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The ²⁴⁹RWMD spike protein insertion in Omicron BQ.1 subvariant compensates the ²⁴LPP and ⁶⁹HV 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 ⁶⁹HV deletion. Whereas ²⁴LPP spike deletion and ³⁶⁷⁵SGF 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 (²⁴⁹RWMD) insertion near the RBD domain of Omicron viruses similar to ²¹⁵EPE 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 (¹⁴⁰Y) 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 ²⁴⁹RWMD 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), 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'-0 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 guite 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 0.954% 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 NH₂ 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 multialignment 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 (NH₂-RWMD-CO₂H) 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: as ORF1	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 Spike nucleotide change (B.0)	BQ.1 Spike BQ.1 Other genetic nucleotide Spike AA change (B.0)		AA change in small proteins			
		(BQ.1 position)		position)					
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	Т9І			
	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		Μ			
	C>T 9344	L3027F	C>T 22674	S366F	C > G 26577	Q19E			
	A > G 9424	No change	T > C 22679	S368P		Μ			
	C > T 9534	T3090I	C>T 22686	S370F	G > A 26709	A63T			
	C>T 10029	T3255I	A > G 22688	T371A		Μ			
	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		Ν
A > G 20055	No change	A > G 23403	D609G	G > A 28881	R201K
		C > T 23525	AAC = AAT		Ν
		T > G 23599	N674K	G > A 28882	G202R
		C > A 23604	P676H		Ν
		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		Ν
		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 subcontinents (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 ³¹ERS deletion in the N-protein. In truth, deadly Delta (B.1.617.2 and AY.103) variants with ¹⁵⁷FR deletion in the spike were generated 1000 times more virus/ml than mild Omicron (BA.1, BA.2) variants. The guestion arises how then more and more Omicron corona virus outbreaks with ²⁴LPP with or without ⁶⁹HV deletion in the spike appearing in the USA and China now [33–35]? Our multi-alignment analysis found that no ³⁶⁷⁵SGF 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 ²⁴⁹RWMD mutant that might cause more serious threat in the future and such mutant was different than previously well characterized ²¹⁵EPE 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 57fold 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 ²⁴⁹RWMD 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, ¹⁴⁰Y 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 O₂ and medicine suppliers. In my opinion, there is no need of concern of Omicron viruses with ²⁴LPP (except BA.1), ⁶⁹HV (except BA.2), ¹⁴³VYY (in BA.1 only) spike protein deletions, ³¹ERS N-protein deletion, 26nt 3'-UTR deletion and ³⁶⁷⁵SGF deletion in ORF1ab including ¹⁴¹KSF deletion in BA.4 variant. But recent compensation of spike deletions in BQ.1 ²⁴⁹RWMD 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, ²⁴⁹RWMD spike insertion BQ.1 mutant may be a new threat where ³⁶⁷⁵SGF deletion in nsp6 protein, ¹³¹ERS 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

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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.

