The ^{249}RWMD spike protein insertion in Omicron BQ.1 subvariant compensates the ^{24}LPP and ^{69}HV deletions and may cause severe disease than BF.7 and XBB.1 subvariants

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Research Article

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Abstract

Alarming antibody evasion properties were documented for new BF, BQ and XBB Omicron subvariants. Most immune-drugs were inactive neutralizing those COVID-19 subvariants and viral titers were exceptionally low as compared to deadly B.1.1.7, B.1.617.2 and B.1.1.529 variants with D614G, N501Y and L452R mutations in spike. The 91% nucleotides changes in spike protein of BQ.1 were resulted in AA changes whereas only 52% nucleotides changes resulted in AAs changes in ORF1ab. The N460K and K444T mutations in BQ.1 may be important driving force for immune-escape similar to F486S and N480K mutations in BA.2.75 subvariant and related XBB.1 subvariant. Further, the R346T mutation as found in BA.4.6 and BF.7, was regained in BQ.1.1 and BA.2.75.2 to enhance immune escape and infectivity (> 80%). The L452R and F486V mutations in spike were main drivers of Omicron BA.2 conversion to BA.4 and BA.5 in presence of ^{69}HV deletion. Whereas ^{24}LPP spike deletion and ^{3675}SGF ORF1ab protein deletion were found in all Omicron viruses including BQ.1 and XBB.1. Interestingly, we found about 211 COVID-19 sequences with four amino acids (^{249}RWMD) insertion near the RBD domain of Omicron viruses similar to ^{215}EPE three amino acids insertion in Omicron BA.1 variant. Such sequences first detected in California and extended to Florida, Washington and Michigan as well as other adjoining US states. An one amino acid deletion (^{140}Y) in spike was also found in BA.4.6, BQ.1.5, BQ.1.8, BQ.1.14, BQ.1.1.5, XBB.1 as well as related AZ.3, BU.1, BW.1, CR.2, CP.1 and CQ.1 subvariants but was not detected in BA.2.75, BF.7, XBD, BQ.1, BQ.1.1, BQ.1.2, BQ.1.6, BQ.1.10, BQ.1.12, BQ.1.16, BQ.1.19, BQ.1.22, BQ.1.1.1, BQ.1.1.4, BQ.1.1.12 and related BK.1, BN.1, BM.1.1.1, BR.2, BU.1, CA.1, CD.2, CH.1.1 subvariants. Thus, BQ.1 insertion was compensated the other deletions and would be more infectious than BA.2.75, BF.7 and XBB.1 subvariants even there was a 26nt deletion in the 3'-UTR. The spike protein R341T one amino acid change in BQ.1.1 and BQ.1.1.1 might be important but no ^{249}RWMD insertion.

Introduction

Corona virus pathogenesis has turn down this Earth with 600 million infections and over a half million deaths worldwide. COVID-19 was first detected in March-2019 and whole genome sequencing was available from December, 2019 onwards but within few months whole world's tragedy was happened [1, 2]. During 2020–2022 period many mutations in the COVID-19 genomes were reported in the NCBI SARS-CoV-2 Database [3, 4]. Truly SARS virus was not new and related respiratory infections happened in 2003 with CoV 229E and in 2012 with MERS virus outbreaks. This led to considerable molecular biology of such viruses were known before 2019 although earlier viruses had only 30–60% homologies [5]. Most astonishing fact was large polyprotein (7096 AAs) synthesis in the infected cells and such protein was proteolytically cleaved into 16 polypeptides with important biological functions. The Nsp1 protein is 180aa (regulatory factor), nsp2 is 638aa (RNA topoisomerase), nsp3 is ~ 1945aa (C3 protease), nsp4 is 500aa (membrane factor), nsp5 is ~ 305aa (C5 protease), nsp6 is 290aa (membrane factor), nsp7 is 183aa (accessory protein to replication), nsp8 is 198aa (accessory protein to replication), nsp9 is 113aa (RNA binding factor), nsp10 is 139aa (RNA binding factor), nsp11 is only 13aa (unknown function), nsp12 is 918aa (RNA-dependent RNA polymerase), nsp13 is 601aa (RNA helicase-capping

methyltransferase), nsp14 is 527aa (exoribonuclease-methyltransferase), nsp15 is 346aa (endoribonuclease-recombinase), nsp16 is 298aa (2'-O Uridine rRNA methyltransferase) [6–14]. On the country, structural spike protein is 1273aa long and other structural proteins (M, N, E) of corona virus are relatively very small (figure-1). Similarly, small regulatory proteins like orf3a, orf7a, orf7b, orf8 and orf10 were also characterized having interacted with many cellular proteins. Further, deletions in the spike, nsp1, nsp6, ORF7a/b, ORF8 and 3'-UTR resulted in defective corona viruses with mild symptoms [15–18]. The spike protein deletions (24LPP, 69HV, 143VYY, 157FR) and point mutations (D614G, N501Y, L452R) were greatly studied [3, 19–21]. However, a cluster of 20 mutations in the RBD domain of Omicron variants cast shadow in there was a new receptor for new viruses. The omicron B.1.1.29 was assigned as BA.0 and then further mutations classified as BA.1, BA.2, BA.3, BA.4 and BA.5 all of which had characteristics mutation in the RBD domain and such viruses hardly were protected by previous infections with Alpha, Delta and Gamma corona viruses [22–25]. Recent outbreaks in India, China and USA suggested that further modification of spike protein resulted in more immune-evasion and more infectious corona viruses like BF.7.4.1, BQ.1.1, XBB.1.5 and BA.2.75.2 with mild symptoms [26–32]. Further sequence variations in the different Omicron corona virus variants led to recent outbreaks of XBB.1.5, BQ.1.1, BA.2.75.2 and BF.7.4.1 subvariants. Here, we showed how a four amino acids deletion in the spike might be increase transmission over related Omicron subvariants.

Methods

We searched PubMed to get idea on published papers on BQ.1, BQ.1.1 and XBB.1 subvariants and genomes were down loaded from SARS-CoV-2 NCBI database. The BLAST-N and BLAST-X search methods were used to compare sequences. Multi-alignment of protein was done by MultAlin software (Corpet, F., 1988; Katoh & Standley., 2013) and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI (Sievers, et al., 2011; Wallace, 2005). The ORF1ab mutants was obtained by Blast-N search of deletion boundary of 60-100nt sequence and then analyzing the sequences with 95–100% similarities (Yang, et al., 2014). The protein 3-D structure of N-protein was determined by SWISS-Model software (Gao, et al., 2022; Waterhouse, et al., 2018; Bienert, et al., 2017; Roy, et al., 2010).

