



# COVID-19 Waves with Associated Variants & Scenario in Pakistan

Ghazala R<sup>1,2\*</sup>, Muhammad B<sup>3</sup>, Tayyaba M<sup>3</sup>, Zainab J<sup>2</sup>, Qayyum M<sup>4</sup> and Raheel N<sup>5</sup>

<sup>1</sup>Post Graduate Medical Institute (PGMI), Ameer-ud-Din Medical College (AMC), Pakistan

<sup>2</sup>Central Research Laboratory, Lahore General Hospital (LGH), Pakistan

<sup>3</sup>Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Pakistan

<sup>4</sup>Professor of Pharmacology, Fatima Jinnah University, Lahore, Pakistan

<sup>5</sup>Associate Professor of Community Medicine, Rashid Latif Medical College, Lahore, Pakistan

## Review Article

Volume 7 Issue 1

Received Date: May 24, 2023

Published Date: July 03, 2023

DOI: 10.23880/oajprs-16000155

**\*Corresponding author:** Ghazala Rubi, Post Graduate Medical Institute(PGMI) and Central Research Laboratory, Ameer-ud-Din Medical College (AMC) and Lahore General Hospital (LGH), Pakistan, Email: rubighazala@yahoo.com

## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped RNA beta-coronavirus virus that is coronaviridae-related. It originated in Wuhan, China in December 2019 and is known to cause COVID-19 illness. SARS-CoV-2 posed a major threat to the world in concern of health along with trade and medical facilities. COVID-19 patients experience comorbid conditions like severe fever, typhoid, myocarditis, and a fatal black fungus attack. World Health Organization declared COVID-19 as an emergency on 30th January 2020 and a pandemic on 11th March. As of right now, COVID-19 has been verified to have infected over 100 million people across 210 nations, and 2 million of those infections have resulted in death. RT-qPCR (Reverse transcriptase qualitative PCR) test is performed if any symptoms exist, and isolation is necessary for three to five days after the confirmation. In Pakistan, it was reported firstly on 26th February 2020 by the Pakistan government when two persons were found infected with the disease. SARS-CoV-2 changes its genome constantly and results in the emergence of different variants in different waves. Pakistan has faced 6 sequential waves since 2020 to date, each associated with different variants and overlapping variants. The first wave was associated with B.1, B.1.471, B.1.36, second with B.1.36.31, B.1.247, B.1.1.1, B.1.160, B.1.471, B.1.562, B.1.1.7, B.1.1.250, B.1.261, B.1.351, P.1, third with B.1.1.7, B.1.351, P.1, B.1.617.2, B.1.36, B.1.468, B.1.1.413, A.27, fourth with P.1, B.1.617.2, fifth with Omicron (B.1.1.529) and sixth with new subvariants of Omicron (BA.4 & BA.5). In terms of severity and infectivity, Omicron and its subvariants differ from Delta and other SARS-CoV-2 variants. In this review, we have discussed SARS-CoV-2 spike mutations, its variants along with their origin & association to wave(s), and its scenario in Pakistan.

**Keywords:** COVID-19; Coronavirus Variants; Waves in Pakistan; Mutations in SARS-CoV-2; Covid Prevalence

**Abbreviations:** CT: Computed Tomography; RBD: Receptor Binding Domain; NIID: National Institute of

Infectious Diseases; NCOC: National Command and Operation Centre

## Introduction and Background

Viral infections create global health challenges due to their severity, high mutation rate, and speedy spread. Coronaviruses affect the respiratory and intestinal tract in humans and animals. They were not known as highly pathogenic and destructive before the outbreaks they created in 2002 in China in the form of the severe acute respiratory syndrome [1,2]. Two highly pathogenic coronaviruses emerged known as severe acute respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory coronavirus (MERS-CoV). MERS-CoV uses DPP4 (dipeptidyl peptidase 4; also known as CD26) as a receptor and infects type-II pneumocytes un-ciliated bronchial epithelial cells [3,4], whereas SARS-CoV primarily infects ciliated bronchial epithelial cells via ACE2 (angiotensin converting enzyme-2) as a receptor [5,6]. MERS-CoV and SARS-CoV were directly transmitted to humans from air droplets or contact with the body fluid of infected organisms such as domestic and dairy animals [7,8]. It's believed that bats are where these viruses first appeared [9,10]. After these two deadly pathogenic strains of coronavirus, the third strain known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in December 2019 in Wuhan, Hubei province of China. This virus is known to cause COVID-19 illness and it posed a major threat to the world in concern of health along with the trade and medical research infrastructure [11,12]. More than 632 million cases and 6.5 million confirmed deaths have been stated globally as of 13th Nov. 2022 [13].

### COVID-19 in Pakistan

World Health Organization declared COVID-19 as an emergency on 30th January 2020 and a pandemic on 11th March [14]. In Pakistan, it was reported firstly on 26th February 2020 by the Pakistan government when two persons were found infected with the disease. Both patients returned from Iran and were declared positive for the disease in big cities of Pakistan, Islamabad, and Karachi [15]. Lahore General Hospital was the first public health institute that immediately started COVID-19 diagnosis and SARS-CoV-2 detection through real-time PCR [16].

Two deaths caused by COVID-19 were observed in Khyber-Pakhtunkhwa on 18th March 2020 [15]. It was observed that 98% of Pakistani patients recovered while the mortality rate was 2% [17].

Pakistani people including healthcare professionals and the public were trained through webinars, seminars, conferences, and other awareness campaigns to fight against this pandemic. Still, this disease created depression and anxiety among people including medical and paramedical staff due to the overload of work, financial and

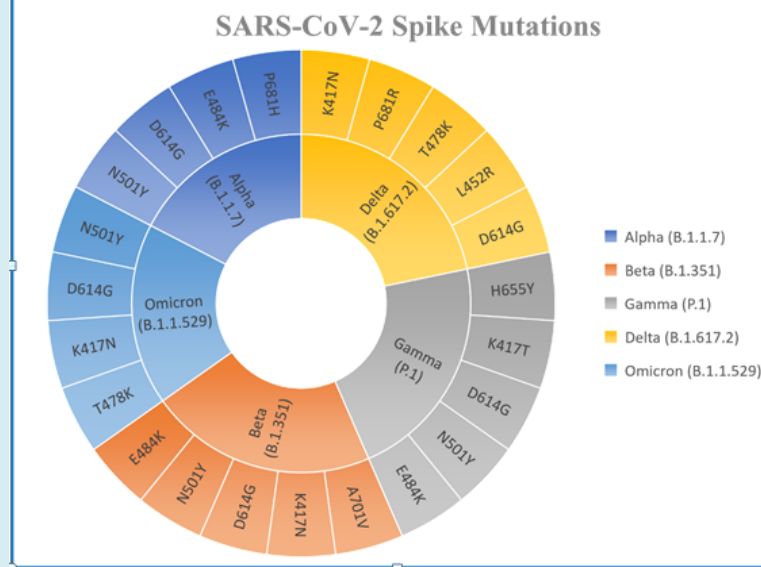
appropriate facility crises. In a survey study, it was observed that 92.6% were familiar with COVID-19 disease, while the prevalence of depression and anxiety was 14.3% and 4.6%, respectively. Healthcare professionals and medical students were observed to have less depression and anxiety when compared with the general public [18].

