*Pakistan J. Zool., pp 1-8, 2024.* **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220911100910

# A Peculiar Mutational Pattern in Omicron Variant of SARS-CoV-2

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**Article Information**
**Received 11 September 2022**
**Revised 05 October 2022**
**Accepted 19 October 2022**
**Available online 01 August 2024 (early access)**

**Authors' Contribution**
**Design of study: YJZ, MTK. Data acquisition: IA, MSL. Data interpretation. MTK, NN. Drafting: YJZ, MTK. MM. Content revision: AR. Approval. Funding YJZ.**

**Key words**
**Omicron, Mutations, VOC, Spike, SARS-CoV-2**



## ABSTRACT

Evolutionary changes are continued in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and new variants of concern are emerging. The emergence of SARS-CoV-2 new Omicron variant and its rapid transmission in many countries ushered in a panic response. Here we analyzed the unique mutational pattern in Omicron through whole-genome sequence analysis, retrieved from different genomic databases worldwide. All the genomic sequences were analyzed for the Omicron variant using GISAID server. Mutations in targets protein were identified using CoVsurver. The most common mutation pattern in virus spike protein was analyzed for stability and flexibility effect using the machine learning algorithm of DynaMut server. A total of 337 genomes were identified as Omicron variants, reported from 23 countries. These sequences harbored some unique patterns of mutations, among which NSP14_I42>V, Spike_N969>K, Spike_N856>K, Spike_S371>L, Spike_L981>F, Spike_Q954>H, Membrane_Q19>E has been detected in 218 genomes. Spike mutations of this pattern, when analyzed for thermodynamic effect through DynaMut server, revealed that N969>K, N856>K, and S371>L are exhibiting stabilizing effects and gain in protein flexibility. Spike_L981>F seems destabilizing, but its impact on molecular flexibility appears to be increasing. The highest gain in flexibility on S proteins mutant has been observed due to mutation Q954>H, which almost affects the entire beta-sheet and loops of the spike C-terminal domain. The gain in flexibility may be supportive of virus adaptation and improved binding affinity with human angiotensin-converting enzyme 2 receptor protein. The second most common pattern NSP14_I42>V, Spike_N969>K, Spike_N856>K, Spike_S371>L, Spike_Q954>H, and Membrane_Q19>E was detected in 42 genomes. The enhanced stabilizing effect and molecular flexibility of most common patterns of Spike_N969>K, Spike_N856>K, and Spike_S371>L may possess higher infectivity and cell-to-cell transport than other SARS-CoV-2 variants. Further experimental validation is needed for a better understanding of the effect of these mutations on Omicron and vaccine efficacy. The current study provides valuable information for further experimental investigation of these mutations' effects on virus transmission and disease severity.

## INTRODUCTION

The emergence of COVID-19 (Coronavirus disease 2019) pandemic raised international health concerns about possible viral evolutionary stages with underlying mutations that have increased the disease severity, transmissibility, and potential immune escape. Different evolutionary stages in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been reported and still continue. New variants of concern/interest (VOC/I) are emerging because of enhanced severity, transmissibility, immune response escape, and reduced vaccine efficacy. Mutations on the viral spike protein may affect SARS-CoV-2 binding to the human cell surface receptor called ACE2 (angiotensin-converting enzyme 2) and antibodies. These VOCs share some important mutations in which N501>Y has been detected in P.1 (Gama), B.1.1.7 (Alpha),

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0030-9923/2024/0001-0001 $ 9.00/0

and B.1.351 (Beta). Mutation E484>K is shared by B.1.351 and P.1 variants, decreasing the binding affinity with ACE2 and leading to immune escape (Janik *et al.*, 2021; Jogalekar *et al.*, 2021; Winger and Caspari, 2021).