Results

Multi-alignment approach is a powerful tool to understand the genetic inter-relationship among different corona virus variants. SARS-CoV-2 Database search identified that BQ.1, BQ.1.1 and BQ.1.1.1 subvariants were astonishingly infecting peoples regardless of their previous exposure to highly transmissible and death promoting B.1.1.7, B.1.617.2 and B.1.1.529 lineages. In truth, Omicron BA.1 and BA.2 infections hardly protected people from notoriously immune-resistant BA.2.75.2, BQ.1.1 and XBB.1.5 subvariants. We performed multi-alignment and phylogenetic analysis to predict the relation among the different BQ subvariants as well as other subvariants like BE, CQ, BW, BG, CM, CR, BU, BN and CA. The BQ.1 had tittle distance to BQ.1.1 or BQ.1.1.1 as well as related BQ.1.1.3, BQ.1.1.6, BQ.1.1.18. It was found that BQ.1.18, BQ.1.22, BQ.1.1.8, BQ.1.1.13 were very close whereas BQ.1.8, BQ.1.12, BQ.1.16, BQ.1.19 were one group

likely due to deletion of one AA in spike at 40 position and BQ.1.1.4 and BQ.1.1.7 were closer. The BQ.1.6, BQ.1.11, BQ.1.12 and BQ.1.14 were closely clustered with BQ.1.2, BQ.1.3, BQ.1.5 and BQ.1.15 but were two distinct groups (figure-2). We found AZ, BK, BT were closely aligned to Wuhan virus (B.0) whereas CR, BU, CD, CP, CA, BR were more related to BA.5.2.1 and BF.7 (BA.5.2.1.7) subvariants than BQ.1. Further analysis suggested CA.1, CA.1.1, BR.2 and XBB were closer to BA.2.75 as well as BN.1, BN.5, CB.1, BM.1.1.1 to BA.2.75.5. Other words common mutations were clustered in those Omicron subvariants and sub-subvariants. Importantly, XBB, XBB.1, XBB.2, XBB.3 and XBD were clustered at same point (figure-2). Multi-alignment showed that all subvariants had 3675SGF three AAs deletion in the nsp6 domain of ORF1ab polyprotein (data not shown) as well as 24LPP three AAs deletion in the spike except AZ.3 subvariant (data not shown). All BQ subvariants had 69HV two AAs deletion and such deletion was also found in related CR.2, BU.1, BK.1, BT.2, CP.1, CP.1.1, CL.1, CQ.2, CR.1.1 as well as well known, BA.5.2.35 and BF.7 variants (Figure-3). However, no 69HV deletion found in the XBB.0/1/2/3 and XBD subvariants as well as CA.1, CB.1, CH.1.1, CM.3, BG.2, BG.5, BN.1, BN.1.3, BN.1.6, BN.1.1.1 and BR.2 subvariants and closer to BA.2.75 and BA.2.75.5 (figure-3). But five common deletions (SGF, LPP, HV, ERS, 26nt 3'-UTR) were located in all BQ.1 subvariants and sub-subvariants (figure-4) suggesting BQ.1 subvariants were derived from Omicron BA.5 variant or BA.5.2.1 variant and very related to BF.7 subvariant (figure-4). The figure-5 showed the nucleotides changed in the RBD domain of spike protein indicating BQ.1 had 31 mutations and quite different than Wuhan virus as well as deadly Alpha and Delta SARS-CoV-2 variants.

In Table-1, we demonstrated the major genetic changes in the BQ.1 genome (AN: OP942855) as compared to Wuhan genome (AN: NC_045512.2). Total 134 nucleotides changes (0.449%) occurred in the BQ.1 genome (59 nucleotides deletions (44%) and 75 nucleotides (56%) point mutations). Total 27 nucleotides changes in the ORF1ab (14 AAs change and 13 silent mutations) whereas a total 36 mutations in spike (33 AA changes and only 3 silent mutations) (table-1). The 91% nucleotides changed into AAs in spike with respect to 51.8% in ORF1ab only when compared with total nucleotides changes. Whereas 2.6% AA changes in spike to only 0.19% in ORF1ab when compared with total AAs (1273AAs and 7096 AAs respectively) content. There was O.954% AA changes in N protein whereas 1.35% in M protein and 1.3% in E protein and 0.363% in ORF3a demonstrating over whelming mutations in smaller proteins of SARS-CoV-2 BQ.1 variant. Overall, huge AA changes in spike and most nucleotide change lead into AA changes suggesting there was a pressure on spike to alter its protein sequence. Thus, conserved nature of receptor was compromised in Omicron variants suggesting if there was an alternate receptor for SARS-CoV-2. The BRD domain of spike binds to ACE-2 receptor of human lung cells. It could be imagined if a new receptor for Omicron viruses possibly helping corona virus to infect more epithelial cells of intestine, kidney or mouth instead lungs and heart! So far, no other new receptor was found for SARS-CoV-2!

Then, we analysed the difference in AAs of ORF1ab and spike proteins of BQ.1, BQ.1.1, BQ.1.8, BQ.1.1.1 as well as related subvariants BA.5.1, BF.7 and XBB.1. The data presented in figure-6 for spike protein and in figure-7 for ORF1ab. There were four AAs changes like D2089E (nsp3), F2173L(nsp3), N5589S (nsp13), A6041V (nsp14) in ORF1ab polyprotein (7093AA) when compared with BQ.1 and BQ.1.1 whereas three common AAs changes (D2089E, N5589S, A6041V) between BQ.1 and BQ.1.1.1 (figure-6). However, total

six AAs variation was observed when compared between BQ.1 and BF.7 like K556Q (nsp2), D2089E (nsp3), F3826 (nsp6), A4120V (nsp8), H4662Y (nsp12) and I5554M (nsp13). However, there were eleven AAs variations between BQ.1 and XBB.1 like K47R (nsp1), P62L(nsp1), K556Q (nsp2), D2089E (nsp3), L3201F (nsp4), F3826L (nsp6), H4662Y (nsp12), G5060S (nsp12), S5357P (nsp13), L5459I (nsp13) and I5554M (nsp13) (in sate we showed the proteins that were derived from ORF1ab polyprotein). In summary, we found there was two AAs variations (K47R, P62L) in the nsp1 moderator protein in XBB.1 subvariant and also similar three AAs variation in the nsp13 RNA helicase-capping methyl transferase (S5357P, L5459I and I555M). The RNA-dependent RNA polymerase (RdRp) variation was not detected when compared among BQ.1, BQ.1.1 and BQ.1.1.1 but H4662Y variation (Y4665 in Wuhan) located between BQ.1 and BQ.7 whereas two AAs variation (H4662Y, G5060S) (G5063 in Wuhan) were found between BQ.1 and XBB.1. Thus, H4662 mutation had occurred in RdRp of BQ.1 subvariant (see, table-1) whereas S5060 mutation could be happened in XBB.1 subvariant, not in BQ.1 subvariant. We knew that excess mutations in the RdRp might be due to dideoxy-nucleotide analogue drug exposure. Usually, RdRp enzyme became insensitive to drugs with time due to such mutations. We found that there was a common K556Q variation (Q556 in Wuhan; see table-1) in nsp2 RNA topoisomerase between BF.7 and XBB.1 although both occurred from different Omicron lineages (BA.5.2.1 and BA.2.75 respectively). As Q556 AA was normally located in Wuhan virus, K556 mutation again located in the BQ.1 subvariant. Such analysis clearly demonstrated more and more mutations in the BQ.1 subvariant as well as in BQ.1.1 and BQ.1.1.1 sub-subvariants (figure-7A/B/C/D).

