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Highly Infectious, Less Pathogenic and Antibody Resistant Omicron Xbb.1, Xbb.1.5 and Xbb.1.5.1-Xbb.1.5.39 Subvariant Coronaviruses Do Not Produce Orf8 Protein Due To 8th Codon Gga=Tga Termination Codon Mutation

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Summary

RNA viruses are very mutation prone. We recently reported SARS-CoV-2 ORF8 gene CAA=TAA and AAA=TAA Termination Codon Mutations in B.1.1.7 variants with no production of viable ORF8 protein. We described here another GGA=TGA termination codon in the 8th codon of ORF8 gene located exclusively in XBB.1 (XBB.1.16 and XBB.1.22) and XBB.1.5 subvariants (XBB.1.5.1 to XBB.1.5.39) but not in XBB.2 variant or Alpha, Beta, Gamma, Delta and Omicron BA.1, BA.2, BA.4, BA.5, BF.7 and BQ.1 subvariants. However, G>T mutation at 27915 also created an alternate ATG codon but the protein product was short due to preceding TAG and TGA termination codons. The originally located following ATG codons were there but in alternate reading frames and ultimately no ORF8 protein was formed in XBB.1.5 subvariants which were spreading highly now over BA.2.75, BA.4.6, BA.5.2.1, BF.7 and BQ.1.1 subvariants. This is a vivid example of three termination codon mutations in the coronavirus ORF8 protein which was implicated as target for many human proteins regulating interferon production, chromosome instability, antibody production, patho genicity and virus clearance. The 30nt deletion in the 3'-UTR, including ²⁴LPP, and ¹⁴⁵Y deletions in Spike protein as well as N-protein ³¹ERS and ORF1ab polyprotein ³⁶⁷⁵SGF deletions made XBB.1.5 Omicron coronavirus weak and less pathogenic so that WHO declared coronaviruses as non-emergency pathogen.

Keywords: Termination codon mutants; ORF8 protein; COVID-19; Immunomodulation; Higher transmission; Lower pathogenicity

Introduction

The SARS-CoV-2 is the causative agent of the coronavirus disease 2019 with severe public health consequences and million deaths [1-9]. The novel SARS-CoV-2 shares nearly 96% similarity to the bat coronavirus isolate RaTG13, suggesting these animals are the likely natural reservoir of the virus [10,11]. Thus, different animal, birds and whale corona viruses were known since 2003 but human corona viruses were appeared in December 2019 at Wuhan province of China. So far million coronaviruses RNA were sequenced and divided into Alpha, Beta, Delta, Gamma etc. as well as Omicron variants with mutations, deletions and insertions [12-18]. There were many differences in pathogenic potential and immunogenicity among those VOCs. SARS-CoV-2 is a large positive-stranded RNA virus with round 30000 nucleotides genome (Figure 1). It has structural proteins Membrane (M), Envelope (E), Nucleocapsid (N), Spike (S) coded from 3'-1/3 part of the virus independently, but RNA-dependent RNA polymerase was coded from nsp12 domain of ORF1ab polyprotein coded from 2/3 of the 5'-parts of the genome and such polyprotein was degraded into sixteen polypeptides (nsp1nsp16). The nsp2 protein is RNA topoisomerase with new therapeutic target whereas Nsp3 and nsp5 are proteases that cleave polyprotein into sixteen polypeptides with diverse functions [18-23]. The nsp6, nsp7, nsp8, nsp9 and nsp10 were small accessory proteins involved in RNA polymerase replication complex. The nsp14 and nsp15 are ribonucleases

and nsp16 is 2'-0 methyltransferase and nsp13 is RNA helicase and may have capping methyl transferase activity. The ORF3a, ORF6,

ORF7a/7b, ORF8 and ORF10 small proteins also coded from 3' end of the genome and have roles in regulating cellular genes.



Figure 1: Structure of Omicron corona virus with polypeptides (A), nucleotide sequence of ORF8 gene (B) and ORF8 protein sequence with three termination codons detected (C).

In USA, Wuhan-D614G mutant first peak between March-August 2020, Alpha (B.1.1.7) 2nd peak with spike ⁶⁹HV deletion immune-escape mutant between January-June 2021 followed by 3rd peak of Delta (B.1.617.2, AY.X) with spike ¹⁵⁷FR deletion mutant between June to December 2021 [24]. Since last week of December 2022 4th peak of Omicron BA.1 variant (B.1.1.519) spread was evident followed by BA.2 variant spread since April 2022 with 29 mutations in the spike. From June-July 2022, Omicron BA.4/BA.5 variants were dominating worldwide followed BF.7, BQ.1, BQ.1.1, XBB.1 and XBB.1.5 subvariants recently (Figure 2).



Figure 2: Distribution and sequencing data comparison for COVID-19 in the United Kingdom.

The Spike protein (1273AA) of COVID-19 had gone extensive mutations and deletions than large polyprotein ORF1ab (7096aa) particularly in Omicron lineages. The spike ²⁴LPP, ⁶⁹HV, ¹⁴³VYY, ¹⁵⁷FR, ²¹²L and ¹⁴⁵Y deletions were detected in different proportion whereas ²¹⁵EPE and ²⁴⁹RWMD insertions were also reported in Omicron BA.1 variant and BQ.1 variant respectively [25-29]. Among the ORF1ab deletions, ¹⁴¹KSF deletion in nsp1 domain was found only in omicron BA.4 subvariants and ³⁶⁷⁴LSG deletion found in omicron BA.1 subvariant whereas ³⁶⁷⁵SGF deletion in nsp6 domain was found in most Omicron (BA.1, BA.2, BA.4, BA.5) and Alpha (B.1.1.7) variants. Dominant point mutations D614G and N501Y were important for higher transmission whereas ~20 mutations in the RBD domain of Omicron were not found in deadly B.1.1.7, B.1.617.2 and AY.103 variants. The E484A, T478K, L452R and K417N/T mutations were very immune-modular and such mutant

viruses were refractory to antibodies of patients.

More than a few dozen spike mutations were recently detected in XBB.1.5 and BQ.1.1.1 subvariants. However, Omicron variants were less pathogenic and usually did not require oxygen support and hospitalization unless co-morbidity [30]. Still pneumonia, chest pain, confusion, and headache with cough and cold, were different symptoms that affected over 650 million people worldwide. The gradual changes in different SARS-CoV-2 variants since 2019 was shown in Figure 3. Presently, infectivity of XBB.1.5 subvariant was dominating worldwide but very mild symptoms due to huge mutations in spike and deletion of 26nt 3'-UTR. However, the titer of such deleted coronaviruses was low and their spread may be due to better interaction with ACE-2 receptor and reinfections of patients [31-36].



Figure 3: Conversion of Wuhan B.0 coronavirus into Omicron viruses like BQ.1.1.1 and XBB.1.5.3 types of sub subvariants.

Accumulating evidence suggested that small regulatory proteins (ORF3a, ORF7a, ORF8) of SARS-CoV-2 interacted highly with many cellular proteins. Preliminary 3-D graphics interactive studies indicated few dozen proteins like PVR, IRF3, ATF6, Beclin 1, FK506-binding protein 10, EDEM, vitronectin, OPJ94, Sec62163, VIP36, TRFT3 and PLAT etc interacted with ORF8 protein regulating protein folding, apoptosis and interferon production. Such process likely favours COVID-19 survival in host cells inhibiting immune control mechanisms [37]. Genetic analysis pointed a severe deletion in ORF8 (Δ 382) caused less severe corona infections likely due to low viral load with increased immune clearance.

However, in cell culture study with such deletion mutant contradicted the finding of lower viral load with no change of cellular transcriptional profile. The ORF8 protein also mediates immune evasion by downregulating MHC-I molecules like HLA-A2 [38]. The IgG domains similarity of ORF8 protein may be important to modulate host immune functions and chromatin structure. The C>T base change at 27972nt and another A>T base change at 28095nt created two termination codons (CAA=TAA and AAA=TAA) to produce 26AA and 67AA long ORF8 truncated proteins. Similar Blast-N search with mutated oligonucleotides detected many ORF8 mutants with distinct S24L, V32L, P38S, R52I, A65V, Y73C, L84S, K92E and V100L mutations with or without TAA termination mutations [39].

During our database search to characterize the XBB.1.5 lineages, we noticed no expression data for ORF8 protein in the genomic sequences. As we already known the two termination codons in ORF8 gene of Alpha variants, we searched the similar termination codon mutation in XBB-related variants which originated from BA.2.10 and BA.2.75 recombination and mutation [39-46]. We detected here a new termination codon mutation in ORF8 gene of XBB.1 lineage but not in XBB.2 lineage. Spike protein mutations and deletions had affected Omicron coronaviruses and for transformation of BA.2 to BA.2.75 required K147E, W152R, F157L, I210V, G257S, D339H, G446S, N460K and Q493R mutations in the spike protein and BA.2.75 was originated in 31.12.2021. Gene rearrangement and deletion in the 5'-UTR and 3'-UTR were also demonstrated in different SARS-CoV-2 variants. The article was deposited in Research Square preprint on 30th May 2023.

Methods

We searched PubMed to get an idea on published papers on ORF8 and searched SARS-CoV-2 NCBI database using BLAST-N and BLAST-X search methods. Multi-alignment of protein was done by Mult Alin software and multi-alignment of DNA by CLUSTAL-Omega software. 1st impression of ORF8 mutants was gained by Blast N searching of deletion boundary of 120nt sequence and analyzing the sequences with 90-100% similarities. Blast X search of ORF8 full length gene used to get mutant ORF8 proteins with or without termination codon. Then, the other ORF8 mutants were detected by Blast-N search of TAA mutant oligos as well as other oligos selected from point mutation boundaries [47,48]. The hairpin structure of ORF8 gene 222nt 5'-terminal sequence was done by Oligo Analyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software [49-61].

Result

In Table 1, we showed XBB.1.5. subvariants specific changes in the coronavirus proteins whereas K304Q and A411S two important mutations in the RBD of spike of XBB.1.5.3 sub-subvariant might be significant. Similarly, XBB.1.5.29 and XBB.1.5.30 had A348V and A348T mutations in the spike but roles of such mutations had to be tested yet. The never-the-less dominant common F486P spike mutation was implicated in antibody evasion and higher transmission in XBB.1.5 subvariants. We found six mutations (P2045S, T2137A, A3697V, T5941I, H5951Y, P6376S) in ORF1ab polyprotein of XBB.1.5.30 subvariant and four different mutations (S1188L, P2045L, P2110S, N6481K) in XBB.1.5.20 which also harboured A398V mutation in the N-protein (Table 1). Three spike mutations (P463S, E554K, P1162S) in XBB.1.5.8 might be significant and the penetration of such subvariant in the database was low. Similarly, four spike mutations (V83S, Y200C, V382L, T573I) in the XBB.1.22.1 subvariant was reported in Table 1. Interestingly, we detected total eight mutations in N-protein of XBB.1.5.sub-subvariants: R10Q in XBB.1.5.8, G25C in XBB.1.5.16, D81H in XBB.1.5.38, I131R in XBB.1.5.3, R195I in XBB.1.5.19, P279L in XBB.1.5.28, S327L in XBB.1.5.9 and A398V in XBB.1.5.20. Mutation rate was higher in recent coronavirus isolates (BQ.1.1.1 and XBB.1.5.1) and we also found four distinct mutations in ORF3A trans-activator protein: G49C in XBB.1.5.21, S92L in XBB.1.5.2, G172D in XBB.1.5.38 and H182Y in XBB.1.5.27 [61-68].

Table 1: Demonstration of major mutations in Omicron XBB.1.5.1-XBB.1.5.39 sub subvariants. XBB.1.5.26 (OQ783732) and XBB.1.5.31 (OQ758970) have no mutation in our search as stated here (ORF1ab, Spike, N, M, E, Orf3A) and also other small ORF proteins. No M protein was conserved but A63T mutation was found in all omicron variants and a D3G mutation in BA.1 variant and a I82T mutation in Iota variant were detected.

