# **Research Article**



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# Conversion of B.0 Lineage of Human Corona Virus (Covid-19) Into Notoriously Infecting Less Pathogenic and Immune Escape Omicron B.1.1.529.2.75.2 or BA.2.75.2 Variant

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## Abstract

Human corona viruses appeared in December, 2019 at China and then within a span of 2 years such viruses (COVID-19) have gone deletions and mutations generating more infectious and death promoting variants like B.1.1.7 (Alpha) and B.1.617.2 (Delta) which were claimed half million deaths worldwide. The D614G and N501Y point mutations in spike protein appeared important for higher transmission and P4715L mutation in RdRP enzyme of ORF1ab polyprotein was also significant. However, since end of November 2021, an Omicron variant with 29 mutations on RBD domain of spike protein appeared in Africa which known as B.1.1.529 lineage which successively generated BA.1 and BA.2 variants. Omicron virus was highly infectious with immune escape properties but caused mild diseases. BA.2 omicron virus changed into BA.2.75.2 with more immune-escape and evasion properties. All omicron viruses had important <sup>31</sup>ERS deletion on N-protein and <sup>3675</sup>SGF deletion on nsp6 domain of ORF1ab which likely borrowed from B.1.1.7 by recombination. However, <sup>69</sup>HV immune-escape deletion in B.1.1.7 and <sup>157</sup>FR deletion in B.1.617.2 were not found in BA.2 variants. Its journey from BA.2.3, BA.2.9, BA.2.12, BA.2.48 etc and finally BA.2.75 variant was happened within a span of 10 months and BA.2.75.2 was highly spreading in India and USA recently. Although BA.2.75.2 variant has unique T607I and D1119N mutations in spike protein, other common N440K, G446S and L452R mutations were necessary for higher immune-escape and transmission including D614G and N501Y mutations. A G44R mutation in ORF3a protein also appeared specific for BA.2.75.2 and a 26 bases deletion in the 3'-UTR (5'-gag gcc acg cgg agt acg atc gag tg-3') found in omicron viruses may be responsible for weak viral load and pathogenicity as such deletion was not found in deadly B.1.1.7 and B.1.617.2 variants. The genetic changes in BA.2.75 sub-variants as well as other emerging omicron variants like BA.4.6, BA.5.2.1, BE.1.1, BQ.1 and BF.7 also have been discussed.

**Keywords:** SARS-CoV-2; large RNA viruses; BA.2.75.2; omicron viruses; alpha and delta variants; rna recombination; respiratory infections; immune escape mutants

## Introduction

Human corona virus appeared in 2019 in the Wuhan province of China although related viruses like CoV-229E, CoV-HKU1, CoV-NL63 and MERS-CoV were known since 2003 [1]. SARS-CoV-2 has caused huge infections worldwide within 2 years and 6.4 million deaths were reported [2]. It caused many point mutations and deletions creating dominant forms like alpha, beta, delta and very recently omicron [3]. COVID-19 is a large positive-sense RNA virus with a compact 29,980 nucleotides-long genome. It had structural proteins (S, M, N, E) at the 3'- end and 5' two (ORF1ab, ORF1a) very large poly-proteins (2/3 of the genome; in same reading frames) which were degraded into sixteen (nsp1-nsp16) non-structural proteins [4] including RNA topoisomerase (nsp2) [5], two proteases (nsp3 and nsp5) [6,7], RNA-dependent RNA polymerase (nsp12) [8], RNA helicase (nsp13) [9], uridine specific endoribonuclease (nsp15) [10] and methyl transferases (nsp16) [11] (figure-1). ORF1ab protein was reported as 7096-7092 AA in different variants due to <sup>141</sup>KSF and or <sup>3675</sup>SGF deletions where Wuhan corona virus was 7096 AA.

Spike protein of B.0 viruses is 1273 AA and stays as trimeric class 1 transmembrane glycoprotein. It's RBD domain (335-515 aa) acts as receptor binding domain to bind ACE-2 receptor of host lung cells for virus entry [12]. Spike protein 1-13 AA acts as signal peptide and

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other two domains, S1 (14-685 AAs) and S2 (686 to 1273 AAs) are also important. S-protein also contains fusion contact peptide (788-806 AA) as well as two hepta-peptide (HPPHCPC) repeats at 1163 and 1213 positions as well as furine cleavage point [13]. RNA sequencing clearly established many variants were generated within 2 years due to point mutations and deletions. In USA Wuhan-D614G mutant first peak between March-August, 2020, Alpha (B.1.1.7) 2<sup>nd</sup> peak

with <sup>69</sup>HV deletion immune-escape mutant between January-June, 2021 followed by 3<sup>rd</sup> peak of Delta (B.1.617.2, AY.X) with <sup>157</sup>FR deletion mutant between June to December, 2021. Since last week of December, 2022 4<sup>th</sup> peak of Omicron BA.1 variant (B.1.1.519) spread was evident followed by BA.2 variant spread since April, 2022. From June-July, 2022, omicron BA.4/BA.5 variants were dominating worldwide.