BLAST-2 analysis between BQ.1 and BQ.1.8 detected a 140Y deletion in spike of BQ.1.8 whereas such Blast-2 homology search detected R341T mutation in BQ.1.1.1. Similarly, Blast-2 homology search between BQ.1 vs. BQ.1.1 and BQ.1 vs. BQ.1.1.1 identified a common variation R341T. Similarly, T439K and K455N two AAs variation located between BQ.1 and BA.5.2.1 while five AAs variation located by Blast-2 search between BQ.1 and BF.7 with two common AAs (T439K, K455N) and one common with BQ.1.1.1 (R341T) and two new AAs variations (S404R and N 412K). Surprisingly, Blast-2 homology search between BQ.1 and XBB.1 identified 18 AAs variations indicating huge difference between spike of BQ.1 whose origin was BA.5 variant and XBB.1 whose origin was BA.2.75. However, all AAs difference located in the NH_2 terminal site (1-500 AAs) (figure-6E). Surprisingly, in XBB variant had no 69HV deletion in spike, but more curiously Y142 one AA deletion located in XBB.1 variant which we also located in BQ.1.8 (Y140 deletion in BQ.1.8 and such position would be 145Y in Wuhan). We knew that 143VYY three AAs deletion was present in Omicron BA.1 variant and 145Y deletion also located in B.1.1.7 Alpha variant indicating a mirror relation among B.1.1.7, BQ.1.8 and Omicron BA.1 subvariants. If such deletion was acquired by recombination or deletion was happened independently, was not clear. To determine the potential of 140Y one AA deletion in spike of BQ.1 sub-subvariants, we checked the genome multi-alignment data. Such data was presented in figure-9 giving very interesting profile of such one AA deletion that originally occurred in B.1.1.17 lineage. The Y140 (5'-TTA-3') one AA deletion located in BQ.1.5, BQ.1.8, BQ.1.1.5, BQ.1.14, BQ.1.18 as well as XBB.1, XBB.2 and XBB.3 and also in AZ.3, CR.1.1, BU.1, CR.2, BW.1 and CP.1 subvariants as well as more surprisingly BA.4.6 subvariants. Similarly, 140Y deletion was not located in BA.2.75, BF.7, XBD, BM.1.1.1, BK.1, BU.3, BN.1, CP.1.1, CA.1, CD.2, CH.1.1,

BE.1.1 as well as other BQ variants like BQ.1.1, BQ.1.2, BQ.1.6, BQ.1.10, BQ.1.11, BQ.1.15, BQ.1.16, BQ.1.22, BQ.1.1.1, BQ.1.1.4, BQ.1.1.5, BQ.1.1.8 and BQ.1.1.12 (figure-8). Interpretation of such data was impossible but one question might be important to discuss, “Why so many variant names? Does such nomenclature necessary to address genetic changes in corona virus for better surveillance and drug design? But it is quite true that we should give a new name to BQ.1 spike insertion mutant!

Importantly, we found three new spike insertion mutants during alignment with SARS-CoV-2 NCBI database (figure-9). Next, spike protein multi-alignment detected the RWMD deletion in BQ.1 subvariant (Figure-101. We made a 45nt oligonucleotide at the deletion boundary and Blast search identified two hundred eleven 100% similar SARS-CoV-2 sequences with four (NH_2-RWMD-CO_2H) amino acids insertions in the spike from US patients only (figure-10). Interestingly, 194 sequences were obtained from California patients only and four from Florida, and two each from Washington and Michigan and one each from Kansas, Colorado, Texas, Pennsylvania, New Mexico, Utah, Georgia, Nevada and Arizona states (figure-12). The most sequences were deposited by Howard D et al. and groups. However, three sequences deposited by Scribnar M, (accession numbers: OQ111964, OQ111965, OQ111966) and one sequence each deposited by Garrigues JM et al. (accession no. OP925220), Matzinger SR et al. (accession no. OQ209704; GISAID: EPI_ISL_16312916) and Linares-Perdomo OJ (accession no. OP998412), The first such mutant virus was isolated from California patient on 2nd November, 2022 and the sequence data deposited on 14th November, 2022 (accession number OP816502). About 124 such sequences were deposited on December, 2022 and more 88 such insertion mutants were deposited into SARS-CoV-2 NCBI Database up to 12th January, 2023. However, during X’MASS and New Year holidays many laboratories were closed and now more and more data would be available worldwide. Very surprisingly, our analysis of recent data suggested such four amino acids insertion was not spread into BQ.1.1 and BQ.1.1.1 subvariants. To overcome the issue, we multi-aligned different mutant spike proteins from COVID-19 isolated by different workers from different US states and also sequenced in the different laboratories. It was found that always the same “RWMD” insertion in the spike pointing the BQ.1 insertional mutant data was correct.

Table-1: SARS-CoV-2 Omicron BQ.1 subvariant point mutations and deletions in the genome as well as ORF1ab, Spike and other proteins

5'-UTR and 3'-UTR	BQ.1 ORF1ab nucleotide change (B.0)	BQ.1 ORF1ab AA change (BQ.1 position)	BQ.1 Spike nucleotide change (B.0)	BQ.1 Spike AA change (BQ.1 position)	Other genetic changes	AA change in small proteins
C > T 241	T > G 670	S135R	C > T 21618	T19I	C > T 25584	T223I
	C > A 1931	Q556K	Del. TACCCCCTG			ORF3a
G > A 29868	C > T 2790	T842I	G > T 21641	A24S	C > T 26060	No change
	T > C 2954	No change	A > C 21766	ATA = ATC		
	C > T 3037	No change	Del. CATGTC		C > T 26270	T9I
	G > A 4184	G1307S	G > A 21987	G142D		E
	C > T 4321	No change	T > G 22200	V213G	G > A 26529	D9N
	G > T 6532	E2089D	G > A 22578	G334D		M
	C > T 9344	L3027F	C > T 22674	S366F	C > G 26577	Q19E
	A > G 9424	No change	T > C 22679	S368P		M
	C > T 9534	T3090I	C > T 22686	S370F	G > A 26709	A63T
	C > T 10029	T3255I	A > G 22688	T371A		M
	C > T 10198	No change	G > A 22775	D400N	C > T 27807	No change
	G > A 10447	No change	A > C 22786	R403S		
	C > A 10449	P3395H	G > T 22813	K412N	C > T 27889	No change
	Del. TCTGGTTTT	No change	T > G 22882	N435K		
	C > T 11750	L3826F	A > C 22893	K439T	A > T 28271	P13L
	G > A 12160	No change	T > G 22917	L447R		N