Acc. no.	Sub Subvariants	ORF1ab	Spike	N	Е	Orf3A
OP699966	BA.2.75	S1221L	K444T			
0Q080316	XBB	G82D	V83A			
0Q244648	XBB.1	T2906I	G252V			
0Q681889	XBB.1.5	D3196G	F486P*			
0Q748396	XBB.1.5.1	K714R	T573I			
0Q748682	XBB.1.5.2	S3309P	K147I, T284I			S92L
0Q748578	XBB.1.5.3	S2048F	K304Q, A411S	I131R		
0Q759166	XBB.1.5.4		T883I			
0Q748550	XBB.1.5.5	T1822I, S2246l	T523A, K1181I			
0Q783432	XBB.1.5.6	S3158G				
0Q748657	XBB.1.5.7	V4649F				
0Q727842	XBB.1.5.8	L3116F	P463S, E554K, P1162S	R10Q		
0Q782510	XBB.1.5.9	R560C		S327L		
0Q748526	XBB.1.5.10	A6044V	F456L			
0Q748511	XBB.1.5.11	G401S				
0Q748664	XBB.1.5.12	S2285F	Q146K			
0Q748399	XBB.1.5.13		Q146K, V1104L			
0Q782367	XBB.1.5.14	R442C				
0Q748855	XBB.1.5.15	P5377S, S5674L	Q146K			
0Q782329	XBB.1.5.16	F6058L	E180V	G25C		
0Q782359	XBB.1.5.17				V62F	
0Q734082	XBB.1.5.18	T2300I, P4619L				
0Q782733	XBB.1.5.19	L3754F, A2128T, R4573C		R195I		
0Q748528	XBB.1.5.20	S1188L, P2045L, P2110S, N6481K,		A398V		
0Q748813	XBB.1.5.21	K322R				G49C
0Q783157	XBB.1.5.22	S167C	S247I			
0Q783778	XBB.1.5.23	N375S, P4220L, T5690A				
0Q802664	XBB.1.5.24	A2584V				
OQ783570	XBB.1.5.25	V665F, A1812D	К97Т			
0Q748846	XBB.1.5.27	E1015G	K478R			H182Y
OQ748750	XBB.1.5.28		K478R	P279L		
0Q759251	XBB.1.5.29	L3808F, A6832V	A348V, G932S			
OQ748271	XBB.1.5.30	P2045S, T2137A, A3697V, T5941I, H5951Y, P6376S	A348T			
0Q748566	XBB.1.5.32	T2823I, Q3966R				
0Q783474	XBB.1.5.33	A138V, S5583L				
0Q748615	XBB.1.5.34	T1754I	T696S, C1253F			
0Q748647	XBB.1.5.35		S98F			
0Q782439	XBB.1.5.36	L293F	A475V, T547K			
0Q782618	XBB.1.5.37	E148G	K1045R			
0Q808416	XBB.1.5.38	T403I		D81H		G172D
OQ783588	XBB.1.5.39	S538L, M3684T, E4388G	Q52H, G1167V			

After the demarcation of XBB.1.5 sub-subvariants, we did multi-alignment analysis to pinpoint the genetic changes and if ORF8 termination codon mutation happened in all those subsubvariants. For multi-alignment, we choose B.o, B.1.1.7, B.1.617.2, BF.7, BA.2.75 and BQ.1 as standard variant and subvariant whereas few XBB.1.5 sub-subvariants as experimental [69]. In truth, we

checked all XBB.1.5.1 to XBB.1.5.39 sub-subvariants for negative ORF8 expression in the database. Figure 4 stated that ORF8 gene mutation (GGA=TGA) in all XBB.1.5.1 sub-subvariants but not in XBB.2 as well as standard variants. We put standard CAA=TAA termination codon mutant (accession no. OP711844) and standard

AAA=TAA termination codon mutant (accession no. OP683545) belonging to Alpha (B.1.1.7) variant for comparison and all three termination codon mutants did not produce viable ORF8 protein to interact and modulate host genes involved in interleukins expression and immune modulation [70-75].

 $\begin{array}{l} BA.2.75-0P699966-30.9.2022\\ XBB.1.16-00748819-30.3.2023\\ XBB.1.9.1-00748878-23.3.2023\\ XBB.1.5.3-00748578-28.3.2023\\ XBB.1.5.3-00748578-28.3.2023\\ XBB.1.5.21-0074899-23.3.2023\\ XBB.1.5.21-0074899-23.3.2023\\ XBB.1.5.21-0074899-23.3.2023\\ XBB.1.5.221-0074859-23.3.2023\\ XBB.1.5.221-0074859-23.3.2023\\ XBB.1.5.12-0074896-23.3.2023\\ XBB.1.5.10-00748526-27.3.2023\\ XBB.1.5.10-00748526-27.3.2023\\ XBB.1.5.10-00748526-23.3.2023\\ BA.4-C6WWV-KSF-00733557-11.10.2022\\ BF.7-0P440319-26.8.2022\\ BD.1-R0MD-00118666-8.1222022\\ BD.1-R0MD-00118666-8.12.2022\\ BD.1-R0MD-00118666-8.12.2021\\ BD.1-R0MD-00118666-8.12.2021\\ BD.1-R0MD-00118666-8.12.2021\\ BD.1-R0MD-00118666-8.12.2021\\ BD.1-R0MD-00118666-8.12.2021\\ BD.1-R0MD-00118660-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-0011860-8.12.2021\\ BD.1-R0MD-001860-8.12.2021\\ BD.1$

BA.2.75-OP699966-30.9.2022
XBB.1.16-00748619-30.3.2023
XBB.1.9.1-00748387-23.3.2023
XBB.1.5.3-00748578-28.3.2023
XBB.1.5-00748845-13.3.2023
XBB.1.5.39-00783588-25.3.2023
XBB.1.5.21-00748813-27.3.2023
XBB.1.5.13-00748399-23.3.2023
XBB-00080316-2.12.2022
XBB.2-00244657-26.12.2022
XBB.1-00244648-26.12.2022
XBB.1.5.10-00748526-27.3.2023
XBB.1.5.1-00748396-23.3.2023
BA.4-GHVMV-KSF-OP733557-11.10.202
BF.7-0P440319-26.8.2022
BQ.1-RWMD-OQ118666-8.12.2022
B117-OP683545-2ndTAA-27.6.2021
Alpha-MZ821602-B117-30.7.2021
B117-OP711844-1stTAA-4.5.2021
B.0-NC_045512-12.2019
Delta-OL317640-13.10.2021

BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.100748377-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.3-00748813-27.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB-0080316-2.12.2022 XBB-00080316-2.12.2022 XBB-00080316-2.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 BA.4-CHVMV-KSF-0P733557-11.10.2022 BF.7-0P440319-26.8.2022 BD.1-RXMD-0011866-8.12.2022 B117-0P683545-2ndTAA-27.6.2021 B117-0P711844-1atTAA-4.5.2021 B.1-NC 045512-12.2019 Delta-0L317640-13.10.2021

$$\begin{split} BA.2.75-0P69\,9966-30.9.2022\\ XBB.1.16-007\,4819-30.3.2023\\ XBB.1.9.1007\,4817-23.3.2023\\ XBB.1.5.3-007\,48578-28.3.2023\\ XBB.1.5.39-00788578-28.3.2023\\ XBB.1.5.39-00788578-28.3.2023\\ XBB.1.5.10-0074899-23.3.2023\\ XBB.1.5.10-0074899-23.3.2023\\ XBB.1.5.10-0074899-23.3.2023\\ XBB.10080316-2.12.2022\\ XBB.1-00244648-26.12.2022\\ XBB.1-00748526-23.3.2023\\ BA.4-CHVMV-KSP-0P733557-11.10.2022\\ Br.1-007483545-23.3.2023\\ Bh.4-CHVMV-KSP-0P733557-11.10.2022\\ Br.1-007683545-20dTAA-27.6.2021\\ B117-00F83545-20dTAA-27.6.2021\\ B117-0P711844-1stTAA-4.5.2021\\ B.01ta-003176 40-13.0.2021 \end{split}$$

ORF8 start GGA=TGA egaacatgaaatttettgttttettaggaateateaeaaetgtagetgeattteaeeaag 27891 egaacatgaaatttettgttttettatgaateateaeaaetgtagetgeattteaeeaag 27888 egaacatgaaatttettgttttettatgaateateaeaaetgtagetgeattteaeeaag 27888

gaacatgaaatttettgttttettatgaateateaeaaetgtagetgeattteaeeaag	27825
gaacatgaaatttettgttttettatgaatcatcacaactgtagetgcatttcaccaag	27855
gaacatgaaatttettgttttettatgaatcatcacaactgtagetgcattteaccaag	27888
gaacatgaaatttettgttttettatgaatcatcacaactgtagetgcatttcaccaag	27877
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gaacatgaaatttettgttttettatgaatcatcacaactgtagetgcatttcaccaag	27888
gaacatgaaatttettgttttettaggaatcatcacaactgtagetgcatttcaccaag	27888
gaacatgaaatttettgttttettaggaatcatcacaactgtagetgcatttcaccaag	27925
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gaacatgaaatttettgttttettatgaateateaeaaetgtagetgeattteaeeaag	27888
gaacatgaaatttettgttttettatgaatcatcacaactgtagetgcatttcaccaag	27825
gaacatgaaatttettgttttettaggaatcatcacaactgtagetgcatttcaccaag	27900
gaacatgaaatttettgttttettaggaateateaeaaetgtagetgeattteaeeaag	27899
gaacatgaaatttettgttttettaggaateateaeaaetgtagetgeattteaeeaag	27864
gaacatgaaatttettgttttettaggaateateaeaaetgtagetgeattteaeeaag	27891
gaacatgaaatttettgttttettaggaatcatcacaactgtagetgcatttcaccaag	27900
gaacatgaaatttettgttttettaggaatcatcacaactgtagetgcattteaccaag	27861
gaacatgaaatttettgttttettaggaateateaeaaetgtagetgeattteaeeaag	27948
gaacatgaaatttettgttttettaggaateateacaaetgtagetgeattteaceaag	27912

