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Correspondence:

Samreen Riaz PTLLS, DTTLS, PGCE (UK), Ph.D (PU), IRSIP PH.D (UK), M.Phil, M.Sc. GCU (Gold Medal), B.SC. PU, (Gold Medal), DCS Institute of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore 54590, Pakistan. Tel: +92 300 435 1979 | +92 42 359 52811 -846 | samreen.mmg@pu.edu.pk | <http://pu.edu.pk/home/department/52/>

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Review Article

A GENERAL OVERVIEW OF COVID'19 STRAINS IN PAKISTAN

Sohaib Imtiaz · Samreen Riaz

Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore. Pakistan.

ABSTRACT

COVID-19 emerged in Wuhan, China in the December of 2019. Ever since then, cases have started springing up in all parts of the world. What start as a disease occupying a particular part of China, progressed to other provinces, nations and then continents. By the time March 2020 began, most of the world had already been exposed to the virus and were initiating lock down modes. It was not long before Covid-19 was declared a pandemic and SOPs were implemented to contain the virus. The emergence of the Omicron variant in Pakistan marked the beginning of 5th wave in Pakistan. Initially the influx was more but then since the vaccination phases started, the transmission of virus on a community level started reducing owing to the herd immunity. The decrease in the number of Omicron positive patients was due to the mass-level immunization strategy of the government. Furthermore, travel restrictions and social gatherings were limited. SOPs were implemented and followed by people. That is why Pakistan was able to cope up with the 5th wave better than most nations and the 5th wave disappeared as quickly as it had originated. The emergence of Covid'19 and its subsequent strains have put the human life as well as social set-ups in jeopardy. With each passing day, the virus propagates in various parts of the world, causing a lot of people to die while others remain infected and struggle to survive. Due to mass immunization programs, billion doses of Pfizer and Moderna have been administered due to which the 5th wave of SARS-CoV-2 has been contained. The lifestyle has returned to normal as WHO and UNICEF has ensured the dispatch of vaccines to all parts of the world. However, despite the preventive measures, we must continue to do research and explore different avenues especially the development of liposome-based mRNA vaccines. The more we know about new therapeutic approaches, the more we are prepared for combating the air-borne viruses and taking appropriate measures timely. Extensive research about all types of variants and their prevalence in Pakistan can help us highlight the areas that were most affected by the pandemic. In future, we can be prepared in case of re-emergence of any viral strains and how to tackle them. This way millions of lives can be saved by implementing SOPs and administering therapeutics timely.

Key words: COVID-19, Corona-Virus, SARS-CoV-2, Outbreak

INTRODUCTION

The novel Virus named as Covid'19 emerged in Wuhan, China is the December of 2019. Ever since then, cases have started springing up in all parts of the world (Weiss, 2020). What start as a disease occupying a particular part of China, progressed to other provinces, nations and then continents. By the time March 2020 began, most of the world had already been exposed to the virus and were initiating lock down modes. It was not long before Covid-19 was declared a pandemic and SOPs were implemented to contain the virus (Meo et al., 2020).

The coronavirus gets its name from its appearance of having spike proteins. There crown like proteins are called as 'Peplomers' as they are connected to the centre (Li, 2016). These proteins help in the identification of the virus inside the host. Though the world came to know about corona viruses in 2020, this family of virus has long been there. In 2002, SARS-CoV-1 emerged in Foshan, China in November (Ceylan, 2020). In the February of 2003, it spread to Hong Kong and from there it spread on a global level. Combined efforts resulted in the containment of virus. However, its re-emergence was reported in Guangdong, Beijing, Singapore as well as Taipei. From 2004 onwards, no cases were reported (Hulswit et al., 2016).

A decade later, MERS (Middle East Respiratory Syndrome) emerged in the April of 2012 in Jordan. It spread to other parts of the Middle East (De Wit et al., 2016). However, after a few precautionary measures, it was contained as well though it was not fully eliminated. All of these viruses belonged to the same family and had the same manifestation of the disease. They all caused respiratory complications leading to the death of the infected individual (Organization, 2019).