Common symptoms in Pakistani patients at the onset of Covid-19 illness were low-grade fever, malaise, cough, chest pain, sore throat, tightness, and shortness of respiratory rate. As SARS-CoV-2 causes respiratory problems therefore computed tomography (CT) scans and radiological examinations also prove helpful for diagnosis [19]. In a study, high-resolution CT scans and Real-Time PCR tests were compared to check the sensitivity for Covid-19 diagnosis, and noted that HRCT has higher sensitivity (92%) than RT-PCR (45%) [20]. But as we know the respiratory complications and symptoms of COVID-19 are also associated with a number of other illnesses therefore molecular especially nucleic acid-based detection of coronavirus is important in COVID-19 diagnosis [21].

### Severe Acute Coronavirus-2 and Mutations in its Genome

SARS-CoV-2 is coronavirus-related beta-coronavirus. The virus is spherical in shape having spikes on the surface, enveloped, and its genome (RNA) can be directly translated into proteins due to its 5'-3' orientation. Non-structural-protein 14 (NSP14) possesses proofreading capability thus keeping the mutation rate slow. Any change in nucleotide sequence is known as mutation and the genomes that possess different genetic sequences are called mutants or variants. There are two ways through which virus change their genomes constantly, antigenic shift and antigenic drift. With antigenic drift, SARS-CoV-2 mutates itself and gets rid of the host immune system's recognition. The COVID-19 variants are classified as variants of concern, variants of interest, and variants of high consequences by Center of Disease Control and Prevention. Variants along with increased spread, illness, and mortality, also escape detection tests and hence pose new challenges in diagnosis and treatment [22]. According to the WHO coronavirus dashboard, 612.72 million COVID-19 cases and 6.51 death reported as of 25th September 2022 [23].

Out of four genera of coronaviruses the alpha and beta genera viruses possess the ability to cause illness in humans. Zoonotic SARS-CoV-2 is very similar to bat coronaviruses [24] RmYN02 and RaTG13 with 93.3% and 96.2% genetic homology, respectively [25,26]. Malayan pangolin coronavirus also found genetic similarity with SARC CoV-2 [27-31].



**Figure 1:** Mutations associated with the SARS-CoV-2 spike protein and the emergence of new variants i.e., Alpha, Beta, Gamma, Delta, and Omicron [32].

Every continent of the world has been affected due to COVID-19. The global health crisis was caused by the emergence of SARS-CoV-2 strains [28]. A number of its variants evolved, and everyone posed destruction in their own way of severity, transmission, drug resistance, and mortality rate. All are associated with decreased immune response from previous infection or immunization resulting in subsequent pandemic outbreaks [29,30]. Different studies reported various mutated genes, E, S, M, N, ORF3a, ORF1ab, ORF6 to ORF10, and spike mutations associated with the emergence of new SARS-CoV-2 variants i.e., alpha, beta, gamma, delta [28], and Omicron [31] as shown in Figure 1.

### Waves of COVID-19 and Associated Variants in Pakistan

The mutation frequency of SARS-CoV-2 increased in the pandemic period from 12.19% to 23.63%, 31.03%, and 41.22% during the 1st wave to the 2nd, 3rd and 4th wave, respectively [33]. Omicron has 5-11 times the mutation frequency than previous variants [34]. The most common strains associated with 1st wave were B.1.160, B.1.255, and B.1.36, 2nd wave B.1.247 and B.1.36, 3rd wave B.1.1.7 and B.1.36, and 4th wave B.1.617.2 (Delta strain) [33]. It was noted that B.1.36 strain was observed during all four waves of COVID-19 which shows their fitness survival and drug & vaccine resistance capability. The 5th wave of this disease was due to the novel variant Omicron B.1.1.529 [35,36], while the new variant BA.5 of omicron is known to be responsible for the sixth wave in Pakistan [37]. The 5th VOC Omicron (B.1.1.529) originated in Botswana, Africa in

November 2021 and surprised the other VOCs with its global dominance [38] and antibody circumvention [39].

Six COVID-19 waves in succession have been reported in Pakistan; the 1st from March to July 2020, 2nd October 2020 to January 2021, 3rd from April to May 2021 [40], 4th from July to September 2021, 5th from December 2021 to May 2022 [36,41], and the sixth wave from June 22 to present [37].

### Corona's first wave in Pakistan

**Duration: February to July 2020:** The first wave of COVID-19 was very uncertain because of the unknown virus nature, pathogenicity, infectivity mechanism, transmission, and treatment options. Pakistan and other countries managed somehow to control the mortality rate because of rapid response and strategies. The initial level of transmission control is very necessary for any kind of outbreak to lower the fatality. Pakistani govt put partial, smart lockdowns, and designed new policies and SOPs for the movement of people from one place to another. The first wave in Pakistan lasted for 7 months from February to September 2020 [42].

The Wuhan strain originated in January 2020 and was of L clade (a viral clade is a group of viruses that share the same genetic changes and belongs to a common ancestor) isolate that later diversified itself into several other clades and subclades such as G, S, and V [43]. During the first wave, S and G clade strains were observed in Pakistan [44]. Initially out of 6 noncoding and 8 coding genes, ORF1B and

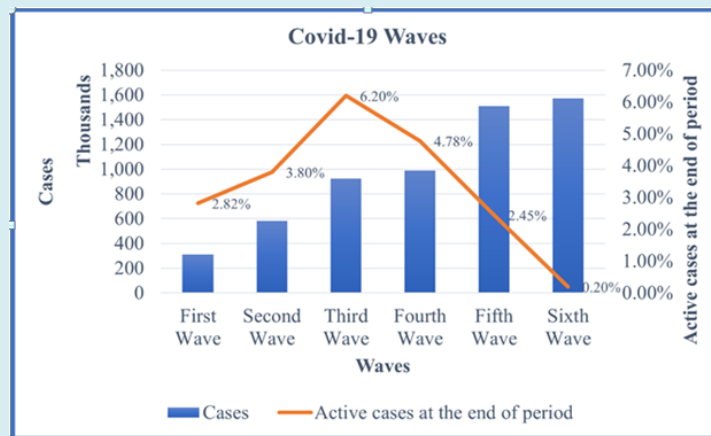
nucleocapsid genes were observed with great variation [45]. Then by the end of 2020 spike glycoprotein genes mutated at a very higher frequency and give rise to a number of variants of concern all around the world [46]. Further mutations in S and N genes, ORFIAB and ORF3A classified strains into G, I, L, S, V, GH, GR, GV, and subclades by GISAID [47,48].