#### Figure 1B





Spike protein in COVID-19 Alpha is 1270 AA due to deletions of <sup>69</sup>HV and <sup>145</sup>Y positions but Delta variant has <sup>157</sup>FR deletion only (S=1271 AA). Spike protein of Omicron BA.1 variant has 69HV, 143VYY and 212L deletions as well as <sup>215</sup>EPE three amino acid insertion but no <sup>24</sup>LPP deletion (1270 AA) [14]. Spike protein of Omicron BA.4 and BA.5 corona viruses are 1268 AA due to deletions of <sup>24</sup>LPP and <sup>69</sup>HV but no <sup>212</sup>L deletion or <sup>215</sup>EPE insertion. Spike protein of omicron BA.2 has 1270 AA due to <sup>24</sup>LPP deletion but no <sup>69</sup>HV and <sup>143</sup>VYY deletions or <sup>215</sup>EPE insertion. <sup>69</sup>HV deletion found in B.1.1.7 also acquired in BA.1/4/5 but BA.2. Among the other structural proteins N-protein (419 AAs) binds to leader RNA of replicating corona virus and also regulates host-pathogen interactions. Three AA deletions (<sup>31</sup>ERS) were found in N-protein (416 AAs) in omicron corona viruses (BA.1/2/4/5) and all BE.1/BK.1/ XE.1/XBB.1/BQ.1) and was very useful for diagnostics [14-16]. Three amino acid deletions (<sup>3675</sup>SGF) were found in ORF1ab protein (nsp6 protein domain) of Alpha and Omicron BA.2/BA.4/BA.5 (ORF1ab=7093) viruses but at the same region <sup>3674</sup>LSG deletion as well as extra <sup>2083</sup>S deletion were found in omicron BA.1 corona virus (ORF1ab=7092 AA) but no such deletions in Delta variant (ORF1ab=7096 AA). Whereas, extra three amino acids (141KSF) deletions

omicron BA.4 variant were found in only (ORF1ab=7090 AAs) and such change was utilized to identify BA.4 omicron variant. Further, D614G mutations were detected in all variants and such mutation increased 80% higher transmission. N501Y mutation was appeared first in alpha variant but also located in omicron variants BA.1/BA.4/BA.5 but not in BA.2 and such mutation increased transmission by more than 20% with more immune escape properties [17-20]. We will discuss the generation of BA.2.75.2 from B.0 Wuhan virus illustrating important mutations and deletions.

## Methods

We searched PubMed to get idea on published papers on BA.2.75 variants and also searched SARS-CoV-2 NCBI database using BLAST-N and BLAST-X search methods to get related sequences. Multi-alignment of protein was done by MultAlin software (Corpet, F., 1988; Katoh & Standley., 2013) and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI (Sievers, et al., 2011; Wallace, 2005); Yang, et al., 2014). Hairpin structure of  $\sim$  120-200nt sequence was done by OligoAnalyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software with normal vs. mutant peptides (Gao, et al., 2022; Waterhouse, et al., 2018; Bienert, et al., 2017).

## Results

We performed multi-alignment of different corona virus genomes since its isolation in December, 2019 to December, 2022 to find the genetic changes with time and to give an idea how BA.2.75.2 omicron corona virus was formed. Severe Delta corona viruses including Alpha and Beta variants generated from Wuhan virus caused havoc deaths between March, 2021-September, 2022 worldwide. However, spike modified omicron viruses since December 2022, had higher transmission and immune-escape but no death usually occurred unless co-morbidity. Figure 1A showed the part of the multi-alignment where we detected <sup>3675</sup>SGF deletion in all BA.2 variant including BA.2.75.2 and such deletion

was first found in B.1.1.7 lineage which highly spread worldwide between March, 2021 to August, 2021. On the other hand, B.1.1.7 had <sup>69</sup>HV deletion on spike which was not located in BA.2 omicron variants (figure-1B). Figure 1C demonstrated a <sup>24</sup>LPP new deletion on the spike NH<sub>2</sub> terminus and such deletion was not found in B.1.1.7 early lineage or afterwards lineages (B.1.1.172; B.1.1.372). Such higher lineages neither had <sup>3675</sup>SGF deletion indicating SGF three amino acids deletion in nsp6 protein might have role in higher transmission due to virus stability. We further showed the portion of Multi-alignment describing <sup>31</sup>ERS Nprotein deletion in BA.2 variant and such deletion was prominent in BA.1/4/5 omicron variants (figure-1D). A 3'-UTR deletion located in omicron BA.2.75.2 as well as omicron BA.4 and omicron BA.5 variants but not in Alpha, Delta and omicron BA.1 variant (figure-2).

