Review

Changing Dynamics of SARS-CoV-2: A Global Challenge

Ananya Chugh 1,†, Nimisha Khurana 1,†, Kangna Verma 1,†, Ishita Sehgal 1, Rajan Rolta 2, Pranjal Vats 1,3, Rajendra Phartyal 1, Deeksha Salaria 2, Neha Kaushik 4, Eun Ha Choi 5, Mansi Verma 1,* and Nagendra Kumar Kaushik 5,*

1 Sri Venkateswara College, South Campus, University of Delhi, New Delhi 110021, India; ananyachugh87@gmail.com (A.C.); nimishakhruna2000@gmail.com (N.K.); kangnaverma1@gmail.com (K.V.); ishi.sehgal2000@gmail.com (I.S.); pranjalvats2000@gmail.com or pranjal-2@postgrad.manchester.ac.uk (P.V.); rajendraphartyal@svc.ac.in (R.P.)
2 Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan 173229, India; roltaranjan612@gmail.com (R.R.); deekshasalaria20@gmail.com (D.S.)
3 School of Biological Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK
4 Department of Biotechnology, College of Engineering, The University of Suwon, Hwaseong 18323, Korea; neha.bioplasma@gmail.com
5 Department of Electrical and Biological Physics, Plasma Bioscience Research Center, Kwangwoon University, Seoul 01897, Korea; ehchoi@kw.ac.kr
* Correspondence: mansiverma@svc.ac.in (M.V.); kaushik.nagendra@kw.ac.kr (N.K.K.)
† These authors contributed equally to this work.

Abstract: Since November 2019, SARS-CoV-2 has been a matter of global concern due to its rapid spread, the millions of deaths it caused, and repeated waves of infections. One after another, many variants of this novel virus have come into existence due to its constant mutability, specifically in the spike glycoprotein region. The tally for variants of concern (VOCs), which already include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617.2), has increased to five with the latest appearance of Omicron (B.1.1.529). In our study, we examine the effect of the transmissibility and infectious potential of the virus due to various mutations of SARS-CoV-2, especially in the receptor-binding domain (RBD). We discuss the role of genome sequencing in tracing all the mutations and the importance of the R value (reproductive number) to understand the virus spread. We also review the effectiveness of the available vaccines on the variants of concern, as the rapid spread of the newly emergent Omicron variant has raised doubts about the usefulness of the current vaccines. The use of a mixed vaccination strategy has proved to be effective, yet the newer variants, such as Omicron, demand booster doses for the population. Multivalent immunogens could be considered as the plausible solution for conferring protection against potential new mutants of the virus in the future.

Keywords: SARS-CoV-2; variants of concern; spike glycoprotein; infectious potential; vaccine efficacy; genome sequencing

1. Introduction

It all started when a media statement on several cases of viral pneumonia was reported by the Wuhan Municipal Health Commission, which was noticed by the World Health Organisation (WHO). This local outbreak of the novel coronavirus disease (COVID-19) spread like wildfire within a short period of time, crippling Europe and other continents, and, on 11 March 2020, COVID-19 was characterized as a pandemic by the WHO. It is caused by a novel human pathogen SARS-CoV-2 from the Coronavirus family [1,2]. The global spread and high virulence of this virus has led to the appearance of many variants in various geographical locations. As stated by the WHO, some of these are emerging variants of concern (VOCs), which include Alpha (lineage B.1.1.7), Beta (lineage B.1.351), Gamma (lineage P.1), Delta (B.1.617.2), and Omicron (lineage B.1.1.529). This virus has reached...
enormous proportions, and as of 15 December 2021, the total number of confirmed cases reached 271,905,597 (Figure 1) with 5,340,623 deaths across the world (Worldometer).

The symptoms of COVID-19 are non-specific and range from patients being asymptomatic to being extremely critical requiring hospitalization in an intensive care unit [3,4]. This disease is easily transmitted by the expulsion of the virus through droplets and aerosols when the lung epithelial cells are irritated. Coughing is one of the most common symptoms reported, in addition to fever, fatigue, headache, shortness of breath, loss of taste and smell and, in some rare cases, diarrhea occurs [5]. Older adults and people with certain comorbidities, such as hypertension and diabetes, show a worse prognosis of this disease [6].

Owing to the mutability of this virus, many variants of SARS-CoV-2 have appeared that may make it either more infectious or more transmissible [7]. Hence, it is necessary to understand the structure of the SARS-CoV-2 virus particle, its major variants and their reproduction rates to understand the infectious potential of this disease.

2. Structure and Sequence of SARS-CoV-2

Similar to SARS-CoVs and MERS-CoVs, which also belong to the Coronaviridae family, SARS-CoV-2 is an enveloped, positive single-strand RNA virus [8–10]. Genome analysis of SARS-CoV-2 has shown that it contains 14 open reading frames (ORFs), which encode 31 proteins [11]. ORF1 and ORF1ab constitute two-thirds of the known 14 ORFs and encode 16 non-structural proteins (nsp). The remaining one-third of ORFs encodes for eleven accessory proteins and four structural proteins, membrane (M), envelope (E), spike (S) and nucleocapsid (N) [12], as shown in Figure 2a. The eleven accessory proteins include ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c and ORF10 [11].
(S) and nucleocapsid (N) [12], as shown in Figure 2a. The eleven accessory proteins include ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c and ORF10 [11].