The research of Pakistani SARC-CoV-2 genomic sequences that were originated during 2020 and 2021 and saved in the PANGOLIN database showed that 58% of sequences from April-June 2020 were of B.1. Along with B.1 the next lineage was A, B.1.1.1 and B.1.36. Whereas, lineage B.1.1.1 and A were studied in sample sequences before August, and B.1.36 and B.1 were seen after August 2020 [49].

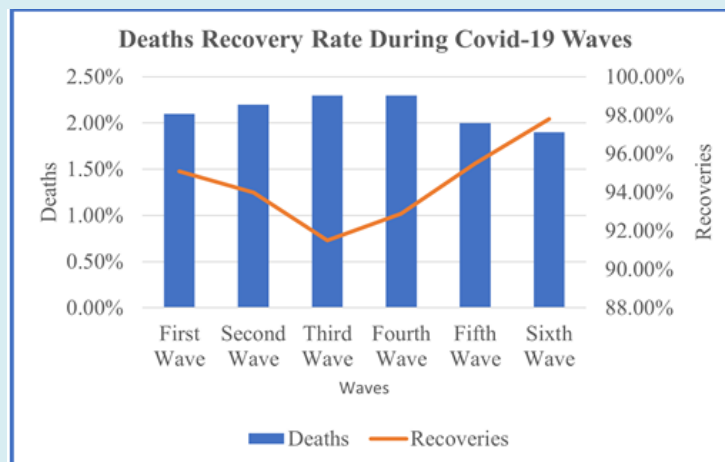
In Pakistan first wave was short with a low mortality rate in which timely management policy has a significant

role. A large number of infected people, active cases, deaths, and recoveries were observed upon the end of the first wave [36,50] as shown in Figure 1 & Figure 2.

The highest mutation incidence recognized during the first wave was in non-structural protein 3 and spike protein. Particularly, the spike protein showed the highest rate of homoplastic sites which includes the D614G mutation, missense SNVs, and Q677H mutation. Among the other frequently repeated variations are 241:C>T, a mutation in the upstream 5'-UTR, the nsp3 silent mutation F924F, missense mutation P4715L in nsp12, silent mutation L227L in nsp13, silent mutations D294D, silent mutation L280L in nsp14, G880G mutation in spike protein, silent mutation Y71Y, missense mutation Q57H in orf3a, and H125Y membrane missense mutation, E39X stop-gain mutation of orf7b, and R209I and S194L nucleocapsid missense mutations [33].



**Figure 2:** The total and active COVID-19 cases obtained from Pakistan's authorized COVID-19 website during the period of six successive waves [51].



**Figure 3:** The overall number of COVID-19 fatalities and survivors was reported on Pakistan's official COVID website over the course of six sequential waves [51].



### Corona second wave in Pakistan

**Duration: October 2020 to February 2021:** To avoid increased treatment and vaccination SARS-CoV learned to resist them and invade the immune system [42]. A second wave of Covid-19 was raised on 28th October 2020 and lasted till February 2021 in Pakistan. This wave infected 579,973 people, with 22,098 active cases, 12,860 mortalities, and 546,371 recoveries [36,50] as shown in Figure 2 & Figure 3. This wave occurred due to B.1.351/20H/501Y.V2 variant of South Africa. Genetic studies of the virus identified different variants of lineage B.1 and B.6 in Pakistan during this period [52].

During the second wave of COVID-19, Rehman et al., 2022 observed B1.562, B1.36.31, and lineages in Pakistan from the PANGOLIN database. It was also observed that from December 2020 to January the sequences were of alpha lineage B.1.1.7. While in Bangladesh beta variant was responsible for COVID-19 second wave.

The B.1.351/501Y.V2 was recognized firstly in October 2020 in Nelson Mandela Bay of South Africa and then in December 2020 in Zambia. The 17 amino-acid changes were found in the S protein with 23 mutations in which notable mutations were N501Y, K417N, and E484K. Antibody escape was performed mainly by the E484K mutation associated with this variant, the main reason for reduced vaccine sensitivity [53]. Studies have shown lower efficacy of Janssen, Novavax, and Astra-Zeneca vaccines against this variant [54].

B.1.1.250 lineage was associated with Covid patients during May and late 2020 in Bangladesh, the United Kingdom, the USA, Australia, and Europe. While lineage B.1.261 was recognized in Saudi Arabia and then disseminated to Pakistan. Other VOC that emerged during the second wave includes UK variant B.1.1.7 [55], South Africa variant B.1.3.51 [56], and P.1 [57] (Japan, 2021) with an increased rate of transmissibility from 50 to 70% [52].

Variants during the COVID wave such as B.1.36.6 and B.1.36 lineages were closely related to subclades from India and the United Arab Emirates. B.1.247 strain was in close association with Iranian sub-clades, B.1.160 strain with India, and B.1.36 with India and Iran. B.1.1 genomes of SARS-CoV-2 during the second wave in Pakistani patients were in close peroxidation with sub-clades from Iran, India, UAE, and Israel [33].

### Corona third wave in Pakistan

**Duration: Late March to May 2021 [40]:** From late March to May 2021, Pakistan experienced the third wave [58]. By

the PANGOLIN database [55] occurrence of B.1.1.7 lineage served as confirmation for the beginning of the third wave and its peak following March. Other B.1.1.7 sub-lineages, like Q.4 and Q.1, were first noticed starting in April 2021 although they haven't consistently been shown in a lot of sequences during the following months. It is interesting to note that none of the sequences examined in the months following April 2021 contained the B.1.36.31 lineage. The Beta strain B.1.351 was detected in Pakistan for the first time in March 2021, and most cases were recorded in May and June of that same year. Meanwhile, the lineage B.1.617.2 (Delta) initially appeared in April 2021, and since then, its numbers have been continuously rising by greater than 100 percent in the month of August 2021 relative to the other sequences observed in July 2021 and onward. The three main lineages identified during the third wave that continued from May to July 2021 were B.1.1.7, B.1.617.2, and B.1.351 [49].

