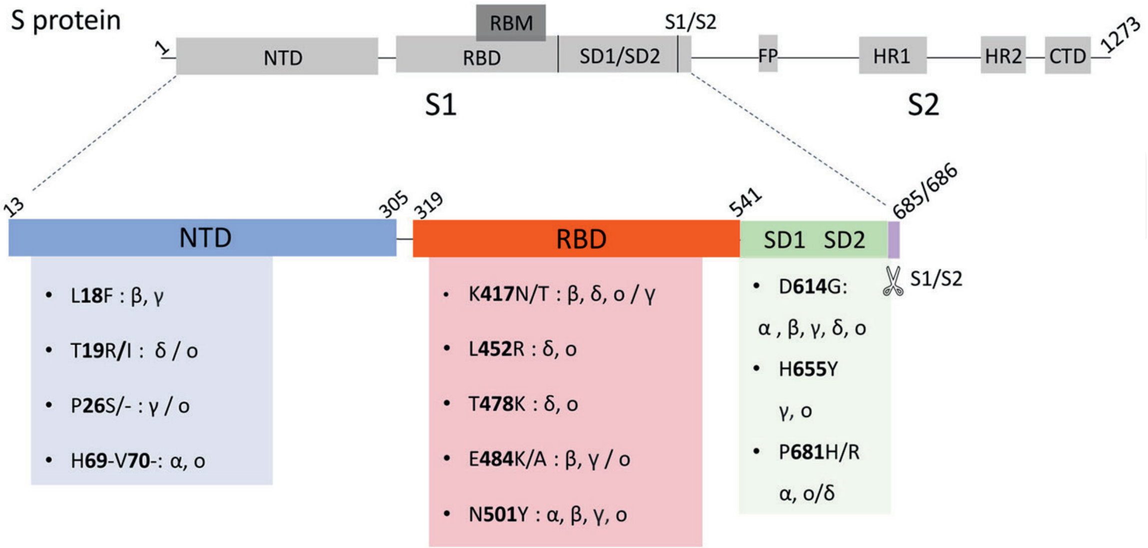


Figure 2.
Shared amino acid mutations of VOCs at the S protein S1 subunit. NTD, amino-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; SD, subdomain; S1/S2, the junction between the exposed S1 attachment domain and the partially buried S2 fusion domain; FP, fusion peptide; HR, heptad repeat; CTD, C-terminal domain.

Beta, Gamma, and Omicron) located at the RBD, increases the binding affinity to the human ACE2 receptor and improves transmissibility [55]. The authors referenced in [56] developed a mouse-adapted strain model (MASCp6) to evaluate the SARS-CoV-2 infectivity and virulence after intranasal inoculation and observed that the N501Y mutation favors interaction with ACE2 and promotes virus entry, and increases infectivity in mouse lung tissue. Recent studies have suggested that the N501Y mutation has a low impact on clinical outcomes and pathogenicity [53, 57] and on the immune response generated by monoclonal antibodies (mAbs), vaccines, or previous infections [58, 59]. Conversely, in the work referenced in [60], the group evaluated more than 2.2 million people with SARS-CoV-2 positive tests and 17,452 related deaths in England and observed a 61% higher mortality rate in those infected with the B.1.1.7 variant than other pre-existing variants. Likewise, another group that assessed the mortality rate of this variant showed Alpha appears to have a 30% higher mortality rate along with other variants of SARS-CoV-2 [61]. Moreover, an increased likelihood of hospitalizations was observed for Alpha cases compared to non-Alpha cases [62]. Thus, this variant presented increased transmissibility and virulence and increased the risk of contracting a more severe disease. The P618H substitution on the S protein is reported as a key determinant for efficient SARS-CoV-2 transmission. It is located immediately adjacent to the furin cleavage site, which is essential for viral entry into the host's cell [63]. Alpha also possesses two amino acid deletions (del69-70) in the S1 of the S protein. It was demonstrated that this variant requires this deletion for efficient cleaved S protein incorporation and infectivity, thus promoting cell entry and cell-to-cell fusion activity [64].