Figure 2. Major amino acid mutations marked on the entire SARS-CoV-2 sequence: (a) Wild-type strain, (b) Alpha variant, (c) Beta variant, (d) Gamma variant, (e) Delta variant and (f) Omicron variant. (M: membrane protein, E: envelope protein, S: Spike protein, N: Nucleocapsid protein, ORF: Open Reading Frame, UTR: Untranslated Region).

The four structural proteins of SARS-CoV-2 and SARS-CoV have amino acid similarity above 90%, excluding the spike glycoprotein, where they diverge. The SARS-CoV-2 spike glycoprotein (1273 Aa) is 18 amino acids longer, compared to the S glycoprotein of SARS-CoV (1255 Aa) [13]. In fact, some of the mutations near the RBD region in the spike glycoprotein are known to make this virus more transmissible; therefore, this protein has been the focus of research to understand the emergence of new variants [14].

Spike glycoprotein is a club-shaped, trimeric class-I viral fusion protein with a length of ~20 nm. These spike glycoproteins form huge protrusions from the virus’s surface that facilitates virus entry into host cells [13,15,16]. It comprises two subunits: subunit S1 mediates receptor recognition, whereas S2 plays a role in membrane fusion [17]. The S1 subunit possesses a receptor-binding domain (RBD), which may be present either at the N-terminal domain (NTD) or C-terminal domain (CTD) or both [18]. The RBD of the virus mediates direct contact with human angiotensin-converting enzyme 2 (hACE2), which is the primary receptor for its entry into the host cell. The mutations in the RBD region of the spike protein are capable of forming closer contact with hACE2, responsible for a higher binding affinity and possibly/probably increased infectivity of VOCs [19]. The S2
subunit plays a role in the fusion of the virus membrane with the cell membrane of the host cell. In comparison to SARS-CoV, the spike glycoprotein of SARS-CoV-2 has four additional amino acid residues (PRRA) at the intersection of S1/S2, generating an S1/S2 protease cleavage site (PRRAR) \[12,13\] (Figures 3i and 4). It is cleaved by cellular cathepsin L and transmembrane protease serine 2 (TMPRSS2), facilitating the virus entry through the plasma membrane or the endosomal membrane \[12,20\]. The spike glycoprotein can acquire two structural states: a closed state (Figure 3(iia)) and an open state (Figure 3(iib–d)) \[21\]. In a closed state, the RBDs are shielded by the N-terminal domains (NTDs) and the three recognition motifs of this trimeric protein do not protrude from the interface. In contrast, in an open state, the RBD is in the “up” conformation where one RBD is exposed upwards, away from the viral membrane, mediating the fusion of viral and host cell membranes (Figure 3(iib)) \[21\]. This open state is essential for SARS-CoV-2 and host cell membrane fusion, as this facilitates the passage of the virus into the host cells. Some mutations in the spike region promote an enhanced open conformation of the RBD \[22\].

![Figure 3](image_url)

**Figure 3.** Spike glycoprotein of SARS-CoV-2; (i): S1/S2 protease cleavage site; SP: signal peptide; NTD: N-terminal domain; RBD: receptor-binding domain; RBM: receptor-binding motif; FP: fusion peptide; HR1: heptapeptide domain 1; HP2: heptapeptide domain 2; TM: transmembrane domain; CD: cytoplasmic domain 3. (ii): SARS-CoV-2 spike glycoprotein trimeric structure showing chains A (pink), B (blue) and C (green) of (a) closed state (7DF3), (b) 1 RBD up (7LWT), (c) 2 RBD up (7LYK) and (d) 3 RBD up (7CAK) (figure visualized using USF Chimera).

Apart from the spike mutations, 13 different ORFs of the SARS-CoV-2 were also found to have mutations. A mutation in the ORF3a protein was observed to be linked to a higher level of infection and mortality rate due to infection caused by this virus \[23\]. The RNA-dependent RNA-polymerase of the SARS-CoV-2 has also been subjected to mutation located at the 14,408th position, which causes improper proofreading activity \[24\].
Throughout the evolution of SARS-CoV-2, it has been observed that mutations and the response of the immune system towards those mutations have become the controlling factors of the pathogenicity of this virus. The host cell membrane has the protein receptor ACE-2, which influences the virus’s passage into the cell [25,26]. The virus enters the host cell by fusion of spike glycoprotein with the ACE-2 receptor on the cell membrane [27]. This induces conformational alteration in the plasma membrane and facilitates receptor-mediated endocytosis (Figure 4) [28]. Once uncoating is performed inside the cell, all proteins are removed and viral RNA is released into the cytoplasm. Then, spike glycoproteins inhibit ACE-2 and downregulate it. The downregulation of ACE-2 leads to the compensatory increase in ACE-1 and causes the excessive production of angiotensin-II, consequently enhancing pulmonary vascular permeability [29,30].

Once the cell is infected by the virus, it stimulates the production of interferon α in infected cells, which alerts the adjacent cells to stimulate the antiviral program. This leads to the production of interferon γ in the immune cells, which, in turn, triggers the immune system to destroy the infected cells. Virus-infected cells lose surface MHC I expression, which leads to a reduced inhibitory signal in NK cells. Additionally, cellular stress due to the viral infection upregulates the activating receptors of NK cells leading to the elimination of infected cells. Simultaneously, cytotoxic T cells are alerted when a fragment of viral protein is transported onto the cell’s surface by MHC I, which presents antigens from the virus.