Since about January 27, 2021, more than 64 nations, including Pakistan [59] had been found to have the novel SARS-CoV-2 strain known as 20I/501Y.V1 and B.1.1.7, which originated in the United Kingdom. B.1.1.7 was associated with a greater death rate in Pakistan as compared to other variants of this wave. The ten major cities in Pakistan; Rawalpindi, Hyderabad, Faisalabad, Islamabad, Bahawalpur, Lahore, Swat, Multan, Muzaffarabad, and Peshawar, were placed under a rigorous lockdown till 11th April 2021, and the provincial administration was instructed to adhere with SOPs. [42] and the total cases, active cases, deaths, and recoveries till the end of this wave were 922,824, 57,336, 20,850, and 844,638 respectively as shown in Figure 1 & Figure 2.

The time of start, which was Spring, was common during the period of the first wave and third waves (from March to April). So, it could be hypothesized that the increased transmission of this SARS-CoV-2 was significantly based on the pollens during this period [42].

In September 2020, B.1.1.7 was found in the UK, and in contrast to the initial strain discovered in Wuhan, China, it includes 23 mutations. The S protein was found to contain eight of all these mutations. N501Y and P681H 69/70 deletion are significant mutations in this gene. The S protein seemed to be able to attach to the ACE2 receptor more firmly because of the N501Y mutation [60]. Compared to the other two, it was 40–80% more transmissible [61] and was responsible for almost 5,000 deaths out of 17,452 deaths due to COVID-19 between September and February. When compared to other variants, it was calculated that the mortality was about 55% greater [62]. Similarly to this, research reports from January 2021 also showed that this variant had a higher fatality rate [63]. 82 different countries have reported this variant [57].

Various research projects have been undertaken to evaluate the efficacy of anti-inflammatory and antiviral drugs in the management of the UK strain [22].

B.1.617.2 and B.1.351 were third-wave variants from Pakistan which are delta and beta variants respectively. As of June 20, 2021, the beta variant B.1.351, analyzed in over 95 nations including Pakistan, was known for having three major mutations at specific residue sites known as receptor-binding domain (RBD) in its spike, specifically N501Y, E484K, and K417N [64]. Furthermore, because of the E484K mutation in the spike protein, the South African variant showed an immunological escape strategy against antibodies [65]. This variant has properties to escape from monoclonal antibodies and serum antibody reactions [66].

B.1.617 variant was 1st time appeared in Maharashtra and then spread to the whole state along with some other countries. This variant has two sub-lineages of B.1.617.1 and B.1.617.2. From these, sub-lineage B.1.617.1 has P681R, E484Q, and L452R mutations whereas B.1.617.2 sub-lineage has P681R, T478K, and L452R mutations in its spike at receptor-binding domain. The mutations in the receptor-binding domain (RBD) boosted the infectivity because of the presence of T478K and L452R by increasing the attachment of its spike protein to the receptors of ACE2 in humans [67,68]. Both mutations lessen the monoclonal antibodies' ability to bind to specific molecules, which reduced their capacity to neutralize. Furthermore, structural examination of mutations in the cleavage site of furin, L452R, E484Q, and P681R demonstrated higher ACE2 interaction and cleavage rate, leading to greater transmissibility [69]. The majority of the patients affected with this variant were found in Islamabad (63%), followed by Azad Kashmir and Peshawar (13% each) as compared to the Beta (B.1.351) variant mostly found in Islamabad (56%) and then in Peshawar (22%), Azad Kashmir (11%) and Rawalpindi (11%).

In this wave, nsp12 & N genes were highly mutated after S and nsp3 genes with 28, 27, 39, and 35 mutational sites respectively. In addition to the D614G, other spike mutations like N501Y, p.68 70del, A570D, p.143 144del were reported in its genome. From these, N501Y and p.68 70del were the distinguishing features of the UK B.1.1.7 variant. These two genomes contained an E484K mutation like the B.1.351 South African variant [33].

The third wave's genomes were predominantly made up of UK variant B.1.1.7 which accounted for sixty-nine percent of the total genomes along with B.1.36, B.1.351, B.1.468, B.1.1.413, and A.27 lineages genome. It was significant to note that there was no Alpha variation in the Quetta genomes and this city has minimal contact with the other provinces. In Karachi, Pakistan's capital and largest

city, the South African variant was seen. ML phylogeny was carried out by using SARS-CoV-2 genomic data from various countries to determine the possible mode of transmission of all these variants. In the case of France, Italy, and England this genomic data was grouped with the South African variant of Karachi. Some B.1.1.7 variants of Mardan and Lahore were also found in England and India whereas others were found in the United States, Italy, and England. These two findings show that Europe and India were the two main entry points for the pandemic's third wave virus [33].

### Corona fourth wave in Pakistan

**Duration: July to September 2021:** The fourth wave started in July 2021 in Pakistan [70]. The Delta variant first appeared in the summer of 2021 and quickly became a global sensation. It was in a worse state when it arrived in Pakistan. Luckily, a vaccine that was effective against the Delta form had been approved. Over 1,245,000 cases with 47,331 active cases, 22,781 deaths, and 919,163 recoveries at the end of this wave were observed as shown in Fig. 1 and Fig 2. Smart restrictions and lockdowns were implemented in key cities including Lahore, Islamabad, Rawalpindi, Muzaffarabad, Faisalabad, Mirpur, Multan, Peshawar, Abbottabad, Karachi, Gilgit, Hyderabad, and Skardu. To prevent the delta virus from spreading, workplaces and schools were shuttered. The construction of several vaccination facilities and the availability of a wide variety of vaccinations helped to advance the immunization process [36].

### Brazilian variant (501Y.V3/P.1 or 20J)

The P.1 variant referred to as B.1.1.28.1, was discovered for the 1st time in Manaus, North Brazil in December 2020. It was discovered in samples examined at the National Institute of Infectious Diseases (NIID) of Japan in January 2021 in Brazilian travelers at the Haneda airport, just outside of Tokyo, during a standard passenger screening test. It seemed to have 35 mutations and 17 alterations to the amino acids. The mutations N501Y, E484K, and K417T were significant ones. According to research, this variant was nearly equally infectious in both older (greater than 60 years old) and younger (18 to 59 years old) individuals. It seemed to be 2.2 times more transmissible and resulted in few occurrences of reinfection after recovering from COVID-19 [71]. The vaccine's effectiveness against the P.1 strain was anticipated to be similar to that of B.1.351 because both strains have identical receptor binding alterations. So, the decreased effectiveness of the vaccine against the B.1.351 variant also resulted in decreased effectiveness against the P. 1 variant. Laboratory tests conducted by Sinovac Biotech have shown that the Corona vaccine has a 50 percent success rate in eliminating P.1 variant acquisition in Brazil [72].