Several studies have demonstrated that existing vaccines remained protective of this variant. For example, the susceptibility of Alpha to mRNA vaccines, specifically the BNT162b from Pfizer Biontech has not been seen to be affected in great measure. Cohort studies from Israel and Qatar – which were the first countries to have a high vaccination rate at the time Alpha was spreading in the population – demonstrated that the vaccine retained more than 90% efficacy against Alpha after a second dose [65]. For the Novavax vaccine NVX-CoV2373, clinical trials showed that the vaccine efficacy against Alpha was 86.3% [66], proving that it could still retain a high efficacy. Regarding the susceptibility to neutralization by commercial mAbs or sera from patients that recovered from COVID-19, Alpha was shown to be susceptible to neutralization [67].

5.2 Beta

The VOC Beta – defined as Pango lineage B.1.351 and Nextstrain clade 20H/V2 – has the earliest clinical sample date of September 2020, and was the cause of a second wave of infections in South Africa. The South African Genomic Surveillance Network (NGS-SA) reported that it was the most prevalent variant sequenced from October 2020 to May 2020 and it was estimated to be around 50% more transmissible than the Wuhan strain [68]. Following its discovery, it was reported in at least 119 countries but it has not been detected since March 2022 [69]. Beta variant contains eight mutations in S protein: three are located at the RBD (mutations K417N, E484K, and N501Y) and five are located in the NTD (substitutions L18F, R246I, D80A, and D215G and three amino acid deletion 242–244). The N501Y mutation is associated with increased affinity to the hACE2 receptor as discussed above for Alpha. The amino acid substitution K417N, also found in Delta and Omicron, replaces a lysine (positively charged) for an asparagine (neutral charge), resulting in a reduced positive-positive charge repulsion between S protein and hACE2 receptor [70] which favors RBD-hACE2 interaction. The E484K substitution extends the amino acid side chain and creates a change in the charge of the amino acid from negative to positive. It was found also in Gamma and Alpha sub-lineages and was important for improving the association of the S protein with hACE2 and it was mostly associated with immune escape [71, 72]. Most of the antibody-based therapies for COVID-19 have shown to decrease the efficacy against Beta. Regarding immune escape from convalescent plasma, the group referenced in [73] showed in studies using a pseudovirus expressing Beta S protein that this variant had almost a complete escape from neutralization, but not binding, by convalescent plasma [73]. Another study showed that the neutralization titers were reduced 13.3-fold for Beta compared with an early Wuhan-related isolate viral strain in 34 convalescent plasma samples from a cohort of patients infected during the first wave of infections in the UK, where precisely 14 of 34 failing to reach the 50% neutralization titer (NT50) at a 1:20 dilution and some showing almost complete knockdown of activity [74]. Furthermore, it was shown that Beta-specific and cross-reactive RBD antibodies from the serum of Beta-infected patients had reduced neutralization of wild-type virus [75]. Regarding immune escape from mAb treatments, the VOC Beta has a widespread escape from mAb neutralization largely driven by E484K. This mutation interferes with the binding of several class 1 and 2 mAbs that target the receptor binding motive. The group referenced in [74] showed that Beta is much more difficult to neutralize than previous circulating viral strains, as the neutralization capacity of 14 out of 20 mAbs used in the study was seriously compromised and some of their neutralization was completely abolished [74]. Regarding vaccines, the immunity acquired by all the available vaccines (Pfizer-BioNTech, Moderna, AstraZeneca-Oxford, Johnson and Johnson, Novavax) has been shown to have reduced neutralization capacity against this variant [76] although vaccine effectiveness against fatal disease from Beta infections has been shown to remain high [77]. Furthermore, there is evidence that Beta may have caused increased disease severity as it was associated with in-hospital mortality increase in the second wave in South Africa compared to the first wave [78]; although the authors state these findings could be attributed to admissions in the second wave being more likely in older individuals and to an increment on the health system pressure.