### 3. Immunopathology of SARS-CoV-2

Throughout the evolution of SARS-CoV-2, it has been observed that mutations and the response of the immune system towards those mutations have become the controlling factors of the pathogenicity of this virus. The host cell membrane has the protein receptor ACE-2, which influences the virus’s passage into the cell [25,26]. The virus enters the host cell by fusion of spike glycoprotein with the ACE-2 receptor on the cell membrane [27]. This induces conformational alteration in the plasma membrane and facilitates receptor-mediated endocytosis (Figure 4) [28]. Once uncoating is performed inside the cell, all proteins are removed and viral RNA is released into the cytoplasm. Then, spike glycoproteins inhibit ACE-2 and downregulate it. The downregulation of ACE-2 leads to the compensatory increase in ACE-1 and causes the excessive production of angiotensin-II, consequently enhancing pulmonary vascular permeability [29,30].

Once the cell is infected by the virus, it stimulates the production of interferon α in infected cells, which alerts the adjacent cells to stimulate the antiviral program. This leads to the production of interferon γ in the immune cells, which, in turn, triggers the immune system to destroy the infected cells. Virus-infected cells lose surface MHC I expression, which leads to a reduced inhibitory signal in NK cells. Additionally, cellular stress due to the viral infection upregulates the activating receptors of NK cells leading to the elimination of infected cells. Simultaneously, cytotoxic T cells are alerted when a fragment of viral protein is transported onto the cell’s surface by MHC I, which presents antigens from the virus.

within the cell [29]. Dendritic cells present antigens on their surface, which interacts with T cells and B cells to produce antigen-specific antibodies (Figure 4). Additionally, phagocytes play a significant role in innate immunity by ingesting the free virus particles and contribute to adaptive immunity by presenting antigens to lymphocytes. Simultaneously, certain chemokines, such as CXCL10 and CCL2, are released in order to guide immune cells towards the infected region and, hence, destroy the infected cells [31,32]. Studies report that the failure of switching between innate to adaptive responses disables the formation of memory cells, which increases the risk of recurring infections. Alluding to the above mechanism, the human body is well designed to protect itself against pathogens. Yet, SARS-CoV-2 successfully escapes and disrupts the immune response by generating various viral proteins, such as 3b and M, which inhibits the natural processes of the cell. One such protein, namely, PLpro, binds with the NF-κβ transcription factor (which is an important response generator of pro-inflammatory cytokines) and downregulates it. This leads to a reduced NF-κβ response and decreased production of pro-inflammatory cytokines [33], thus disabling the timely destruction of infected cells and leads to symptoms. Although the symptoms of infection by variants of SARS-CoV-2 are similar, their transmissibility varies due to mutations primarily in the spike glycoprotein [14,34].

The genomic sequencing of the virus from various locations at a larger scale has played a vital role in tracing the mutations (Figure 5). Evidence shows that some of these mutations, such as K417N and E484K, elude the immune system, i.e., they can escape the neutralizing antibodies, even after a person is vaccinated (Table 1) [35,36]. This integration of genome sequencing on a global scale, with routine sanitation practices and good quality clinical management, can equip the world against any upcoming crisis of this type. Therefore, it is important to consider the mutations currently known, and predict emerging prospective mutations that should be considered when testing and developing vaccines.

![Figure 5](image_url)

**Figure 5.** Maps showing global country-wise sequence count of all variants of concern as of 13 September 2021. (a) Sequence count of B.1.1.7 (Alpha variant), (b) sequence count of B.1.351 (Beta variant), (c) sequence count of P.1 (Gamma variant), (d) Sequence count of B.1.617.2 (Delta variant) and (e) sequence count of B.1.1.529 (Omicron variant) (data source: GISAID, Figure redrawn using GeoMaps).
Table 1. SARS-CoV-2 variants of concern (VOCs) with their date and place of emergence and important spike mutations, along with their effect on the variant’s overall transmissibility.