Table-1: SARS-CoV-2 Omicron BQ.1 subvariant point mutations and deletions in the genome as well as ORF1ab, Spike and other proteins						
	C > T 12880	No change	T > A 22942	N455K	C > T 28310	No change
	T > C 14247	Y4662H	G > A 22992	S472N		
	C > T 14408	P4712L	C > A 22995	T473K	C > T 28311	No change
	C > T 15714	No change	A > C 23013	E479A		
	G > A 16935	No change	T > G 23018	F481V	Del. AGAACGCAG	
	C > T 17410	R5712C	A > G 23055	Q494R		
	A > G18163	No change	A > T 23063	N496Y	G > T 28681	E133D
	C > T 19965	T6561I	T > C 23075	GTT = GTC		N
	A > G 20055	No change	A > G 23403	D609G	G > A 28881	R201K
			C > T 23525	AAC = AAT		N
			T > G 23599	N674K	G > A 28882	G202R
			C > A 23604	P676H		N
			C > A 23854	N759K	G > C 28883	No change
			G > T 23948	D791Y		
			A > T 24424	Q949H	A > C 29510	S410R
			T > A 24469	N964K		N
			C > T 25000	GAC = GAT		

Discussion

The genetic changes in RNA viruses are obvious due to cellular resistance and targeted drug action. Molecular biology of SARS-CoV-2 viruses were elucidated in great details and bioinformatics approach was aimed here to get vivid demonstration of genetic changes in SARS-CoV-2 BQ.1 subvariants (figure-6 and figure-7). An October, 2022 study indicated that about 5% COVID-19 infection in the USA was BF.7 variants and that of in the UK was about 7.3%. While the immune-resistance properties of BQ.1 was 10

times lesser than BF.7 indicating more transmission might be possible with BF.7 variant. Interestingly, study reported that a recombinant variant XBB (Omicron BA.2.10.1 and BA.2.75) was found in Indian sub-continents (65.5% of COVID-19 infections). The 26nt deletion in the 3'-UTR likely 10–20 times reduced viral titer in those BA.5 subvariants as also with ^{31}ERS deletion in the N-protein. In truth, deadly Delta (B.1.617.2 and AY.103) variants with ^{157}FR deletion in the spike were generated 1000 times more virus/ml than mild Omicron (BA.1, BA.2) variants. The question arises how then more and more Omicron corona virus outbreaks with ^{24}LPP with or without ^{69}HV deletion in the spike appearing in the USA and China now [33–35]? Our multi-alignment analysis found that no ^{3675}SGF three AA deletion in nsp6 domain of ORF1ab polyprotein was found in Delta variants but was present in all Omicron variants (BA.1/2/4/5) and subvariants (BF.7, BQ.1, XBB.1) as well as early Alpha (B.1.1.7) variant. Study indicated that the December, 2022 daily infections might be exceed 200000–500000 daily that was much higher than 20000–25000 daily infections occurred in April-May, 2022 serge. Scientists predicted that mRNA vaccine or Adeno-vector based spike vaccine was more potential to develop antibody than whole virus vaccine that was used in China and India [36–38]. However, India first largely used UK-based DNA vaccine of spike gene origin (Covishild) and might be in a better situation than China. On the other hand, China achieved 100% vaccination to people whereas in India only 90% people got vaccination once and 70% got twice (assuming 135 crores total population). Perhaps such calculation has no effect on Omicron infections which occurred in people those were infected with Alpha and Delta variants because spike protein in Omicron has ~ 30 mutations. Otherwise, all people are susceptible to reinfection except those are taking new Omicron vaccine if available. Thus, Omicron BF.7, BQ.1 and XBB.1 subvariants infections in mass people were happening! We explained here a new spike insertion ^{249}RWMD mutant that might cause more serious threat in the future and such mutant was different than previously well characterized ^{215}EPE insertion mutant in Omicron BA.1 variant (figure-10, 12). We BLAST-N searched to get 211 such spike insertion mutants using the unique oligo at the insertion boundary (5'-ACA TAG AAG TTC AAG ATG GAT GGA TTT GAC TCC TGG TGA TTC TTC-3').

Abeyardhana et al. found that the binding affinity of ACE-2 receptor and RBD domain increased in the order of Wuhan < Beta < Alpha < BA.5 < Gamma < Delta < BA.2.75 < BA.1 < BA.3 < BA.2. Interactions between docked complexes revealed that the RBD residue positions like 452, 478, 493, 498, 501, and 505 were crucial in creating strong interactions with ACE-2 [25]. Omicron BA.2 shows the highest binding capacity to the ACE-2 receptor among all the mutant complexes studied. The L452R, F486V, and T478K mutations in the spike of BA5 significantly impacted the interaction network in the BA.5 RBD-ACE2 interface [25].

In a simulation study, Zappa et al. reported that, compared to the BA.5 variant, BA.2.75 showed about 57-fold increased receptor binding affinity (ACE2 receptor). The subvariant also showed markedly higher receptor binding affinity (more than 3000-fold) compared to the Alpha (B.1.1.7) variant [34]. Shaheen et al. defined the BA.2.75 subvariant with the spike protein mutations: the R493Q, G446S, W152R, and K147E. They also reported that R493Q and G446S were alarming mutations. Similarly, the G446S mutation might have a role in immune resistance or ACE2 receptor binding [35]. Recently, Sheward et al.

illustrated that nine additional mutations are found in the spike protein of BA.2.75 compared to BA.2, which are R493Q, N460K, G446S, G339H, G257S, I210V, F157L, W152R, and K147E. The XBB isolate had nine more changes (G339H, R346T, L368I, V445P, G446S, N460K, F486S, F490S, and the wild-type amino acid at position 493) in its receptor-binding domain than a BA.2 (hCoV-19/Japan/UT-NCD1288-2N/2022) isolate [32]. We showed that BQ.1 had N460K and K444T important mutations and ^{249}RWMD insertion in spike was never discussed in the PubMed literature (table-1).

Imai et al. recently reported that immune-antibody drugs like imdevimab, casirivimab, tixagevimab, cilgavimab, and sotrovimab did not neutralize the BQ.1.1 or XBB subvariants. The similar drug bebtelovimab which effectively neutralizes Omicron BA.1, BA.2, BA.4, and BA.5 variants, had no efficacy against BQ.1.1 or XBB subvariants. Further, both combinations of monoclonal antibodies tested (i.e., imdevimab–casirivimab and tixagevimab–cilgavimab) failed to neutralize either BQ.1.1 or XBB subvariants [39]. The BQ.1.1 and BQ.1.1.1 had unique R341T mutation but surprisingly 249RWMD insertion yet was not inserted into BQ.1.1 and BQ.1.1.1 sub-subvariants (data not shown)! However, ^{140}Y deletion was distributed in the BQ.1, BQ.1.1 and BQ.1.1.1 variants disproportionally (figure-8).

Indian Government has issued alert warrant to medical authorities and hospitals as well as O_2 and medicine suppliers. In my opinion, there is no need of concern of Omicron viruses with ^{24}LPP (except BA.1), ^{69}HV (except BA.2), ^{143}VYY (in BA.1 only) spike protein deletions, ^{31}ERS N-protein deletion, 26nt 3'-UTR deletion and ^{3675}SGF deletion in ORF1ab including ^{141}KSF deletion in BA.4 variant. But recent compensation of spike deletions in BQ.1 ^{249}RWMD insertion mutant may cast a shadow. Surely, if Delta variant corona virus somehow reappears, there will be catastrophic again worldwide. If SGF deletion in nsp6 domain, ERS deletion in N-protein and 26nt deletion in 3'-UTR were also repaired like spike in BQ.1 RWMD insertion mutant! We argue that similar consequence may occur because we are doing experiments with corona viruses in different cell lines and we are taking immune drugs unnecessary for the treatments of Omicron infections where the main culprit for disease severity is co-morbidity! However, more and more drug discovery efforts should be targeted against SARS-CoV-2 proteins and BQ.1 specific peptide vaccine may be welcome [40–42].