<ul> <li>B.1.1.7-OK341253-3.2021</li> <li>B.1.1.7-MZ821602-7.2021</li> <li>B.1.1.372-OA990799-9.2020</li> <li>B.1.1.370-OA990797-9.2020</li> <li>B.1.1.32-OA990199-9.2020</li> <li>B.1.1.196-OA990199-9.2020</li> <li>B.1.1-OA982934-9.2020</li> <li>B.1.1-OA981400-10.2020</li> <li>B.0-NC-045512-12.2019</li> <li>B.1.1-OP703145-1.2021</li> <li>B.1.1.529.2.12.1-ON386383</li> <li>B.1.529.2.9-ON386395-4.</li> <li>B.1.1.529.2.75-OP699966-5</li> <li>B.1.1.529.2.75.2-OP567923</li> </ul>	-4.2022 022 2022 9.2022 9-9.2022	3' UTR region gggaggacttgaaagagcaccacattttcaccgaggccacgcggagtacgatcgat	tgt -29741 tgt -29760 tgt -29760 tgt -29760 tgt -29760 tgt -29760 tgt -29760 tgt -29760 tgt -29760 tgt -29714 mmt -29682 -t -29682 -t -29682 -t -29682 -t -29657 *
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BA.2-05067923 BA.2-09901219 BA.4-0N907393 BA.5.2-0P579714 BA.5.1-0P579710 BA.5-0P579711 BA.5.2.1-0p647004 BF.7-0P440319 BA.1-0M003665 B.1.17-M2821602 B.1.617.2-0M542166 B-NC 045512 BA.2-75.2-0P567923 BA.2-0M901219 BA.4-0N907293 BA.5.2-0P579714 BA.5.2-0P579710 BA.5-0P579711 BA.5.2.1-0p647004 BF.7-0P440319 BA.1-0M003685 B.1.1.7-M2821602	gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt staacatt ataacatt	Agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcacc agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg agggaggacttgaaagagccaccacattttcaccgaggccacgcggagtacg tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt tacagtgaacaatgctagggagagctgcctatatggaagagccctaatgtgt	29681 29641 29650 29650 29598 29675 29702 29702 29704 29704 29751 29708 29708 29708 29702 29702 29702 29702 29702 29702 29702 29702 29750 29779 29762
BA. 2-04901219 BA. 2-04901219 BA. 4-00901219 BA. 4-00901219 BA. 5-09579714 BA. 5-09579710 BA. 5-09579711 BA. 5-0.04003685 B. 1. 1. 7-M2821602 B. 1. 617. 2-0M542166 B-NC 045512 BA. 2-04901219 BA. 4-00907393 BA. 5. 2-09579714 BA. 5. 1-09579710 BA. 5-09579711 BA. 5. 2. 1-09647004 BF. 7-09440319 BA. 1-0M003685 B. 1. 1. 7-M2821602 B. 1. 617. 2-0M542166	gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt gtaacatt staacatt ataacatt	Agggaggatttgaaggagtactatattttattttacc agggaggatttgaaggggcaccacatttttacc agggaggatttgaaggggcaccacatttttacc agggaggatttgaaggggcaccacatttttacc agggaggatttgaaggggcaccacatttttacc agggaggattgaaggggcaccacattttacc agggaggattgaagggcaccacattttacc agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggcaccacattttaccgaggcacgggggtacg agggaggattgaagggggggggagtgctatatggaagggcactaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggccctaatgtgt tacagtgaacaatgctagggagggggctgcctatatggaagggcctaatgtgt tacagtgaacaatgctagggagggtgcctatatggaagggccctaatgtgt tacagtgaacaatgctagggagggaggtgcctatatggaagggccctaatgtgt tacagtgaacaatgctagggaggggaggtgcctatatggaaggccctaatgtgt tacagtgaacaatgctagggagaggagtgcctatatggaagggccctaatgtgt tacagtgaacaatgctagggagaggtgcctatatggaagggccctaatgtgt	29681 29641 29650 29650 29598 29598 29575 29702 29704 29704 29704 29705 29702 29708 29702 29702 29702 29702 29702 29702 29702 29702 29750 29762

**Figure 2**: 3'-UTR deletion located in BA.2.75.2 as well as other BA.2 variant but not in B.1.1.7 and B.1.1.372 (2A) as well as in highly transmissible Delta, Omicron BA.1, BA.4, BA.5, BF.7 and BA.5.2.1 corona viruses (2B).