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Figure 1

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



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

NC 045512.2B.0-12-2019	cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac	21780
00096683-AZ.3-25-3-2021	cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac	21735
OQ080609-BQ.1.1.8-3-12-2022	cttgttcttacctttttttttccaatgttacttggttccatgctatctctgggac	21322
OQ080226-BQ.1.1.13-6-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21657
OP987645-CR.1.1-22-11-2022	cttgttcttacctttcttttccaatattacttggttccatgctatctctgggac	21754
OQ08108€-BQ.1.18-5-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21684
OP942662-BQ.1.8-16-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21756
OP944129-BQ.1.14-14-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21706
OP945540-BQ.1.5-14-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21706
OP936264-BQ.1.1.13-15-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21624
OP936469-BQ.1.1.5-15-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21731
OQ110174-CQ.2-11-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21678
OQ080289-BQ.1.16-1-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21678
OP936263-BQ1.1.5-13-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21539
OQ080880-BQ.1.11-4-12-2022	cbbgbbcbbaccbbbcbbbccaatgbbacbbggbbccatgcbabcbcbgggac	21621
OQ080144-BQ.1.6-4-12-2022	cbtgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21657
OP436374-BE.1.1-2-8-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21731
OP943063-BQ.1.19-17-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21754
OQ081088-BQ.1.22-5-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21621
OQ080575-BQ.1.1.7-3-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21621
OP926190-BQ.1.12-16-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21716
OQ080629-BQ.1.1.13-3-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21684
OP945732-BQ.1.1.3-19-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21706
OP944130-BQ.1.3-14-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgccatctctgggac	21706
OP943262-BQ.1.1.4-20-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21756
OP942658-BQ.1.1.1-16-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21754
OP942852-BQ.1.1-17-11-2022	cttgttcttacctttctttcctatgttacttggttccatgctatctctgggac	21756
OP944132-BQ.1.1.18-14-11-2022	cttgttcttacctttctttcctatgttacttggttccatgctatctctgggac	21706
OQ080772-BQ.1.10.1-4-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21621
OQ080229-BQ.1.1.6-6-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21657
OP944337-BQ.1.11-14-11-2021	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21706
OQ080628-BQ.1.2-3-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21717
OP943060-BQ.1.15-17-11-2022	cttqttcttacctttcttttccaatqttacttqqttccatqctatctctqqqac	21754
OP942855-BQ.1-17-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21756
OP942661-BQ.1.12-16-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21756
OP944135-BQ.1.12-14-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21706
OQ110340-CL.1-12-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21684
OP753852-BA.4.€-12-10-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21645
OP987705-CP.1.1-23-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21754
0Q080271-CP.1-30-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21717
OQ000309-BT.2-13-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21722
OP987641-BK.1-22-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21754
OP440709-BK.1-4-7-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21723
OP436293-BK.1-6-7-2022	cbbgbbcbbaccbbbccbbbccaatgbbacbbggbbccatgcbabcbcbgggac	21731
OP948588-BU.2-27-10-2022	cbtgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21702
OP936875-BF.7-14-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21731
OP753838-BA.5.2.1-11-10-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21684
OQ096950-BW.1-9-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21710
OQ109844-BA.5.2.35-9-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21533
OQ097098-CD.2-14-9-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21744
OP987600-BU.1-22-11-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21756
00081068-CR.2-5-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatctctgggac	21717
0Q000207-CA.1-28-11-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21723
OQ109613-XBB.2-8-12-2022	cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac	21722
OQ032315-XBB-3-12-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21319
OP987686-XBB.3-23-11-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21762
OP999808-XBB.2-23-11-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21664
OP999965-XBB.1-22-11-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21627
OQ109633-XBB.1-8-12-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21723
OP987795-CM. 3-23-11-2022	$\tt cttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21762
00031139-CM. 2-5-12-2022	$\tt tttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21705
OP439923-BG.5-30-€-2022	$\tt t \tt t$	21737
OQ033138-BG.2-27-6-2022	$\tt tttgttcttacctttcttttccaatgttacttggttccatgctatacatgtctctgggac$	21760

Multi-alignment of SARS-CoV-2 Omicron subvariants to demonstrate all BQ.1 subvariants had ⁶⁹HV 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 ⁶⁹HV deletion.

в.0	11281	TAGTTTGTCTGGTTTTAAGCTAAAAGACTGTGTTATGTATG	11340
BQ.1	11281	TAGTTTGAAGCTAAAAGACTGTGTTATGTATGCATCAGCTGTAGTGTTACT	11331
в.0	21601	TCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCCTGCATACACTAATTCTTTCAC	21660
BQ.1	21592	TCAGTGTGTTAATCTTATAACCAGAACTCAATCATACACTAATTCTTTCAC	21642
B.0	21721	CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGAC	21780
BQ.1	21703	CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATCTCTGGGAC	21756
в.0	28321	GTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCG	28380
BQ.1	28297	GTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGTGGGGCGCG	28347
в.0	29701	GGGAGGACTTGAAAGAGCCACCACACTTTCACCGAGGCCACGCGAGTACGATCGAGTGT	29760
BQ.1	29668	GGGAGGACTTGAAAGAGCCACCACATTTTCACCT	29701

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.

B.0	22561	AAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTG	22620
BQ.1	22537	AAACTTGTGGCCTTTTGATGAAGTTTTTAAGGCCACCAGATTTGCATCTGTTTATGCTTG	22596
B.0	22621	${\tt GAACAGGAAGGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATT{\tt C}{\tt C}{\tt GCATC}$	22680
BQ.1	22597	GAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTTCGCACC	22656
B.0	22681	ATTTTCCACTTTTAAGTGTTATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTAC	22740
BQ.1	22657	ATTTTTCGCTTTTAAGTGTTATGGAGTGTCTCCCTACTAAATTAAATGATCTCTGCTTTAC	22716
B.0	22741	$\texttt{TAATGTCTATGCAGATTCATTTGTAATTAGAGGT}{\texttt{GATGAAGTCAG}{\texttt{A}}\texttt{CAAATCOCTCCAGG}$	22800
BQ.1	22717	TAATGTCTATGCAGATTCATTTGTAATTAGAGGT AATGAAGTCAGOCAAATCGCTCCAGG	22776
B.0	22801	GCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGT	22860
BQ.1	22777	GCAAACTGGAAATATTGCTGATTATAAATTATAAATTACCAGATGATTTTACAGGCTGCGT	22836
B.o	22861	TATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTA	22920
BQ.1	22837	${\tt TATAGCTTGGAATTCTAACAAGCTTGATTCTACGGTTGGTGGTAATTATAATTACOGGTA$	22896
B.0	22921	${\tt TAGATTGTTTAGGAAGTCTAA{\tt T}CTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTA}$	22980
BQ.1	22897	TAGATTGTTTAGGAAGTCTAAACTCAAACCTTTTGAGAGAGA	22956
B.0	22981	TCAGGCCGGTAGCACACCTTGTAATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACA	23040
BQ.1	22957	TCAGGCCGGTAACAACCTTGTAATGGTGTTGCAGGTGTTAATTGTTACTTTCCTTTACA	23016
B.0	23041	ATCATATGGTTTCCAACCACTAATGGTGTTGGTTACCAACCA	23100
BQ.1	23017	ATCATATGGTTTCCGACCCACTTATGGTGTTGGTCACCAACCA	23076