<table>
<thead>
<tr>
<th>WHO Label</th>
<th>Pango Lineage</th>
<th>Potential Emergence</th>
<th>Number of Countries Affected</th>
<th>Important Spike Mutations</th>
<th>Sequenced Genomes (GISAID)</th>
<th>Impact of Mutations</th>
<th>Transmissibility of Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>B.1.1.7</td>
<td>UK, September 2020</td>
<td>170</td>
<td>N501Y P681H</td>
<td>11,49,443</td>
<td>P681H: Slightly increases the S1–S2 cleavage [37]. N501Y: Enhances binding affinity between spike RBD domain and hACE2 receptor [38]. E484K *, L452R *</td>
<td>~70% increase with respect to wild type</td>
</tr>
<tr>
<td>Beta</td>
<td>B.1.351</td>
<td>South Africa, May 2020</td>
<td>112</td>
<td>K417N E484K N501Y</td>
<td>39,604</td>
<td>K417N: Responsible for antibody-escape property of the virus [39]. N501Y: Same as above. E484K: Reduces antibody neutralisation and is responsible for increased infectivity [40].</td>
<td>20–113% increase with respect to wild type</td>
</tr>
<tr>
<td>Gamma</td>
<td>P.1</td>
<td>Brazil, November 2020</td>
<td>80</td>
<td>K417T E484K N501Y</td>
<td>1,17,675</td>
<td>K417T: Responsible for antibody escape property of the virus [39]. N501Y: Same as above. E484K: Same as above.</td>
<td>~161% increase with respect to wild type</td>
</tr>
<tr>
<td>Delta</td>
<td>B.1.617.2</td>
<td>India, October 2020</td>
<td>105</td>
<td>L452R T478K P681R</td>
<td>34,37,588</td>
<td>T478K: Helps evade neutralising antibodies. Enhances viral infectivity [41]. L452R: Impairs antibody neutralization. Increases viral infectivity [39,42]. P681R: Increases the rate of S1–S2 cleavage, and hence results in enhanced transmissibility [40].</td>
<td>- 40–60% more transmissible than Alpha. - Twice as transmissible as the original Wuhan strain of SARS-CoV-2.</td>
</tr>
<tr>
<td>Omicron</td>
<td>B.1.1.529</td>
<td>Multiple countries, November 2021</td>
<td>-</td>
<td>H655Y N679K P681H E484A Q498R N501Y</td>
<td>5352</td>
<td>N501Y: Same as above. H655Y: May be associated with increased transmissibility as it is proximal to the Furin cleavage site, hence aids in increased spike cleavage (CoVariants-GISAID). N679K: Associated with increased transmissibility (CoVariants-GISAID). P681H: Slightly increases the S1–S2 cleavage [37]. E484A: May be associated with immune escape (CoVariants-GISAID). Q498R: Enhances binding affinity with ACE2 receptor in combination with N501Y (CoVariants-GISAID).</td>
<td>-</td>
</tr>
</tbody>
</table>

(hACE2: human angiotensinogen-converting enzyme 2, RBD: receptor-binding domain) ([33], GISAID, WHO [14,43]).

*: Found only in some of the sequenced alpha strains.

4. Variants of SARS-CoV-2

Mutations cause viruses to change and new viral variants are expected to arise. Some mutations emerge, but fail to prevail, whereas others persist since they are beneficial to the virus, in terms of immune evasion, viral fitness and receptor binding, and become dominant over time in a host population (Table 1) [37]. The analysis of variants computationally led to the validation of important variants, which have a high impact and may have led to severe consequences, and hence increase the transmission, infectivity or pathogenesis of the virus (gov.uk, accessed on 20 December 2021). Even though continuous and rigorous studies are being performed to attain better comprehension of all aspects of SARS-CoV-2, controlling the spread of the virus and its variants is still a major issue. Since SARS-CoV-2
keeps mutating, new variants are arising in every corner of the world, which could be one of the reasons why many countries are experiencing the second or even third wave of this deadly virus [44].

4.1. The D614G Variant

The D614G variant came into existence in around January 2020 [45], but now it has become the predominant mutation found in all variants of concern. According to the studies, the D614G variant has higher infectivity and transmissibility than the wild-type sequence that originated in Wuhan [46]. The D614G mutation modulates S-protein-cleavage efficiency, promotes favorable conformation for RBD-ACE2 interaction, facilitates more efficient S-protein incorporation into the virion and stabilizes prefusion spike-trimer association [47]. In the D614 spike trimer, an average of one RBD is open; whereas, in the G614 spike trimer, two or all three RBDs are open [37]. There is almost a 2.4-to-7.7-fold increase in the transmission in G614 from that observed in the D614 variant [48]. All these factors make the D614G mutation a very significant one in the evolution of SARS-CoV-2. Given the continuous evolution, some of the new variants have proved to be a great threat to public health, which has prompted the need for the characterization of specific variants into variants of concern (VOCs) (Table 1) and variants of interest (VOIs) (Table 2), in an attempt to monitor the evolution of the variants on a global level by WHO.

Table 2. Emergence of SARS-CoV-2 variants of interest (VOIs) and mutation in spike glycoprotein of each variant (expasy, CoV lineage, [49,50]).

<table>
<thead>
<tr>
<th>WHO Label</th>
<th>Pango Lineage</th>
<th>Emergence</th>
<th>RBD Mutations</th>
<th>Other Spike Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eta *</td>
<td>B.1.525</td>
<td>Multiple countries, December 2020</td>
<td>E484K</td>
<td>O52R, Q677H, F888L, D614G, A67V</td>
</tr>
<tr>
<td>Kappa *</td>
<td>B.1.617.1</td>
<td>India, October 2020</td>
<td>E484Q, L452R, G142D, D614G, T95I, E154K, Q1071H, M153I</td>
<td></td>
</tr>
<tr>
<td>Lambda</td>
<td>C.37</td>
<td>Peru, December 2020</td>
<td>L452Q</td>
<td>G75V, T76I, F490S, D614G, T859N</td>
</tr>
</tbody>
</table>

* These variants have now been re-categorized under variants under monitoring.