Conclusion

The Omicron corona viruses greatly impacted society even with mild symptoms. Recently, such viruses diverged into BQ.1, XBB.1, BA.2.75 and BF.7 with higher infections and immune-invasive. Thus, ^{249}RWMD spike insertion BQ.1 mutant may be a new threat where ^{3675}SGF deletion in nsp6 protein, ^{131}ERS deletion in N-protein and 26nt 3'UTR deletion may be compensated in the future with generation of deadly Delta-like (B.1.617.2 and AY.103) new SARS-CoV-2.

Declarations

Acknowledgement

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Conflict of interest

The author has no conflict of interest to any agency or company. The data provided here were computer generated.

References

[1]. Rockx B, Kuiken T, Herfst S, Bestebroer T, Lamers MM, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. Science, 2020; 368: 1012–1015. doi: 10.1126/science.abb7314.

[2]. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019; 17: 181–192. doi: 10.1038/s41579-018-0118-9.

[3]. Chakraborty AK. Hyper-variable Spike protein of Omicron corona virus and its differences with Alpha and Delta variants: Prospects of RT-PCR and new vaccine. J Emerg Dis Virol. 2022; 7 (1):1-13. dx.doi.org/10.16966/2473-1846.166.

[4]. Araf Y, Akter F, Tang YD, Fatemi R, Parvez MSA, Zheng C, Hossain MG. Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines.J Med Virol. 2022 May;94(5):1825-1832. doi: 10.1002/jmv.27588.

[5]. Ota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science, 2003; 300: 1394–1399. doi: 10.1126/science.1085952.

[6]. Chakraborty AK, Chanda A. New Biotechnological Exploration on COVID-19 Proteins: Functions, Mutational Profiles and Molecular Targets for Drug Design. Sun Text Rev Virol.2021; 2(1):115. Doi:10.51737/2766-5003.2021.015.

[7] Chakraborty AK. Coronavirus Nsp2 Protein Homologies to the bacterial DNA Topoisomerase I and IV Suggest Nsp2 Protein is a unique RNA Topoisomerase with novel target for drug and vaccine development. Virol Mycol. 2020; 9: 185. DOI: 10.35248/2161-0517.20.09.185.

[8]. Nguven TT, Pathirana PN, Abdelrazek M. Genomic mutations and changes in protein secondary structure and solvent accessibility of SARS-CoV-2. Scientific Reports, 2021; 11(3487). Doi: https://doi.org/10.1038/s41598-021-83105-3.

[9]. Noske GD, Nakamura AM, Gawriljuk VO, Fernandes RS, et al. A Crystallographic Snapshot of SARS-CoV-2 Main Protease Maturation Process. J Mol Biol. 2021; 433: 167118.

[10]. Gao Y, Yan L, Huang Y, Liu F, Zhao Y, et al. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science, 2020; 368: 779-782.

[11]. Slanina H, Madhugiri R, Bylapudi G, et al. Coronavirus replication–transcription complex: Vital and selective NMPylation of a conserved site in nsp9 by the NiRAN-RdRp subunit. Proc Natl Acad Sci. USA, 2021; 118(6): e2022310118. doi: 10.1073/pnas.2022310118.

[12]. Chakraborty AK. Multi-Alignment comparison of Coronavirus non-structural proteins Nsp13-16 with ribosomal proteins and other DNA/RNA modifying enzymes suggested their roles in the regulation of host protein synthesis. International J Clini Med Informatics, 2020; 3: 7-19. https://doi.org/10.46619/ijcmi.2020.1024.

[13]. Chakraborty AK. Clinical, Diagnostic and Therapeutic implications of Coronavirus ORFab Polyprotein associated Nsp16 Protein-A bioinformatics approach. Acta Scientific Medical Sciences, 2020; 4(5): 97-103. DOI: 10.31080/ASMS.2020.04.0629.

[14]. Addetia A, Xie H, Roychoudhury P, Shrestha L, Loprieno M, et al. Identification of multiple large deletions in ORF7a resulting in in-frame gene fusions in clinical SARSCoV-2 isolates. J Clin Virol. 2020; 129: 104523. https://doi. org/10.1016/j.jcv.2020.104523. PMID:

[15]. Al-Rashedi NAM, Alburkat H, Hadi AO, et al. High prevalence of an alpha variant lineage with a premature stop codon in ORF7a in Iraq, winter 2020–2021. PLoS One, 2022; 17(5): e0267295. Doi: 10.1371/journal.pone.0267295.

[16]. Hachim A, Gu H, Kavian O, Mori M, Kwan MYW, et al. SARS-CoV-2 accessory proteins reveal distinct serological signatures in children. Nat Commun. 2022; 13: 2951. doi: 10.1038/s41467-022-30699.

[17]. Chakraborty AK. Dynamics of SARS-CoV-2 ORF7a Gene Deletions and Fate of Downstream ORF7b and ORF8 Genes Expression. SunText Rev Biotechnol. 2022; 3(1): 142. Doi: https//doi.org/10.51737/2766-5097.2022.042.

[18]. Chakraborty AK. SARS-CoV-2 ORF8 gene CAA=TAA and AAA=TAA termination codon mutations found mostly in B.1.1.7 variant was independent of popular L84S mutations. Int J Clini Med Edu Res. 2022; 1(6): 192-208.

[19]. Meng B, Kemp SA, Papa G, et al. Recurrent emergence of SARS-CoV-2 Spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. Cell Reports, 2021; 35(13): 109292. Doi: 10.1016/j.celrep.2021.109292.

[20]. Guruprasad K. Geographical Distribution of Amino Acid Mutations in human SARS-CoV-2 Orf1ab polyprotein compared to the equivalent reference proteins from China. ChemRxiv. July, 2021. Preprint. Doi: https://doi.org/10.26434/chemrxiv.12951617.v1.

[21]. Liu Z, Zheng H, Lin H, Li M, Yuan R, et al. Identification of Common Deletions in the Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2. J Virol. 2020; 94(17): e00790-20. doi: 10.1128/JVI.00790-20.

[22]. Ju B, Fan Q, Wang M, Liao X, Guo H, Wang H, Ge X, Liu L, Zhang Z. Antigenic sin of wild-type SARS-CoV-2 vaccine shapes poor cross-neutralization of BA.4/5/2.75 subvariants in BA.2 breakthrough infections. Nat Commun. 2022; 13(1): 7120. doi: 10.1038/s41467-022-34400-8.

[23]. Wang Q, Guo Y, Iketani S, et al. Antibody evasion by SARS-CoV-2 omicron subvariants BA.2.12.1, BA.4 and BA.5. Nature, 2022; 608: 603-608.