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

(A): 8	pike p	rotein difference (WAD75079 vs. WAD72773)	1.00
D'2- 4	191	VVIK/CEFOFCHDFFLDV/ HKINKSWHESEFRVISSANCTFEYVSOFFLDLEGROS	100
BQ.1.8	121	VVIK/CEFQFCNDFFLD/Y-HXINKSWMESEFR/YSSANNCIFEYVSQFFLMDLEGRQGN	179
(D): 9 BQ.1	pike p 301	<pre>xotein difference (WAD75079 vs. WAD72725) FTVENGIYOISNFRNOPTESIVRFRNITNLOFFDEVENAIRFASVYAMNRERISNCVADY</pre>	360
		FTVERGINGTSNFRUOPTES IVR FENITNLCPFDE VFNAT FASVY AWNRER I SNCVADY	
82.1.1	.1 301	FIVERCITQISSINGPIESIVALETINLOPDEVENTITENSVIAMNAALEBOOMDI	3.60
(O): 5	pike p	rotein difference (WAD75079 VR. BD502358)	400
P.4. 1	451	EDDFTCCVIAWNSNELDS VCCNYNYRYDLFRKS LEPFERDISTEIYOACNERCNCVAC	480
BA.5.2	.1 421	EDDFTGCVIAMNSIKLDSKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGNKECNG/AG	480
(=):	Spike	protein difference (WAD75079 vs. UWV75786)	
BQ.1	301	PTVERCIYQISMFRUQPIESIVRFINITNLCPFDEVFINITRFASVYAWNERDISNCVADY FTVERCIYOTSMFRUQPIESIVRFINITNLCPFDEVFINIT, FASUYAWNERDISNCVADY	360
BF.7	301	FIVENGIYOISNFRNOPTESIVRFENITNLCPFDEVFNRITFASVYAWNFKRISNCVADY	360
BQ.1	361	SVLYNFAPFEAFKCYCVSPTKLNDLOFTNYYADSEVIRCNEVSQ1APOQTCMINDYNYKL	420
-		SVLYNFAPFFAFKCYGVSPTKLMDLCFTNVYADSEVIRGNEV QIAPQQIG IMDYWYKL	
BF_7	361	SVLYNFAPFFAFKCYGVSFTKLMDLCFTNVYADSEVIRGNEVROIA PROIGKIADYNYKL	420
BQ.1	421	eddftgcviannsnkldstvggnynyryrlfrksklkpferdiste iygagnkrcng/ag	400
BF.7	421	PDDFIGCVIAWNSNKLDS VGGNYNYRYRLFRKS LKPFERDISIEIYQAGNKRCNG/AG PDDFIGCVIAWNSNKLDSKVGGNYNYRYRLFRKSNLKPFERDISIEIYQAGNKRCNG/AG	480
(2): 8	pike p	rotein difference (WAD78079 vs. WAYC8898)	
BQ.1	1	HFVFLVLLPLVSSGCVNLITRTQSYINSFIRGVYYPDKVFRS5VLHSTQLFLPFFSNVT MFVFLVLLPLVSSCCVNLITRTOSYINSFIRGVYYPDKVFRS5VLHSTOELFLPFFSNVT	60
XBB.1	1	MFVFLVLLPLVSSQCVNLITRTQSYINSFIRGVYYPDKVERS5VLHSTQDLFLPFFSNVT	60
BQ.1	61	WFHAISGINGTERFDNPVLPFNDGVYFASTERSNIIRGWIFGTTLDSETQSLLIVNNA	118
X88.1	€1	WFHAI SGINGTKRFDNP LPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNA WFHAIRVSGINGTKRFDNPALDFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNA	120
		_	
BQ.1	119	INVVIEWCEEQFCNDFFLDVY THENNESSMESEFRVYSSANNCIFEYVSQFFLMDLEGRQ INVVIEWCEEQFCNDFFLDVY KINKSWESEFRVYSSANNCIFEYVSQFFLMDLEGR4	178
X38.1	121	INVVIKVCEPQFCNDPFLDVY-QKNNES%MESEFRVYSEANNCTFEYVSQDFLMDLECKE	179
BQ.1	179	GNFNNLRE FVFRNIDGY FRIYSKHIP INLGRDLPQGF SALE PL/DLPIGINITRFQTLLR	238
X28.1	180	GNFWILPFFVFKNIDGYFKIYSKHIDINL RDLDCGFSALFDLUDLDIGINITPFOTLLA CNFWILDFFVFKNIDGYFKIYSKHIDINLEDDLDCGFSALFDLUDLDIGINITPFOTLLA	225
BQ. 1	239	LHRSYLTPGDSSSGNTAGARAYYVGYLQERTFLLKYNENGTITDAVDCALDPLSETRCTL THDSVLTD DSSSGNTAGARAYYVGYLQERTFLLKYNENGTITDAVDCALDPLSETRCTL	298
XBD.1	240	LIIR SYLTPVECS S GNTA GAAAY YVGYLQPRT FLLWYNENGT I TDAVDCALD PLGET KOTL	200
BQ.1	299	KSFTVENSIYOTSNERVORTESIVREPNITNLCPEDEVENATREASVYAMVRKRISNOVA	355
200 1	200	KSFTVERGINGTSNERVQDTES+VDEPNIINLODE EVENAT FASVYAMERKDISNOVA	200
A.M	300	NOT IVERNING OPEN YEAR THE FRANK STEE FERT LEROTING KORDIONOVE	965
BQ. 1	359	DYSULYNFAPFFAFRCYGJS FTKLNDLCFTNVYADSFVIRGNEJSQI APGOTGNI ADYNY DYCIA VNEADERA ENCYCLO DEFU NDLCFTNIN D GEUTIDCHEDGO DA DOGTCHI A DYNY	418
XBB.1	360	EYSVIYAFAFFFASWCYGYC PTKLNDLCFINVYAESFVIRGNEVEQIAEQCTGNIADYN	419
BQ.1	419	KLDDDFTGCVIANNENKLDSTVGGNYNYPYRLFRKSKLKDFFRDISTEIYQAGNEDCNCV KLDDDFTGCUIANNENKLDS CNNNY VDLFDKSKLKDFFRDISTEIYQAGNEDCNCV	478
XBB.1	420	KLPEDFT@CVIAWNSNKLDSEPSGNYNYLYRLFRKSKLK#FERDISTEIYOAGNEPCNGV	475
BQ.1	479	AGVNCYFPLQSYGERPTYGVGEQPYRVVVLSFELLHADAIVCGDKKSTNLVKNKCVNFNF	538
2000 ·	480	AG NOY PLOSTGERPTYSVGIQPYRVVULSFELLIAPATVOSPRIKSTNLVINKOVNTNE BOSMOVERI OSVORBTYSVGIQPYRVVULSFELLIAPATVOSPRIKSTNLVINKOVNTNE	6.30
ADD - 1	- C U O I	NORMAL FOR MUCH REPORT A CONTRACT OF A CONTR	043