5.3 Gamma

VOC Gamma – defined as Pango lineage P.1 and Nextstrain clade 20 J/V3 – was the third VOC identified. It was first detected in Japan in travelers returning from the Amazonas state in Brazil in January 2021 [79]. It originated in Brazil where the earliest documented samples are associated with an outbreak in Manaus, capital of the Amazonas state, in November 2020 which was preceded by a period of faster molecular evolution [80]. Its high transmissibility became rapidly evident after the observation of a short period between its emergence and its high prevalence in the reported cases from the Amazonas state [81]. Gamma introduction in Brazil and its neighboring countries in South America was followed by the displacement of previously circulating SARS-CoV-2 variants and a rapid increase in prevalence in the entire continent. As an illustration of this phenomenon, Uruguay, which shares 600 miles of dry border with Brazil, is a clear example of how Gamma worsened the COVID-19 pandemic as this country experienced an exponential increase in COVID-19 cases after the variant was introduced. By June 2021, Uruguay was among the countries with the highest number of daily cases and deaths per million persons [82] which could be attributed to an almost 100% Gamma prevalence. Also, Gamma accounted for the high number of infections in several South American and Caribbean countries by June 2021 - the most affected region by this VOC. It has been reported in more than 86 countries since its discovery but is no longer detected since December 2021. Gamma is characterized by mutations N501Y, E484K (also seen in VOCs Beta and Omicron), and K417T in the RBD. It also contains five mutations in the NTD, among which the substitution L18F has a known impact of interfering with the binding of neutralizing antibodies targeting NTD. There is also a mutation in nucleocapsid (N) protein P80R and the deletion in ORF1a(Nsp6) gene at positions 3675–3677 (also present in Alpha and Beta). Having the mutation N501Y confers Gamma with an increased affinity for the hACE2 receptor as mentioned before for Alpha and Beta [83]. Gamma was proved to be between 1.4 and 2.2 times more transmissible than the Wuhan strain [80]. The E484K mutation, associated with immune evasion, supports that this variant is able to infect and cause illness in persons previously infected with other variants, which explained the resurgence of COVID-19 cases in Manaus despite the high seroprevalence in its population [84, 85]. In addition, Gamma was associated with increased mortality risk and severity of COVID-19 cases in younger age groups – corresponding to the unvaccinated population at that time in Brazil [86]. The mortality rate associated with Gamma infections was estimated to be 1.1-fold to 1.8-fold higher than with earlier variants and people infected with Gamma showed to have approximately 3 to 4 times higher viral loads [80].