On November 25, 2021, Omicron (B1.1.529), a new SARS-CoV-2 VoC (WHO, 2021), was reported. This VOC appeared at a time when vaccine immunity was enhancing. The other VOCs of SARS-CoV-2 were highly devastating worldwide (Fontanet *et al.*, 2021). The increased transmissibility of the Delta (Luo *et al.*, 2021) was associated with decrease in binding and prolong infection duration, increased rates of reinfection, and the capability of natural immunity (Townsend *et al.*, 2021; Sharawi, 2023), which resulted in a globally dominant variant. The delta remains dominant during the fourth wave.

The Omicron's first sequenced genome was published in Botswana on November 11, 2021 (Kamil, 2021). Several SARS-CoV-2 genome sequences have been reported from South Africa after the early identification of S-gene target failure on PCR assay due to a 69–70del, similar to the alpha variant (Volz *et al.*, 2021). The earliest Omicron case in South Africa has some deletions and more than 30 spike mutations, overlap with other VOCs (Kamil, 2021). These mutations have already been known for increased transmissibility and antibody escape (Greaney *et al.*, 2021; Harvey *et al.*, 2021; Rehman et al., 2023). Experimental verification to understand the effects of remaining mutations on Omicron virulency and the immune response is still unknown.

In the current investigation, about six million genome sequences have been screened for Omicron variant up to December 8, 2021, in GISAID. A total of 337 isolates were Omicron SARS-CoV-2 variant. These isolates harbor unique mutational patterns which may be investigated for their role in virus pathogenicity and COVID-19 disease severity.

## MATERIALS AND METHODS

*Whole-genome sequences retrieval*

The whole-genome sequences (WGS) of SARS-CoV-2 Omicron variants were searched using the EpiCov>Search option on the global science initiative on sharing all influenza data (GISAID) (Dec 2019- December 8, 2021) (Elbe and Buckland-Merrett, 2017) and NCBI virus repository (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/). GISAID is the leading and primary source of SARS-CoV-2 genomic data. The database is freely accessible to over 42,000 researchers from 198 countries. NCBI Virus is also freely accessible for retrieving and analyzing the virus whole genome sequence datasets. All the genomic data was retrieved with the default option and complete genome tab of SARS-CoV-2 in GISAID.

*Variant identification*

GISAID enables several web tools to perform SARS-CoV-2 variant identification. At that time (December 2019-December, 2021), a total of six million genomes of SARS-CoV-2 at default tabe option of variants (including all variants) were submitted from all over the world. GISAID has a built-in function on the variant's 'tab' option for screening variants among all the WGS, where different variants of SARS-CoV-2, including Omicron, can be screened. On the analysis tab of GISAID, only ten WGS could be analyzed at a time. Therefore, all the complete WGS files of Omicron variants in FASTA format were downloaded.

*Omicron WGS analysis*

The retrieved WGS files in FASTA format were uploaded to CoVsurver application (https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/). The server has a built-in function to align hundreds of query sequences with Wuhan reference (NC_045512) for variant identifications and mutations in all structural and non-structural proteins. The results of amino acid mutations are displayed along with specific locations and number of mutations in each target protein, clade, % identity, and coverage in all structural and NSPs proteins. The summary of all the genomes and the frequencies of mutations in each viral target can be exported to Excel on the CoVsurver query summary report option. The server has a built-in search engine that filters and searches each mutation along with frequencies in all the WGS isolates present in GISAID. Unique and reported mutations are also screened in the query sequences and can be observed in Excel along with their frequencies, length of genomes, etc.

*Spike protein structure retrieval*

To analyze the effect of unique mutational patterns on S protein structure, the crystal structure data in PDB format was retrieved from PDB (Berman *et al.*, 2000) (PDB ID: 7l09). The missing residues in the structure were modelled using SWISS-MODEL (https://swissmodel.expasy.org/) using the same structure as template. The PDB is a repository of crystal structures containing DNA, proteins, and RNA. The S proteins structure was subject to DynaMut server for mutation effect on protein thermodynamic properties.