SARS-CoV-2 is readily transmissible via the respiratory droplets in the form of sneezing and coughing. However general symptoms like fever, fatigue, dry cough, muscular pain and lethargy have been observed (Alimohamadi et al., 2020). Along with these, several complications are there as well. Once the virus had settled well inside the lungs of an individual it starts spreading to other organs in case of viremia. SARS-CoV-2 has the potential of binding to other receptors as well and can infect the immune complexes to generate a cytokine storm inside the human body (Thomson, 2020). As a result, many parts of the body experience inflammation due to the incitement of Inter-leukins. Ultimately, due to hypoxia and lung fibrosis, a patient is unable to cope up with the stress induced in the body and dies (Cavezzi et al., 2020).

Structure of Corona-Virus

For having a better idea about the pathology of any virus, it is important to have studied the detailed structure of the virus. The Structural as well as the non-structural proteins play a great role in the interaction of the viral proteins as well as the host-cell receptors (Yadav et al., 2021). Covid-19 is an RNA virus whose nucleic acid sequence is about 30kb long. It is a single stranded virus. The entire genome has been categorized on the basis of next-generation sequencing. It is 29,881 base pairs to be exact. It encodes about 9860 proteins. The genes: S,E,M and N are responsible for encoding the structural proteins (Naserghandi et al., 2020).

The non-structural proteins include the papain-like protease, protease as well as RNA-dependent RNA polymerase. They all are encoded by the ORF region. The surface of SARS-CoV-2 is covered by the glycosylated S proteins. They have a high affinity to bind to the host-cell receptor (ACE-2). It mediates the viral entry into the host-cell (Lu et al., 2020).

Upon the binding of S proteins to the TM protease serine 2 (TMPRSS2), viral entry is promoted inside the host cell through the activation of these S-proteins. The viral RNA is now released and virus manipulates the host-cell machinery to begin transcription of polyproteins (Wang et al., 2020). Then protein cleavage

occurs, after which assembly of the replicase-transcriptase complex is done. Now, the life cycle of the virus is completed and it assembles its synthesized proteins, packages them and releases them into the extra-cellular environment where they function by infecting the other neighboring cells and destroying them too so that their own progeny can be propagated (Moreira et al., 2020).

Origin of SARS-CoV-2

The virus is known to have originated from the animals. Such viruses are called zoonotic viruses. Their primary host is an animals and then they are able to thrive in the human host and cause disease. However, they do not cause any infection in their primary host (Morens et al., 2020). It has been established that the virus was found in bats and from animal trade markets it spread in the population of Chinese where it got transmitted to so many other humans via respiratory droplets. The death toll increased exponentially as the transmission increased day by day. The scientists traced its origin to know more about its pathophysiology as well as epidemiology (Berekaa, 2021).

Manifestation of the virus in Three Waves

A three-wave pattern has been observed throughout the world. The first wave of infection originated from China and spread to all parts of the world. The second and third wave originated due to the emergence of various strains and how they had acquired better replicating and transmissible properties (Basheer and Zahoor, 2021). Generally, the first wave occurred in the spring of 2020 while the second wave emerged in the summers of June and July. The third wave struck many countries at the beginning of 2021 (Lu et al., 2020).

The first wave of infection hit many countries in the March 2020 where the countries were put in lockdown mode. All kinds of socializing were banned and no one was allowed to leave homes. The medical resources and facilities were exhausted as the countries struggled to ensure their patients received timely aid (Challen et al., 2021). There was a shortage of ventilators as well as hospital beds. Millions around the world could not even get hospitalized and were instead treated in their own homes (Kaye et al., 2021). Even the operating rooms were turned into the ICUs (intensive care units). By implanting the SOPs, many countries were able to contain the virus temporarily before the second wave initiated and caused the world to revert back to lockdown and isolating mode (Yang and Shaman, 2021).

During the second wave, smart lockdowns were implemented so that the economy could be run in a cautious manner (Thompson et al., 2020). During the first wave, a lot of strain was put on the economy and many countries struggled to keep up with the pace. However, even countries like China and America who are considered well-equipped to deal with medical health emergencies could not be at the same footing as corona's destruction worldwide (Thompson et al., 2020).