5.4 Delta

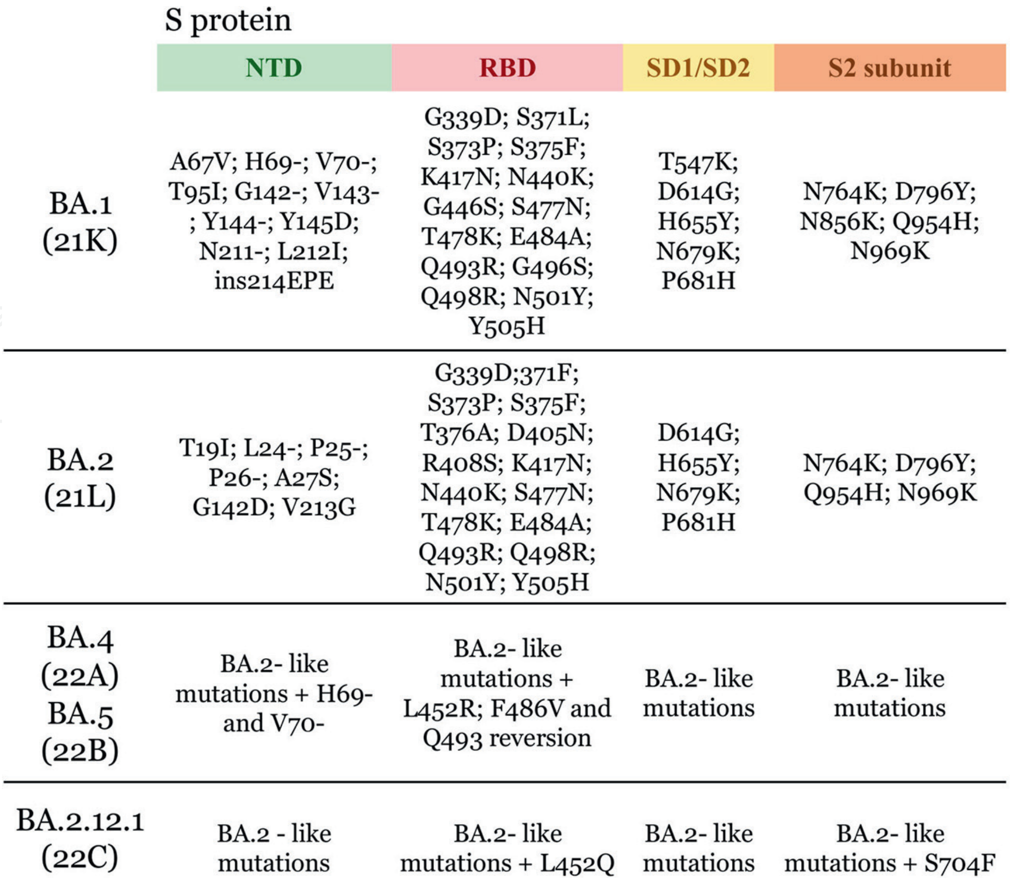
VOC Delta – defined as Pango lineage B.1.617.2 and Nextstrain clade 21A – is the fourth VOC identified. It was first detected in India during an uncontrollable surge of COVID-19 infections that hit the country in May 2021 [87] and spread rapidly worldwide causing a second global wave by mid-2021. Delta rapidly outcompeted Alpha and was determined to be approximately 60% more transmissible [88], leading the WHO to classify Delta as a Variant of Concern on May 11, 2021. It has a total of 10 amino acid mutations in the S protein: T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N. Both L452R and T478K are located at the RBM of the S protein and have been shown to enhance the binding affinity of the virus to the host cell leading to increased infectivity [89] and reduce the neutralizing activity of monoclonal antibodies and convalescent plasma [90, 91]. The mutation L452R when present together with the T478K mutation was shown to increase the stability of the spike protein, which could alter the interaction capacity of neutralizing antibodies [92]. The T478K mutation substitutes the non-charged amino acid threonine with a positively charged lysine, which alters the electrostatic surface in the RBD, affecting the protein interaction with the cellular receptor [93]. The P618R is located adjacent to the polybasic furin cleavage site between S1 and S2 and makes the sequence less acidic. This alteration has been demonstrated to be key in enhancing Delta infectivity, as facilitates the cleavage by the furin protease giving the virus a higher fusogenicity and pathogenicity [91]. COVID-19 cases caused by Delta infections have been associated with a shorter incubation period before disease onset and with a viral load of about 1000 times greater compared to earlier infections by previous viral variants [94]. Regarding the impact on immunity, Delta exhibited some resistance to immunity acquired either by previous natural infection or by first-generation vaccines. The neutralizing ability of convalescent serum from unvaccinated individuals was shown to be significantly decreased against Delta, by four-fold when compared to Alpha and by six-fold when compared to B.1 strains [91] Other studies conducted using data collected by the Scotland-wide COVID-19 surveillance platform have shown that the vaccines from Oxford-AstraZeneca and Pfizer-BioNTech could still reduce the risk of infection or hospitalizations, although this reduction was considerably less when compared to infections with Alpha [95]. Importantly, two doses of these vaccines can still safeguard their effectiveness, as it was shown to decrease only from 87.5% with the Alpha variant to 79.6% with the Delta variant.

The accumulation of more mutations overtime gave rise to 133 Delta sub-lineages, identified with the AY alias by Pangolin nomenclature (Delta AY.1 to AY.133) [96]. One of these sub-lineages was reported to be more transmissible, referred to by the media as 'Delta plus', which was first identified in Nepal. It carried an additional K417N mutation which is also found in Beta and has been related to decrease neutralization by antibodies.