### Double mutant variant (B.1.617)

It was India where this variant was discovered first. This variant was referred to as a “double mutant” because two mutations occur in this variant. On April 5, 2021, its first case was reported in San Francisco, USA. L452R and E484Q were two significant mutations. These variants had a higher likelihood of spreading and were also immune to immunization.

According to the PANGOLIN database [55], B.1.617.2 and B.1.617.2 variants along with their sub-lineages were among the most common lineages during the fourth wave. These sub-lineages initially appeared in the month of May and then in August 2021. In December 2021, a new BA.1 lineage of Omicron was discovered in Pakistan. It was interesting to see that the isolates were having lineages A and B. A.1. [49].

During the fourth wave, the delta 21J (known as B.1.617.2) and 21I variants of the genome were discovered. The Delta variant was the most lethal and highly contagious with severe illness signs. As of June 24th, 2021, 77 countries have identified this variant [29,73]. The Delta variety has had severe effects in the UK. More comprehensive molecular testing technologies and strict quarantine regulations were developed for international travelers to stop the rapid spread of this strain in the population. On September 15–20, 2021, GISAID CoV-Surver analysis of all the examined genomic samples revealed that they were all positive for Delta variants. The most prevalent mutations found in all genomic isolates were T478K, L452R, T19R, E156G, F157del, P681R, R158del, D614G, and G142D. The S protein's RBD showed a T478K mutation that affected how it interacts with human ACE2 [74,75]. The T478K, which was present in the epitope area of powerful neutralizing monoclonal antibodies, was specific to the Delta variant [76].

Delta variant has affected >100 countries. Humans were seriously harmed by the delta variant. Many people died as a result of this variant. Additionally, a lot of people experienced difficulties in getting medical care and dealing with oxygen cylinders. However, the delta version of the coronavirus proved particularly dangerous for humans. This virus caused a lot of losses. A very large death rate was associated with this wave [77].

South Africa's 4th wave was mostly caused by three Omicron variants (BA.3, BA.2, and BA.1). The most destructive Delta variant was succeeded by the BA.1 variant in late 2021, and it quickly rose to prominence. At the onset of March 2022, the BA.2 variant of Omicron succeeded it, and by late April 2022, it had overtaken it as the most prevalent variant globally [78,79].

### Corona Fifth Wave in Pakistan

**Duration:** December 2021 to May 2022 [36,41]: In the fifth wave of COVID-19, a new variant of Omicron (or B.1.1.529) was reported in Botswana, South Africa in November 2021. Fig. 2 and Fig. 3 show the total number of cases, active cases, deaths, and recoveries of patients from COVID-19 experienced during this wave. Omicron has destroyed every city in its path. Compared to the delta virus, Omicron was more (double) transmissible but not as deadly as earlier variants were. All ages, more men, both vaccinated and unvaccinated individuals were affected, and re-infection was observed [36]. This variation has undergone 50 mutations, while the spike protein has undergone more than 30 modifications [80].

This variant was reported in Israel, the UK, Australia, the Netherlands, Germany, Italy, Belgium, Hong Kong, and Botswana after its discovery in South Africa [81]. The human immune cells release antibodies that primarily affect its spike protein in order to treat COVID infection. This variant showed multiple mutations which played a great role in its escape from neutralization and the immune system. That's why researchers were not clear about the effectiveness of its vaccination. According to studies, these mutations were in the spike that enable it to escape from antibodies brought up by either a prior coronavirus infection or immunization [82]. Omicrons' new subvariant BA.2, highly contagious, has been discovered. The BA.2 was the most contagious but the least lethal of all the variants that were in existence. BA.2 variant has vaccine resistance, and the majority of illnesses in the fifth wave were breakthrough infections [83].

### Corona Sixth Wave in Pakistan

**Duration:** June 22 to present: On June 22, 2022, a new rapidly – moving Omicron sub-Variant was discovered in Pakistan, particularly in Karachi, and is currently being transmitted locally. Health experts have issued a warning that the increase in new infections could become the sixth wave of the pandemic based on recent changes in the spike of Omicron variant cases around the nation. According to the figures, Karachi has been the province's most impacted city with 340 confirmed cases. A 10.69% positive rate was registered in the port city within seven days, as reported by the health authority. Agha Khan University reported that Hospitalization has grown in the sixth wave, although not as significantly as it happened in the Delta (4th) wave. However, some people who exhibited severe symptoms were admitted to hospitals [37]. Nine deaths and 872 cases were reported in Pakistan on July 8, 2022. Additionally, the national positive rate reached over 4% for one week before falling to 3.77 percent as the number of tests administered rose by 30 percent. This wave experienced a much lower Active cases &

death rate and a much higher recovery rate than the previous waves as shown in Figure 2 & Figure 3. Contrary to earlier waves, which were stopped by effective lockdowns and tight commitment to SOPs in public places, there were no such preventive measures in place nationwide. Because of over crowdedness, transportation hubs like train and bus stations turned into the hotspot for super-spreader outbreaks.

According to data from the National Command and Operation Centre (NCOC), nine persons died because of this variant. More information from the data revealed that the number of daily Covid tests—which had previously been at just over 15,000—had jumped to 23,125. Due to more testing, the positivity rate in Pakistan remained below 7 percent, with Karachi topping the list with 6.49 percent. Although the NCOC had not formally announced the 6th wave of the pandemic, it was clear that cases were increasing both in Pakistan and globally. The lifting of travel restrictions abroad was the cause. The sub-variant BA.5 of Omicron is currently circulating throughout the world, but the good news is that it

only causes bronchitis and does not progress to pneumonia. Additionally, there are very few illnesses and deaths that aren't just virus-related but are caused by comorbidity. Lung bronchitis is an illness that irritates and inflames the lungs' major airways. Comorbidity seems to be the simultaneous presence of multiple diseases, such as diabetes, high blood pressure, and cardiac issues [84].

Two novel Omicron subvariants (B.4 and B.5) have emerged in the sixth wave because of negligence from SOPs. Resuming the practice of socially isolating yourself in busy public spaces whilst wearing face masks should be advised. Otherwise, the advent of these two new Omicron subvariants could put the nation in a bad situation. The National Institute of Health in Islamabad has used genome sequencing to identify these new sub-variants. These two new sub-variants, which are causing almost all the infections countrywide, are rapidly spreading throughout the nation.