4.2. Variants of Concern

The VOCs are those variants that are more contagious or have a COVID-19 epidemiology, which is more detrimental. They are characterized by a higher virulence, different symptoms, a lower response to public health measures or difficult diagnosis, therapeutics and vaccines (WHO, 2021). To date, five variants, namely, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529), and their sub-lineages, are categorized as the variants of concern (VOCs), as shown in Table 1 (WHO, 2021). Many countries have made rigorous efforts in sequencing the genome of these variants to determine the recurring mutations in each variant (Figures 5 and 6 and analyze their effect on the pathogenesis of the virus.
4.2.1. The Alpha Variant (B.1.1.7)

This variant was first reported in the United Kingdom in late September 2020 and has since spread to almost 180 countries, some of them being the USA, Germany, Denmark and Sweden (15 December, GISAID). The Alpha variant has a total of 15 mutations (Figure 6), out of which 7 mutations lie in the spike region. The N501Y is an RBD mutation (Figure 7) that causes a 10-fold increase in the binding affinity of spike glycoprotein to the hACE2 receptor, and this mutation, in combination with D614G, results in an increased number of open RBDs making the virus more contagious and deemed as a variant of concern (Table 1 and Figure 2b) [37,51]. The P681H mutation near the furin site slightly increases the S1/S2 cleavage [52] (Figures 2b and 3i). The spike also has 2 deletions, ∆H69-V70, which increase the rate of S1/S2 cleavage and cell–cell membrane fusion. This deletion increases the infectivity of B.1.1.7 and it can also compensate for immune escape mutations that reduce infectivity [53]. Recent sequencing of some more strains of the Alpha variant shows new mutations in the spike region, E484K and L452R, as mentioned in Table 1 (WHO).

4.2.2. The Beta Variant (B.1.351)

The Beta variant was first reported in South Africa in August 2020, and its prevalence escalated from 11% in October to 87% by December 2020 [54]. Currently, the variant is present in about 117 countries, including the United States, Sweden, Germany and France (15 December, GISAID).

It has a total of 11 mutations and shares D614G and N501Y with the Alpha variant (Figure 6). Apart from those in the spike region, this variant contains successive deletions of the amino acids L242, A243 and L244, and replacements D80A, D215G, A701V, K417N and E484K (Figure 2c) [55]. Two lineages of B.1.351 were detected in November 2020, denoted as variant B.1.351-V2 (with an extra N-terminal mutation, L18F), and variant B.1.351-V3 (it possesses an additional R246I mutation in loop N5; however, the D215G mutation is missing) [37].
Figure 7. Alignment between sequences of spike glycoprotein of SARS-CoV-2 variants. Amino acid variations within each variant are marked at the particular positions. (In order from up to down; wild type (PDB ID: 6VYB), Alpha (PDB ID: 7LWT), Beta (PDB ID: 7LYQ), Gamma (accession no.: QQX12069.1), Delta (accession no.: QWO57033), Eta (accession no.: QWO17721.1), Iota (accession no.: QTP80309.1), Kappa (accession no.: QTY54081.1), Lambda (accession no.: QRX62290.1)) (alignment of sequences using Bioedit).
Due to D614G and the three RBD mutations, N501Y, E484K and K417N (Figures 6 and 7), the affinity of B.1.351-V1 for hACE2 magnifies by 19-fold [37]. The K417T/N mutation lowers the hACE2 binding affinity, but helps the virus to evade neutralizing antibodies [56]. The K417N destabilizes the RBD-down conformation, hence promoting the open conformation similar to D614G and N501Y. E484K lacks interactions with the neighboring RBD required for stabilizing the down conformation, hence the mutant favors only RBD-up conformation; it also has the ability to prevent the binding of neutralizing antibodies to the virus [37]. As the cumulative effect of all these mutations, the transmissibility of Beta has increased by ~20–113% when compared to the wild-type strain [14] (Table 1).

4.2.3. The Gamma Variant (P.1 Variant; a Descendent of B.1.1.28)

The third VOC, Gamma variant was first detected in Manaus, the capital city of Amazonas State by the end of 2020. The Gamma variant fueled the second and largest epidemic wave in the Amazonas state in Brazil [57]. Since then, it has spread in almost 91 countries, including the USA, Canada, Mexico and Chile (15 December, GISAID). More than 75% of the population in Amazonas was already infected by the time the epidemic was brought under control in March 2021. The transmissibility of the Gamma variant has been estimated to have increased by ~161% as compared to the wild-type strain [14] (Table 1). It has 2 mutations in common with Alpha variant, while it has 4 in common with the Beta variant (Figure 6) and a total of 17 mutations with respect to the wild-type strain. The three mutations, K417T, E484K and N501Y, are in the RBD domain (Figures 6 and 7) [58]. This trio of mutations in the spike glycoprotein is responsible for an increase in its binding affinity to the hACE2 receptor [37].

4.2.4. The Delta Variant (B.1.617.2)

Yet another variant, Delta, emerged in India in December 2020 and was primarily accountable for India’s deadly second COVID-19 wave, which spanned from March to July. On 7 May 2021, India reported the highest number of COVID-19 cases at 4,14,433 in a day, while the highest figure of 5015 deaths was reported on 23 May (Worldometer). The variant rapidly spread to almost 184 countries, including the UK, the USA, Israel, South Africa, Denmark, Germany, Spain and Italy (15 December, GISAID). The highly contagious Delta variant of SARS-CoV-2 has largely driven several waves of infection. Earlier this year, the Delta variant was responsible for 91% of new cases in the UK, displacing the Alpha strain, which previously dominated. The number of reported cases peaked on 17 July 2021, at 54,674 in the United Kingdom. Around the same time, the Delta variant was associated with 83% of all newly reported cases in the USA [59]. In Amazonas (Brazilian state) during September 2021, Delta surpassed the previously circulating variants of concern [57].