CAA=TAA

a atgtagtttacagtcatgtactcaaccatcaaccatatgtagttgatgacccgtgtccta	27951
a atgtagtttacagtcatgtactcaaccatcaaccatatgtagttgatgacccgtgtccta	27948
a atgtagtttacagtcatgtactcaacatcaaccatatgtagttgatgacccgtgtccta	27885
a atgtagtttacagtcatgtactcaaccatcaaccatatgtagttgatgacccgtgtccta	27915
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aatgtagtttacagtcatgtactcaacatcaaccatatgtagttgatgacccgtgtccta	27959
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$a atgtagtttacagtcatgtact {\tt taacatcaaccatatgtagttgatgacccgtgtccta}$	27921
a atgtagtttacagtcatgtactcaaccatcaaccatatgtagttgatgacccgtgtccta	28008
a atgtagtttacagtcatgtactcaacatcaaccatatgtagttgatgacccgtgtccca	27972
${\tt tteacttet} attetaa atggta {\tt tattag} ag {\tt tag} ag {\tt ag} a {\tt aa} a {\tt teacttet} a {\tt tteacttet} a {\tt tt$	28011
ttea ettetattetaa atggtatattag ag taggag etaga aaateag ea eetttaattg	28008
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	27945
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	27975
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	28008
tteaettetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	27997
tteaettetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	28047
the activity of a terrary of a calcular and a second terrary of a calculation of of a calcula	29009
tteacttetattetaaatggtatattagagtaggagetagaaaateageacetttaattg	28045
tteaettetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	28047
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	28008
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	27945
${\tt tteacttet} attetaaatggtatattagagtaggagetagaaaateageaeetttaattg$	28020
tteaettetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	28019
tteacttetattetaaatggtatattagagtaggagetagaaaateageaeetttaattg	27984
tt cacttet attet a a atggt at attag ag tag g ag et a tag a a teag cacett ta attggt a tag ag tag a g a state a tag ag tag a state a s	28011
tteaettetattetaaatggtatattagagtaggagetataaaateageaeetttaattg	28020
tteaettetattetaaatggtatattagagtaggagetataaaateageaeetttaattg	27981
the set of the table and the table again and a second the set of t	29022
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222=T22	
ast totootootootootootootootootootootootoo	29071
ant cycycycyg cygacyng y contain control actor actor y cartegy	29069
aattgtgcgtggatgaggetggttetaaatcacccattcagtacatcgatatcggtaatt	28005
aattgtgggtggatgagggtggttetaaatcacccattcagtacatcggtaatt	28035
aattgtgggtggatgaggetggttetaaateaceeatteagtaeategatateggtaatt	28068
aattgtgegtggatgaggetggttetaaateaeceatteagtacategatateggtaatt	28057
a attgtgegtggatgaggetggttetaaateaceeatteagtacategatateggtaatt	28107
a attgtgegttgatgaggetggttetaaateacceatteagtacategatateggtaatt	28068
a attgtgegtggatgaggetggttetaaateaeceatteagtaeategatateggtaatt	28068
a attgtgegtggatgaggetggttetaaateaeceatteagtaeategatateggtaatt	28105
a attgtgegtggatgaggetggttetaaateaeceatteagtaeategatateggtaatt	28107
aattgtgegtggatgaggetggttetaaateaeeeatteagtaeategatateggtaatt	28068
aattgtgegtggatgaggetggttetaaateaeceatteagtaeategatateggtaatt	28005
aattgtgegtggatgaggetggttetaaateaeeeatteagtaeategatateggtaatt	28080

Figure 4: Localization of three termination codon mutants in ORF8 gene of SARS-CoV-2.

aattgtgcgtggatgaggetggttetaaa

aattgtgegtggatgaggetggttgtaata aattgtgegtggatgaggetggttgtaata aattgtgegtggatgaggetggttetaaata

aattgtgegtggatgaggetggttetaaattacceatteagtgeategatateggtaatt aattgtgegtggatgaggetggttetaanteacceatteagtgeategatateggtaatt

aattgtgcgtggatgaggctggttctaaatcacccattcagtacatcgatatcggtaatt

aattgtgegtggatgaggetggttetaaateaceeatteagtaeategatateggtaatt

We checked the ³⁶⁷⁵SGF dominant deletion in the nsp6 domain of ORF1ab polyprotein and except in Wuhan and Delta, all Omicron

(BA.1/2/4/5, BF.7, BQ.1, XBB.1) and Alpha (B.1.1.7) lineages had such deletion (Figure 5). Similarly, we checked the nsp1 deletions

acceatteagtacategatateggtaatt

accenticagtacateg atateggtaatt accenticagtgeateg atateggtaatt accenticagtgeateg atateggtaatt 28079

28044

28071 28080

28041

28128

28092

in XBB.1.5 and no such deletion was found (Figure 6). Astonishingly, we detected a B.1.1.7 alpha variant with GHVMV deletion which also had ORF8 termination codon mutation (accession no. OP711844). Next, we determined if there was any Spike deletion in XBB.1.5 subvariants. Spike ²⁴LPP deletion was found in BA.2 lineages including BA.2.75, XBB, XBB.1, XBB.1.5 (Figure 7) but no

⁶⁹HV deletion. On the contrary, both ²⁴LPP and ⁶⁹HV spike deletions were found in BQ.1, BF.7, BA.4/5 but not in Delta variant whereas only ⁶⁹HV deletion found in Alpha variant. We also demonstrated that BA.2.75, XBB, XBB.1 and XBB.1.5 subvariants had N501Y mutation (Figure 8) and D614G mutation both of which increased transmission to over 100% than Wuhan virus.

Variant/Acc. No. /Date of virus isolation	SGF deletion in nsp6 gene	
BA.2.75-OP699966-30.9.2022	tagtttg\$aageta.aaagaetgtgttatgtatgeateag.etgtagtgttaet	11292
XBB.1.16-00748619-30.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
XBB.1.9.1-00748387-23.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11229
XBB.1.5.3-00748578-28.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11259
XBB.1.5-00748845-13.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
XBB.1.5.39-00783588-25.3.2023	tagtttgaagetaaaagaetgtgttaegtatgeateagetgtagtgttaet	11281
XBB.1.5.21-00748813-27.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11331
XBB.1.5.13-00748399-23.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
XBB-00080316-2.12.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
XBB.2-00244657-26.12.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11329
XBB.1-00244648-26.12.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11331
XBB.1.5.10-00748526-27.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
XBB.1.5.1-00748396-23.3.2023	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11229
BA.4-GHVMV-KSF-OP733557-11.10.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11307
BF.7-OP440319-26.8.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11306
BQ.1-RWMD-OQ118666-8.12.2022	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11259
B117-OP683545-2ndTAA-27.6.2021	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11292
Alpha-MZ821602-B117-30.7.2021	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11301
B117-OP711844-1stTAA-4.5.2021	tagtttgaagetaaaagaetgtgttatgtatgeateagetgtagtgttaet	11262
B.0-NC_045512-12.2019	tagtttgtetggttttaagetaaaagaetgtgttatgtatg	11340
Delta-OL317640-13.10.2021	tagtttgtetggttttaagetaaaagaetgtgttatgtatg	11310
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Figure 5: Demonstration of all omicrons including XBB, XBB.1, XBB.2 and XBB.1.5 subvariants as well as Alpha variant had SGF deletion in nsp6 protein but Delta and Wuhan.

	Grivity deletion region Nsp1	
BA.2.75-OP699966-30.9.2022	acgtteggatgetegaactgeaccteatggteatgttatggttgagetggtageagaact	501
XBB.1.16-00748619-30.3.2023	acgtteggatgetegaactgeaceteatggteatgttatggttgagetggtageagaact	501
XBB.1.9.1-00748387-23.3.2023	acqttcqqatqctcqaactqcacctcatqqtcatqttatqqttqaqctqqtaqcaqaact	438
XBB.1.5.3-00748578-28.3.2023	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	468
XBB.1.5-00748845-13.3.2023	acgtteggatgetegaactgeaceteatggteatgttatggttgagetggtageagaact	501
XBB.1.5.39-00783588-25.3.2023	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	490
XBB.1.5.21-00748813-27.3.2023	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	540
XBB.1.5.13-00748399-23.3.2023	acgttcggatgetcgaactgcacctcatggtcatgttatggttgagetggtagcagaact	501
XBB-00080316-2.12.2022	acgtteggatgetegaactgeaceteatgateatgttatggttgagetggtageagaact	501
XBB.2-00244657-26.12.2022	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	538
XBB.1-00244648-26.12.2022	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	540
XBB.1.5.10-00748526-27.3.2023	acgttcggatgetcgaaetgeaeeteatggteatgttatggttgagetggtageagaaet	501
XBB.1.5.1-00748396-23.3.2023	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	438
BA.4-GHVMV-KSF-OP733557-11.10.2022	acgtteggatgetegaaetgeaeeteatgagetggtageagaaet	525
BF.7-OP440319-26.8.2022	acgtteggatgetegaaetgeaeeteatggteatgttatggttgagetggtageagaaet	515
BQ.1-RWMD-OQ118666-8.12.2022	acgttcggatgetcgaactgeaceteatggteatgttatggttgagetggtageagaact	468
B117-OP683545-2ndTAA-27.6.2021	acgtteggatgetegaactgeaceteatggteatgttatggttgagetggtageagaact	501
Alpha-M2821602-B117-30.7.2021	acgttcggatgetcgaactgcacetcatggtcatgttatggttgagctggtagcagaact	510
B117-OP711844-1stTAA-4.5.2021	acgtteggatgetegaaetgeaeeteatgagetggtageagaaet	471
B.0-NC_045512-12.2019	acgttcggatgctcgaactgcacctcatggtcatgttatggttgagctggtagcagaact	540
Delta-OL317640-13.10.2021	acgttcggatgetcgaactgeaceteatggteatgttatggttgagetggtageagaact	510
	KSF deletion region Nsp1	
BA.2.75-OP699966-30.9.2022	KSF deletion region Nsp1 tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gettggeaetga	681
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023	KSF deletion region Nsp1 tggeeataggtaeggegeegatetaaagteatttgaettaggegaegaggttggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaegaggttggeaetga	681 681
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023	KSP deletion region Nsp1 tggccataggtacggcgccgat ctaaagtcattgacttaggcgacga gettggcactga tggccataggtacggcgccgat ctaaagtcatttgacttaggcgacga gettggcactga tggccataggtacggcgccgat ctaaagtcatttgacttaggcgacga gettggcactga	681 681 618
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023	KSF deletion region Nsp1 tggccataggtacggcgccgatetaaagteattgacttaggcgacgaggtggcactgg tggccataggtacggcgccgatetaaagteattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagteatttgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagteatttgacttaggcgacgaggttggcactga	681 681 618 648
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023	KSF deletion region Nsp1 tggeeataggtaeggegeegatetaaagteattgaettaggegaega.gettggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gettggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gettggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gettggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gettggeaetga	681 681 618 648 681
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023	KSF deletion region Nsp1 tggeeataggtaeggeegeatetaaagteattgaettaggegaega.gattggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gattggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gattggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaega.gattggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaeg.gagttggeaetga tggeeataggtaeggegeegatetaaagteatttgaettaggegaeg.gagttggeaetga	681 618 648 681 670
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023	KSP deletion region Nsp1 tggccataggtacggcgccgat ctaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgat ctaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgat ctaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgat ctaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgat ctaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgat ctaaagtcattgacttaggcgacg agcttggcactga tggccataggtacggcgcgat ctaaagtcattgacttaggcgacg agcttggcactga tggccataggtacggcgcgat ctaaagtcattgacttaggcgacg agcttggcactga	681 618 648 681 670 720
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00788813-27.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023	KSF deletion region Nsp1 tggccataggtacggcgccgat etaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgat etaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgat etaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgccgat etaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggat etaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggat etaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgcggat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgcggat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgccgat etaaagtcatttgacttaggcgacg agettggcactga	681 618 648 681 670 720 681
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.13-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB-00080316-2.12.2022	KSF deletion region Nsp1 tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcggatetaaagtcatttgacttaggcgacgaggttggcactga	681 618 648 681 670 720 681 681
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748819-23.3.2023 XBB-0080316-2.12.2022 XBB.2-00244657-26.12.2022	KSF deletion region Nsp1 tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga	681 618 648 681 670 720 681 681 718
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748845-13.3.2023 XBB.1.5.39-00748813-27.3.2023 XBB.1.5.13-00748813-27.3.2023 XBB.1.5.13-00748819-23.3.2023 XBB.00080316-2.12.2022 XBB.2-00244657-26.12.2022 XBB.1-00244648-26.12.2022	KSP deletion region Nsp1 tggccataggtacggcgcgat etaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgate taaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgate taaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacgaggttggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcattgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcatttgacttaggcgacg agettggcactga tggccataggtacggcgcgat etaaagtcatttgacttaggcgacg agettggcactga	681 681 648 681 670 720 681 681 718 720
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BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.00748845-13.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.2-00244657-26.12.2022 XBB.2-00244648-26.12.2022 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.1-00748396-23.3.2023 XBB.1.5.1-00748396-23.3.2023 XBB.1.5.1-00748396-23.3.2023 BA.4-CHVMV-KSP-0P733557-11.10.2022 BF.7-0P440319-26.8.2022 B0.1-KWMD-00118666-8.12.2022 B117-0P683545-2ndTAA-27.6.2021	KSF deletion region Nsp1 tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggeeataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga	681 618 648 670 720 681 681 718 720 681 618 696 695 648 681
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.13-007884813-27.3.2023 XBB.1.5.13-00748819-23.3.2023 XBB.0080316-2.12.2022 XBB.2-00244657-26.12.2022 XBB.1-00244648-26.12.2022 XBB.1.5.10-00748396-23.3.2023 RBB.1.5.10-00748396-23.3.2023 RBB.1.5.10-00748396-23.3.2023 BA.4-CRVMV-KSF-0P733557-11.10.2022 BP.7-0P440319-26.8.2022 BJ.1-RWMD-00118666-8.12.2022 BJ17-0P683545-2ndTAA-27.6.2021 Alpha-MZ821602-B117-30.7.2021	KSF deletion region Nsp1 tggceataggtacggegegatetaaagteattgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteattgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagtaeggegegatetaaagteattgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteattgaettaggegaegagettggeaetga tggceataggtaeggegegatetaaagteattgaettaggegaegagagettggeaetga tggceataggtaeggegegatetaaagteattgaettaggegaegagagaga	681 618 648 670 720 681 718 720 681 618 696 695 648 695 648 690
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748845-13.3.2023 XBB.1.5.9-00748845-25.3.2023 XBB.1.5.13-00748813-27.3.2023 XBB.00080316-2.12.2022 XBB.2-00244657-26.12.2022 XBB.1-00244648-26.12.2022 XBB.1.5.10-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-27.3.2023 XBB.1.5.1-00748326-23.3.2023 BA.4-CHVMV-KSP-0F733557-11.10.2022 BF.7-0P440319-26.8.2022 BJ.17-0P683545-2ndTAA-27.6.2021 Alpha-MZ821602-B117-30.7.2021 BJ17-0P711844-1stTAA-4.5.2021	KSP digition rogion Nsp1 tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceataggtacggegeegatetaaagteatttgaettaggegaegaggettggeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegagettgeaetga tggceatagttacggegeegatetaaagteatttgaettaggegaegaggettgeaetga tggceatagttaeggegeegatetaaagteatttgaettaggegaegaggettgeaetga tggceatagtaeggegeegatetaaagteattgaettaggegaegaegaggettgeaetga tggceatagtaeggegeggeegatetaaagteattgaettaggegaegaggettgeaetga tggceatagtaeggegegegegatetaaagteattgaettaggegaegaggettgeaetga tggceatagtaeggegegegatetaaagteattgaettaggegaegaggaggettgeaetga	681 648 648 670 720 681 718 720 681 618 696 695 648 691 690 651
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748845-13.3.2023 XBB.1.5.39-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.00080316-2.12.2022 XBB.2-002446457-26.12.2022 XBB.1-00244648-26.12.2022 XBB.1.5.10-00748396-23.3.2023 BB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 BB.1.5.10-00748526-27.3.2023 BB.4-CHVMV-KSF-0P73557-11.10.2022 BF.7-0P440319-26.8.2022 BQ.1-RWMD-00118666-8.12.2022 BI.17-0P683545-2ndTAA-27.6.2021 Alpha-MZ821602-B117-30.7.2021 B117-0P711884-1atTAA-4.5.2021 B.0-NC_045512-12.2019	KSP diction rogion Nsp1 tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccataggtacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccatagttacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccatagttacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccatagttacggcgccgatetaaagtcatttgacttaggcgacgaggttggcactga tggccatagttacggcgcgatetaaagtcatttgacttaggcgacgaggttgcactga tggccatagttacggcgcgatetaaagtcatttgacttaggcgacgaggttggcactga tggccatagtacggcgcgatetaaagtcatttgacttaggcgacgaggttgcactga tggccatagttacggcgcgatetaaagtcatttgacttaggcgacgaggttgcactga tggccatagttacggcgcgatetaaagtcatttgacttaggcgacgaggttgcactga tggccatagttacggcgcgatetaaagtcatttgacttaggcgacgaggttgcactga tggccatagtacggcgcggcgcgatetaagtcatttgacttaggcgacgaggttgcactga tggccatagtacggcgcggcggatetaagtcatttgacttaggcgacgaggttgcactga tggccatagttacggcgcgggcgatetaagtcatttgacttaggcgacgaggttgcactga tggcca	681 618 648 681 670 681 681 718 720 681 618 696 695 648 695 648 691 651 720
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748845-13.3.2023 XBB.1.5.13-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.00080316-2.12.2022 XBB.2-00244657-26.12.2022 XBB.1-00244657-26.12.2022 XBB.1.5.10-00748326-27.3.2023 BB.4-CHVMV-KSF-0073557-11.10.2022 BF.7-0P440319-26.8.2022 BQ.1-RWMD-00118666-8.12.2022 BJ.17-0P683545-2ndTAA-27.6.2021 Altho-MZ21602-B117-30.7.2021 B117-0P11884 -1stTAA-4.5.2021 B.0-NC_045512-12.2019 Dalta-0L317640-13.10.2021	KSF diction rogion Nsp1 tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceataggtacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttacggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagttaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagtaeggegegatetaaagteatttgaettaggegaegagettggeaetga tggceatagtaeggegegegatetaaagteatttgaettaggegaegagagttggeaetga tggceatagtaeggegegatetaaagteatttgaettaggegaegagagttggeaetga tggceatagtaeggegegegatetaaagteatttgaettaggegaegagagettggeaetga tggceatagtaeggegegatetaaagteatttgaettaggegaegagagettggeaetga tggce	681 618 648 681 670 720 681 681 718 720 681 696 695 648 695 648 691 651 720 690