*Stability and flexibility effect*

The five most common unique mutations of S protein were selected for computing the stability and flexibility effect on S protein using DynaMut server (Rodrigues *et al.*, 2018). The impact of mutations is projected in both graph-based signatures and normal mode. The results in the form

of a good resolution protein structure are displayed. This approach is more significant (P-value < 0.001) than other techniques to predict the mutation's effect on biomolecular structure. To compute the impact of each mutation on S protein stability and flexibility, the PDB file (IDs: S=7l09) of S protein was uploaded to the DynaMut server, and a point mutation was inserted at a specific site. Wild types and mutants' total and vibrational entropy energies were recorded. The high-resolution structures of wild-type and mutant S proteins were retrieved for further processing.

In DynaMut, changes upon point mutation in free energy of protein folding, combining the impacts of mutation stability of protein and the dynamics properties, were computed by ENCoM, Bio3D, and DUET computational servers, generating a more robust predictor of energies.

The PyMOL session of S protein structure was retrieved in the download resource tab. Residues were labeled at a location where unique mutations have been detected, using PyMOL version 2.5.1. The domain of S protein has also been colored using the color (C) option in PyMOL. A flowchart methodology has been shown in Figure 1.

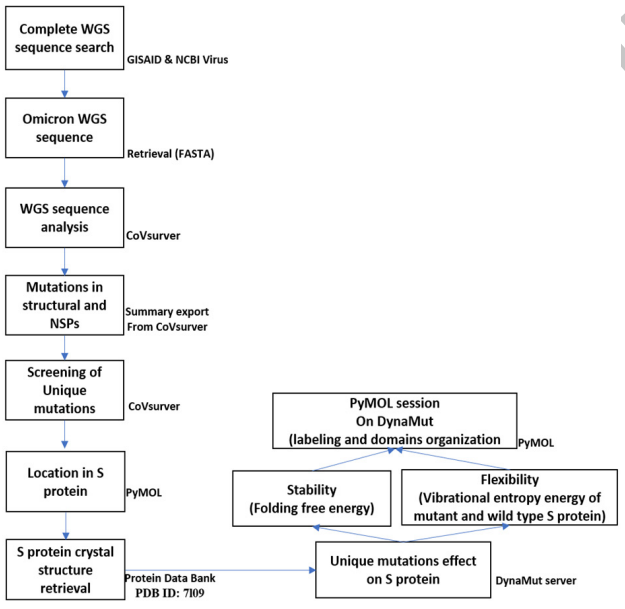


Fig. 1. Flowchart methodology.

*Statistical analysis*

Simple statistical analysis was performed using EpiData Analysis (Singh, 2009), suitable for epidemiological and other quantitative studies, to identify the unique and most common mutational patterns in all structural and non-structural proteins.

## RESULTS

Among the six million SARS-CoV-2 whole-genome isolates, 337 were Omicron variants. All these genomic isolates were collected from 23 countries (Supplementary File S1). The majority have been reported from South Africa (n=170), Australia (n=14), Botswana (n=19), and Ghana (n=13). Locations of unique and most common mutation patterns in spike, NSP14, and membrane proteins of Omicron variants have been shown in Figure 2. Mutations in these domains and motifs may affect the virus binding with human ACE2.