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.

(A): CR	Flab p	protein AA difference (WAD75077 vs. WAY14400)	
BQ.1 2	2041	CEDIKPVSEEWENFTIQKDVLECNVKTIEVWGDIILKPANNSLKITEDVGHTDLMAAYV CEDIKPVSEEWENFTIQKDVLECNVKTTEVWGDIILKPANNSLKITE+VGHTDLMAAYV CEDIKPUSEFUUENDTIQKDULECNVKTTEVUGDIILKPANNSLKITESUEHTDIMASVV	2100
BQ.1 :	2161	LNRVCTNYMPYF FTLLLOLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS	2220
BQ.1.1 :	2161	LNRVCTNYMPYF TLLLOLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS LNRVCTNYMPYFLTLLLOLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS	2220
BQ.1 8	5581	DEFSSNVANYQKVGMQKYSTLQGPRGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE	5640
BQ.1.1 8	5581	DEFSSNVA+YCKVQMCKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE DEFSSNVA <mark>S</mark> YCKVQMCKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE	5640
BQ.1	6001	AIRHVRAWIGEDVEGCHATREAVGINLPIQLGESTGVNLVAVPTGYVDTPNNTDESRVSA	6060
BQ.1.1	6001	AIRHVRAWIGFDVEGCHAIREAVGINLPIQLGFSTGVNLV VPIGYVDIPNNTDFSRVSA AIRHVRAWIGFDVEGCHAIREAVGINLPIQLGFSTGVNLVVVPIGYVDIPNNTDFSRVSA	6060
(B): ORI	Flab p	protein AA difference (WAD75077 vs. WAD72723)	
BQ.1 BQ.1.1.1	2041	CEDLKØVSEEVVENPTIQKDVLECNVKITEVVGDIILKPANNSLKITEVGHDIMAAV CEDLKØVSEEVVENPTIQKDVLECNVKITEVVGDIILKPANNSLKITEVGHDIMAAV CEDLKØVSEEVVENPTIOKDVLECNVKITEVVGDIILKPANNSLKITEZVGHDIMAAV	2100
BQ.1	5581	DEFSSNVANYOKVGMCKYSTLOGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE	5640
BQ.1.1.1	1 5581	DEFSSNVA+YQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE DEFSSNVASYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE	5640
BQ.1	6001	AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGVVDTPNNTDFSRVSA	6060
BQ.1.1.1	1 6001	AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTG NLVAVPTGYVDTPNNTDFSRVSA AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGANLVAVPTGYVDTPNNTDFSRVSA	6060
(C) BQ.1	541	ab protein AA difference (WAD75077 vs. UWV75784) ARVVRSIFSRILETAKNSVRVLQKAAITILLOGISQYSLRLIDAMMFTSDIATNNLVVMAY	600
BF.z	541	ARVVRSIFSRTLETA+NSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAY ARVVRSIFSRTLETAQNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAY	600
BQ.1	2041	CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEDVGHTDLMAAYV	2100
BF.7	2041	CEDLKPVSEEWENFTIGKDVLECNVKTTEVVGDIILKPANNSLKITE+VGHTDLMAAYV CEDLKPVSEEWENPTIGKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV	2100
BQ.1	3781	CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSGGLFPPKNSIDAFKLNIK CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTOEFRYMNSGGL PPKNSIDAFKLNIK	3840
BF.7	3781	CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQGLLPPKNSIDAFKLNIK	3840
BQ.1	4081	CDGTT FTYASALWEIQQVVDADSKIVQLSE I SMDNSPNLAWPLIVTALRANSAVKLQNNE CDGTT FTYASALWEIQQVVDADSKIVQLSE I SMDNSPNL WPLIVTALRANSAVKLQNNE	4140