It was only a matter of time before millions of cases started springing up all over the country with the emergence of the second-strain. It was at this point the WHO (world health organization) realized that the fight against corona was far from over and a vaccine must be established immediately to counteract the effects of this deadly virus (Shahcheraghi et al., 2021). The virus was acquiring positive mutations as a result of which it was replicating at a much faster rate than the previous strains (Pooladanda et al., 2020).

Table 1: A general Comparison of Three Waves of Covid-19

Subject	1 st Wave	2 nd Wave	3 rd Wave
Time-Frame	March 2020	July 2020	Dec/Jan 2021
Symptoms	Sore throat, cough, fever, muscular pain, headache, confusion, insomnia, dyspnea, chest pain	Fever, pneumonia, dyspnea, communal cold, Sudden death	Neurological effects along with fever, immediate death
Mortality Rate	Intermediate	Very High	Lower as compared to the 1 st and 2 nd
Effect on Economy	Complete Lockdowns	Greatly Affected	Less Affected
Development of Vaccination	Not-available, primary research carried out	Prototypes were created, work-in-progress	Phase 1 and 2 trials were initiated
Re-infection	Not present	Yes	More common
Mutations	Not present	Yes	New variants emerged steadily.

COVID 19 and its Impact in Pakistan

Pakistan is an under-developed country. It has a population of about 220 million. There are five provinces: Punjab, Sindh, Balochistan, Khyber-Pakhtunkhwa and Gilgit Baltistan. The first case of Covid-19 was confirmed on 26th February by the Ministry of Health in Pakistan, (Govt. of Pakistan). From then onwards, the disease spread among the population like wild-fire. The virus spread among common population through the entry of a large group of pilgrims that returned from Iran from the border of Taftan (Mubeen et al., 2020). Moreover, Pakistanis that were in isolation in overseas were called back in special flight for the nationals. Till March 2021, about 1,024,737 tests were carried out, out of which 672,931 turned out to be positive. Around 14, 530 died. As per the available data, 300 medical workers died in the pandemic (Kamran and Ali, 2021).

2nd Wave and its implications in Pakistan

The non-compliance with the SOPs made the second wave hit even harder in some parts of the world. Now the number of cases were even greater than the ones experienced in the first wave. To make things worse, the new mutant had properties that worsened the condition of people in less time (Shahid and Zeb, 2020). More than 750 cases were reported each day and the activated cases rose up to 11,000. It was not long before the figures crossed 410,000 threshold with more than 8,260 fatalities. The national command and operation center of Pakistan confirmed that the rate of spread was three-fold than the first wave (Shahid and Zeb, 2020).

3rd wave and its implications in Pakistan

Pakistan was warned by WHO that by mid-july 2020, the no of patients could increase to 0.2 million. However, a new variant emerged (B.1.1.7) in the United-Kingdom and travelled all the way to Pakistan (Imran et al., 2021). (Volz et al., 2021a)It was detected in 64 countries of the world by January 27, 2021 (Volz et al., 2021b). This particular variant was associated with the increase in death rate as compared to the other two previous variants which had an average death rate of 100 patients per day. The third variant put the major cities of Pakistan: Faislabad, Rawalpindi, Islamabad, Bahawalpur, Lahore, Multan, Peshawar, Muzaffarbad, Karachi and Swat under strict lockdown till April 11,2021. The provincial governments did all they could to regulate health facilities among people (Umair et al., 2021)