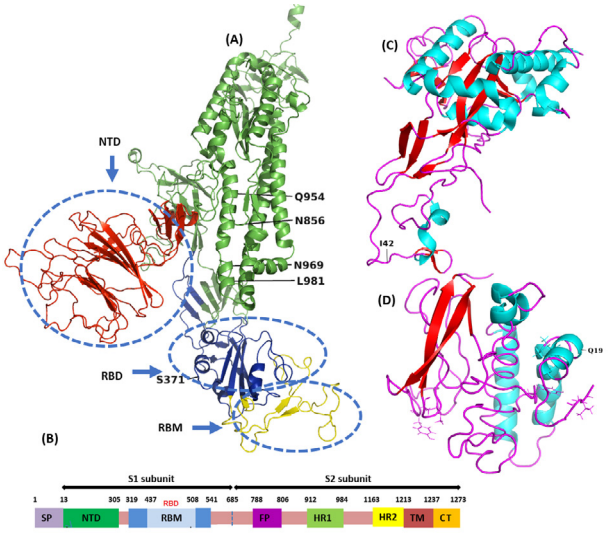


Fig. 2. Locations of unique and most common mutation patterns in spike, NSP14, and membrane proteins of Omicron variants. (A) Spike protein. (B) Domain organization of spike protein. NTD: N-terminal domain, RBD: receptor binding domain, RBM: receptor binding motif, FP: fusion peptide, HR: Heptapeptide repeat sequence, TM: transmembrane region, CT: cytoplasmic tail. (C) NSP14 and mutation in the loop region of Omicron. (D) Membrane protein and location of Q19 mutation.

*Unique mutations in omicron variant*

All the whole genomes were analyzed for unique patterns of mutations. The complete set of patterns has been provided in Supplementary File S1, some of the most common in Table I. Among the 337 genomes, most of these mutation patterns share one non-synonymous mutation of non-structural protein 14 (NSP14_I42V) and one in membrane protein (Q19E). The I42>V of NSP14 is present in the loop region. The unique patterns NSP14_I42>V, Spike_N969>K, Spike_N856>K, Spike_S371>L, Spike_L981>F, Spike_Q954>H, Membrane_Q19>E were detected in 218 genomic isolates which have been

**Table I. Mutation frequency in 337 SARS-CoV-2 Omicron variant.**

| S. | Unique Mutation's pattern | Freq* | Major prevalent countries |
|---|---|---|---|
| 1 | (NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, Spike_Q954H, Membrane _Q19E) | 218 | South Africa (115), Australia (9), England (8), Botswana (14), Ghana (28), Scotland (7) |
| 2 | (NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_Q954H, Membrane _Q19E) | 42 | Netherlands (13), South Africa (22), Botswana (3) |
| 3 | (NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, Spike_Q954H) | 29 | South Africa (18), Ghana (2), Germany (7), |
| 4 | (NSP14_I42V, Spike_N969K, Spike_N856K, Spike_L981F, Spike_Q954H, Membrane_Q19E) | 8 | South Africa (2) |
| 5 | (Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, Spike_Q954H) | 4 | South Africa (3) |
| 6 | (NSP5_P132Y, NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, Spike_Q954H, Membrane _Q19E) | 4 | Ghana |
| 7 | (NSP14_I42V, Spike_N969K, Spike-N856K, Spike-Y145D, Spike_S371L, Spike_L981F, Spike_Q954H, Membrane _Q19E) | 4 | South Africa |
| 8 | (NSP14-I42V, Spike-S371L, M-Q19E) | 2 | Australia, Botswana |
| 9 | (NSP6-L105I, NSP6_S106L, Spike_S371L, Membrane_Q19E) | 2 | Australia and Canada |
| 10 | (NSP14-I42V, Spike-N856K, Spike-S371L, Spike-Q954H, Membrane-Q19E) | 2 | Portugal, Italy |

*Freq: Frequency of mutation pattern.

detected in South African, Ghana, and Botswana. This pattern includes one non-synonymous Mutation in NSP14 (I42>V), membrane proteins (Membrane_Q19E), and five mutations in spike (Table I). Among the spike unique mutations, four were present in the S2 subunit (N856>K, Q954>H, N969>K, L981>F) and one in the receptor binding domain (RBD) (S371L) (Fig. 2). Some of the other countries isolates also harbored this pattern of mutations. However, the effect on disease severity still needs experimental validation.

The second most common unique pattern of mutations (NSP14_I42>V, Spike_N969>K, Spike_N856>K, Spike_S371>L, Spike_Q954>H, Membrane_Q19>E) has been detected in genomic isolates from South Africa (n=22), Netherlands (n=13), Botswana (n=3), Scotland (n=1), Hong Kong (n=1), Israel (n=1), Scotland (n=1). Mutations Spike_L981>F is missing in this pattern when compared with the first unique pattern (Table I).