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In figure-3, multi-alignment (3A) and BlastX analysis (3B) were performed to locate distinct three spike mutations (N440K, G446S, L452R) in BA.2.75.2 including other variants. The L452R mutation was also located in B.1.617.2 Delta variant and might be the source of such variation in BA.2.75.2 and such

mutation also carried on to omicron variants lately (BA.4 and BA.5). Similarly, G446S mutation also located in omicron BA.1 variant and N440K mutation was found in most omicron variants (data not shown). Thus, none of these mutations were specific for BA.2.75.2.

B.1.1.7-OK341253-3.2021	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22902	
B.1.1.7-MZ821602-7.2021	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22872	
B.1.1.372-OA990799-9.2020	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22920	
B.1.1.70-OA994797-9.2020	tatagettggaattetaacaatettgattetaaggttggtggtaattataattacetgta=22920	
B.1.1.33-OA982939-9.2020	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22920	
B.1.1.196-0A990199-9.2020	tatagettggaattetaacaatettgattetaaggttggtggtaattataattacetgta=22920	
B.1.1-0A982934-9.2020	tatagettggaattetaacaatettgattetaaggttggtggtaattataattacetgta=22920	
B.1.1.1-0A991400-10.2020	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22920	
B.0-NC-045512-12.2019	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22920	
B.1.1-OP703145-1.2021	tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22882	
B.1.1.529.2.12.1-ON386383-4.2022	tatagettggaattetaacaagettgattetaaggttggtggtaattataattaccagta=22852	
B.1.1.529.2-CM901219-2.2022	tatagcttggaattctaacaagcttgattctaaggttggtggtaattataattacctgta=22877	
B.1.1.529.2.9-ON386395-4.2022	tatagettggaattetaacaagettgattetaaggttggtggtaattataattacetgta=22852	
B.1.1.529.2.75-OP699966-9.2022	tatagettggaattetaacaagettgattetaaggttagtggtaattataattacetgta=22863	
B.1.1.529.2.75.2-OP567923-9.2022	tatagcttggaattctaacaagcttgattctaaggtt <mark>a</mark> gtggtaattataattacc <mark>g</mark> gta=22852	
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Figure 3(A): Multi-alignment to show the BA.2.75 and BA.2.75.2 distinct mutations in the spike.