Table 2. Time frame of all three waves in Pakistan

Time Frame	1 st Wave (March-Mid July 2020)	2 nd Wave (Oct- Mid February 2021)	3 rd Wave (May/June 2021)
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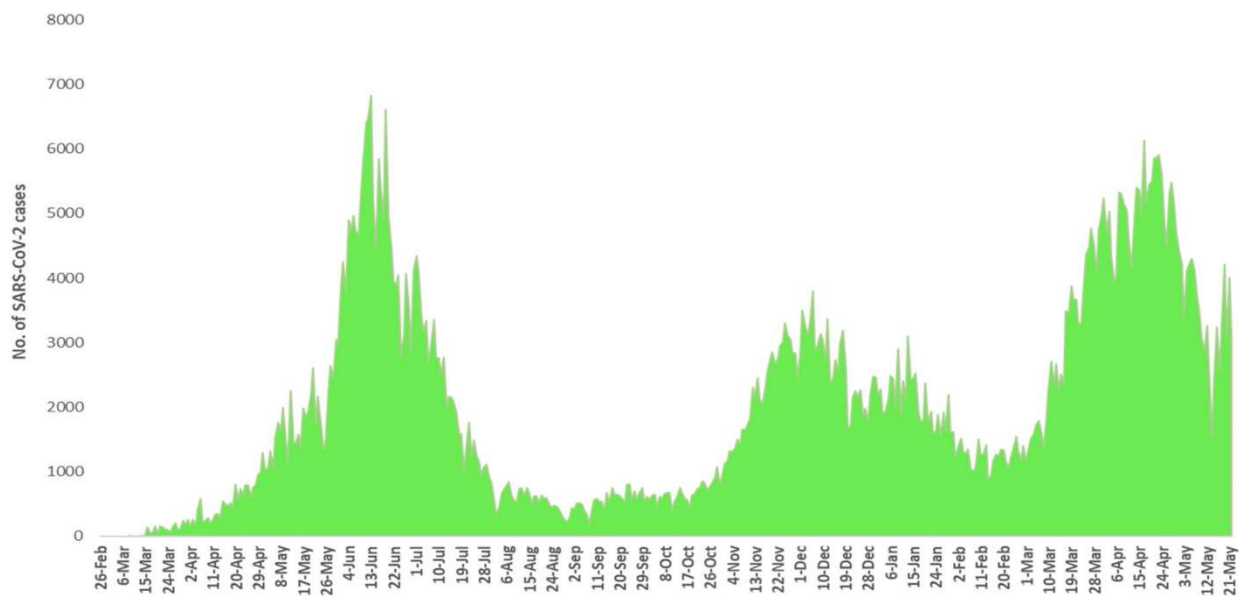


Figure. 1 Month wise cases representation of SARS-CoV-2 from February 2020 to May 2021. The data has been taken from www.covid.gov.pk

Genetic Epidemiology of SARS-CoV-2 and its lineages

An extensive study was conducted on the lineages of SARS-CoV-2 along with its seven clades G, GH, GR, GRY, L, O, and S in the three waves. In the first wave, 16 genomic lineages were identified with B.1(24.7%), B.1.36(18.8%), and B.1.471(18.8%) as the as widely prevalent lineages respectively. In the second wave, 18 lineages were identified but 10 of them overlapped with the first wave variant (Davies et

al., 2021). The small mutations clearly showed that the acquired changes made the viral strains more transmissible. In this particular wave, a new lineage was reported from the cases of Pakistan AE.4 for the first time. The most important lineages of the 2nd wave were B.1.36 (17.8%), B.1.36.31 (11.9%), B.1.1.7 (8.5%), and B.1.1.1 (5.9%). The third wave confirmed the presence of nine lineages with with Alpha/B.1.1.7 (72.7%), Beta/B.1.351 (12.99%), and Delta/B.1.617.2 (10.39%) as the most predominant variants in the native population (Volz et al., 2021a).