The third pattern of unique mutations was NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, and Spike_Q954H was detected in 29 genomic isolates in which Q19E in membrane proteins is missing. This pattern was found in five countries isolates (Australia (n=1), Norway (n=1), South Africa (n=18), Ghana (n=2), Germany (n=7).

The fourth pattern of unique mutations NSP14_I42>V, Spike_N969>K, Spike_N856>K, Spike_L981>F, Spike_Q954>H, Membrane_Q19>E) was detected in eight genomic isolates from South Africa, Japan, France, England, Scotland, India, and Australia in which Spike_S371>L is missing. All known and unique mutations and their frequencies in each target protein have been provided in Supplementary File S1.

However, the effect on virus transmission behind Omicron variants is largely unknown. Geographic-specific drugs and diagnostic techniques may be designed based on mutations in the virus proteins for better management of COVID19.

According to South Africa's National Institute for Communicable Diseases (NICD), the Omicron variant is now becoming the dominant strain and has surged the coronavirus cases in South Africa. The data collected by NICD showed that Omicron might evade some immunity, but existing vaccines may protect against severe disease. Although South African scientists observed no sign of severe illness behind the Omicron variant, further data is needed from hospitalized patients for better future disease management. S protein NTD, RBD, and RBM encircled (Fig. 2).

Spike_N969>K, Spike_N856>K, Spike_S371>L, and Spike_Q954>H exhibited stabilizing effect (Fig. 3) when subjected DynaMut server. The total energies and vibrational entropy energy show that Omicron S protein mutations may stabilize the virus binding with human ACE2. In mutant Spike _N969>K, the vibrational entropy energy between wild types and mutant S protein may cause an increase in molecular flexibility, which shows a stabilizing effect when a total change in free energy was calculated (Figs. 3, 4). The residues which gain flexibility in surrounding spike _N969>K mutant has been shown in red.

The same effect has been observed with the Spike_S371>L mutant. However, the level of gain in flexibility is low.

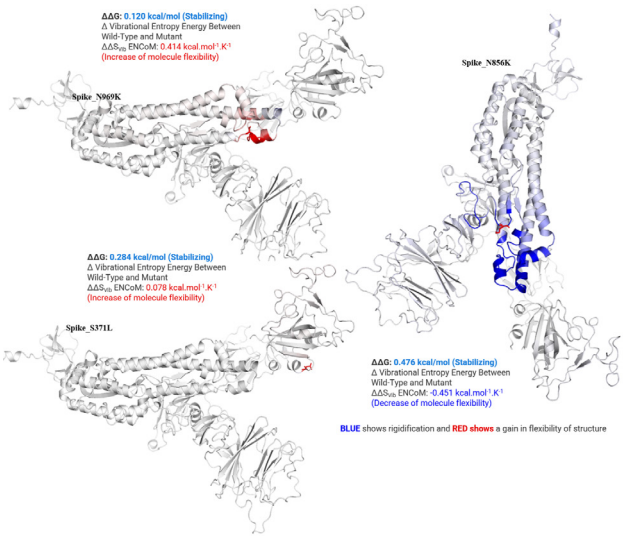


Fig. 3. Mutation effect on spike protein stability and flexibility. Blue shows rigidification and red shows gain in flexibility of structure. Red regions show gain in flexibility and blue reveals a decrease in protein flexibility. Mutations Spike_N969K, Spike_N856K, and Spike_S371L exhibited stabilizing effect. These mutations exhibited increased molecular flexibility except in N856K.