Figure 6: Demonstration of absence of GHVMV and KSF deletions in nsp1 protein of XBB.1.5 subvariants.

Variant/Acc. No./Date of virus isolation	²⁰ LPP deletion in Spike protein	
BA. 2.75-0P699966-30.9.2022	teagtgtgttaatettataaccagaacteaateatacactaattettteac	21603
XBB 1 16-00748619-30 3 2023	teagtgtgttaatettataaccagaacteaateatacactaattettteac	21603
XBB. 1. 9. 1-00748387-23. 3. 2023	teagtgtgttatettataaceagaacteaateatacaetaattettteae	21540
YRR 1 5 3-00748578-28 3 2023	teagtgtgttatettataaccagaactcaateatacactaattettteac	21570
XBB.1.5-00748845-13.3.2023	teagtgtgttaatettataaceagaacteaateatacaetaattettteae	21603
XBB.1.5.39-00783588-25.3.2023	teagtgtgttaatettataaccagaactcaateatacactaattettteac	21592
XBB.1.5.21-00748813-27.3.2023	teagtgtgttaatettataaccagaactcaateatacactaattettteac	21642
XBB.1.5.13-00748399-23.3.2023	teagtgtgttaatettataaccagaactcaateatacactaattettteac	21603
XBB-00080316-2.12.2022	teagtgtgttaatettataaceagaacteaateatacaetaattettteae	21603
XBB.2-00244657-26.12.2022	teagtgtgttaatettataaceagaacteaateataeaetaattettteae	21640
XBB.1-00244648-26.12.2022	teagtgtgttaatettataaceagaacteaateatacaetaattettteae	21642
XBB.1.5.10-00748526-27.3.2023	teagtgtgttaatettataaceagaacteaateatacaetaattettteae	21603
XBB.1.5.1-00748396-23.3.2023	teagtgtgttaatettataaccagaactcaateatacaetaattettteac	21540
BA.4-GHVMV-KSF-OP733557-11.10.2022	teagtgtgttaatettataaccagaactcaateatacactaattettteac	21618
BF.7-OP440319-26.8.2022	teagtgtgttaatettataaceagaacteaateatacaetaattettteae	21617
BQ.1-RWMD-OQ118666-8.12.2022	teagtgtgttaatettataaccagaactcaateatacactaattettteac	21570
B117-OP683545-2ndTAA-27.6.2021	teagtgtgttaatettacaaccagaactcaattaccccctgcatacactaattetttcac	21612
Alpha-MZ821602-B117-30.7.2021	teagtgtgttaatettaeaaceagaacteaattaccccctgcatacactaattettteac	21621
B117-OP711844-1stTAA-4.5.2021	t eagtgtgttaatettaeaaceagaacteaattaececetgeataeaetaattettteae	21582
B.0-NC_045512-12.2019	teagtgtgttaatettaeaaceagaacteaattaccccctgcatacactaattettteac	21660
Delta-OL317640-13.10.2021	teagtgtgttaatettagaaeeagaaeteaattaeeeeetgeatataetaattettteae	21630
	^{tti} HV deletion in Spike prote	in
BA.2.75-OP699966-30.9.2022	¹¹¹ HV deleion in Spike prote ettyttettaeetttettteeaatyttaettygtteeatgetataeettytteetgggae	in 21723
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023	^{EB} HV deletion in Spike prote ettgttettaeetttettteeaatgttaettggtteeatgetataeatgtetetgggae ettgttettaeetttettteeaatgttaettggtteeatgetataeatgtetetgggae	in 21723 21723
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023	HV delejon in Spike prote ettgttettaeetttettteeaatgttaettggtteeatgetataeatgtetetgggae ettgttettaeetttettteeaatgttaettggtteeatgetataeatgtetetgggae ettgttettaeetttettteeaatgttaettggtteeatgetataeatgtetetgggae	21723 21723 21660
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023	"HV deleion in Spike prote ettyttettaeetttetttee aatyttaettygtteeatgetataeatyteetgggae ettyttettaeetttetttteea atyttaettygtteeatgetataea tyteetgggae ettyttettaeetttettteea atyttaettygtteeatgetataea tyteetgggae ettyttettaeettetttteea atyttaettygtteeatgetataea tyteetgggae	21723 21723 21660 21690
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BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748378-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00748578-28.3.2023 XBB.1.5.21-0074899-23.3.2023 XBB.1.5.13-0074899-23.3.2023 XBB.2.5.21-0074899-23.3.2023 XBB.2.5.202244657-26.12.2022 XBB.2-00244658-26.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 RA.4-GRWM-KSP-0P733557-11.10.2022 BP.7-0P440319-26.8.2022 BD.1-F0MD-00118666-8.12.2022 BIT-0P683545-20dTAA-27.6.2021 Alpha-M2821602-BIT-30.7.621 BIT-0P71844-1atTAA-4.5.2021 B17-0P71844-1atTAA-4.5.2021	"Welegion in Spike prote ctgttettaeetttetttee aatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetataeatgteetgggae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgttettaeetttetttteeaatgttaettggtteeatgetatae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgttettaeetttetttteeaatgttaettggtteeatgetatae ctgggae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgggae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgggae ctgttettaeetttettteeaatgttaettggtteeatgetatae ctgggae ctgttettaeetttettteeaatgttaettggtteeatgetate ctgggae ctgttettaeettetttteeaatgttaettggtteeatgetate ctgggae ctgttettaeettettteeaatgttaettggtteeatgetate ctgggae ctgttettaeettettteeaatgttaettggtteeatgetate ctgggae ctgttettaeettettteeaatgttaettggtteeatgetate ctgggae ctgttettaeettettteeaatgttaettggtteeatgetate ctgggae ctgttettaeettettteeaatgttaettggtteeatgetae ctgggae ctgttettaeettettteeaatgttaettggtteeatgetae ctgggae ctgttettaeettettteeaatgttaettggtteeatgetae ctgggae ctgttettaeettettteeaatgttaettggtteeatgetae ctggae ctgttettaeettettteeaatgttaettggtteeatgetae ctggae ctggae ctggtettaeettettt	n 21723 21760 21690 21723 21762 21712 21762 21762 21762 21762 21760 21762 21760 21762 21723 21660 21732 21731 21664 21735 21696
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BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9-OQ748378-23.3.2023 XBB.1.5-OQ748578-28.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5.21-OQ748399-23.3.2023 XBB.1.5.21-OQ748399-23.3.2023 XBB.2.00244657-26.12.2022 XBB.2-OQ244657-26.12.2022 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-OQ748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748526-27.3.2023 XBB.1.5.10-0Q748390-23.2022 B0.1-RWM-KSP.000733557-11.10.2022 B0.1-RWM-KSP.00733557-11.10.2022 B0.1-RWM-C011866-8.12.2022 B0.1-RWM-C011866-8.12.2022 B0.1-RWM-0011866-8.12.2021 B117-0P71844-1stTAh-27.6.2021 B117-0P71844-1stTAh-27.5.2021 B117-0P71840-1317.640-13.10.2021	"HV deleion in Spike prote ettyttettaeettetttee aatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteea atyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatyttaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetataeatyteettyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettyttettaeettetttteeaatytaettygtteeatyetate=tetyggae ettytettaeettetttteeaatytaettygtteeatyetate=tetyggae ettytettaeettetttteeaatytaettygtteeatyetate=tetyggae	21723 21723 21660 21690 21723 21712 21722 21723 21723 21723 21723 21723 21723 21723 21723 21723 21731 21684 21735 21735 21796 21735

Figure 7: Demonstration of Spike deletion in XBB.1.5 subvariants. Spike ²⁴LPP deletion found in BA.2 lineages including BA.2.75, XBB, XBB.1, XBB.1.5 (A) but no spike ⁶⁹HV deletion (B). The ⁶⁹HV deletion was also found in Alpha, BQ.1, BF.7, BA.4 but not in Delta variant.