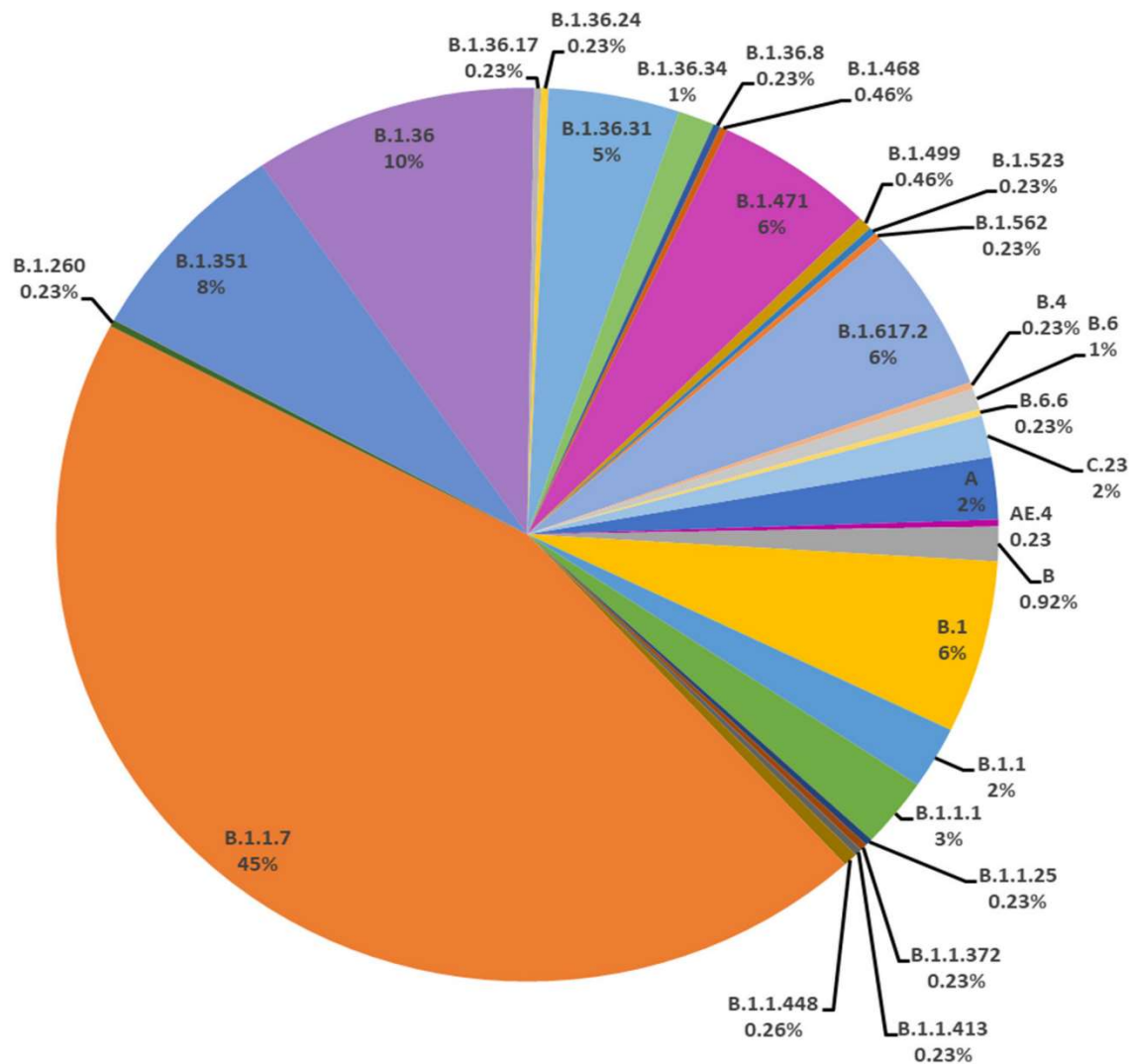


Figure 2. All the lineages observed from Pakistan sequences downloaded on 10 July 2021. The image has been taken from Genomic Epidemiology of SARS-CoV-2 Divulge B.1, B.1.36, and B.1.1.7 as the Most Dominant Lineages in First, Second, and Third Wave of SARS-CoV-2 Infections in Pakistan