[24]. Tan CW, Lim BL, Young BE, Yeoh AY, et al. Comparative neutralisation profile of SARS-CoV-2 omicron subvariants BA.2.75 and BA.5. Lancet Microbe. 2022; 3(12): e898. doi: 10.1016/S2666-5247(22)00220-8.

[25]. Abeywardhana S, Premathilaka M, Bandaranayake U, et al. In silico study of SARS-CoV-2 spike protein RBD and human ACE-2 affinity dynamics across variants and omicron subvariants. J Med Virol. 2023; 95(1): e28406. Doi: 10.1002/jmv.28406.

[26]. Qu, P., Evans, J.P., Faraone, J.N., Zheng, Y.-M., Carlin, C., et al. Enhanced neutralization resistance of SARS-CoV-2 Omicron subvariants BQ.1, BQ.1.1, BA.4.6, and BA.2.75.2. Cell, 2022;
Doi: https://doi.org/10.1016/j.chom.2022.11.012.

[27]. Wang Q, Iketani S, Li Z, Liu L, Guo Y, et al. Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. Cell. 2022 Dec 14: S0092-8674(22)01531-8. doi: 10.1016/j.cell.2022.12.018.

[28]. Kurhade C, Zou J, Xia H, Liu M, Chang HC, Ren P, Xie X, Shi PY. Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1 and XBB.1 by parental mRNA vaccine or a BA.5 bivalent booster. Nat Med. 2022; Dec 6. doi: 10.1038/s41591-022-02162-x.

[29]. Callaway E. Coronavirus variant XBB.1.5 rises in the United States - is it a global threat? Nature. 2023; 613(7943): 222-223. doi: 10.1038/d41586-023-00014-3.

[30]. Davis-Gardner ME, Lai L, Wali B, Samaha H, et al. Neutralization against BA.2.75.2, BQ.1.1, and XBB from mRNA Bivalent Booster. N Engl J Med. 2022; NEJMc2214293. doi: 10.1056/NEJMc2214293.

[31]. Scarpa F, Sanna D, Benvenuto D, Borsetti A, et al. Genetic and Structural Data on the SARS-CoV-2 Omicron BQ.1 Variant Reveal Its Low Potential for Epidemiological Expansion. Int J Mol Sci. 2022; 23(23): 15264. doi: 10.3390/ijms232315264.

[32]. Sheward DJ, Kim C, Fischbach J, Sato K, et al. Omicron sublineage BA.2.75.2 exhibits extensive escape from neutralising antibodies. Lancet Infect Dis. 2022; 22(11): 1538-1540. doi: 10.1016/S1473-

3099(22)00663-6.

[33]. Chakraborty C, Bhattacharya M, Dhama K. Cases of BA.2.75 and recent BA.2.75.2 subvariant of Omicron are increasing in India: Is it alarming at the global level? Ann Med Surg (Lond). 2022; 84: 104963. doi: 10.1016/j.amsu.2022.104963.

[34]. Shaheen N., et al. Could the new BA.2.75 sub-variant lead to another COVID-19 wave in the world? - Correspondence. Int. J. Surg. 2022;105

[35]. Zappa M., Verdecchia P., Angeli F. Knowing the new Omicron BA.2.75 variant ('Centaurus'): a simulation study. Eur. J. Intern. Med. 2022; S0953.

[36]. Lin J, Anjum Huma F, Irfan A, Ali SS, Waheed Y, Mohammad A, Munir M, Khan A, Wei DQJ. Structural plasticity of omicron BA.5 and BA.2.75 for enhanced ACE-dependent entry into cells. Biomol Struct Dyn. 2022:1-12. doi: 10.1080/07391102.2022.2158944.

[37]. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet, 2020; 396: 479- 488

[38]. Anderson EJ, Rouphael NG, Widge AT, et al. (2020) Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. New Engl J Med. Doi: https://doi.org/10.1056/NEJMoa2028436.

[39]. Imai M, Ito M, Kiso M, Yamayoshi S, Uraki R, Fukushi S, et al. Efficacy of Antiviral Agents against Omicron Subvariants BQ.1.1 and XBB. N Engl J Med. 2023; 388(1): 89-91. doi: 10.1056/NEJMc2214302.

[40]. Bacha, U., Barrila, J., Gabelli, S.B., Kiso, Y., et al., (2008). Development of broad-spectrum halomethyl ketone inhibitors against corona virus main protease 3CL(pro). Chem. Biol. Drug Des. 72:34–49.

[41]. Chakraborty, AK. (2020c). Multi-Alignment Comparison of Coronavirus Non-Structural Proteins Nsp13-16 with Ribosomal proteins and other DNA/RNA modifying Enzymes Suggested Their Roles in the Regulation of Host Protein Synthesis. International J Clinical Medical Informatics 3(1):7-19.

[42]. Wu C, Liu Y, Yang Y, Zhang P, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmaceutica Sinica B. 2020; Doi:

https://doi.org/10.1016/j.apsb.2020.02.008.

Figures

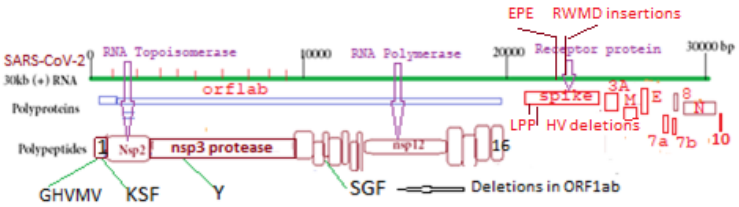

Figure 1

Genetic structure of SARS-CoV-2 and highly deletions, insertions and mutations in spike of Omicron variants.

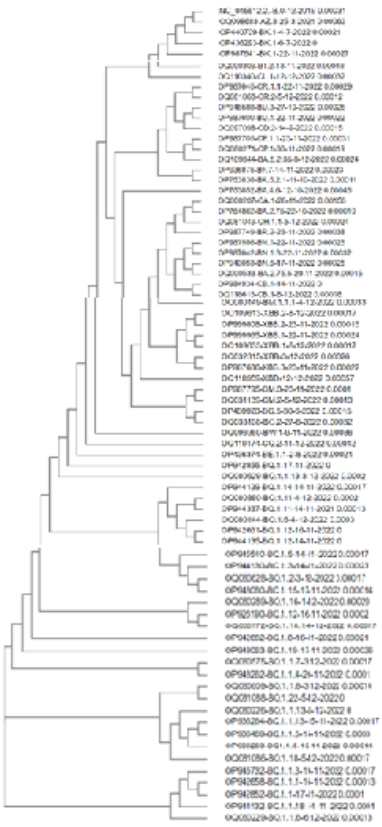

Figure 2

Multi-alignment (CLUSTAL Omega) and then phylogenetic analysis of recently appeared Omicron subvariants.