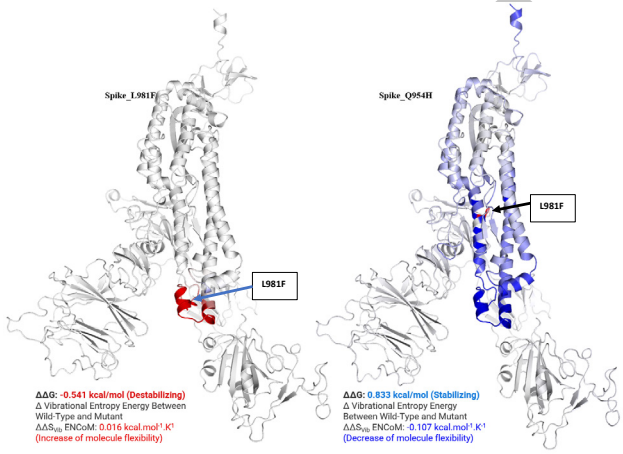


Fig. 4. Mutation effect on spike protein stability and flexibility. Spike_Q954H and Spike_L981F mutations locations and flexibility effect has been shown with arrow. Effect on surrounding residues are red (increase flexibility) and blue (decrease flexibility).

Spike_N856>K mutant exhibited a more stabilizing effect (0.476 kcal/mole) when compared with N969>K (0.120 kcal/mol) and S371L (0.284 kcal/mol). However, in contrast with N969>K and S371L mutants, the N856K exhibited a high flexibility loss in surrounding residues of β-sheets (Blue). Mutant L981F seems destabilizing, but its effect on molecular flexibility is increasing (Fig. 3). The highest flexibility gain on the S proteins mutant has been observed in Q954>H, which almost affects the entire beta-sheet and loops of CTD (blue).

## DISCUSSION

Omicron harbors three furin cleavage site mutations and 15 on the RBD of S protein, which may impact virus infectivity. This may increase the evolutionary adaptation and strengthen the ACE2-RBD binding stability by mutations (Wang *et al*., 2021). Different VOCs from different countries has been reported with some specific mutations in RBD of the spike protein. The virus characteristics always evolve via mutations in structural and non-structural proteins. Omicron emerged in South Africa and caused a sudden increase in cases per day. This sudden increase may be due to variations in virus proteins which may help in immune escape, transmission rate, and disease severity, or this variant is more resistant to vaccines and drugs than other VOCs.

The presence of a unique pattern of mutations in virus structural and non-structural (Table I) may have some consequences on Omicron transmissibility. The high frequency (218/337) of a unique pattern of Mutation (NSP14_I42V, Spike_N969K, Spike_N856K, Spike_S371L, Spike_L981F, Spike_Q954H, Membrane_Q19E) need further studies to explore the pattern's effect on virus transmission and immune escape. In a previous study, a positive correlation was observed between mutations NSP14_I42V, NSP2_T153M, and Spike_L18F and case fatality reports (Al-Awaida *et al*., 2021). N856K, Q954H, N969K, and L981F have been detected in the S protein's S2 domain (aa 691 to 1273) (Fig. 2). Previously, mutations in the S2 domain of Omicron (N764K, D796Y, N856K, Q954H, N969K, and L981F) were rarely detected (Alkhatib *et al*., 2022) before the emergence of Omicron, prevalent in less than 0.04%. The S2 domain of S protein has an essential role in membrane fusion. However, the effect of these mutations in this domain has been poorly investigated. In the current study, mutations, Q954H and L981F in S proteins exhibited a stabilizing effect (Fig. 3) along with flexibility change on surrounding residues and blue. Similar to our study, Zeng *et al*. (2021) analyzed two critical mutations (T547K and L981F) present close to RBD of S proteins. Both of these mutations stabilize the RBD by enhancing the hydrophobic interactions. The Omicron variant displayed higher transmission in cell-to-cell, an efficient mechanism facilitating the virus spread

within a host, and Omicron immune evasion (Sattentau, 2008; Mothes *et al*., 2010; Shafiq *et al*., 2022; Zeng *et al*., 2022). Zeng *et al*. (2021) reported vaccine efficacy against the Omicron, along with molecular of Omicron S protein, in parallel with other variants. The study found that vaccine recipients of two doses have minimal neutralization of the Omicron, and a booster dose, has a much stronger neutralizing capacity. Omicron S protein demonstrated low binding affinity to soluble ACE2, which may show a fitness cost after the accumulation of a large number of mutations in RBD (Zeng *et al*., 2021; Khan *et al*., 2022a)

Similarly, mutations spike_N969K and Spike_N856K present in the S2 domain of S protein and Spike_S371L present in the S1 domain have also been investigated for stabilizing effect. These mutations exhibited increased molecular flexibility except in N856K.