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B.0-NC-045512-12.2019 (B.1.1.7 same)
                                    tatagcttggaattctaacaatcttgattctaaggttggtggtaattataattacctgta=22920
blastX
surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]Sequence ID: QTZ40111.1Length: 1273.
Score
                    Expect
                               Method
                                                                   Identities
                                                                                       Positives
45.8 bits(107)
                    3e-04
                                Composition-based stats.
                                                                   19/19(100\%)
                                                                                       19/19(100%)
Query 2 IAWNSNNLDSKVGGNYNYL 58
            IAWNSNNLDSKVGGNYNYL
Sbjct 434 IAWNSNNLDSKVGGNYNYL 452
B.1.1.529.2.12.1-ON386383-4.2022
                                   tatagettggaattetaacaagettgattetaaggttggtggtaattataattaccagta=2852
blastX
surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]Sequence ID: UYD62730.1Length: 1273.
                               Method
Score
                    Expect
                                                                   Identities
                                                                                       Positives
44.7 bits(104)
                     8e-04
                                Composition-based stats.
                                                                   19/19(100\%)
                                                                                      19/19(100%)
Query 2 IAWNSNKLDSKVGGNYNYQ 58
             IAWNSNKLDSKVGGNYNYO
Sbjct 434 IAWNSNKLDSKVGGNYNYQ 452
B.1.1.529.2.9-ON386395-4.2022
                                   tatagettggaattetaacaagettgattetaaggttggtggtaattataattacetgta=22852
blastX
surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] Sequence ID: UPN11814.1Length: 1273.
                Expect Method
                                                                   Identities
Score
                                                                                       Positives
45.4 bits(106)
                    5e-04
                                Composition-based stats.
                                                                   19/19(100%)
                                                                                       19/19(100%)
Query 2 IAWNSNKLDSKVGGNYNYL 58
             IAWNSNKLDSKVGGNYNYL
Sbjct 434 IAWNSNKLDSKVGGNYNYL 452
B.1.1.529.2.75-0P699966-9.2022
                                   \texttt{tatagcttggaattctaacaagcttgattctaaggttagtggtaattataattacctgta=22863
blastX
surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] Sequence ID: UK S04805.1 Length: 1275.
Score
                    Expect
                               Method
                                                                   Identities
                                                                                       Positives
44.3 bits(103)
                     0.001
                                Composition-based stats.
                                                                   19/19(100%)
                                                                                       19/19(100%)
Query 2 IAWNSNKLDSKVSGNYNYL 58
             IAWNSNKLDSKVSGNYNYL
Sbjct 436 IAWNSNKLDSKVSGNYNYL 454
B.1.1.529.2.75.2-OP567923-9.2022
                                   \texttt{tatagcttggaattctaacaagcttgattctaaggttagtggtaattataattaccggta=22852}
blastX
surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] Sequence ID: ULE98956.1Length: 1270.
              Expect Method
Score
                                                                  Identities
                                                                                       Positives
44.3 bits(103)
                    0.001
                                                                   19/19(100%)
                                                                                       19/19(100%)
                                Composition-based stats.
Query 2 IAWNSNKLDSKVSGNYNYR 58
             IAWNSNKLDSKVSGNYNYR
Sbjct 431 IAWNSNKLDSKVSGNYNYR 449
```

**Figure 3(B):** BlastX search to get mutant AAs. All three spike mutations (N440K, G446S, L452R) was found in BA.2.75.2 and such changes might be responsible for high transmission and immune-escape which were not located in B.0 as well as high transmissible B.1.1.7. variant.

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However, figure-4 (A, B and C) demonstrated unique BA.2.75.2 variant specific mutations T607I (4A), D1199N (4B) in spike protein and G44R (4C) in ORF3a protein. No other variant has such mutation till now (Alpha, Delta, BA.1, BA.2, BA.4, BA.5 and BF.7).