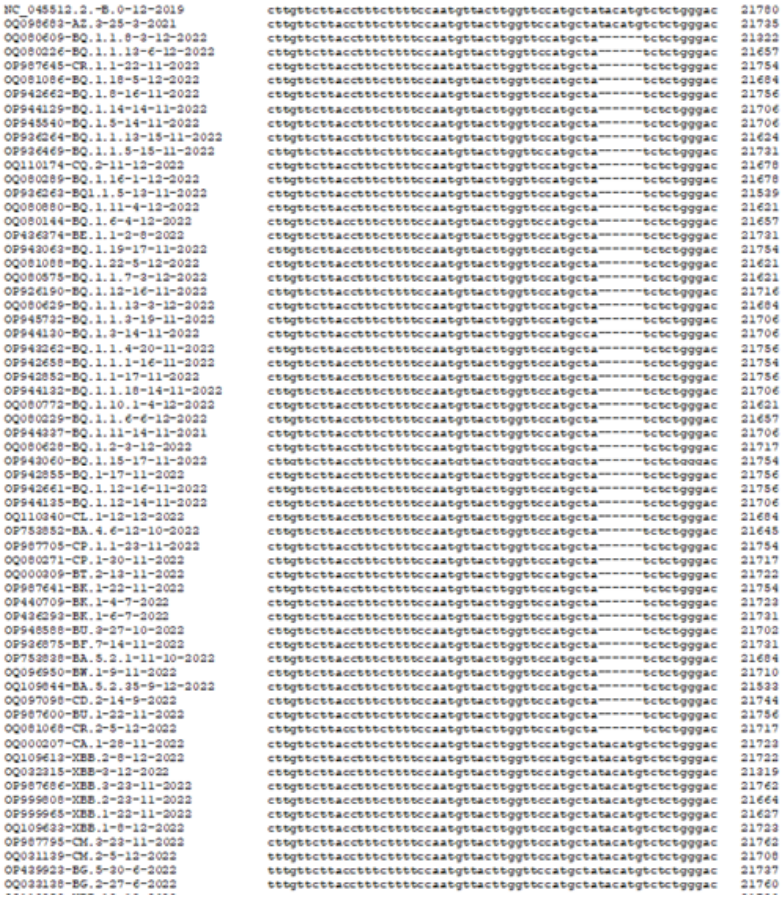

Figure 3

Multi-alignment of SARS-CoV-2 Omicron subvariants to demonstrate all BQ.1 subvariants had ^{69}HV deletion including BK, BW, CD, CR, CQ, and important BF.7 subvariants. But BG, BN, BR, CA, CB, CM, XBB, XBD are related to BA.2.75 subvariants and had no ^{69}HV deletion.

Figure 4

Major deletions in the BQ.1 Omicron subvariant as compared to Wuhan virus genome. Only deletion portions of the BLAST-2 alignment were shown. The Wuhan virus genome accession number is NC_045512.2 and BQ.1 variant genome accession number is OP942855.

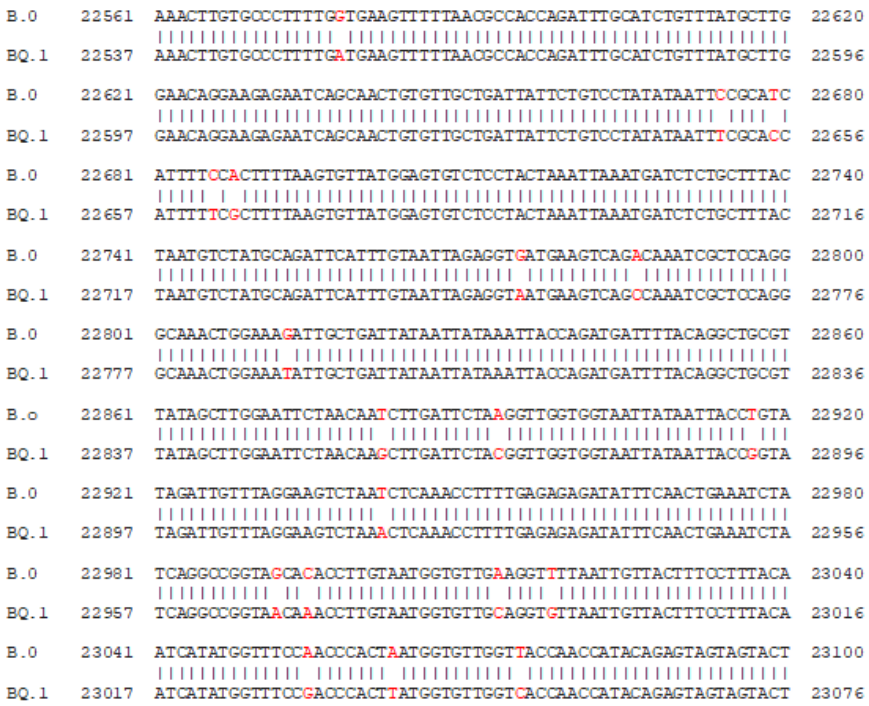

Figure 5

Major point mutations in the RBD domain of Spike protein of Omicron BQ.1 subvariant as compared to Wuhan corona virus (B.0).

Figure 6

BLAST-2 homology to demonstrate the Spike protein differences in SARS-CoV-2 Omicron BQ.1 variant with BQ.1.8, BQ.1.1.1, BF.7 and XBB.1 subvariants. The alignment portions with AA difference only shown here in each case.