The Delta variant has only one mutation, D614G, which is common with the Alpha, Beta and Gamma variants. The other 18 mutations are unique to the variant (Figure 6). The spike mutations include G142D, D614G, T19R, R158G, D950N and P681R (Figure 2e), and the specific RBD mutations are L452R and T478K (Figures 6 and 7). Both these RBD mutations help the virus escape from the neutralizing antibodies, hence making it fit and more infectious as shown in Table 1 [37]. Additionally, it has deletions at positions E156 and F157. According to the Centers for Disease Control and Prevention (CDC), the Delta variant is two times more contagious than other variants. Two new sub-lineages of the Delta variant were reported, AY.1 and AY.2 (Delta Plus variant), which had an additional spike glycoprotein mutation, K417N, which exists in the Beta variant [58,60].

4.2.5. The Omicron Variant (B.1.1.529)

The Omicron variant was reported in Botswana during early November 2021. The first case from South Africa was reported on 24 November 2021, and the WHO named this variant B.1.1.529 COVID-19 variant, followed by its declaration as VOC on 26 November 2021 [61]. Since then, cases due to B.1.1.529 have been reported in 89 countries, including the United Kingdom, Australia, Israel and the United States [62]. The appearance of this variant
aggravated the situation concerning the COVID-19 pandemic, and has been persistent since 2019. Early sequence analysis suggests that this variant contains over 32 mutations in the spike glycoprotein, many of which are found in the RBD and NTD regions; therefore, some of these mutations are linked to the increased interaction of the SARS-CoV-2 spike glycoprotein with hACE-2 [63,64] and neutralizing capability of SARS-CoV-2 antibodies and nanobodies [64,65] (Table 1).

Omicron has 3 common mutations with Alpha, Beta and Gamma, respectively, and 2 common mutations with Delta (Figure 6). Some of the prominent mutations found in the Omicron variant, N679K, P681H (next to the furin cleavage site), N501Y (inside the RBD) and D614G (spike glycoprotein), are among the polymorphisms that have been previously identified in other variants and shown to be more transmissible [66]. Most recently, genomic sequence analysis suggests that the 21K (Omicron BA.1) has sister clades, 21L (BA.2) and 21M (BA.3), thus indicating the presence of 3 sub-lineages. All of them comprise the Pango Lineage B.1.1.529. The spike glycoproteins of BA.2 and BA.1 (Omicron) share 21 amino acid mutations, but 21L carries 6 additional mutations and a deletion (CoVariants.org, accessed on 30 December 2021; GISAID).

Moreover, the computational analysis suggests that Omicron and its sub-lineages were discovered to have less stable and closely packed RBD structures, which impeded antibody interactions more than wild-type and Delta (AY.1 and AY.2) variants, potentially leading to increased pathogenicity and immune escape [67]. Hence, it is likely that Omicron will spread quicker and elude antibodies more easily than prior variations, leading to a higher risk of re-infection [65,68,69]. Thus, multiple efforts are being made around the world to assess the comprehensive effect of the Omicron variant. Currently, the total number of sequenced Omicron strains are 5352, out of which the UK and South Africa have sequenced 2957 and 900 genomes, respectively, as of 15 December 2021 (Figure 5e) (GISAID).

4.3. Variants of Interest

The VOIs are those variants that have been identified to cause community transmission, multiple COVID-19 cases or have been detected in multiple countries based on the assessment by the WHO. The VOIs as named by the WHO are Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37) and Mu (B.1.612) (Table 2 and Figure 6). These variants have some common mutations with VOCs, such as E484K, N501Y and D614G (Figure 6).

However, certain variants do not pose a major risk to global health anymore, when compared to other existing variants. Such variants have been reclassified into variant under monitoring (VUM) by the WHO. Due to emerging variants, it is necessary to study the infectious potential of this virus, which may help in designing efficient vaccines and in avoiding or managing community spread.

5. Infectious Potential of SARS-CoV-2

The pattern of infection of the virus can be studied based on certain parameters, such as $R_o$, $r$ and $d_t$. The reproduction number is found useful in determining the epidemic potential and viral spread of a virus in a population with less efficacious control and vaccine coverage [70,71]. The higher transmission rate of the virus has consequently led to a high R value globally; for instance, the $R_o$ of the Delta variant (~5.08) is much higher than the $R_o$ of the wild-type strain (~2.79), reflecting a higher viral load as of October 2021 [70]. Most recently, between November and December 2021, the reproduction number of SARS-CoV-2 was found to be >1 in some countries, as shown in the graph (Figure 8), indicating higher infections and growth of an epidemic (CMMID nCoV working group), possibly due to the emergence of the Omicron variant. The trend of the R value highly depends on the geographical area, the number of tests performed and the number of cases reported in a time frame, and these measures also vary within regions because of population density and immunization in a group and strongly depend on measures taken to control the spread of the disease, such as certain mitigation strategies of imposing a lockdown, maintaining social distance and wearing a mask [72]. As the number of infections increases, the opportunity
for SARS-CoV-2 to mutate also increases, leading to the emergence of new variants with even higher virulence [73]. It is also an important indicator of vaccine coverage required in a population [70].

![Effective Reproduction No. (R-Value) Overtime](image)

**Figure 8.** Effective reproduction number of SARS-CoV-2 of ten selected countries from 8 November 2021 to 28 December 2021 (data source: CMMID).