All those above mutations in the spike were responsible for greater immune escape but role of ORF3a G44R mutation was not clear. However, ORF3a may be involved in RNA binding and Arginine (R) may increase RNA binding efficiency.



(4b). BlastX search to find AA change was shown in figure-4C.

	(A)Multi-alignment to locate specific genetic changes in spike of omicron BA.2	.75.2 variant		
	B.1.1.7-0K341253-3.2021 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25182	
	B.1.1.7-MZ821602-7.2021 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25152	
	B.1.1.372-OA990799-9.2020 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc 25200	
	B.1.1.70-OA994797-9.2020 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25200	
	B.1.1.33-OA982939-9.2020 asatgastctctcstcgstctccasgascttggsasgtat	gagcagtatataaa	atggcc25200	
	B.1.1.196-0A990199-9.2020 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25200	
	B.1.1-0A982934-9.2020 asatgastctctcstcgstctccasgascttggssagtat	gagcagtatataaa	atggcc25200	
	B.1.1.1-0A991400-10.2020 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa:	atggcc 25200	
	B.0-NC-045512-12.2019 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25200	
	B.1.1-OP703145-1.2021 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25162	
	B.1.1.529.2.12.1-ON386383-4.2022 asatgastctctcstcgstctccasgascttggssagtate	gagcagtatataaa	atggcc25132	
	B.1.1.529.2-0M901219-2.2022 asatgastctctcstcgstctccasgascttggasagtat	gagcagtatataaa	atggcc 25157	
	B.1.1.529.2.9-ON386395-4.2022 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc 25132	
	B.1.1.529.2.75-OP699966-9.2022 aaatgaatctctcatcgatctccaagaacttggaaagtat	gagcagtatataaa	atggcc 25143	
	B.1.1.529.2.75.2-OP567923-9.2022 aaatgaatctctcatcaatctccaagaacttggaaagtat	gagcagtatataaa	atggcc25132	
	***************************************	*************	*****	
	(B) Blast% search to find specific AA change in BA.2.75.2 variant.			
	B.1.1.529.2.75.2-OP567923-9.2022 aaatgaatctctcatcaatctccaagaacttggaaagtat	gagcagtatataaa:	atggcc=25132	
	blastX(BA.2.75.2)			
	surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]Sequence ID:	UYN35199.1Len	gth: 1273.	
	Score Expect Method Identit	ies	Positives	
	45.8 bits(107) 3e-04 Composition-based stats. 19/19(	100%)	19/19(100%)	
	Cuerty 2 NESTINIOFICEVEOVIEW 58	200.0)	15, 15 (100 /0)	
	Vdery 2 NESDINDELENTRY 30			
	SBJCt 1194 NESLINLQELGKYEQYIKW 1212			
	B.0-NC-045512-12.2019 aaatgaateteteategateteeaagaaettggaaagtate	gagcagtatataa	aatggcc-25200	
	blastX (B.0, B.1.1.7 and BA.2.75)			
	eurface give opportein IS evere soute reepiratory eurodrome compavirus 21 Seguence ID I	UKC 24720 4L on	ath: 4276	
	surface glycoprotein [severe acute respiratory syndrome coronavirus 2] sequence in.	<u>UKUJ47JJ.1</u> LCII	gui. 12/0.	
	Score Expect Method Identit	les	Positives	
	45.8 bits(107) 3e-04 Composition-based stats. 19/19(	100%)	19/19(100%)	
	Query 2 NESLIDIOFLCKVFOVIEW 58			
	Viery - Assembly Derivery Vier			
	NESLIDLQELGKYEQYIKW			
	SDJCT 1197 NESLIDLQELGKYEQYIKW 1215			
	GAT=D and AAT=N. So, D1199N AA change was occurred in BA.2.75.2, not present is	n B.O, B.1.1.7	and BA.2.75.	
T: 4T	$\mathbf{P}$ Male length of $\mathbf{P}$ is a second se			
rigure 41	<b>D</b> : Multi-alignment and blastA search to demonstrate D1199N g	genetic char	ige in spike f	protein of

BA.2.75.2 variant.



The figure-5 demonstrated the time course of generation of BA.2.75 which in our assay found July, 2022 but other report showed May, 2022. It also

showed how  $^{24}LPP$  deletion in spike was important for BA.2.75 sub-lineages but not  $^{69}HV$ , neither  $^{143}VYY$ , nor  $^{157}FR$ .

	mivilvilplvssqcvnittrtqippaytnsitigvyypdkvirssvinstqdiilpiis	60
NC 045512.2-B.0-12-2019	mfvflvllplvssqcvnlttrtqlppaytnsftrgvyypdkvfrssvlhstqdlflpffs	60
OM003685-B.1.1.529-27-11-2021	mfvflvllplvssgcvnlttrtglppavtnsftrgvvvpdkvfrssvlhstgdlflpffs	60
OP943055-XBB.1-17-11-2022	mfvflvllplvssgcvnlitrtgsvtnsftrgvvvpdkvfrssvlhstgdlflpffs	57
OP567922-BA 2 75 2-12-9-2022	mfuflullplusacunlitrta	57
OD567022-B3 2 75 2-12=0-2022		57
0250/528 DR.2./5.2 18-5 2022	mivilipiosideville of synatolicy yyparvilipiosi	57
OP740593-BA.2.75-21-9-2022	mivilyliplyssdcoulfieredsynsifieddolybarolissoluseddilbils	57
OP943256-BN.1.2-19-11-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP828120-BA.2.75-3-11-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP438188-BA.2.75.2-28-8-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP754862-BA.2.75-22-10-2022	mfvflvllplvssqcvnlitrtqsytnsftrqvyypdkvfrssvlhstqdlflpffs	57