BF.7	4081	CDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLVWPLIVTALRANSAVKLQNNE	4140
BQ.1	4621	PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKNDLLKHDFTEERLKLFDRYFKYWU PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKNDLLK+DFTEERLKLFDRYFKYWU	4680
BF.7	4621	PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWD	4680
BQ.1	5521	FEKGDYGDAVVYRGITIYKLNVGDYFVLTSHIVIPLSAPILVPQEHYVRIIGLYPIAIS FEKGDYGDAVVYRGITIYKLNVGDYFVLTSHIV+PLSAPILVPQEHYVRIIGLYPIAIS	5580
B2./	5521	PERGDIGRAVVIRGIIIIKLNUGDIFVLISHIVNPLSAPILVRQEHIVRIIGLIPILNIS	5560
(1)	-		
(D): (BQ.1	1	A difference (WAD/5077 VS. WATU5996) MESIVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKDGTCGLVEVEKGV	60
XBB.1	1	MESLVFGPNEKTHVQLSLFVLQVRUVLVRSFGDSVEEVLSEARQHL+DGTCGLVEVEKG MESLVFGFNEKTHVQLSLFVLQVRUVLVRGFGDSVEEVLSEARQHLRDGTCGLVEVEKGV	60
BQ.1	61	LPOLEOPYVFIKRSDARTAPHCHVMVELVAELEGIQYGRSGETLGVLVPHVGEIPVAYR L OLEOPYVFIKRSDARTAPHCHVMVELVAETEGICVGDSGETLGULVDHVGETPUAVD	120
XBB.1	61	LLQLEQPYVFIKRSDARTAPHCHVMVELVAELEGIQYGRSGETLGVLVPHVGEIPVAYR	120
BQ.1	541	ARVVRSIFSRTLETAKNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMA) ARVVRSIFSRTLETA+NSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMA)	600
XBB.1	541	ARVVRSIFSRTLETAQNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMA	600
BQ.1	2041	CEDLKPVSEEWENPTICKDVLECWKTTEVVGDIILKPANNSLKITEDVGHTDLMAAY CEDLKPVSEEWENPTICKDVLECWKTTEVVGDIILKPANNSLKITE+VGHTDLMAAY	2100
X88.1	2041	CEDIKPVSEEVVENPTICKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDIMAAYV	2100
BQ.1	3181	CIFLENKEMYLKLRSDVILPLTQYNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALNU CIFLENKEMYLKLRSDVILP TQYNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALNU	3240
X88.1	3181	CELEVITAL CONTRACTOR OF A CONTRACT OF A CONT	3240
YPD 1	3781	CFLOFFCICIFGLFCLLNRYFRLTLGVIDTUSTQEFRINNSQLFPPRNSIDAFKINI CFLGYFCTCYFGLFCLLNRYFRLTLGVUDYLVSTQEFRINNSQGL PPRNSIDAFKINI CFLGYFCTCYFGLFCLLNRYFRLTLGVUDYLVSTQFFDVANSQCL DDAFFINI	3840
BO 1	4621	DVUDSYYSLIMPILTLTDALTARSHUDTDLTKDYIWDLLKHDFTERDLYF.	4680
XBB.1	4621	PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLK+DETEERLKLFDRYFKYWI PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWI	4680
BQ.1	5041	FYRLANECAQVLSEMVMOGGSLYVKPGGTSSGDATTAYANSVENICQAVTANVNALLSTI	5100
X88.1	5041	FYRIANECAQVLSEMVMOG SLYVKPGGTSSGDATTAYANSVENICQAVTANVNALLST FYRIANECAQVLSEMVMOGSSLYVKPGGTSSGDATTAYANSVENICQAVTANVNALLST	5100
BQ.1	5341	IRRPFLOCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVIDVIQLYLGGMSYYCKSHKPPI	5400
XBB.1	5341	IRRPFLOCKCCYDHVI TSHKLWLSWNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKPPI IRRPFLOCKCCYDHVIPTSHKLWLSWNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKPPI	5400
BQ.1	5401	SFPLCANGQVFGLYKNTCVGSDNVTDFNALATCDWINAGDYILANTCTERLKLFAAETL	5460
XBB.1	5401	SFPLCANGQVFGLYKNICVGSENVIDFNALAICDWINAGDYILANICTERLKLFAAET+ SFPLCANGQVFGLYKNICVGSENVIDFNALAICDWINAGDYILANICTERLKLFAAETI	5460