### 6. Impact of Variants on the Vaccines

The current goal of immunization against COVID-19 is to provide protection against hospitalization, severe illness and death. The spike glycoprotein mutates at a very high frequency, which modifies its interaction with the hACE2 receptor, thereby increasing the infection rate or negatively affecting the potential of neutralizing antibodies, ultimately compromising the vaccine’s efficacy [74,75]. To date, numerous COVID-19 vaccines have been licensed by the WHO for use against SARS-CoV-2, such as Covishield, BNT162b2 (Pfizer), Ad26.COV2.S (Janssen—Johnson and Johnson), AZD1222 (AstraZeneca/Oxford), BBIBP-CorV (CDC, 2021), CoronaVac (CDC, 2021) and mRNA-1273 (Moderna) [43,76–81]. The efficacies of these vaccines against VOC are listed in Table 3.

Immune imprinting has become a double-edged sword. The memory of infection is cardinal to initiate immune response, but at the same time, for SARS-CoV-2, it becomes a barrier. Studies indicate a correlation between the vaccines’ effectiveness against infection and their neutralizing antibody titters, indicating that vaccine efficacy tends to vary with different variants. The waning of immunity over time towards new VOCs demands urgent attention as the outbreak of the Omicron variant has again become a cause for global concern. The rapid spread of the Omicron variant makes it evident that the virus is able to bypass the immune system, even when an individual has had both vaccination shots, because of the multiple mutations at the RBD region [82] (Table 1).
Table 3. Vaccines licensed to date for use against SARS-CoV-2, with their respective efficacies against different variants [43,78–80,83–93].

<table>
<thead>
<tr>
<th>Name of Vaccine</th>
<th>Vaccine Type</th>
<th>Efficacy</th>
<th>Effectiveness against Variants (B.1.1.7/alpha (United Kingdom), B.1.351/beta (South Africa), P.1/gamma (Brazil), B.1.617/delta (India))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfizer-BioNTech (BNT162b2)</td>
<td>mRNA</td>
<td>First Dose</td>
<td>92.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second Dose</td>
<td>95.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–87.0% Beta (B.1.351)–75.0% Gamma (P.1)–82.0% Delta (B.1.617.2)–95.0%</td>
</tr>
<tr>
<td>Moderna (mRNA-1273)</td>
<td>mRNA</td>
<td>First Dose</td>
<td>81.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second Dose</td>
<td>94.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–81.6% Beta (B.1.351)–95.7% Gamma (P.1)–89.0% Delta (B.1.617.2)–95.0%</td>
</tr>
<tr>
<td>AstraZeneca, University of Oxford (AZD1222)</td>
<td>Adenovirus-based</td>
<td>First Dose</td>
<td>90.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second Dose</td>
<td>62.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–70.4% Beta (B.1.351)–77.0% Gamma (P.1)–82.0% Delta (B.1.617.2)–67.0%</td>
</tr>
<tr>
<td>Johnson &amp; Johnson (Ad26.COV2.S)</td>
<td>Non-replicating viral vector</td>
<td>First Dose</td>
<td>66.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second Dose</td>
<td>73.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–74.0% Beta (B.1.351)–52.0% Gamma (P.1)–66.0% Delta (B.1.617.2)–60.0%</td>
</tr>
<tr>
<td>BBIBP-CorV</td>
<td>Inactivated virus vaccine</td>
<td></td>
<td>79.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Efficacious in preventing PCR-confirmed COVID-19 in adults. No vaccine effectiveness studies yet to inform protection against variants of concern (CDC, 2021)</td>
</tr>
<tr>
<td>Novavax (NVX-CoV2373)</td>
<td>Protein-based vaccine</td>
<td>83.4%</td>
<td>89.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–86.3% Beta (B.1.351)–51.0% Non-B.1.1.7–96.0%</td>
</tr>
<tr>
<td>Covishield (ChAdOx1 nCoV-19)</td>
<td>Adenovirus-based vaccine</td>
<td>64.0%</td>
<td>70.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha (B.1.1.7)–70.4% Non-B.1.1.7–81.5% Gamma (P.1)–N.A Delta (B.1.617.2)–87.0%</td>
</tr>
<tr>
<td>CoronaVac</td>
<td>Inactivated virus vaccine</td>
<td>50.0%</td>
<td>50.0–91.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>More data are needed on its efficacy against emerging SARS-CoV-2 variants</td>
</tr>
</tbody>
</table>

The use of existing vaccines to boost previously infected individuals has been shown to increase protection against COVID-19 infection and enable a higher neutralization of SARS-CoV-2 variants than the primary vaccination alone [94]. The extent of waning immunity and the necessity for booster doses may vary between different populations, vaccine products, circulating SARS-CoV-2 variants (VOCs in particular) and the intensity of exposure. As reported above, the Omicron variant is highly transmissible, spreading at a higher rate than Delta, and can cause infection in people who have already been immunized [94]. This variant reduces the effect of neutralizing antibodies to a greater extent than any other variant of SARS-CoV-2, which suggests that vaccines’ efficacy is likely to be remarkably altered by Omicron. The Pfizer-BioNTech study found that people vaccinated with the third dose of its vaccine developed neutralizing antibodies against the variant at levels similar to those against other existing variants triggered by two doses of the vaccine [67]. A mixed vaccination strategy is now being tested in several countries to combat SARS-
CoV-2 infections [89]. Currently, a mix-and-match approach using COVID-19 vaccines administered as heterologous prime-booster doses are being tested in the UK to estimate the effectiveness of Moderna (mRNA-1273), Pfizer (BNT162b2), Oxford (AZD1222) and Novavax (NVX-CoV2373) vaccines in ‘Com-Cov2 trial’, and Oxford and Pfizer vaccines in ‘CombivacS trial’ in Spain [95]. With the appearance of different strains of SARS-CoV-2, a multivariant vaccine could also be developed. Additionally, COVID-19 can be prevented by using trivalent as well as quadrivalent vaccines, which target three and four strains of the virus, respectively [83]. COVID-19 could be addressed with a significant modification in immunization policies and through the implementation of the suggested approaches. We need to understand that an individual with partial protection conferred from vaccines is better off than an individual without any protection. However, the problem of whether vaccine development would be able to keep up with the evolution of the virus still remains unsolved [96].