Variant/Acc. No. / Date of virus isolation	N501Y mutation in Spike	
BA.2.75-OP699966-30.9.2022	ettteetttaeaateatatggttteegaeeeaettatggtgttggteaeeaaeea	23031
XBB.1.16-00748619-30.3.2023	eteteetttacaateatatggttteegaceeaettatggtgttggtcaceaaceatacag	23028
XBB.1.9.1-00748387-23.3.2023	eteteetttacaateatatggttteegacceaettatggtgttggteaccaaceatacag	22965
XBB.1.5.3-00748578-28.3.2023	et et e et t t a caate at at ggtt t c e gace caet t at ggtg t t ggt c a c caace at a cag	22995
XBB.1.5-00748845-13.3.2023	$eteteetttaeaateatatggttteegaeeeaet\\ tatggtgttggteaeeaaeeataeag$	23028
XBB.1.5.39-00783588-25.3.2023	$eteteetttaeaateatatggttteegaeeeaet \\ tatggtgttggteaeeaaeeataeag$	23017
XBB.1.5.21-00748813-27.3.2023	$eteteetttaeaateatatggttteegaeeeaet\\ tatggtgttggteaeeaaetaeag$	23067
XBB.1.5.13-00748399-23.3.2023	$eteteetttaeaateatatggttteegaeeeaet\\ tatggtgttggteaeeaaeeataeag$	23028
XBB-00080316-2.12.2022	eteteetttacaateatatggttteegaceeaettatggtgttggteaecaaeeataeag	23028
XBB.2-00244657-26.12.2022	$eteteetttaeaateatatggttteegaeeeaet\\ tatggtgttggteaeeaaetaeag$	23065
XBB.1-00244648-26.12.2022	eteteetttaeaateatatggttteegaceeaettatggtgttggteaeeaaeeataeag	23067
XBB.1.5.10-00748526-27.3.2023	eteteetttacaateatatggttteegaceeaettatggtgttggteaecaaceatacag	23028
XBB.1.5.1-00748396-23.3.2023	eteteetttaeaateatatggttteegaeeeaettatggtgttggteaeeaaeea	22965
BA.4-GHVMV-KSF-OP733557-11.10.2022	ettteetttaeaateatatggttteegaeeeaettatggtgttggteaeeaaeea	23040
BF.7-0P440319-26.8.2022	ettteetttaeaateatatggttteegaceeaettatggtgttggteaeeaaeeataeag	23039
BQ.1-RWMD-CQ118666-8.12.2022	ettteetttaeaateatatggttteegaceeaettatggtgttggteaecaaeeataeag	23004
B117-0P683343-2ndTAA-27.6.2021	ettteetttaeaateatatggttteeaaceeaettatggtgttggttaeeaaeeataeag	23031
Alpha-M2821602-8117-30.7.2021	ettteetttacaatcatatggttteeaacceaettatggtgttggttaccaaccatacag	23040
B 0-NC 045512-12 2019	ettteetttacaateatatggttteesaaceaetaatggtgttggttaccaaceataeag	23088
Dolto=01317640=13 10 2021	at the of the approximation of	22052
DALLA-(00317040-13.10.2021	** ***********************************	AL W. A
	DOMO de viscost en de la Calles en de la	
	Do 14G dominant mutation in Spike protein	
BA.2.75-0P699966-30.9.2022	tgttetttateaggtgttaaetgeacagaagteeetgttgetatteatgeagateaaet	23391
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023	Dol4G domnar mutadonin Spike proken Egittetthateaggigigitaaetgeaeagaagteeetgitgetatteatgeagateaaet Egitetthateaggigitaaetgeaeagaagteeetgitgetatteatgeagateaaet	23391 23388
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023	Uol4G domnar mutadonin Spike prokin tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet	23391 23388 23325
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-0074837-23.3.2023 XBB.1.5.3-00748378-28.3.2023	UoiaG domnar mutaonn Spike proxin tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet	23391 23388 23325 23355
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023	Dolad domnar musaonin Spike prokin tgttetttateagggtgttaactgeacagaagteeetgttgetatteatgeagateaact tgttetttateagggtgttaactgeacagaagteeetgttgetatteatgeagateaact tgttetttateagggtgttaactgeacagaagteeetgttgetatteatgeagateaact tgttetttateagggtgttaactgeacagaagteeetgttgetatteatgeagateaact tgttetttateagggtgttaactgeacagaagteeetgttgetatteatgeagateaaet	23391 23388 23325 23355 23388
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748819-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-007488578-28.3.2023 XBB.1.5.007488578-13.3.2023 XBB.1.5.39-00783588-25.3.2023	Upide domnar mutabonn Spike prokin tyttetttateaggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeagaagteeetgttgetatteatgeagateaaet	23391 23388 23325 23355 23388 23377
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.09700783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023	Upied domnam mutadonin Spike prokin tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet tgttetttateagggigttaaetgeaeagaagteeetgttgetatteatgeagateaaet	23391 23388 23325 23355 23388 23377 23427
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BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.00748878-28.3.2023 XBB.1.5.39-00785788-25.3.2023 XBB.1.5.39-00788578-25.3.2023 XBB.1.5.31-00748813-27.3.2023 XBB.00980316-2.12.2022 XBB.2-002466457-26.12.2022 XBB.2-002466457-26.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.2.5.10-00748526-27.3.2023 XBB.2.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.2.5.10-00748526-27.3.2023 XBB.2.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBD.1.5.10-00748365-2022 B117-0P683545-20dTAA-27.6.2021 A1pha-M282602-B117-30.7.2021 B.0-NC_045512-12.2.201 B.0-NC_043512-12.2.201	tyttettateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttettateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaactgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet tyttetttateagggtgttaaetgeaeggagteetgttgetatteatgeagateaaet	23391 23388 23325 23355 23355 23325 23427 23427 23427 23427 23427 23427 23427 23427 23427 23427 23427 23427 23427 23427 23364 23391 23449 23447 23447

Figure 8: Multi-alignment demonstration that genomes of BA.2.75, XBB, XBB.1 and XBB.1.5 subvariants had N501Y mutation similar to B.1.1.7 (A) and D614G mutation similar to B.1.1.7, B.1.617.2 and others (B). Delta variant had D614G mutation but not N501Y mutation. Wuhan related early corona viruses (B.0, B.1, B.1.1) had no both D614G and N501Y mutations.

The deadly Delta varianN501YG mutation but no N501Y mutation whereas control 2019 Wuhan virus (B.0) had no D614G and N501Y both mutations. Previously, we showed that N-protein ³¹ERS three amino acids deletion was prominent in Omicron variants starting from B.1.1.529 variants including BA.2, BA.2.9, BA.2.75 and BA.2.75.2 [76-78]. However, Wuhan, BA.1.1, B.1.1.1, BA.1.1.172, B.1.1.7, B.1.617.2 and other early coronavirus lineages had no such deletion. Figure 9 showed that BA.4, BA.5, BF.7, BQ.1, XBB.1 lineages also had such deletions which caused N-protein three amino acids

shorter (216AAs). Most importantly, previously we reported 26nt deletion in 3'-UTR in many Omicron lineages (BA.2, BA.4, BA.5) which made coronavirus very replication defective. Interestingly, we did not find 26nt deletion in BA.1 early omicron lineages or old Wuhan, Alpha, Beta, Gamma, Delta coronavirus variants. In Figure 10, we showed that XBB.1.1 lineages retained such deletion including XBB.1, XBB.2, XBB.1.16, XBB.1.22.1, BF.7, BQ.1, BQ.1.1 and BQ.1.1.1 whereas spread of such variant likely increased than BA.2.75, BF.7 and BQ.1.1.1 subvariants.