5.5 Omicron

Omicron was the fifth VOC to emerge in late 2021, undermining all predictions about where the next variant would come from. Most scientists believed it would descend out of Delta or one of its sub-lineages, but instead it evolved to a completely different lineage from previous ones as it is shown in **Figure 1**. Omicron currently refers to a larger group of descendent variants of the Pango lineage B.1.1.529 or Nextstrain clade 21 M. They are currently classified as VOC-Linages Under Monitoring (LUM) by the WHO due to their high prevalence of transmission in the population and concomitant increase in viral sequence diversity. This large group consist of variants BA.1 (clade 21 K), BA.2 (clade 21 L) and its descendent lineages BA.4 (clade 22A), BA.5 (22B), BA.2.12.1 (22C), BA.2.9.1, BA.2.11, BA.2.13, and BA.2.75 [8].

The first identification of Omicron (BA.1) was on November 24, 2021 from an immunocompromised individual in South Africa. It caused a sharp increase in daily cases, which rose rapidly from 273 on November 16 to more than 1200 by November 25, more than 80% of which were in the northern province of Gauteng, where the first cases were seen. Genomic sequencing revealed it acquired an astounding number of 62 amino acid mutations, where 36 of them are located in the S protein, particularly at the N-terminal and RBD as shown in **Figure 3**. The BA.1 sub-lineage was the first variant to displace Delta, becoming the most prevalent variant worldwide by December 2021. BA.2 variant gradually replaced BA.1, becoming the most prevalent worldwide by May 2022. BA.1 and BA.2 have 21 amino-acid mutations in common at the S protein, with BA.1 having 12 additional unique mutations and BA.2 having 7 (including a three amino acid deletion) which is shown in **Figure 3**. BA.4 and BA.5 arose in mid-January 2022 displacing BA.2, have an identical S protein amino acid composition (**Figure 3**), and are differentiated only by mutations in the following positions: BA.4 has the mutation L11F at the ORF7b gene and the mutation P151S at the N gene whereas BA.5 has the mutation D3N at the membrane (M) protein [97]. Both have estimated growth advantages over BA.2 and can also display immune evasion acquired after a previous infection with Omicron sub-lineages. BA.2.12.1 is a sub-lineage of BA.2 that differs from it only for the mutations in S protein L452Q at the RBD and the S704F at the S2 (**Figure 3**), but it continues to be closely monitored due to its high prevalence and its capacity to evade the immunity acquired by vaccines and previous infection with other Omicron sub-lineages [98]. Although Omicron possesses high transmissibility, it was the first VOC to show signs of viral attenuation which helped to decrease the disease burden [78].



S protein

	NTD	RBD	SD1/SD2	S2 subunit
BA.1 (21K)	A67V; H69-; V70-; T95I; G142-; V143-; Y144-; Y145D; N211-; L212I; ins214EPE	G339D; S371L; S373P; S375F; K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; N501Y; Y505H	T547K; D614G; H655Y; N679K; P681H	N764K; D796Y; N856K; Q954H; N969K
BA.2 (21L)	T19I; L24-; P25-; P26-; A27S; G142D; V213G	G339D;371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; S477N; T478K; E484A; Q493R; Q498R; N501Y; Y505H	D614G; H655Y; N679K; P681H	N764K; D796Y; Q954H; N969K
BA.4 (22A) BA.5 (22B)	BA.2- like mutations + H69- and V70-	BA.2- like mutations + L452R; F486V and Q493 reversion	BA.2- like mutations	BA.2- like mutations
BA.2.12.1 (22C)	BA.2 - like mutations	BA.2- like mutations + L452Q	BA.2- like mutations	BA.2- like mutations + S704F

Figure 3.
Spike amino acid mutations in the most prevalent Omicron sub-lineages circulating during the first half of 2022.

6. Conclusion

Since its discovery in 2020, the SARS-CoV-2 virus has been circulating in the human population evolving in genomic variants with distinct evolutionary advantages, such as increased transmissibility, immune evasion, and increased virulence, causing outbreaks and new waves of infections worldwide. By monitoring the evolution of SARS-CoV-2 scientists provided valuable information about newly emerging variants over the course of the COVID-19 pandemic. Thus, the implementation of comprehensive real-time genomic surveillance programs is vital for monitoring, detecting, and characterizing new variants, helping sanitary authorities to better manage the crisis.

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