7. Conclusions

Ever since SARS-CoV-2 came into existence, new patterns and thousands to millions of new cases have arisen with every new mutation. Our thorough literature review found that each variant has different combinations of mutations that have strengthened the virus, brought about second and third waves of infection and increased mortality rates due to cases of COVID-19 in almost all parts of the world. There is a need to track the transmission pattern of the infection in an attempt to prepare for future adversities caused by the pandemic. More importantly, to tackle the infection, it is necessary to predetermine the potential mutations and to keep track of all the new mutations and their spread in the population. Some of these variants are now known to escape the neutralizing antibodies that bring into question the efficacy of the vaccines. Perfecting the vaccine composition and administration strategy with the increasing number of new mutations of SARS-CoV-2 is the need of the hour in hope of bringing this virus to its knee. Pursuing new variants on time, determining the efficacy of existing vaccines and designing new vaccines against them is key in this fight against COVID-19.


Funding: This study was supported by the National Research Foundation (NRF) of Korea, funded by the Korean government (NRF-2021R1C1C1013875, 2021R1A6A1A03038785 and 2021R1F1A1055694). This work is also supported by the Kwangwoon University Research Fund 2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement:
- Global Initiative on Sharing All Influenza Data (GISAID). EpiCoV: https://www.epicov.org/epi3/frontend#3a1b04 (accessed on 30 December 2021).
• CoVariants (c) 2020–2021 (Enabled by GISAID)—https://covariants.org/ (accessed on 30 December 2021).
• Investigation of SARS-CoV-2 variants of concern: Variant risk assessments—gov.uk—(accessed on 30 December 2021).

Acknowledgments: The authors acknowledge C. Sheela Reddy, Principal, Sri Venkateswara College, University of Delhi, India for providing the necessary logistic support. The authors also extend their gratitude to Alice Boldrini and Eden Conroy for checking the English language throughout the manuscript.

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

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>Severe Acute Respiratory Syndrome Coronavirus 2</td>
</tr>
<tr>
<td>COVID-19</td>
<td>Coronavirus Disease 2019</td>
</tr>
<tr>
<td>VOC</td>
<td>Variant of Concern</td>
</tr>
<tr>
<td>VOI</td>
<td>Variant of Interest</td>
</tr>
<tr>
<td>ORF</td>
<td>Open Reading Frame</td>
</tr>
<tr>
<td>Nsp</td>
<td>Nonstructural protein</td>
</tr>
<tr>
<td>hACE-2</td>
<td>human Angiotensin-Converting Enzyme 2</td>
</tr>
<tr>
<td>RBD</td>
<td>Receptor Binding Domain</td>
</tr>
<tr>
<td>Aa</td>
<td>Amino Acid</td>
</tr>
<tr>
<td>NTDs</td>
<td>N Terminal Domains</td>
</tr>
<tr>
<td>CTDs</td>
<td>C Terminal Domains</td>
</tr>
<tr>
<td>Nt</td>
<td>Nucleotide (s)</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Middle East respiratory syndrome—Coronavirus</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>Severe acute respiratory syndrome—Coronavirus</td>
</tr>
<tr>
<td>R0</td>
<td>Basic reproduction number</td>
</tr>
<tr>
<td>Rₜ</td>
<td>Effective reproduction number</td>
</tr>
<tr>
<td>r</td>
<td>Epidemic growth rate</td>
</tr>
<tr>
<td>dₜ</td>
<td>Doubling time</td>
</tr>
</tbody>
</table>

References


40. Barton, M.J.; MacGowan, S.A.; Kutuzov, M.A.; Dushek, O.; Barton, G.J.; van der Merwe, P.A. Effects of common mutations in the SARS-CoV-2 Spike RBD and its ligand, the human ACE2 receptor on binding affinity and kinetics. eLife 2021, 10, e70658. [CrossRef]


43. Teo, S.P. Review of COVID-19 mRNA Vaccines: BNT162b2 and mRNA-1273. [PubMed] [CrossRef]


70. Liu, Y.; Rocklöv, J. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. *J. Travel Med.* 2021, 28, taab124. [CrossRef]


81. Chang, X.; Augusto, G.S.; Liu, X.; Kündig, T.M.; Vogel, M.; Molsen, M.O.; Bachmann, M.F. BNT162b2 mRNA COVID-19 vaccine induces antibodies of broader cross-reactivity than natural infection, but recognition of mutant viruses is up to 10-fold reduced. *Allergy* 2021, 76, 2895–2998. [CrossRef]


88. Sanderson, K. COVID Vaccines Protect against Delta, but Their Effectiveness Wanes. Nature 2021. [CrossRef]