Variants/Accession/Date of virus	isolation N-gene region ERS	
BA.2.75-OP699966-30.9.2022	geactecgeattacgtttggtggaccetcagattcaactggcagtaaccagaatg	28304
XBB.1.16-00748619-30.3.2023	geactecgeattacgtttggtggacceteagatteaactggcagtaaccagaatg	28301
XBB.1.9.1-00748387-23.3.2023	gcactccgcattacgtttggtggaccctcagattcaactggcagtaaccagaatg	28238
XBB.1.5.3-00748578-28.3.2023	geactecgeattacgtttggtggacceteagatteaactggcagtaaccagaatg	28268
XBB.1.5-00748845-13.3.2023	geactecgeattacgtttggtggaccetcagattcaactggcagtaaccagaatg	28301
XBB.1.5.39-00783588-25.3.2023	geactecgeattacgtttggtggacectcagattcaactggcagtaaccagaatg	28290
XBB.1.5.21-00748813-27.3.2023	geactecgeattacgtttggtggaceeteagatteaactggeagtaaccagaatg	28340
XBB.1.5.13-00748399-23.3.2023	geaetecgeattacgtttggtggaeeeteagatteaaetggeagtaaeeagaatg	28301
XBB-00080316-2.12.2022	geactecgeattacgtttggtggacceteagatteaactggeagtaaccagaatg	28301
XBB.2-00244657-26.12.2022	geacteogeattacgtttggtggaceeteagatteaactggeagtaaccagaatg	28338
XBB.1-00244648-26.12.2022	geactecgeattacgtttggtggaceeteagatteaactggcagtaaccagaatg	28340
XBB.1.5.10-00748526-27.3.2023	geactecgeattacgtttggtggaceetcagattcaactggcagtaaccagaatg	28301
XBB.1.5.1-00748396-23.3.2023	geactecgeattacgtttggtggacceteagattcaactggcagtaaccagaatg	28238
BA.4-GHVMV-KSF-OP733557-11.10.20	122 geactecgeattacgtttggtggaceetcagattcaactggcagtaaccagaatg	28313
BF.7-OP440319-26.8.2022	geacteogeattacgtttggtgggeeeteagatteaactggeagtaaccagaatt	28312
BQ.1-RWMD-OQ118666-8.12.2022	geacttegeattaegtttggtggaeeeteagatteaaetggeagtaaeeagaatg	28277
B117-OP683545-2ndTAA-27.6.2021	geaccecegeattaegtttggtggacceceagatteaactggcagtaaccagaatggagaa	28310
Alpha-MZ821602-B117-30.7.2021	geaccecegeattaegtttggtggaccecteagatteaactggcagtaacceagaatggagaa	28317
B117-OP711844-1stTAA-4.5.2021	geacceegeattaegtttggtggacceteagatteaactggeagtaaceagaatggagaa	28278
B.0-NC_045512-12.2019	geacceegeattacgtttggtggaccetcagattcaactggcagtaaccagaatggagaa	28366
Delta-OL317640-13.10.2021	geacceegeattaegtttggtggacceteagatteaaetggeagtaaccagaatggagaa	28323
	eRS N-gene region	
BA.2.75-OP699966-30.9.2022	ERS N-gene region	28360
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023	ERS N-gene region	28360 28357
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ74887-23.3.2023	ERS N-gene regiongtgggggeggateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtgggggeggateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtgggggeggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet	28360 28357 28294
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023	ERS N-gene regiongtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtetgtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtetgtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtetgtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet	28360 28357 28294 28324
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023	ERS N-gene region gtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet gtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet gtgggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet gtggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet gtggggeggateaaaacaacgteggeeceaaggtttaceeaataataetgegtet	28360 28357 28294 28324 28357
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5-OQ748845-13.3.2023 XBB.1.5-OQ748845-13.3.2023 XBB.1.5.39-OQ783588-25.3.2023	ERS N-gene region gtgggggggggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet gtgggggggggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet gtgggggggggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet gtggggggggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet gtggggggggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet gtgggggeggateaaaacaacgteggeeceaaggtttaceeaataatactgegtet	28360 28357 28294 28324 28357 28346
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.15.21-00748813-27.3.2023	ERS N-gene region gtggggeggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaeeeaataataetgegtet	28360 28357 28294 28324 28357 28346 28396
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023	ERS N-gene region gtgggggeggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggegggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet gtggggeggateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet	28360 28357 28294 28324 28357 28346 28396 28357
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB-00080316-2.12.2022 YBB.202244651-26	ERS N-gene regiongtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeeeaaggtttaceeaataatactgegtet	28360 28357 28294 28324 28357 28346 28396 28357 28357
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.13-OQ748813-27.3.2023 XBB.1.5.13-OQ748399-23.3.2023 XBB-OQ080316-2.12.2022 XBB.2-OQ244657-26.12.2022 XBB.2-OQ244657-26.12.2022	ERS N-gene regiongtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtetgtggggegegateaaaacaacgteggeeceaaggtttaceeaataatactgegtet	28360 28357 28294 28324 28357 28346 28356 28357 28357 28357 28357
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ7483878-28.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.13-OQ748813-27.3.2023 XBB.1.5.13-OQ748399-23.3.2023 XBBOQ080316-2.12.2022 XBB.2-OQ244657-26.12.2022 XBB.1-OQ244648-26.12.2022 XBB.1-OQ244648-26.12.2022 XBB.1-OQ244648-26.12.2022	ERS N-gene region	28360 28357 28294 28324 28357 28346 28356 28357 28357 28357 28394 28356
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.21-OQ748813-27.3.2023 XBB.1.5.13-OQ748813-27.3.2023 XBB.1.5.13-OQ748399-23.3.2023 XBB-OQ080316-2.12.2022 XBB.2-OQ244657-26.12.2022 XBB.1-OQ24468-26.12.2022 XBB.1.5.10-OQ748526-23.3.2023 XBB.1.5.10-OQ748526-23.3.2023	ERS N-gene region	28360 28357 28294 28324 28357 28346 28357 28357 28357 28394 28396 28357
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.2-00244657-26.12.2022 XBB.2-00244657-26.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748596-23.3.2023 XBB.1.5.10-00748596-23.3.2023 XBB.1.5.10-0074	ERS N-gene region	28360 28357 28294 28324 28346 28396 28357 28357 28394 28396 28357 28394 28396 28357 28294 28357
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.21-00748899-23.3.2023 XBB.2-00244657-26.12.2022 XBB.1-00244657-26.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748396-23.3.2023 BA.4-GHVMV-KSF-0P733557-11.10.20 BE.7-0P440319-26.8.2022	Pers N-gene region	28360 28357 28294 28324 28357 28346 28357 28357 28354 28394 28394 28394 28369 28369 28369
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.13-00748813-27.3.2023 XBB.00080316-2.12.2022 XBB.1-00244667-26.12.2022 XBB.1-00244667-26.12.2022 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.1-00748396-23.3.2023 BA.4-CHVMV-KSP-0P733557-11.10.20 BF.7-0P440319-26.8.2022	Pers N-gene region	28360 28357 28324 28324 28357 28346 28357 28357 28357 28357 28357 28357 28357 28357 28357 28369 28369 28368
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5-OQ748845-13.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.13-OQ748399-23.3.2023 XBB.0Q080316-2.12.2022 XBB.1-OQ244657-26.12.2022 XBB.1-S.10-OQ748526-27.3.2023 XBB.1.5.1-OQ748396-23.3.2023 XBB.1.5.1-OQ748396-23.3.2023 BA.4-CHVMV-KSF-OP733557-11.10.20 BF.7-OP440319-26.8.2022 BO.1-RWMD-OQ118666-8.12.2022 BD.1-RWMD-OQ118666-8.2022 BD.1-CP68355-20dTAA-27.6.2021	Pers N-gene region	28360 28357 28324 28357 28346 28357 28357 28357 28357 28357 28354 28357 28368 28369 28368 28333
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.5.3-00748387-23.3.2023 XBB.1.500748845-13.3.2023 XBB.1.5-00748845-13.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB00284657-26.12.2022 XBB.1-00244657-26.12.2022 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 XBB.1.5.10-00748396-23.3.2023 BA.4-GHVMV-KSF-0P733557-11.10.20 BF.7-0P440319-26.8.2022 B117-0P683545-20dTAA-27.6.2021 Albba-MZ821602-B117-30.7.2021	Pers N-gene region	28360 28357 28294 28324 28357 28346 28357 28357 28356 28357 28394 28357 28294 28369 28368 28333 28377
BA.2.75-OP699966-30.9.2022 XBB.1.16-OQ748619-30.3.2023 XBB.1.9.1-OQ748387-23.3.2023 XBB.1.5.3-OQ748578-28.3.2023 XBB.1.5-OQ748845-13.3.2023 XBB.1.5.39-OQ783588-25.3.2023 XBB.1.5.21-OQ748813-27.3.2023 XBB.0Q080316-2.12.2022 XBB.2-OQ244657-26.12.2022 XBB.1-5.10-OQ748526-27.3.2023 XBB.1.5.1-OQ748526-27.3.2023 XBB.1.5.1-OQ748526-27.3.2023 BA.4-GHVMV-KSF-OP733557-11.10.20 BF.7-OP440319-26.8.2022 BO.1-RWMD-OQ118666-8.12.2022 B117-OP741844-1stTAA-4.5.2021	Pers N-gene region	28360 28357 28294 28357 28346 28357 28357 28357 28394 28357 28394 28357 28394 28357 28358 28357 28358 28357 28370 28338
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.9.1-00748387-23.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.2-00284657-26.12.2022 XBB.2-00244657-26.12.2022 XBB.1-5.10-00748526-27.3.2023 XBB.1.5.10-00748526-27.3.2023 XBB.1.5.10-00748396-23.3.2023 BA.4-GHVMV-KSF-0F73557-11.10.20 BF.7-0P440319-26.8.2022 B0.1-RWMD-00118666-8.12.2022 B117-0P683545-2ndTAA-27.6.2021 Alpha-MZ821602-B117-30.7.2021 B117-0P711844-1stTAA-4.5.2021 B.0-NC 045512-12.2019	Pers N-gene region	28360 28324 28324 28357 28346 28357 28357 28357 28357 28357 28394 28357 28294 28368 28333 28370 28377 28377 28377
BA.2.75-0P699966-30.9.2022 XBB.1.16-00748619-30.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.3-00748578-28.3.2023 XBB.1.5.39-00783588-25.3.2023 XBB.1.5.21-00748813-27.3.2023 XBB.1.5.13-00748399-23.3.2023 XBB.2-00244657-26.12.2022 XBB.2-00244657-26.12.2022 XBB.1-5.10-00748396-23.3.2023 BA.4-GHVMV-KSF-0F73557-11.10.20 BF.7-0F440319-26.8.2022 B117-0F683545-20dTAA-27.6.2021 Alpha-MZ821602-B117-30.7.2021 B10-NC_045512-12.2019 Delta-0L317640-13.10.2021	Pers N-gene region	28360 28294 28324 28357 28346 28357 28394 28357 28394 28357 28394 28369 28369 28369 28369 28369 28369 28369 28377 28338 28370 28377

Figure 9: Multi-alignment demonstration of ³¹ERS N-protein deletion in Omicron corona viruses but not in Alpha, Delta or Wuhan early coronaviruses. The other early VOCs like Beta, Gamma, Epsilon, Zeta also had no such deletion (data not shown).

Variants	Accession /	/Date of v	virus iso	lation
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26nt 3'-UTR deletion

BA.2.75-OP699966-30.9.2022	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29667
XBB.1.16-00748619-30.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29664
XBB.1.9.1-00748387-23.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29601
XBB.1.5.3-00748578-28.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29631
XBB.1.5.39-00783588-25.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29653
XBB.1.5.21-00748813-27.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29703
XBB.1.5.13-00748399-23.3.2023	agtgtgtaacattagggaggacttgaaagagceaceacatttteace	29664
XBB-00080316-2.12.2022	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29664
XBB.2-00244657-26.12.2022	agtgtgtaacattagggaggacttgaaagagceaceacatttteace	29701
XBB.1-00244648-26.12.2022	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29703
XBB.1.5.10-00748526-27.3.2023	agtgtgtaacattagggaggacttgaaagagceaceacatttteace	29664
XBB.1.5.1-00748396-23.3.2023	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29601
BA.4-GHVMV-KSF-OP733557-11.10.20	22 agtgtgtaacattagggaggacttgaaagagceaceacatttteace	29676
BF.7-OP440319-26.8.2022	agtgtgtaacattagggaggacttgaaagagccaccacattttcacc	29675
BQ.1-RWMD-OQ118666-8.12.2022	agtgtgtaacattagggaggacttgaaagagceaccacattttcacc	29640
B117-OP683545-2ndTAA-27.6.2021	agtgtgtaacattagggaggacttgaaagagccaccacattttcaccgaggccacgcgga	29690
Alpha-MZ821602-B117-30.7.2021	agtgtgtaacattagggaggacttgaaagagccaccacattttcaccgaggccacgcgga	29697
B.0-NC_045512-12.2019	agtgtgtaacattagggaggacttgaaagagccaccacattttcaccgaggccacgcgga	29746
Delta-OL317640-13.10.2021	agtgtgtaacattagggaggacttgaaagagccaccacattttcaccgaggccactcgga	29703

	B'-UTR region of SARS-CoV-2	
BA.2.75-OP699966-30.9.2022	tacagtgaa.caatgctagggagagctgcctatatggaagagccctaa	29714
XBB.1.16-00748619-30.3.2023	tacagtgaacaatgctagggagagctgcctatatggaagagccctaa	29711
XBB.1.9.1-00748387-23.3.2023	tacagtgaacaatgctagggagagctgcctatatggaagagccctaa	29648
XBB.1.5.3-00748578-28.3.2023	tacagtgaa.caatgctagggagagctgcctatatggaagagccctaa	29678
XBB.1.5.39-00783588-25.3.2023	tacagtgaa.caatgetagggagagetgeetatatggaagageeetaa	29700
XBB.1.5.21-00748813-27.3.2023	tacagtgaa.caatgctagggagagetgeetatatggaagageeetaa	29750
XBB.1.5.13-00748399-23.3.2023	tacagtgaacaatgetagggagagetgeetatatggaagageeetaa	29711
XBB-00080316-2.12.2022	tacagtgaa.caatgctagggagagetgeetatatggaagageeetaa	29711
XBB.2-00244657-26.12.2022	tacagtgaa.caatgetagggagagetgeetatatggaagageeetaa	29748
XBB.1-00244648-26.12.2022	tacagtgaa.caatgetagggagagetgeetatatggaagageeetaa	29750
XBB.1.5.10-00748526-27.3.2023	tacagtgaa.caatgetagggagagetgeetatatggaagageeetaa	29711
XBB.1.5.1-00748396-23.3.2023	tacagtgaa.caatgctagggagagetgeetatatggaagageeetaa	29648
BA.4-GHVMV-KSF-OP733557-11.10.20	22tacagtgaacaatgetagggagagetgeetatatggaagageeetaa	29723
BF.7-0P440319-26.8.2022	tacagtgaa.caatgctagggagagetgeetatatggaagageeetaa	29722
BQ.1-RWMD-OQ118666-8.12.2022	tacagtgaacaatgctagggagagctgcctatatggaagagccctaa	29687
B117-OP683545-2ndTAA-27.6.2021	gtaegategagtgtaeagtgaaeaatgetagggagagetgeetatatggaagageeetaa	29750
Alpha-MZ821602-B117-30.7.2021	${\tt gtacgatetagtgtacagtgaacaatgetagggagagetgeetatatggaagageeetaa$	29757
B.0-NC_045512-12.2019	gtacgatcgagtgtacagtgaacaatgctagggagagctgcctatatggaagagccctaa	29806
Delta-OL317640-13.10.2021	${\tt gtacgatcgagtgtacagtgaacaatgctagggagagctgcctatatggaagagccctaa$	29763

Figure 10: Multi-alignment demonstration of 26nt 3'-UTR deletion in most Omicron coronaviruses (BA.2.75, BA.4, BF.7, BQ.1, XBB.1.5.1) but such deletion was not reported in Alpha, Delta and Wuhan early coronaviruses.