 BQ.1
 5521
 FEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVIPLSAPTLVPQEHYVRITGLYPTLNIS
 5580

 FEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTV+PLSAPTLVPQEHYVRITGLYPTLNIS
 S580

 XEB.1
 5521
 FEKGDYGDAVVYRGTTYKLNVGDYFVLTSHTVHPLSAPTLVPQEHYVRITGLYPTLNIS
 S580

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.

NC_045512.2B.0-12-2019	bcaabbbbgbaabgabccabbbbbggggbgbbbabbaccacaaaaacaacaacaacagbbggab	22020
OOD56683-AZ.8-25-3-2021	bcaabbbbgbaabgabccabbbbbgggbgbbbaccacaaaaagbbggab	21972
Q0050609-B0.1.1.8-3-12-2022	to as to the store to a to the top of the traces as a sand to dat	21562
00050226-80 1 1 12-6-12-2022		21696
OD027645eCR 1 1e22e11e2022		21991
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	concerences and a second and a second s	
GP9426C2-BQ.1.0-16-11-2022	bcastobbgtastgabccatotboggabgtbtaccacasascascastagbbggab	21992
OP544125 BQ.1.14-14-11-2022	tic as UUU glastigatics altitutiggs tigt Ulacs as a sacaas aa sagtiliggati	21942
CP548540-BQ.1.5-14-11-2022	bcaabbbbgbaabgabccabbbbggabgbbbaccacaaaaacaacaaaagbbggab	21,943
OP934264-BQ.1.1.12-15-11-2022	ccattetdtategatetattttggatgtttaccacaaaaacaatagttggat	21661
OP926469-B0.1.1.5-15-31-2022	teaststeptastgatesatttttggatgtttassacaaaaaaaaaaaaaaaaaaa	21968
CO110174-CC.2-11-12-2022	bcaabbbbgbaabgabccabbbbbggabgbbbaccacaaaaacaacaacaaaagbbggab	21915
00010089-80.1.18-1-12-2028	bcaabbbbcbaabcaabbbbbbbbbbbbbbbbbccacaaaaacaac	21915
GP99-C2-C2-D01.1.5-19-13-2022	to as to the aboat of all the top so the traces as a san though	21779
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00010144-80 1 6-4-12-2022		11867
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GQ056878-BQ.1.1.7-8-12-2022	tcasttttgtastgstccatttttggatgtttattaccacaasaacaacaasagttggat	21061
OP924190-BQ.1.12-16-13-2022	ne as thing to sugare extra trading the strategy as a second as softing gat.	21956
OQ050529-BQ.1.1.13-3-12-2022	tcasttetgtastgatecattetggatgtttastaccacasastasttggat	21924
CP945722-BQ.1.1.2-19-11-2022	tcasttttgtastgatecatttttggatgtttsttaccacaaaaacaacaasagttggat	21946
CP954130-BC.1.2-14-11-2022	teasttttetasteaterattttteeestttstasracaaaasaacaasaeteest	21946
OP913063-B0.1.1.4-20-11-2022	bcaabbbbgbaabgabccabbbbbggabgbbbabbaccacaaaaacaacaacaacaabbccab	21996
OP942658-B0.1.1.1-16-11-2020	bcaabbbbcbaabcabbbbbbcoabbbbbbbbbbbbbbccacaaaaacaacaaaaabbbooab	21994
QP942052-D0.1.1-17-11-2022	to as bit to be about to a bit to be bound of the black ac as a section of	21996
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GP914037-80.1.11-11-11-0031	acurate lange lange and a set all a share a set	21910
00010638-80.1.2-3-13-2022	bcaabbbbgbaabgabccabbbbbggabgbbbabbaccacaaaaacaacaasagbbggab	21987
GP942060-BQ.1.15-17-11-2022	tcasttttgtaatgatccatttttggatgtttsttaccacasaacaacaasagttggat	21994
OP542055-BQ.1-17-11-2022	b carb b b b g bar a b g a b c carb b b b g g a b g b b b a b b a c carca a a a a c a a c a a a g b b g g a b - b b b b b b b b g g a b - b b b b b b b b b b b b b b b b b	21996