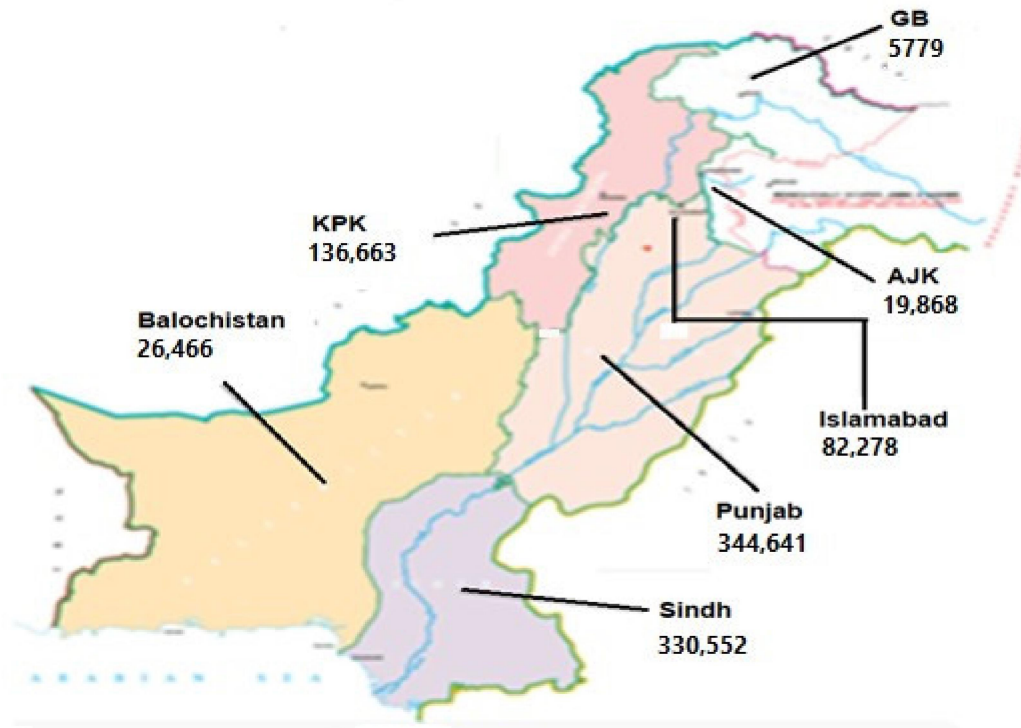


Figure 3. Number of total Covid-19 cases in different provinces of Pakistan up till 16th June, 2021. The map has been adopted from <https://covid.gov.pk/>.

Various Mutations and Its Impact

The persistence of corona virus over the time span of two years resulted in the emergence of various strains. The higher the infection rate, the more the chances of mutation are there. This way a virus is able to sustain itself longer in the population. SARS-CoV-2 and its variants have been thoroughly studied by the researchers all around the world (Banerjee et al., 2020). The most common mutation was those that occurred in the NSP3 and S proteins. By the end of December 2020, more than 5000 mutations had occurred in the sequence. The lineage of B.1.1.7 and variant 20I/501Y.V1 emerged from UK and this particular variant involved the spike protein changes including the deletions of 145, N501Y, deletion 69–70,

D614G, A570D, T716I, P681H, D1118H, and S982A (El-Shabasy et al., 2022). This lineage became so prevalent that it gave rise to the second wave of the pandemic.

Another lineage was discovered in South Africa: B.1.351, variant 20H/501Y.V2. It accumulated about eight mutations in its S protein: D80A, L18F, R246I, D215G, E484K, K417N, A701V, and N501Y. Three other mutations in the S protein, N501Y, E484K, and K417N were seen in lineage P.1 in Brazil. That is why the S-protein has been targeted for vaccine development (Graichen, 2021).