OP813522-BA.2.75-28-10-2022	mfvflvllplvssgcvnlitrtgsvtnsftrgvvvpdkvfrssvlhstgdlflpffs	57
OP699966-B3 2 75-20-9-2022	wfuflullplusacunlitta	57
OD567876-B3 2 75 2-12-0-2022		57
OF507070 DR.2.75.2 12 9 2022	synsterious and a synsterious and a single second and a second se	57
OP360/32-BR.2./3.2-13-9-2022	mivilipiossdconitered syensterdoyypakorissoinsedariipiis	57
OP825919-BA.2.75-1-10-2022	mivilvllplvssqcvnlitrtqsytnsitrgvyypdkvirssvlhstqdlilpiis	57
OP571747-BA.2.75-18-9-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP579410-BA.2.75.1-16-9-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP582071-BA.2.75.1-14-9-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP437363-BA.2.75-31-8-2022	mfvflvllplvssqcvnlitrtqsytnsftrqvvvpdkvfrssvlhstadlflpffs	57
ON999228-BA 2 75-2-7-2022	mfuflyllplyssacyplityta===sutpsftrayymdkyfrssylbstadlfloffs	57
OD828219-B1 2 75-4-11-2022	mfuflullalussacualitutanesutastastastastastastastastastastastastast	57
OF020819-DR.2.73-4-11-2022	miviiviipivssqcvniitroq synsitrigvyypakvirssvinstqdlflpffs	57
OF430771-BA.2.75-31-8-2022	mrvriviipivssqcvnlitrtqsytnsitrgvyypdkvirssvihstqdlflpffs	57
ON624670-BA.2.12.1-12-5-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP941167-BQ.1-18-11-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP943660-CK.1-21-11-2022	mfvflvllplvssqcvnlitrtqsytnsftrgvyypdkvfrssvlhstqdlflpffs	57
OP753852-BA.4.6-12-10-2022	mfvflvllplvssqcvnlitrtqsytnsftrqvyypdkvfrssvlhstqdlflpffs	57
OP942856-CN.1-17-11-2022	mfvflvllplvssgcvnlitrtgsvtnsftrgvvvpdkvfrssvlhstgdlflpffs	57
OP812324-BE 7-28-10-2022	mfvflyllplysacynlitrtasytnsftrayymdkyfrasylhatadlflpffa	57
OP752828-B3 5 2 1-11-10-2022	mfuflullalusacunlitta	57
01700000 Dationali 11 10 2022	*****	· · ·
OP581694-B.1.1.7-2-4-2021	nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	118
NC_045512.2-B.0-12-2019	nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	120
OM003685-B.1.1.529-27-11-2021	nvtwfhvisqtnqtkrfdnpvlpfndqvvfasieksniirqwifqttldsktqslliv	118
OP943055-XBB.1-17-11-2022	nvtwfhaihvsotngtkrfdnpalpfndgvvfasteksniirgwifgttldsktgslliv	117
OP567922-BA. 2. 75. 2-12-9-2022	nytwfhaihysotnotkrfdnyylpfndoyyfasteksniirowifottldsktoslliv	117
OP567922-BA 2 75 2-12=9-2022	nutwfhaihusetnetkrfdnyulnfndeuufasteksnijreuifettlekteslliv	117
07749500-73 0 75-01-0-0000		117
OF/40393-DA.2./5-21-9-2022	nvtwinainvsgengtkiinnpvipinngvyiasteksniingwingttidsktqsiiiv	117
OP943256-BN.1.2-19-11-2022	nvtwihaihvsgtngtkridnpvlpindgvyfasteksniirgwifgttidsktqsiliv	117
OP828120-BA.2.75-3-11-2022	${\tt nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv}$	117
OP438188-BA.2.75.2-28-8-2022	nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	117
OP754862-BA.2.75-22-10-2022	nvtwfhaihvsgtnvtkrfdnpvlpfndgvyfasteksniirgwifgttldsktgslliv	117
OP813522-BA.2.75-28-10-2022	nvtwfhaihvsqtnqtkrfdnpvlpfndqvvfasteksniirgwifgttldsktgslliv	117
OP699966-BA 2 75-30-9-2022	nvtwfhaihvsotnotkrfdnpvlpfndovvfasteksniirowifottldsktoslliv	117
OP567876-BA 2 75 2-12-9-2022	nytwfhaibysotnotkrfdnnylnfndgyyfastekeniirgyifgttldektgellig	117
OD569752-B3 2 75 2-15-0-2022	and the single of the single for the state of the single state of	117
OF300/32-DR.2./3.2-13-9-2022	nvowinginvsgengekrranpviprnagvyrgsteksniirgwirgttidsktq5111v	117
OF625919-BA.2.75-1-10-2022	nvtwinainvsgingtkridnpvlpindgvyfasteksniirgwifgttldsktqslliv	117
OP571747-BA.2.75-18-9-2022	${\tt nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv}$	117
OP579410-BA.2.75.1-16-9-2022	nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	117
OP582071-BA.2.75.1-14-9-2022	nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktgslliv	117
OP437363-BA.2.75-31-8-2022	nvtwfhaihvsgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktgslliv	117
ON999338-BA. 2.75-3-7-2022	nvtwfhaihvsotnotkrfdnpvlpfndqvvfasteksniirgwifgttldsktgslliv	117
OP828219-BA 2 75-4-11-2022	nutschaibusetnetkrfennulnfnetsetskaniirewifettlettesliir	117
OD408771-B3 2 75-21-0-2022	note the site of the stand of the standard stand sta	117
0F100//1-DR.4./0-81-0-4044	nvowingingstridepvipingsvistersniigwigttidsttdslliv	117
ONE04500-01 0 10 1-10-F-0000	nvtwinainvsgtngtkridnpvipindgvyfasteksniirgwifgttidsktqslliv	117
ON624670-BA.2.12.1-12-5-2022	nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	115
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022		115
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022 OP943660-CK.1-21-11-2022	nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv	113
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022 OP942660-CK.1-21-11-2022 OP753852-BA.4.6-12-10-2022	nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktgslliv	115