Variant name (s)	Associated wave (s)	Origin place & date
SARS-CoV2	1 <sup>st</sup> wave	Wuhan, China/ December 2019
B.1	1 <sup>st</sup> wave	Northern Italian/1 <sup>st</sup> January 2020
B1.36.31	2 <sup>nd</sup> wave	UK/8 <sup>th</sup> March 2020
B.1.247	2 <sup>nd</sup> wave	India/4 <sup>th</sup> May 2020
B.1.1.1	2 <sup>nd</sup> wave	England/2 <sup>nd</sup> March 2020
B.1.160	2 <sup>nd</sup> wave	European, Switzerland, Denmark, UK, and Hungary lineage/2 <sup>nd</sup> February 2020
B.1.1.471	1 <sup>st</sup> 2 <sup>nd</sup> wave	UK/25 <sup>th</sup> March 2020
B.1.562	2 <sup>nd</sup> wave	England base, to Ireland/10 <sup>th</sup> October 2020
B.1.1.7 (Alpha)	2 <sup>nd</sup> , 3 <sup>rd</sup> wave	United Kingdom/1 <sup>st</sup> September 2020
B.1.1.250	2 <sup>nd</sup> wave	United Kingdom and Bangladesh/April 2020
B.1.261	2 <sup>nd</sup> wave	South Africa/1 <sup>st</sup> September 2020
B.1.351(Beta)	2 <sup>nd</sup> , 3 <sup>rd</sup> wave	South Africa/ 1 <sup>st</sup> September 2020
P.1 (Gamma)	2 <sup>nd</sup> , 3 <sup>rd</sup> , 4 <sup>th</sup> wave	North Brazil city of Manaus in the state of Amazonas/22 <sup>nd</sup> September 2020
B.1.617.2(Delta)	3 <sup>rd</sup> , 4 <sup>th</sup> wave	India/1 <sup>st</sup> March 2021
B.1.36	1 <sup>st</sup> , 3 <sup>rd</sup> wave	Global many sub-lineages/16 <sup>th</sup> February 2020
B.1.468	3 <sup>rd</sup> wave	Indonesia, Singapore/13 <sup>th</sup> February 2020
B.1.1.413	3 <sup>rd</sup> wave	Europe, Turkey, Iran/7 <sup>th</sup> September 2020
A.27	3 <sup>rd</sup> wave	Mayotte, European /27 <sup>th</sup> November 2020
B.1.1.529 (Omicron)	5 <sup>th</sup> wave	South Africa and Botswana/24 <sup>th</sup> November 2021
BA.4 & BA.5 (Sub-variant of Omicron)	6 <sup>th</sup> wave	South Africa/7 <sup>th</sup> January 2022 South Africa/15 <sup>th</sup> November 2021

**Table 1:** COVID-19 Variants along with their origin & association to wave (s) reported on the official SARS-CoV-2 lineages website [86].



Due to successive genetic variations, new variants of SARS-CoV-2 emerged. Table 1 summarizes the variants experienced or observed in all six waves along with their origin and earliest date. The present Omicron variant has the most diverged genetic variations. Other factors contributing to its fast spreading include the fact that existing COVID vaccines lose their effectiveness 6 months after the second dose, the public's resistance to receiving a booster dose, and the general public's lack of preventative behavior [85]. During October 2022 BA.5 and its descendent lineages became dominant around the globe [13].

## Conclusions

Numerous research has shown that at the time of inception SARS-CoV-2 spread very fast in Pakistan due to poor management facilities. Meanwhile, it was mutating gradually. There was no proper vaccination system against it because of the lack of proper medical research facilities. It took a lot of time to make a vaccine against this disease. Initially, its effectiveness resulted in a decline in terms of preventing fatal and serious illnesses because of its diverged genetic variations and evasion from the immune system. The latter COVID-19 vaccinations were having good protection qualities, but they are no longer effectively working. To produce powerful medications to combat such natural outbreaks or bioterrorism, a robust medical research infrastructure is required. Additionally, we must consider developing biological means of controlling such pandemics while staying within the realm of ethics. New SOPs must be developed, and caution must be exercised if any new diseases are discovered.

## Conflicts of Interest

It is declared that authors do not have any conflict of interest.

## References

- Cui J, Li F, Shi ZL (2019) Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 17: 181-192.
- Zhong NS, Zheng B, Li Y, Xie ZH, Chanet KH, et al. (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362: 1353-1358.
- Lu G, Hu Y, Wang Q, Jianxun Q, Feng G, et al. (2003) Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature* 500: 227-231.
- Raj VS, Mou H, Smits SL, Dick HWD, Marcel AM, et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495: 251-254.
- Li W, Moore MJ, Vasilieva N, Jianhua S, Swee KW et al. (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426: 450-454.
- Qian Z, Travanty EA, Oko L, Karen E, Andrew B, et al. (2013) Innate immune response of human alveolar type ii cells infected with severe acute respiratory syndrome-coronavirus. *Am J Respir Cell Mol Biol* 48(6): 742-748.
- Alagaili AN, Briese T, Mishra N, Vishal K, Stephen CS, et al. (2014) Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *MBio* 5(2).
- Hemida M, Perera R, Wang P, Alhammadi MA, Siuet LY, al. (2013) Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. *Euro Surveill* 18(5): 20659.
- Ithete NL, Stoffberg S, Corman VM, Veronika MC, Leigh RR, et al. (2013) Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. *Emerg Infect Dis* 19(10): 1697-1699.
- Hu B, Zeng LP, Yang XL, Xing Yi G, Wei Z, et al. (2017) Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog* 13(11): e1006698.
- Wu JT, Leung K, Leung GML (2020) Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. *Lancet* 395(10225): 689-697.
- Hui DS, Azhar EI, Madani TA, Francine N, Richard K, et al. (2020) The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis* 91: 264-266.
- WH (2022) COVID-19 Weekly Epidemiological Update. 118.
- WH (2020) WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020. Geneva, Switzerland.
- Waris A, Atta U, Ali M, Asmat A, Baset A (2020) COVID-19 outbreak: current scenario of Pakistan. *New Microbes New Infect* 35: 100681.
- Rubi G, Malik I, Javaid ZJ (2020) COVID-19 in Pakistan. *BioMedica* 36(2): 33-34.