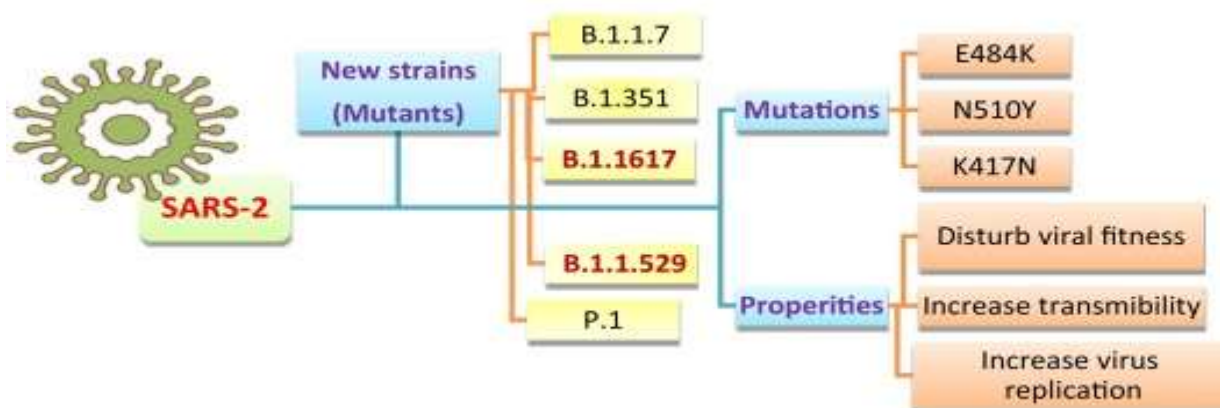


Figure. 4 COVID-19 and its common variants in all three waves. This image has been taken from Three wave changes, new variant strains, and vaccination effect against COVID-19 pandemic. International Journal of Biological Macromolecules.

Delta & Omicron

Delta (B.1.617.2) was isolated from the infected patients in India in May 2021. It then spread to about 176 locations worldwide. It became the leading strain that caused a lot of deaths in several people. Delta has D614G mutation (Alexandar et al., 2021). It has not been previously discovered in any strains. This included the L452R mutation, which steadily increased the infectivity of the virus by many fold. This particular mutation made the virus capable of escaping immune response. The P681R mutation triggers a severe disease in humans. The 'Delta plus variant' was identified in Nepal with the K417N mutation. As compared to the alpha variant, delta variant is 40%- 60% more transmissible. It also had double risk of hospitalization. Initially the vaccines that were developed were not effective against the delta variant (He et al., 2021).

Omicron (B.1.1.529) was identified by November 2021 in Guateng province. WHO was notified immediately. The sample was confirmed by 9th November (Thangaraj et al., 2022). By 3rd December, it was detected in 22 locations all over the world. This included parts of Europe, Africa, Asia, Australia as well as America. Omicron has a lot of mutations in its genome. This included the T478K mutations, K417N mutation, D614G as well as N501Y. these mutations have also been identified in the other variants worldwide. However, many of them have not yet been characterized (Shiehzaidegan et al., 2021)

It was suggested that Omicron had an increased risk of re-infection. It was also partly resistant towards the vaccine prototypes. Though, ongoing research is been done on this matter. However, the variant is by far the most transmissible one. On the other hand, it is less lethal. Moreover, the symptoms associated with omicron are quite different from the rest of the variants (Burki, 2021).

Omicron and Pakistan

By December 27, only 75 cases were reported by the National Institute of Health. 33 were reported in Karachi, 13 in Lahore and 17 in Islamabad. By the end of January and start of February in Pakistan, the cases surged up (Umair et al., 2022). Policies and Sops were implemented to contain the spread of virus. Total 19,404 samples were testes and RT-PCR was carried out by using the TaqPath Covid'19 kit. Only 3% of the samples tested positive for the omicron variant and 15 samples were further selected for the whole genome sequencing. All of the tested positive for the omicron variant. The median age was 36 years. The age range was between 20 to 67 years. The male: female was 2:3 with 40% males and 60% females. About 33% of the samples that tested positive were the ones that came from the passengers (Basheer and Zahoor, 2021). Nearly all of them had a travel history of the UK, one was from Maldives and one from South Africa. In these samples the characteristic mutations of omicron variant were observed. In two isolates, rare spike protein mutations of the R346K were seen. From there onwards, community level transmission started (Anser et al., 2020).

The emergence of the Omicron variant in Pakistan marked the beginning of 5th wave in Pakistan. Initially the influx was more but then since the vaccination phases started, the transmission of virus on a community level started reducing owing to the herd immunity (Rambaut, 2020). The decrease in the number of Omicron positive patients was due to the mass-level immunization strategy of the government. Furthermore, travel restrictions and social gatherings were limited. SOPs were implemented and followed by people. That is why Pakistan was able to cope up with the 5th wave better than most nations and the 5th wave disappeared as quickly as it had originated (Ejaz and Syed, 2021).