OP942661-BQ.1.12-16-13-2022	tcasttttgtastgatecatttttggatgtttattaccacasascascassgttggat	21996
GP944138-BQ.1.13-14-11-2022	bcaabbbbgbaabgabccabbbbbggabgbbbabbaccacaaaaacaacaacaacaacaacaa	21915
OQ110040-CL.1-12-12-2022	bcaabbbbcbaabgabccatbbbbggabcbbbabbaccacaaaaacaacgaaacbbggab	21924
OP753052-BA. 4. 6-12-10-2022	to as biblio bastorate call biblionated black at as and an antiblionati-	21002
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CQ0000009-51.2-18-11-2022	ocanobedeanedaeceneeeddaedeeeneereeneereeneereeneereeneeree	21902
OP95/641-EK.1-22-11-2022	tearteequargaterateetetgqatgeterreacasaascasaasgetgqat	11334
CP440709-EK. 1-4-7-2022	traiteretatgateratittiggatgittiltizeraraaaaaaaaaagbtegat	27965
CP436293-EK.1-6-7-2023	\$CAR\$\$\$\$\$;\$AA\$\$R\$\$CCA\$\$\$\$\$\$\$\$\$\$\$\$\$\$\$\$A\$\$ACCACACAAAACAACA	21971
OP918588-BU.2-27-10-2022	bcaabbbbgbasbgabccabbbbbggabgbbbabbaccacaabaacaacaasagbbggab	21942
OP\$14075-BF.7-14-11-2022	tcasttitigta abgatccattititiggatgitita the ccacasa as cases a case and the gat the statement of the second secon	21971
OP753588-Eh.5.2.1-11-30-2022	bc aabbbbgbaabgabee abbbb bggabgbbb abbace ac a a aac aac aa agbbggab	21924
OQ094980-BW.1-9-11-2022	tcasttttgtastgstccatttttggstgtttsccscasasascascasagttggst	21947
OQ109844-EA.5.2.25-9-12-2022	teasttttqtastgatecasttttqqatgtttittaccacaaaaacaacaaiagttqqat	21772
CC097098-CD, 2-14-9-2022	Scantesterascenterastititerrasettitettastascancancancancancancancancancancancancanc	21984
GP987800-80.1-00-11-0000	brasbbbbbbs should abbbbb on sol bharra cas sascas cas saobboost	01990
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05357666-7885.8-28-11-2022	pcaropopderspdapecaresolddapdepercerareserereredpoddap	21995
OP999000 RDD.2 28 11 2022	Comparing the style book and the bygge byt blaccame an and an angli bygge b	21901
GF555568-XHB.1-33-11-2022	bcasbbbbgbasbgsbccabbbbbggsbgbbbaccassassascascassgbbggsb	21064
OQ109632-XBB.1-8-12-2022	tcasttttgtastgstccatttttggstgtttaccasssascascassgttggst	21960
OP957795-CM. 8-28-11-2022	beaatonegtaanganeeannenggangtonannaceaeaaaaacaacaaaagunggae	55005
OQ031139-CM.2-5-12-2022	tcaattttgtaatgatecatttttggatgtttattaccacaaaaacaacaaaagttggac	21945
CP429922-85.5-20-6-2022	tcasttttqtaatgatecatttttggstgtttittaccacaiaaacaacaaiagttggat	21977
OQ033138-BG.2-27-6-2022	SCALSSS TAASTASCASSSS TOTAST STATES SALAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	22000
00118059-KBD-12-12-2022	bcaabbbbgbaabgabccabbbbbggabgbbbabbaccacgaaaacaacaacaasagbcggab	01960
00010149-59.1.1.1-4-12-2022	bcastbtbgtasbgstccattbbbggstgtbbstbsccacgaaacaacaacaasagbcogab	21902
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Og110613-CB.1-8-12-2022	ocaroosogeasegatecatettetggstgttttatescacgaassaacaacaasgteggat	21968
OP957642-EH.1.2-22-11-2022	teastettegtastgatecattttetggatgtttattaccacgasaacaacaasagteggat	22002
OP942052-BH. 6-17-11-2022	eerreeederregerregeereeeddrefeereeredrereererrerereedrere	22002
OQ000633-BA.2.75.5-20-11-2022	peweppederversersersersered and page show the terestry of the second sec	21977