We showed the XBB.1.5.1 to XBB.1.5.39 specific mutations in most COVID-19 proteins in Table 1. We showed the part of the multi-alignment of spike protein RBD domain in Figure 11. Figure 11 also demonstrated Omicron BA.2 specific mutations (green arrows), XBB.1.5 specific mutations (blue arrows), N501Y mutation (green circle) and XBB.1.5.1 sub subvariants mutations (grey circles). Further, A411S (XBB.1.3), P463S (XBB.1.5.8), F456l (XBB.1.5.10), A475V (XBB.1.5.36) including A520S (XBB.1.22) and S408W (XBB.1.22.1) sub subvariant specific mutations were demonstrated (Table 1). Those sub sub variant specific mutations will be utilized to make subvariant specific oligonucleotides for the detection of unknown COVID-19 variants as well as to demonstrate the database penetration of those sub subvariants by BLAST search.

	391	400	410	0420	430	440	11 450	460	470	6480	, al	500	510	520
8.0-NC_045512.2-12.2	CETNVY	ROSEVIRG	EVEQIAPED	TEXTROYNY	1POOFTG	CVIRHNSNN	LOSKVGGNYN	LYRLFRISSLKP	FERDISTEI	YORGSTPCN	GVEGENCYEP	OSYGEOPT	GVGTOPYRVVN	SFELLHR
BR.2-00028416-28.4.2	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKVGGNYN	LYRLFRISMLKP	FERDISTEI	YQRENKPCN	GVRGENCYFP	RSYGERET	GTEHOPYRVVVL	SFELLIN
BR.2.75.2-00(28860-2	CF THVY	HOSE VIRGE	EVSQIAPGQ	TENTROYNYX	LPOOFTG	CVIRINSING	LOSKVSGNYN	LYRLFRICSRLKP	FERDISTEI	YORGNOPCH	GVHGSNCYF P	QSTGERPT	GIGIOPYRYMI	SFELLIN
XBB-00032315-3.12.20 XBB.2-00244657-26.12	CETNVY	HUSP VIRGE	EVSOTRPGO	TENTROYNYK	LPOOF TG	CVTRUNSING	LOSKPSGNYN	L YRL FRISKLKP	FERDISTET	YOREN PUN	GVRGSNCYSP	OSYGERPTY	GUGHOPYRVVVI	SEELLING
XB8.2.4-00681818-10.	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSRLKP	FERDISTEI	YURGNOPCH	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVI	SFELLHR
X88.1-00000169-28.11	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YQREMCPCN	GVHGSNCYSPI	QSYGERPTY	GYCHOPYRYVYL	SFELLIN
X88.1.1-00165343-17. X88.1-00109633-8.12	CETNYY	HUSE VIEW	E V SQIDPGQ	IGNIDIOTRYS.	LPOOF IG	CVIDENSING	DSKPSGNYN	THEFRESH KP	FERDISTET	YORGANEPUN	SVHUSINE TSP	OSVEEPPTY	GYGRUP TRYVIL	SPELLING
XB8.1-00248544-3.1.2	CETNVY	HOSEVIRG	EVSQIAPGO	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YURENPEN	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVI	SFELLHA
XB8.1.5-00681889-11.	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LDSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YQRENCPCN	GVRGPNCYSP1	QSYGFRPTY	GVEHQPYRVVVL	SFELLHR
X88.1.5.9-00681211-9	CF THVY	HOSE VIRGE	E VSQIAPGQ	TENTROYNYX	LPOOFTG	CVIRENSING	LOSKPSGNYN	LYRLFRICSRLKP	FERDISTEL	YORGNOPCN	GVHGPNCYSP	QSTEFRPT	GIGIQPYRVIN	SFELLIN
X88.1.5.14-00782367-	CETNVY	HOSE VIRGE	EVSOTAPGO	TENTROYNYK	1 POOFTG	CVTRANSING	LOSKPSGNYN	TYREFRICS TOP	FERDISTET	YOREN PCN	GVRGPNCYSPI	OSYGERPTY	GVGHOPYRVVVI	SFELLMA
XB8,1,5,16-00782329-	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YURGNOPCH	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVL	SFELLHA
XB8.1.5.17-00782359-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSK <mark>PS</mark> GNYN	LYRLFRKSRLKP	FERDISTEI	YQRENCPCN	GVRGPNCYSP1	QSYGERPTY	GVEHQPYRVVVL	SFELLHR
X88.1.5.18-00734082- X88.1.5.19-00759002-	CO TRVY	HUSE VIRGE	e vsqinecq	TENDROYNYK	LPOOF TG	CVIDBINSING	LOSKPSGNYN	LYRLFRICSIL KP	FERDISIEI	YUHENCPCN	SVHGPNUTSP	OSTGERPTY	GYGRUPYRYYNL	SFELLING
X88.1.22.2-00748547-	CETNVY	HOSE VIRGE	EVSOTAPGO	TENTROYNYK	1 POOFTG	CVTRANSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTET	YOREN PCN	GVRGPNCY SPI	OSYGERPTY	GVGHOPYRVVVI	SFELLMA
X88,1,5,20-00748528-	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOF TG	CVIRMNSNM	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YURGNEPCN	GVRGPNCYSP1	QSYGERPTY	GVGHQPYRVVVL	SFELLHR
X88.1.5.21-00748813-	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSK <mark>PS</mark> GNYN	LYRLFRKSRLKP	FERDISTEI	YQREMCPCN	GVHGPNCYSP1	QSYGERPTY	GYCHOPYRYYYL	SFELLHA
X88.1.5.23-00/8133/- X88.1.5.24-00749539-	CETRAN	HUSE VIRGE	e vsemeco	TENTROYNYK T	LPOOF TG	CVIDBINSING	LOSKP SUNTRY	T AND EDUCATION	CERDISTET	YORGARPEN	SVIIG NUTSI	OSYGERPTY	GYORD TRYNT	SPELLING
X88.1.5.31-00758970-	CETNVY	HDSF VIRG	EVSQIAPGO	IGNIBOYNYK	LPOOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRKSRLKP	FERDISTEI	YORGNEPCN	GVRGPNCYSPI	OSYGERPTY	GYGHOPYRYYYI	SFELLHA
X88.1.5.32-00748566-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YQREMCPCN	GVRGPNCYSP	QSYGFRPTY	GVGHQPYRVVVL	SFELLHR
X88.1.5.33-00783474-	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSRLKP	FERDISTEI	YQRENKPCN	GVRGPNCYSPI	QSYGFRPTY	GYCHOPYRYYYL	SFELLHA
X88.1.5.6-00681316-1 V00 1 5 20-00000416-	CETRAN	HUSE VIEW	E VSQINPGQ	TON TROY WYN	LPOOF TG	CVIDENCS NO.	DOMPSON IN	THE FRENCH	CERDISTET	YORGANERCH		OST CEPPT	GYGRUP TRYVIL	SPELLING
XB8.1.22-00748542-27	CETNVY	ROSEVIRG	EVSQIAPGO	TENTROYNYK	LPOOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YURGNEPCN	GVRGPNCYSPI	OSYGERPTY	GVGHOPYRVVVI	SFELLINS
X88.1.5.37-00782618-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YQREMCPCN	GVRGPNCYSPI	QSYGFRPTY	GVGHQPYRVVI	SFELLUR
X88.1.5.27-00748846-	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIDBINSNO	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YQREMRPCN	GVRGPNCYSPI	QSYGERPTY	GYGHQPYRVVIL	SFELLIN
X88.1.5.28-00/48/50- X88.1.5.30-00748221-	CETNYY	HUSE VIEGE	EVS010PG0	IGNINUTATA.	LPOOF IG	CVTRANSING	LUSKPSGNYN	THEFRESH KP	FERDISTET	YORGANEPUN	GVHGPNL TSP	OSVEERPTY	GYGROPYRYYYI	SEELLING
XB8.1.9.2-00681424-1	CETNVY	ROSEVIRG	EVSQIAPGO	TENTROYNYK	LPOOFTG	CVIRIANSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YURGNEPCN	GVRGPNCYSPI	QSYGERPTY	GVGHQPYRVVVI	SFELLHA
XB8.1.5.12-00748664-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVININSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YQRENCPCN	GVRGPNCYSP1	QSYGERPTY	GVEHQPYRVVVL	SFELLHR
X88.1.5.15-00748855-	CF THVY	HOSE VIRGE	E VSQINPGQ	TENDROYNYK	LPOOFTG	CVIDBINSING	LOSKPSGNYN	LYRLFRICSRLKP	FERDISIEI	YURGNOPCN	GVHGPNCYSP	QSTGERPTY	GYGHQPYKYYYL	SFELLING
X888.1.5.39-00758967	CETNVY	ROSEVIRG	EVSOIAPGO	IGNIBOYNYK	1 POOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRKSRLKP	FERDISTET	YOREN PCN	GVRGPNCY SPI	OSYGERPTY	GVGHOPYRVVVI	SFELLHA
X88,1,5,25-00783570-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOF TG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YORGNEPCN	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVL	SFELLHA
X88.1.5.1-00682449-1	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSK PS GNYN	LYRLFRISMLKP	FERDISTEI	YQRENKPCN	GVHGPNCYSPI	QSYGERPTY	GYCHOPYRYYYL	SFELLHA
X88.1.5.4-04681323-1 X88.1.5.7-00681221-9	CETNYY	HUSE VIEW	E VSQIIPPGQ	TONTROY WY	LPOOF TG	VIDENSAR	DSKP5GRTR1	TRUF RESELRP	CERDISTET	YORGANEPCN		OSYGEPPTY	COUNTRY TRY TR	SECTION
XB8.1.5.10-00681275-	CETNVY	ROSEVIRG	EVSQIAPGO	TENTROYNYK	LPOOF TG	CVIRMNSNO	LOSKPSGNYN	LYNLERKSKLKP	FERDISTEI	YURGNEPCN	GVRGPNCYSPI	QSTGFRPT	GVGHQPYRVVVI	SFELLHA
XB8.1.5.22-00783157-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSK PS GNYN	LYRLERKSRLKP	FERDISTEI	YQRGNCPCN	GVRGPNCYSP1	QSYGERPTY	GVEHQPYRVVVL	SFELLHA
XB8.1.5.26-00738311- V00.1.9.2-00249231-2	CO TRVY	HOSE VIRGE	E VSQINPGQ	TENDROYNYK	LPOOF TG	CVIDBINSING	LOSKPSGNYN	LYRLFRICSKLKP	FERDISIEI	YUHENCPCN	SVHGPNCYSP	QSTGERPTY OCYCERPTY	GYGHQPYRYYN	SFELLING
X88.1.5.3-00681234-9	CETNVY	ROSEVIRG	EVSOLSPIO	IGNIBOYNYK	1 POOF TG	CVIRINSING	DSKPSGNYN	LYRLFRKSRLKP	FERDISTEI	YORGNEPCN	GVRGPNCYSPI	OSYGERPTY	GYGHOPYRYYYI	SFELLHA
X88.1.5.35-00748647-	CETNVY	ROSEVIRG	EVSQINPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSRLKP	FERDISTEI	YURGNOPCH	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVL	SFELLHA
XB8.1.9.1-00748387-2	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRHNSNO	LOSK ps gnyn	LYRLFRISKLKP	FERDISTEI	YQREMCPCN	GVRGPNCYSP1	QSYGERPTY	GYCHOPYRYYYL	SFELLHA
X88.1.5.8-00783108-2 V88.1.5.29-00759251-	CETRAN	HUSE VIRGE BOCK VIRGE	e vsqineuq	TENDOTHYS:	LPOOF TG	CVIDBINSING	LUSKP SUNTRY	T TRUE RESIDENT	CERDISIEI	YUENDER	SVHGPNIL TSP	OSVEEPPTY	GYGRUP TRYVIL	SPELLING
X88.1.5.36-00782439-	CETNVY	HDSEVIRG	EV SOTAPGO	IGNIBOYNYK	1POOFTG	CVIRMINSING	DSKPSGNYN	LYRLFRKSKLKP	CERDISTEI	TUVGOPEN	GVRGPNCY SPI	OSTGERPTY	GYGHOPYRYYY	SFELLINA
X88.1.5.8-00727842-1	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOF TG	CVIRMNSNO	LOSKPSGNYN	LYRLFRKSKLKS	FERDISTEI	YREENPEN	GVRGPNCYSPI	QSYGFRPTY	GVGHQPYRVVVL	SFELLHR
X88.1.5.5-00681243-1	CETNVY	HOSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	EERDISTEI	YQREMCPCN	GVRGPNCYSPI	QSYGERPTY	GYCHOPYRYYYL	SFELLHA
X88.1.16-00748619-30 X88.1.16.1-00748605-	CETNYY	HUSE VIEW	E VSQIDPUQ	IGNIDIOTRTS.	LPOOF TG	CVIDENSING	DISKPSONTN'	THE FREEDOM	FERDISTET	YORGANEPUN	CARGE NO. 1511	OSVICE RPTY	GYGROPYRYYY	SEELUNE
X88.1.5.2-00681158-6	CETNVY	ROSEVIRG	EVSQIAPGO	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRISKLKP	FERDISTEI	YURGNEPCN	GVRGPNCYSPI	OSTGERPTY	GYGHOPYRYYYI	SFELLHA
X88,1,5,13-00748399-	CETNVY	ROSEVIRG	EVSQIAPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSK PS GNYN	LYRLFRKSKLKP	FERDISTEI	YQREMEPCN	GVRGPNCYSPI	QSYGFRPT	GVGHQPYRVVVL	SFELLIN
X88.1.5.39-0082261-7	CETNVY	HOSEVIRGE	EV QIRPGQ	TENTROYNYK	LPOOFTG	CVIRINSING	LOSKPSGNYN	LYRLFRKSKLKP	FERDISTEI	YURENKPCN	GVHGPNCYSPI	QSYGERPTY	GTGHQPYRVTT	SFELLINA
X88.1.22.1-00749512-	CETNYY	RESEVICE	E VEOLIPCO	TENTROYNYY	1 PODETO	CVTRANSAR	DISKPSGRYW	TYRE FRICSRE KP	FERNISTET	YDRENCPUN	GVRGPNCY CP	OSYGERPTY	GUGHOPYRY VIL	SEELUM
Consensus	CETNVY	ROSEVIRG	EV QIAPGO	TENTROYNYK	LPOOF TG	CVIRINSING	LOSKPEGNYN	LYRLFRISSLKP	FERDISTEI	YURGN PCN	GVRG NCY PI	OSTGERPTY	GVGHQPYRVVVI	SFELLHA