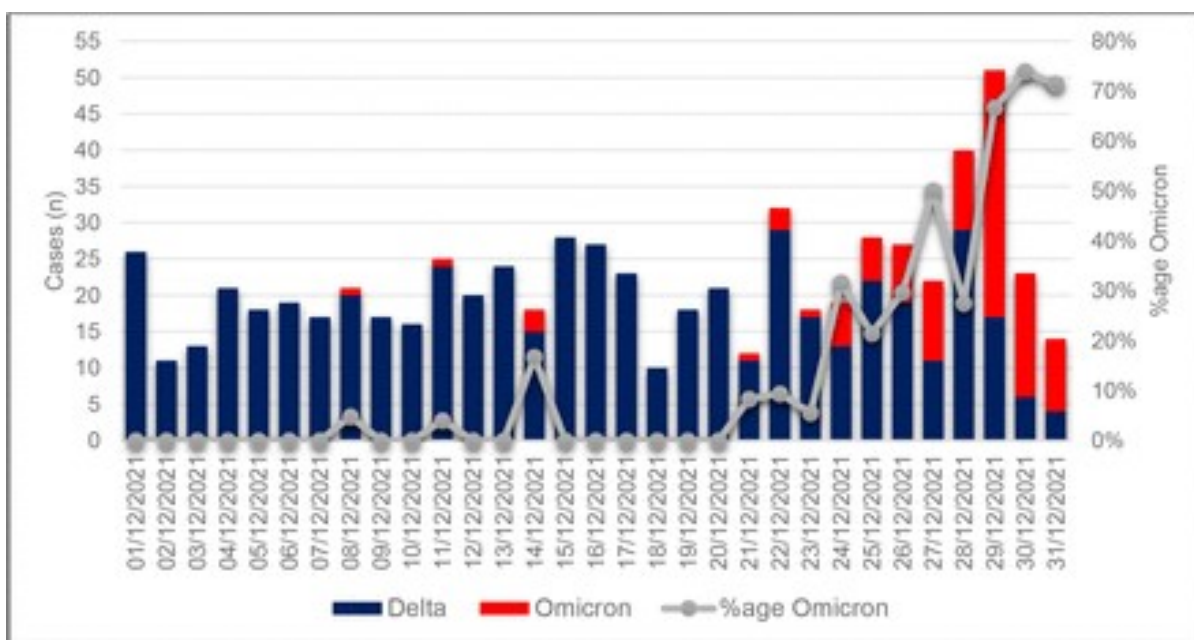


Figure. 5 Data Genotyping of patients with Omicron and Delta strains. The increase in omicron positive cases was seen after December 22, 2021 and January 2022. This data has been retrieved from *Detection and upsurge of SARS-CoV-2 Omicron variant in Islamabad Pakistan. medRxiv.*

CONCLUSION

The emergence of Covid'19 and its subsequent strains have put the human life as well as social set-ups in jeopardy. With each passing day, the virus propagates in various parts of the world, causing a lot of people to die while others remain infected and struggle to survive. Due to mass immunization programs, billion doses of Pfizer and Moderna have been administered due to which the 5th wave of SARS-CoV-2 has been contained. The lifestyle has returned to normal as WHO and UNICEF has ensured the dispatch of vaccines to all parts of the world. However, despite the preventive measures, we must continue to do research and explore different avenues especially the development of liposome-based mRNA vaccines. The more we know about new therapeutic approaches, the more we are prepared for combating the air-borne viruses and taking appropriate measures timely.

Future Prospects

Extensive research about all types of variants and their prevalence in Pakistan can help us highlight the areas that were most affected by the pandemic. In future, we can be prepared in case of re-emergence of any viral strains and how to tackle them. This way millions of lives can be saved by implementing SOPs and administering therapeutics timely.

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