17. Sarfaraz S, Shaikh Q, Saleem SG, Anum R, Fivzia FH, et al. (2021) Determinants of in-hospital mortality in COVID-19; a prospective cohort study from Pakistan. *PLOS ONE* 16(5): e0251754.
18. Zafar SMA, Tahir MJ, Malik M, Malik MI, Akhtar FK, et al (2020) Awareness, anxiety, and depression in healthcare professionals, medical students, and general population of Pakistan during COVID-19 Pandemic: A cross sectional online survey. *Med J Islam Repub Iran* 34: 131.
19. Cozzi D, Albanesi M, Cavigli E, Chiara M, Alessandra B, et al. (2020) Chest X-ray in new Coronavirus Disease 2019 (COVID-19) infection: findings and correlation with clinical outcome. *Radiol Med* 125(8): 730-737.
20. Hanif N, Rubi G, Irshad N, Ameer S, Habib U, et al. (2021) Comparison of HRCT Chest and RT-PCR in Diagnosis of COVID-19. *J Coll Physicians Surg Pak* 31: 1-6.
21. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, et al. (2020) Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA* 26(7): 771-783.
22. Vasireddy D, Vanaparthi R, Mohan G, Malayala SV, Atluri P (2021) Review of COVID-19 variants and COVID-19 vaccine efficacy: what the clinician should know? *J Clin Med Res* 13:317.
23. WHO (2022) Coronavirus (COVID-19) Dashboard.
24. Hodcroft EB (2021) CoVariants: SARS-CoV-2 mutations and variants of interest.
25. Zhou P, Yang XL, Wang XG, Ben H, Lei Z, et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *579*: 270-273.
26. Li W, Shi Z, Yu M, Wuze R, Craig S, et al. (2005) Bats are natural reservoirs of SARS-like coronaviruses. *Science* 310(5748): 676-679.
27. Lam TTY, Jia N, Zhang YW, Marcus HHS, Jia Fu J, et al. (2020) Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *583*: 282-285.
28. Umair M, Ikram A, Salman M, Nazish B, Syed Adnan H, et al. (2021) Detection and whole-genome sequencing of SARS-CoV-2 B. 1.617. 2 and B. 1.351 variants of concern from Pakistan during the COVID-19 third wave.
29. Planas D, Veyer D, Baidaliuk A, Isabelle S, Guivel Benhassine F, et al. (2021) Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *596*: 276-280.
30. Stefanelli P, Trentini F, Petrone D, Alessia M, Luigina A, et al. (2022) Tracking the progressive spread of the SARS-CoV-2 Omicron variant in Italy, December 2021-January 2022. *27*(45).
31. Chenchula S, Karunakaran P, Sharma S, Chavan M (2022) Current evidence on efficacy of COVID-19 booster dose vaccination against the Omicron variant: A systematic review. *J Med Virol* 94(7): 2969-2976.
32. VW (2021) From Alpha to Omicron: Everything you need to know about SARS-CoV-2 variants of concern.
33. Shakeel M, Irfan M, Nisa Zu, Saba F, Noor UA, et al. (2022) Genome sequencing and analysis of genomic diversity in the locally transmitted SARS-CoV-2 in Pakistan. *Transbound Emerg Dis* 69(5): 2418-2430.
34. Kannan S, Shaik Syed Ali P, Sheeza A (2021) Omicron (B. 1.1. 529)—Variant of concern—Molecular profile and epidemiology: A mini review. *Eur Rev Med Pharmacol Sci* 25(24): 8019-8022.
35. Tegally H, Moir M, Everatt J, Marta G, Cathrine S, et al. (2022) Emergence of SARS-CoV-2 Omicron lineages BA. 4 and BA. 5 in South Africa. *Nat Med* 28(9): 1785-1790.
36. Bukhari MH (2022) Is Omicron a Tsunami? What should be the strategy to prevent the loss? *J Pak Med Assoc* 72(5): 798-799.
37. Khan DS (2022) Sixth wave of COVID-19 may hit Pakistan, fear health experts. In *The News International*.
38. Parums DV (2022) The 2022 World Health Organization (WHO) Priority Recommendations and Response to the Omicron Variant (B. 1.1. 529) of SARS-CoV-2. *Med Sci Monit* 28: e936199.
39. Liu L, Iketani S, Guo Y, Jasper FWC, Maple W, et al. (2022) Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *602*: 676-681.
40. Dil S, Dil N, Maken ZHJC (2020) COVID-19 trends and forecast in the eastern mediterranean region with a particular focus on Pakistan. *Cureus* 12(6): e8582.
41. Link Gelles R, Levy ME, Gaglani M, Stephanie AI, Melissa S, et al. (2022) Effectiveness of 2, 3, and 4 COVID-19 mRNA vaccine doses among immunocompetent adults during periods when SARS-CoV-2 Omicron BA. 1 and BA. 2/BA. 2.12. 1 sublineages predominated—VISION Network, 10 states, December 2021–June 2022. *MMWR Morb Mortal Wkly Rep* 71(29): 931-939.
42. Kamran K, Ali A (2021) Challenges and strategies for Pakistan in the third wave of covid-19: A mini review.

Frontiers in Public Health 9.

1407.