Figure 11: Multi-alignment of spike protein RBD domain region to demonstrate Omicron BA.2 specific mutations (green arrows), XBB.1.5 specific mutations (blue arrows), N501Y mutation (green circle) and XBB.1.5.1 sub subvariants mutations (grey circles).

Discussion

Five VOCs of SARS-CoV-2 mainly caused million deaths worldwide and named as B.1.1.7 (U.K.), B.1.351 (South Africa), P.1 (Brazil), B.1.617.2 (India), and B.1.1.529 (Africa). The B.1.1.529 variant was named Omicron which diverged into different BA.1, BA.2, BA.3, BA.4 and BA.5 lineages worldwide during 2022 with mild infections. Viral transactivator proteins regulate cellular genes to modulate immunogenicity and pathogenicity. As for example, in HIV retrovirus mediated pathogenesis, TAT, NEF and REV small proteins modulate its own transcription as well as human cellular genes. Similarly, preliminary reports indicated that corona virus ORF8 protein (121 AAs) acts as histone mimics disrupting chromatic structure with many epigenetic changes and immune modulator functions. ORF8 protein could inhibit MHC-1 and IFNbeta functions due to some similarities to immunoglobulin domains and also modulate spike protein. A 382-nucleotide deletion (Δ 382) in the ORF8 region of the corona virus genome causes weak virus load and weak pathogenicity (accession no.MT374101) [78-83]. Previously, we showed that C>T base change at 27972nt and another A>T base change at 28095nt created two termination codons (CAA=TAA and AAA=TAA) to produce 26AA and 67AA long ORF8 truncated proteins. Further, S24L, V32L, P38S, R52I, A65V, Y73C, L84S, K92E and V100L mutations in the ORF8 gene located with or without TAA termination mutations. Thus, ORF8 gene is very proven to mutation disrupting its function which controls immunogenicity and virus clearance.

Over time, the coronavirus has undergone mutations and deletions and different variants reported in different parts of the world with different time since December 2019. We found no GGA=TGA termination codon mutation in Alpha (B.1.17), Beta (B.1.351), Gamma (P.1/B.1.128.1), Delta (B.1.617.2), Kappa (B.1.617.1), Epsilon (B.1.427/B.1.429), Zeta (P.2); Eta (B.1.525), Iota (B.1.526) and B.1.1.298 (Mink Variant). Spike mutations are of major health concerns, as they reportedly exacerbate the infectious rate of the virus as in D614G and N50Y mutations. We found here major ORF8 truncated mutant in XBB.1 and XBB.15

lineages which were rapidly spreading now. Interestingly, RNA recombination generated few omicron lineages similar to XBB which was generated due to recombination between BA.1.10 and BA.2.75. Thus, XBB to XBB.1 and then XBB.1 to XBB.1.5 may be important but we characterized up to XBB.1.5.39 and in all we detected GGA=TGA termination codon mutation.

Thus, present outbreaks occurred with a corona virus with ORF8 gene product deficient. We also know that in most Omicron corona viruses, a 26nt deletion was prominent and we do not find any report that an insertion in that 3' UTR region has happened yet in Omicron lineages! Dominant mutation and deletion of small regulatory protein ORF7a limits viral suppression of the interferon response. We recently reported such deletions abolishing the production of both ORF7a and ORF7b proteins (see, accession no. OP711842). A WA1/BA.5 bivalent mRNA vaccine was inactive to kill BQ.1 and XBB.1 coronaviruses and titre against BQ and XBB subvariants were lower by 13-81fold and 66-155fold respectively as compared to Omicron BA.2 and BA.5 variants. Antiviral COVID-19 medications such as Paxlovid (Nirmatrelvir/Ritonsvir) and Remdesivir should still be effective against XBB.1.5 variant and both of these drugs will prevent virus replication as RdRp enzyme crucial function is not altered in XBB.1.5 [29,37].

The most sequences in SARS-CoV-2 database we analyzed were from USA (Howard, D. et al group) and we characterized some Bangladesh omicron data which confirmed that GGA=TGA termination codon was found in XBB.1 variant (accession no. OQ075411) but not in XBB variant (accession no. OQ075381) or XBB.2 variant (accession no. OQ075400). Interestingly, we also recently pinpointed round 400 ¹⁴⁷RWMD spike insertion mutants in US patients. We found three Northern Ireland (UK) based patients where such RWMD insertion was observed (accession numbers; OX527225, OX520545, OX486753). The 5'-UTR of SARS-CoV-2 forms five hairpin structures and nsp1 protein bound to first stem and loop structure helping transport of nsp1 to ribosome. Thus, 26nt 3'-UTR (29534-29870nt) S2M long stem-loop structure implicated in replication and other function in the coronavirus biology. The size of the genome has been changed from 29903nt to 29782nt. Thus, point mutation, deletion and insertion greatly affected coronavirus biology and WHO declared that recent isolates were no more so dangerous to cause death in COVID-19 infected individual [31,83].

HIV transactivator protein, TAT (71 AAs) binds to promoters of genes that are bound by the ETS1 transcription factor, the CBP histone acetyltransferase suggesting its role in regulating genes involved in T cell biology and immune response. Tat protein binds to TAR region where other cellular protein (puralpha) also docks. Further, Tat binds to breast cancer resistant protein implying its role in cancer pathogenesis. Nef protein (216 AAs) activates cellular Src and Tec tyrosine kinases after binding Nef-dimer to SH3-SH2 domain of tyrosine kinases activating transcription of HIV. The Rev protein (129 AAs) was suggested as HIV RNA transporter and such function was inhibited by nuclear factor 90, a dsRNA binding host protein whereas other host proteins XPO1 (CRM1) and RBM14 may be involved [9,69].

Strikingly, ORF8 protein 62-77 residues has Ig-like domain and binds CD16a (FcyRIIIA) with high affinity. Such interaction lowers the activity of CD16 at the surface of monocytes and NK cells reducing the capacity of PBMCs and monocytes to mediate antibody-dependent cellular cytotoxicity and humoral responses The crystal structure of SARS-CoV-2 ORF8 reveals a ~60-residue core polypeptides has potential similarity to ORF7a interface containing two dimerization interfaces and a covalent disulfidelinked dimer through an N-terminal sequence, while a separate noncovalent interface is formed by a carboxy-terminal SARS-CoV-2-specific sequence, $_{73}$ YIDI $_{76}$. We found many deletions in the ORF7a protein also and thus both should be transactivator proteins absence of which markedly lower the pathogenicity. Unfortunately, although ORF7a and ORF8 NH2-terminal 40 AAs has 25% scattered similarities, no such minor similarity with TAT protein or other HIV transactivator proteins like Nef and Rev (data not shown). Never-the-less, the roles of ORF8 protein in COVID-19 replication and pathogenesis were emerging and absence of ORF8 protein due to termination codon mutation was now well established in XBB.1.5 and B.1.1.7 variants. Thus, translational suppression of ORF8 protein is a therapeutic method to control Corona virus pathogenicity [29,37].

Conclusion

Presently circulating XBB.1.5 sub subvariants (XBB.1.5.1-XBB.1.5.39, XBB.1.16, XBB.1.22, XBB.1.9.1, XBB.1.9.2 etc) have GGA=TGA termination codon mutation in the ORF8 gene and such ORF8-deficent coronaviruses are less pathogenic. The 26nt deletion in the 3'-UTR, ²⁴LPP and ¹⁴⁰Y deletions in spike and ³¹ERS deletion in N-protein may be significant in such a process.

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