Omicron with this pattern of mutations needs further experimental studies on vaccine efficacy for better management of the infection in the future. According to the WHO update on this variant, there may be more risk of reinfection than other VOCs. However, data is very limited, and further experimental information is needed on an urgent basis.

Omicron isolates with these different unique patterns of mutations (Table I) may be subjected to current treatment with corticosteroid and IL-6 receptor blockers to observe whether they are effective against this variant. Further, diagnostic tests may also be verified, harboring all these common unique mutation patterns. As shown in Figures 3 and 4, mutations Spike_Q954>H, Spike_N969>K, Spike_N856>K, and Spike_S371>L exhibited stabilizing effect and also showed an impact on the flexibility of S proteins (Fig. 2), which may increase Omicron virulence factors. In support of this stability prediction in the current study, a more recent study (Chen *et al*., 2022), used an artificial intelligence model to reveal that Omicron may be 2.8 times more infectious than the Delta variant. The mutation effect on S protein binding with human ACE2 was also investigated using molecular dynamic simulation (Khan *et al*., 2022b). The binding pattern between the wild-type and Omicron variant with substitutions N417, S446, R493, and R498 in RBD of S protein may cause more interactions with human receptor ACE2. Further, a stable dynamic was observed in mutant S protein of Omicron variants. In addition, hydrogen bonding and binding free energy also supported these results. The results in these recent studies and the stability and flexibility analysis in the current study provide evidence that Omicron may possess higher infectivity than other SARS-CoV-2 variants. However, further experimental studies may be useful to find whether this variant has more adoptive properties and transmission than other VOC.

Several measures are needed to be taken for better management of COVID19 Omicron variant. Countries should make sure the maximum sequencing from field infections and sharing the sequences to NCBI databases for investigations on virus mutation rate and pattern, field studies and laboratory tests, their proper assessments to observe the impacts on vaccines efficacy, diagnosis, or social precautions. Early sequences from patients' samples may help evaluate vaccine efficacy and antibody investigation on sera.

Vaccine efficacy was little reduced in previous VOC; however, BNT162b2 retained its potency against Beta VOC (Abdool-Karim and de Oliveira, 2021). Omicron harbored several known and unique mutation patterns (Supplementary file S1) compared to other VOCs; therefore, vaccine efficacy against Omicron infections is not yet apparent. Some observational investigations in Qatar and Kaiser Permanente (Chemaitelly *et al*., 2021; Tartof *et al*., 2021) found more than 90% vaccine efficacy during Delta-variant. Some data in New York indicates a good efficacy in 65 years and older people, exhibiting a different level of efficacy for other vaccines (Rosenberg *et al*., 2021).

## CONCLUSION

In conclusion, the Omicron variant harbored some unique patterns of mutations that may affect virus transmissibility from cell to cell. Spike_N969K, Spike_N856K, Spike_S371L, and Spike_Q954H is the most common unique pattern of mutations, exhibiting stabilizing effect which may facilitate a higher transmission to the host receptor. The Omicron could have faster transmissibility and immune escape based on the known mutations than previous SARS-CoV-2 variants. Based on mutational and other data from previous VOCs, vaccinated people may need a booster dose for better management of Omicron infections. Continuous molecular epidemiology may be an effective strategy for further analysis of virus evolutionary stages.

## DECLARATIONS

*Funding*

Frontier Research Planning Project (cstc2018jcyjA2487). The National Natural Science Foundation of China (31871274, 32060077), Chongqing Natural Science Foundation.

*Supplementary material*

There is supplementary material associated with

this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/.....

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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