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022 OP943660-CK.1-21-11-2022 OP753852-BA.4.6-12-10-2022 OP942656-CN.1-17-11-2022	<pre>nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvvfasteksniirgwifgttldsktgslliv</pre>	115
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022 OP943660-CK.1-21-11-2022 OP753852-BA.4.6-12-10-2022 OP942856-CN.1-17-11-2022 OP813224-BF.7-28-10-2022	<pre>nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv</pre>	115 115 115
ON624670-BA.2.12.1-12-5-2022 OP941167-BQ.1-18-11-2022 OP942660-CK.1-21-11-2022 OP753852-BA.4.6-12-10-2022 OP942856-CN.1-17-11-2022 OP942856-CN.1-17-11-2022 OP813224-BF.7-28-10-2022 OP75288-BB.5.2.1-11-00-2022	<pre>nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv nvtwfhaisgtngtkrfdnpvlpfndgvyfasteksniirgwifgttldsktqslliv</pre>	115 115 115 115

**Figure 5:** Multi-alignment of spike proteins of BA.2.75 variants and time course of BA.2.75 generation with <sup>24</sup>LPP deletion but no <sup>69</sup>HV deletion. The Date of Generation of BA.2.75 was estimated here as July, 2022 based on NCBI SARS-CoV-2 Database. Parts of the alignment were shown.

The figure-6 showed the important passage of BA.2.75.2 from BA.2 which was initiated from B.0

Wuhan corona virus after so many mutations since December, 2019.



# Discussion

Corona virus transmission was huge and cell to cell transmission was reported whereas saliva may be a route of such transmission [19,20]. Alpha and Delta corona viruses caused million deaths whereas Omicron spread also havoc but it caused mild disease and no oxygen requirement or hospitalization was necessary unless comorbidity. Many mutations and deletions were reported in most genes of SARS-CoV-2. However, D614G spike mutation was very necessary for deadly disease. Analysis found that ORF1ab protein had less mutations compared to spike protein. The Nsp2 RNA topoisomerase I120F mutants were significant in Australia [16]. COVID-19 Nsp15 protein H235Y mutation was a marker for Delta clade C and K260R mutation was a marker for Delta clade E. Among the many mutations reported, N74N, D79D, L214L, L217L and N278N were synonymous and one (D220Y) was non-synonymous found in more than 10,000 isolates worldwide [21]. Ziegler et al (2021) demonstrated that interferon production of nasal epithelial cells was highly impaired in severe corona virus infection with weak antibody production. Huang et al (2022) showed that SARS-CoV-2 viral entry factors such as ACE2 and TMPRSS members were highly enriched in epithelial cells of oral cavity and saliva may be a potential route of virus transmission. Multiple variants were generated by RNA recombination and thus single human might carry multiple species due to multiple infections at different time [22]. We found that D614G and N501Y mutation were carried into omicron viruses including BA.2.75, BA.2.75.1 and BA.2.75.2 variants. HIV RNA virus is only 9.8kb which infects CD4+ lymphocytes through gp120 spike protein causing immune-deficiency (AIDS) so that other pathogens can grow in host. It contains reverse transcriptase enzyme that coverts RNA into doublestranded DNA which then integrates into host chromosome [24.25]. Such process was not reported for COVID-19. The RdRp enzyme of COVID-19 had P4715L mutation and such enzyme was a target for many drugs like remdesivir and favipiravir. However, so far, few vaccines were approved against COVID-19 and very effective to generate antibodies against corona viruses [26,27]. Serum generated in COVID-19 infected host is very good drug to treat other SARS-CoV-2 infected person but in case of omicron virus infections old serum seems not effective [28]. Thus, BA.2.75 variant's therapy was not successful yet using human serum of previously infected person [29,30]. The BA.2.75 variant hCoV-19/Japan/TY41-716/2022 (TY41-716) had nine amino acid changes (K147E, W152R, F157L, I210V, G257S, D339H, G446S, N460K, and Q493 [reversion]) in its S protein as compared with a BA.2 isolate (hCoV-19/Japan/UT-NCD1288-2N/2022) and completely resistant to Imdevimab [31]. The BA.2.75.2 and BA.4.6 variants both showed complete escape from Cilgavimab and a combination of Cilgavimab and Tixagevimab but was Bebtelovimab [32,33]. sensitive to However, accumulation of so many distinct mutations and deletions in the spike and ORF1ab proteins from earlier deadly B.1.1.7 and B.1.617.2 variants signals that BA.2.75.2 variant may gain more dominant marker to stay as important omicron corona viruses [34,35].

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## **Competing interest**

The author declares no conflict of interest.

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