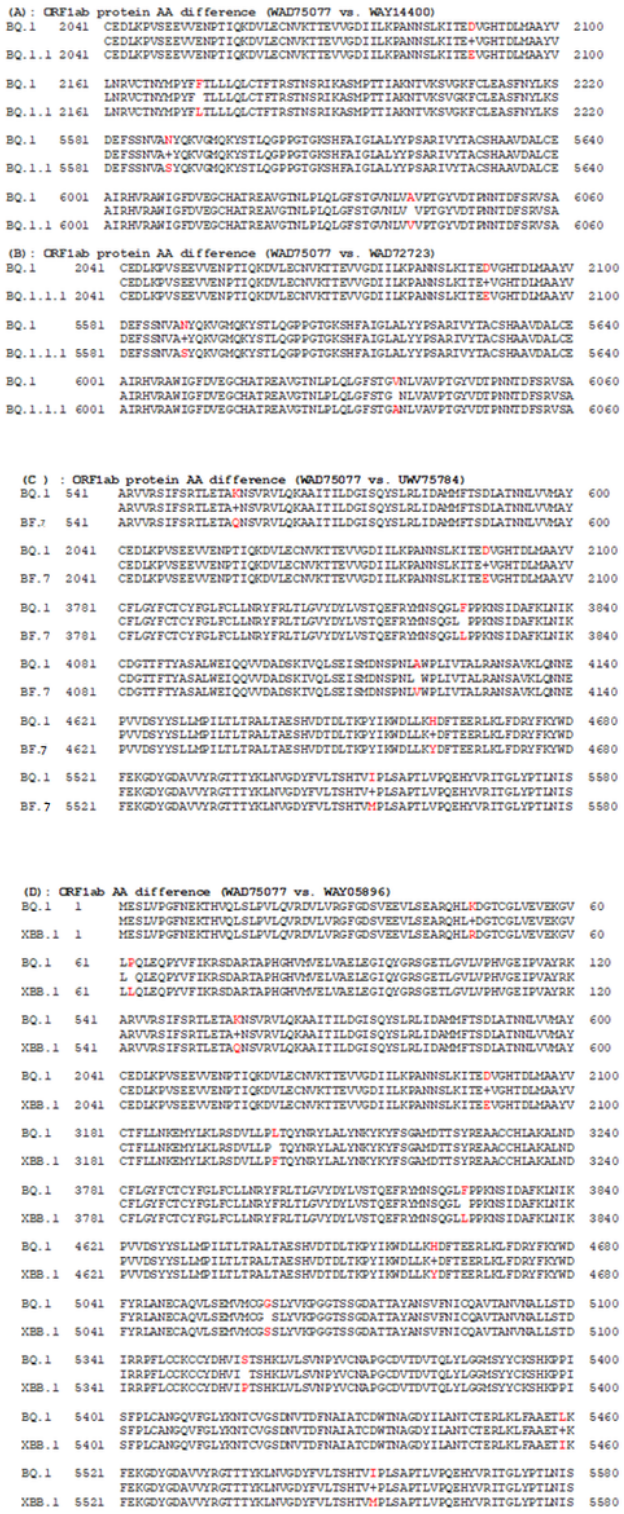

Figure 7

BLAST-2 homology between BQ.1 and BQ.1.1(A), BQ.1 and BQ.1.1.1 (B), BQ.1 and BF.7 as well as BQ.1 and XBB.1 to demonstrate the difference in amino acids of spike protein. It was found that a profound difference in AAs between BQ.1 and XBB.1.

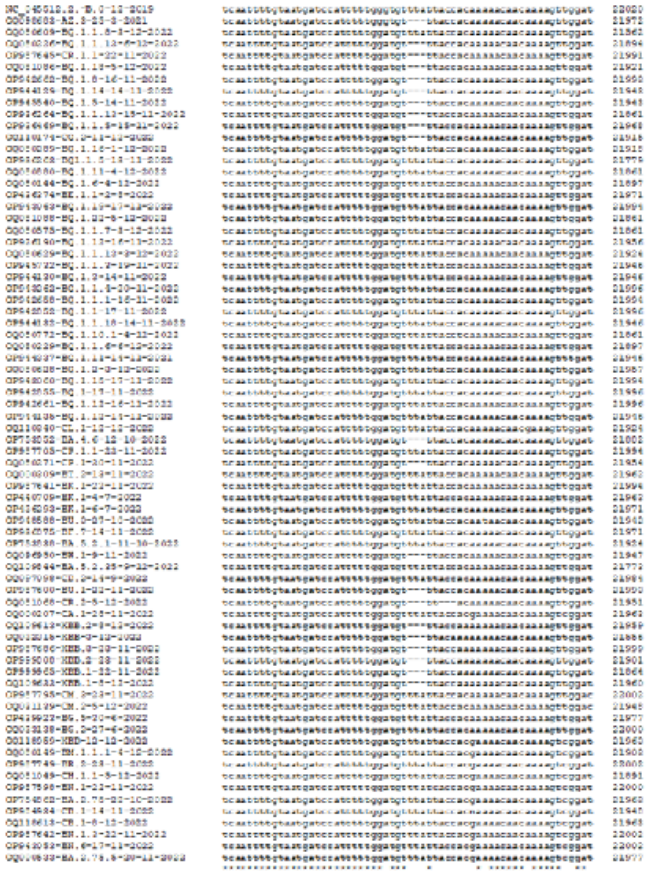

Figure 8

Multi-alignment of different SARS-CoV-2 subvariant genomes recently identified in NCBI database to demonstrate the ^{140}Y deletion in spike protein of many BQ.1 sub-subvariants.

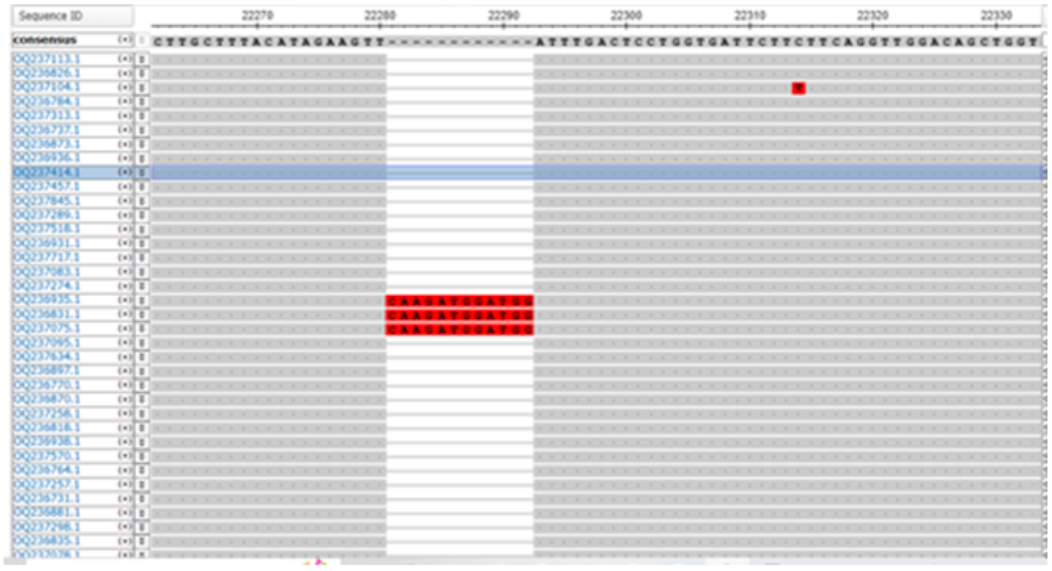

Figure 9

Detection of COVID-19 second insertion mutants in spike of Omicron BQ.1 subvariants. The selected BQ.1 variant sequences in the SARS-CoV-2 NCBI portail were aligned and scanned to insertion point and photographed.

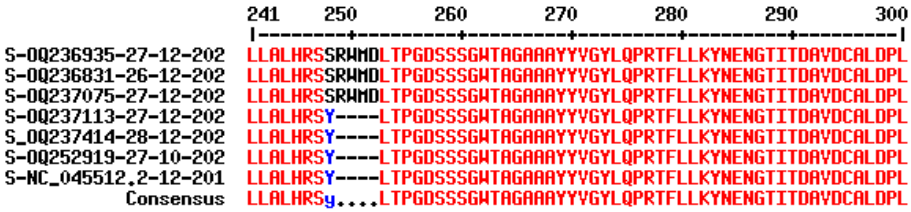

Figure 10

Multi-alignment of few Omicron BQ.1 spike protein sequence with or without four amino acids insertion as compared to Wuhan (NC_045512.2) and BA.5.2.1 (OQ252919).

Figure 11

BLAST-2 homology between NC_045512.2 Wuhan virus and BQ.1 insertion mutant to find an oligonucleotide (red underline) at the insertion boundary for BLAST-N search to get related insertion BQ.1 mutants.

Figure 12

Multi-alignment of spike proteins from RWMD insertion mutants of Omicron SARS-CoV-2 isolated from the different US states and sequenced in the different laboratories as compared to Wuhan virus.