43. Yang X, Dong N, Chan EWC, Chen S (2020) Genetic cluster analysis of SARS-CoV-2 and the identification of those responsible for the major outbreaks in various countries. *Emerg Microbes Infect* 9(1): 1287-1299.
44. Umair M, Ikram A, Salman M, Adnan K, Masroor A, et al. (2021) Whole-genome sequencing of SARS-CoV-2 reveals the detection of G614 variant in Pakistan. *16(3): e0248371*.
45. Laha S, Chakraborty J, Das S, Soumen KM, Sampa B, et al. (2020) Characterizations of SARS-CoV-2 mutational profile, spike protein stability and viral transmission. *Infect Genet Evol* 85: 104445.
46. Li Q, Wu J, Nie J, Li Z, Huan H, et al. (2020) The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell* 182(5): 1284-1294.
47. Yang HC, Chen Ch, Wang JH, et al. (2020) Genomic, geographic and temporal distributions of SARS-CoV-2 mutations.
48. Hamed SM, Elkhatib WF, Khairalla AS, Noreddin AM (2021) Global dynamics of SARS-CoV-2 clades and their relation to COVID-19 epidemiology. *11(8435): 1-8*.
49. Rehman Z, Umair M, Ikram A, Fahim A, Salman M (2022) Footprints of SARS-CoV-2 genome diversity in Pakistan, 2020–2021. *Virol Sin* 37(1): 153-155.
50. Abid K, Bari YA, Younas M, Tahir Javaid S, et al. (2020) <? covid19?> Progress of COVID-19 Epidemic in Pakistan. *Asia Pac J Public Health* 32(4): 154-156.
51. (2022) COVID-19 Situation.
52. Tamim S, Trovao NS, Thielen P, Tom M, Brian M, et al. (2022) Genetic and evolutionary analysis of SARS-CoV-2 circulating in the region surrounding Islamabad, Pakistan. *Infect Genet Evol* 94: 105003.
53. Planas D, Bruel T, Grzelak L, Guivel Benhassine F, Isabelle S, et al. (2021) Sensitivity of infectious SARS-CoV-2 B. 1.1. 7 and B. 1.351 variants to neutralizing antibodies. *27: 917-924*.
54. Rubin RJJ (2021) Figuring out whether COVID-19 vaccines protect against variants\_ etermining How Much Immunity Is Enough. *JAMA Network*325(13): 1241-1243.
55. Rambaut A, Holmes EC, O'Toole Á, Verity H, John TM, et al. (2020) A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *5: 1403-*
56. Tegally H, Wilkinson E, Giovanetti M, Arash I, Vagner F, et al. (2021) Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* 592: 438-443.
57. Toole Á O, Hill V, Pybus OG, Alexander W, Issac IB, al. (2021) Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2 with grinch. *Wellcome Open Res* 6: 121.
58. Imran M, Khan S, Khan S, Uddin A, Khan MS, et al. (2021) COVID-19 situation in Pakistan: A broad overview. *Respirology* 26(9): 891-892.
59. Umair M, Ikram A, Salman M, Muhammad Masroor A, Nazish B, et al. (2021) Importation of SARS-CoV-2 variant B. 1.1. 7 in Pakistan. *J Med Virol* 93(5):2623-2625.
60. CDC (2022) How Flu Viruses Can Change: Drift and Shift.
61. Galloway SE, Paul P, MacCannell DR, Michael AJ, John TB, et al. (2021) Emergence of SARS-CoV-2 b. 1.1. 7 lineage—united states, december 29, 2020–january 12, 2021. *MMWR Morb Mortal Wkly Rep* 70(3): 95-99.
62. Davies NG, Jarvis CI, Edmunds WJ, Jewell NP, Diaz Ordaz K, et al. (2021) Increased mortality in community-tested cases of SARS-CoV-2 lineage B. 1.1. 7. *593:270-274*.
63. Choi JY, Smith DM (2021) SARS-CoV-2 variants of concern. *Yonsei Med J* 62(11): 961-968.
64. Nelson G, Buzko O, Spilman P, Niazi K, Rabizadeh S, et al. (2021) Molecular dynamic simulation reveals E484K mutation enhances spike RBD-ACE2 affinity and the combination of E484K, K417N and N501Y mutations (501Y. V2 variant) induces conformational change greater than N501Y mutant alone, potentially resulting in an escape mutant.
65. Wibmer CK, Ayres F, Hermanus T, Mashudu M, Prudence K, et al. (2021) SARS-CoV-2 501Y. V2 escapes neutralization by South African COVID-19 donor plasma. *27: 622-625*.
66. Greaney AJ, Starr TN, Gilchuk P (2021) Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. *Cell Host Microbe* 29(1): 44-57e49.
67. Starr TN, Greaney AJ, Dingens AS, Bloom JDJCRM (2021) Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. *bioRxiv* 2: 100255.
68. Zahradník J, Marciano S, Shemesh M (2021) SARS-CoV-2

- RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor.
69. Cherian S, Potdar V, Jadhav S (2021) SARS-CoV-2 spike mutations, L452R, T478K, E484Q and P681R, in the second wave of COVID-19 in Maharashtra, India. *Microorganisms* 9(7): 1542.
  70. Nasir N, Habib K, Khanum I, Khan N, Muhammad ZA, et al. (2021) Clinical characteristics and outcomes of COVID-19: Experience at a major tertiary care center in Pakistan. *J Infect Dev Ctries* 15 (4): 480-489.
  71. (2021) P.1 Variant, Dominant Strain In Brazil, Reported In New York.
  72. Mentzer AJ, Ginn HM, Duyvesteyn HM, Liu C, Slon-Campos J, et al. (2021) Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* 184(11): 2939-2954.
  73. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, et al. (2021) SARS-CoV-2 B. 1.617. 2 Delta variant replication and immune evasion. *Nature* 599(7883): 114-119.
  74. Gurung AB, Ali MA, Lee J, Farah MA, Al-Anazi KM, et al. (2022) Structural and functional insights into the major mutations of SARS-CoV-2 Spike RBD and its interaction with human ACE2 receptor. *J King Saud Univ Sci* 34(2): 101773.
  75. Wang Y, Liu C, Zhang C, Wang Y, Hong Q, et al. (2022) Structural basis for SARS-CoV-2 Delta variant recognition of ACE2 receptor and broadly neutralizing antibodies. *Nature communications* 13(871): 1-12.
  76. Barnes CO, Jette CA, Abernathy ME, Dam KMA, Esswein SR, et al. (2020) SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* 588(7839): 682-687.
  77. Chaubey G (2020) Coronavirus (SARS-CoV-2) and Mortality Rate in India: The Winning Edge. *Front Public Health*. *Front Public Health* 8: 397.
  78. Khandia R, Singhal S, Alqahtani T, Kamal MA, El-Shall NA, et al. (2022) Emergence of SARS-CoV-2 Omicron (B. 1.1. 529) variant, salient features, high global health concerns and strategies to counter it amid ongoing COVID-19 pandemic. *Environ Re* 209: 112816.
  79. Mohapatra RK, Kandi V, Verma S, Dhama KJC (2022) Challenges of the omicron (B. 1.1. 529) variant and its lineages: a global perspective. *Chembiochem* 23(9): e202200059.
  80. Organization WHO (2022) Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern.
  81. Livemint (2021) Omicron outbreak: List of countries where new Covid-19 variant has been detected.
  82. Stoltz E (2021) New Covid-19 variant is a concern as cases show a sharp increase in Gauteng.
  83. Cheung P HH, Chan CP, Jin DY (2022) Infections: Lessons learned from the fifth wave of COVID-19 in Hong Kong in early 2022. *Emerg Microbes Infect* 11(1): 1072-1078.
  84. Tribune P (2022) Pakistan moving towards sixth wave of Covid-19. *Pakistan News Service*.
  85. (2022) Experts Warn of Another Coronavirus Wave Due to New Variants in Pakistan. In *ProPakistani*.
  86. (2022) Cov-lineages.