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

Sequence ID		22270	22260	22290	22300	22310	22320	22330
consensus	(·) : CTTG	CTTTACATAG	AAGTT		TTGACTCCTG	GTGATTCTTCT	TTCAGGTTGG	ACAGCTGGT
00237113.1	(+)							
OQ236826.1	(*) 8 * * * *							
OQ237104.1	(*) \$ 1 1 1 1		A & A & A & A					
OQ236784.1	(*) 3 3 3 3 3		A. A. A. A. A.					
OQ237313.1	(*) 8 2 2 2 3		A. A. A. A. A.					
OQ236737.1	(*) 8 2 2 2 2		A. A. A. A. A.					
OQ236873.1	(*) \$ 2 2 2 2		A. A. A. A. A.					
OQ236936.1	(*) \$ 2 2 2 3							
0Q237414.1	(*) 🖡 👘 👘							
OQ237457.1	(*) 8		A. A. A. A. A.	2.3				
OQ237845.1	(*) 8 2 2 2 2		A. A. A. A. A.					
OQ237289.1	(*) 8 8 8 8 8		A A A A A					
OQ237518.1	(*) 1 1 1 1 1		A. A. A. A. A.					
OQ236931.1	(*) 8 2 2 2 2			2.3				
OQ237717.1	(*) 1 1 1 1 1			20				
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OQ237274.1	(*) 3 2 2 2 2		A A A A A A					
OQ236935.1	(*) 1 1 1 1 1		CARGE CARGE	ATCOATCO				
OQ236831.1	(*) 8 2 2 2 3		CARG	ATCOATCO				
OQ237075.1	(*) 1			ATCOATCO				
OQ237095.1	(•) 1 1 1 1 1		A. A. A. A. A.					
OQ237634.1	(*) 8 2 2 2 2		A. A. A. A. A.					CONTRACTOR OF STREET
OQ236897.1	(*) 3 3 3 3 3							
OQ236770.1	(*) 8 2 2 2							
OQ236870.1	(*) 🖡 🗉 🗉 🖉							
OQ237258.1	(*) 1 1 1 1 1		A. A. A. A. A.					CONTRACTOR OF STREET
OQ236818.1	(*) 8 2 2 2 2		A. A. A. A. A.					
OQ236938.1	(*) 🛢 🗉 🗉 🖉		A A A A A					
OQ237570.1	(*) § [1] 1] 1							
OQ236764.1	(*) 🖡 🛛 🖉		A A A A A					
OQ237257.1	(*) 1 (0.000)		X X X X X					
OQ236731.1	(*) 8 2 2 2 2							
OQ236881.1	(*) 2 2 2 2 3							
OQ237298.1	(•) 8 8 8 8 8							CONTRACTOR OF STREET,
OQ236835.1	(*) 8 2 2 2 2		A. A. A. A. A.					
1002323326.1	(a) x 1 + + + +			10.00				

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.

	241	250	260	270	280	290	300
	1	+	+	+	+	+	1
5-00236935-27-12-202	LLALH	RSSRHHDL	TPGDSSSGHT	AGAAAYYYGYL	QPRTFLLKYN	IENGTITDAYE	CALDPL
5-00236831-26-12-202	LLALH	RSSRAMD	TPGDSSSGHT	AGAAAYYYGYL	OPRTFLLKYN	IENGTITDAYE	CALDPL
S-0Q237075-27-12-202	LLALH	RSSRAMD	TPGDSSSGHT	AGAAAYYYGYL	QPRTFLLKYN	IENGTITDAYE	CALDPL
S-0Q237113-27-12-202	LLALH	RSYL	TPGDSSSGHT	AGAAAYYYGYL	QPRTFLLKYN	IENGTITDAVE	CALDPL
S_0Q237414-28-12-202	LLALH	RSYL	TPGDSSSGHT	Agaaayyygyl	QPRTFLLKYN	IENGTITDAYD	CALDPL
S-0Q252919-27-10-202	LLALH	RSYL	TPGDSSSGHT	Agaaayyygyl	QPRTFLLKYN	IENGTITDAVE	CALDPL
S-NC_045512.2-12-201	LLALH	RSYL	TPGDSSSGHT	Agaaayyygyl	QPRTFLLKYN	IENGTITDAYD	CALDPL
Consensus	LLALH	RSyl	TPGDSSSGHT	AGAAAYYYGYL	QPRTFLLKYN	IENGTITDAYE	CALDPL

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 (0Q252919).

B.0	22261	Splike gene region of SARS CoV 2 TAACATCACTAGGTTTCCAAACTTTACTTGCTTTACATAGAAGTTATTT	22308
BQ.1	22237	TAACATCACTAGGTTTCAAACTTTACTTGCTTT <u>ACATAGAAGTTCAAGATGGATGGATTT</u>	22296
в.0	22309	GACTCCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTA	22368
BQ.1	22297	GACTCCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTA	22356

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.

Author/Acc. no./date of virus isolation/ state Mu-NC 041512-12.2019-China Wuhan Garrigues-OP925220-2.11.2022-California Moline-02244055-27.12.2022-California Howard-0228169-29.12.2022-Michigan Matsinger-0209704-28.11.2022-Colorado Howard-02178625-14.12.2022-Florida Howard-OQ17369-14.12.2022-Florida Howard-OQ085095-3.12.2022-Georgia Howard-OQ085095-3.12.2022-Washington Howard-OR816502-2.11.2022-California Howard-OQ193239-20.12.2022-Texas Howard-OQ193239-22.12.2022-Texas Howard-OQ193239-22.12.2022-Texas

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.