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Research Article

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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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Summary

Alarming antibody evasion properties were documented for new BF, BQ and XBB Omicron subvariants. XBB was originated from BA.2.75 lineage with no 69HV deletion whereas BQ was originated from BA.5 variant with 69HV deletion which also detected in Alpha variant but not in Delta. Most immune-drugs were inactive neutralizing those COVID-19 subvariants and viral titers were exceptionally low as compared to deadly B.1.1.7 (Alpha) and B.1.617.2 (Delta) 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 or related recent lineages CH.1, BM.1 and CA.1 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 and 30nt deletion in 3'-UTR. Whereas 24LPP spike deletion and 3675SGF ORF1ab protein deletion were found in all Omicron viruses including BQ.1, XBB.1 and other new omicron lineages. Interestingly, in January 2023, 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, Michigan, New York as well as other adjoining US states. As in August, we detected more than 448 such sequences which also appeared in Europe. Data analysis detected one amino acid deletion (140Y=TAT; 145Y in B.0) in spike 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 spike 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.

Keywords: Omicron BQ.1, RWMD Spike Insertion, Immune-Escape, Higher Infectivity, SARS-CoV-2, XBB.1 and BF.7 Subvariants.

1. 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 insertion in the spike might be increase transmission over related Omicron subvariants. The finding was deposited to Research Square Preprint Server on 17th January, 2023.

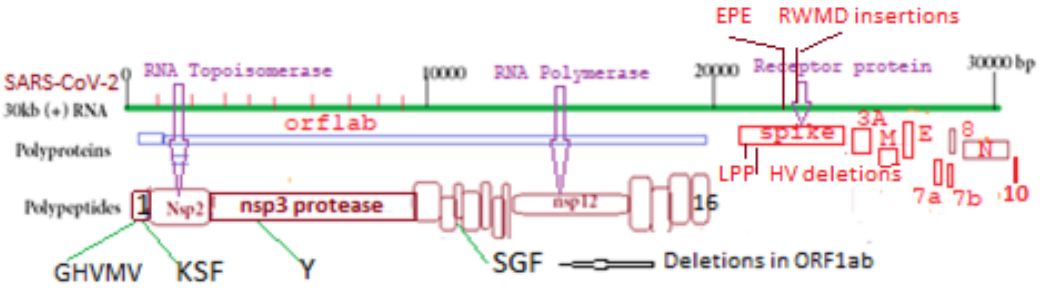

Figure1: Genetic structure of SARS-CoV-2 and highly deletions, insertions and mutations in spike of Omicron variants.

2. 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 and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI [33-36]. 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 [37]. The protein 3-D structure of N-protein was determined by SWISS-Model software [38,39].

3. 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).

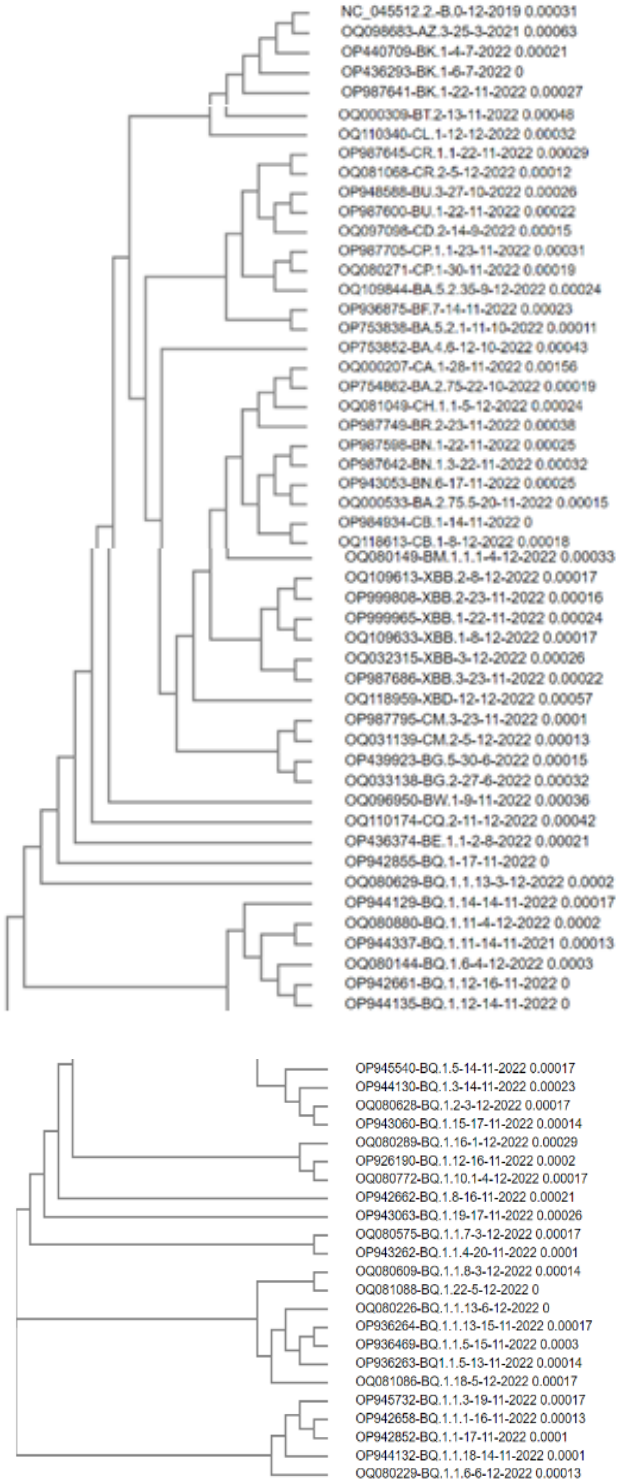

Figure 2: Multi-alignment (CLUSTAL Omega) and then phylogenetic analysis of recently appeared Omicron subvariants.

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.

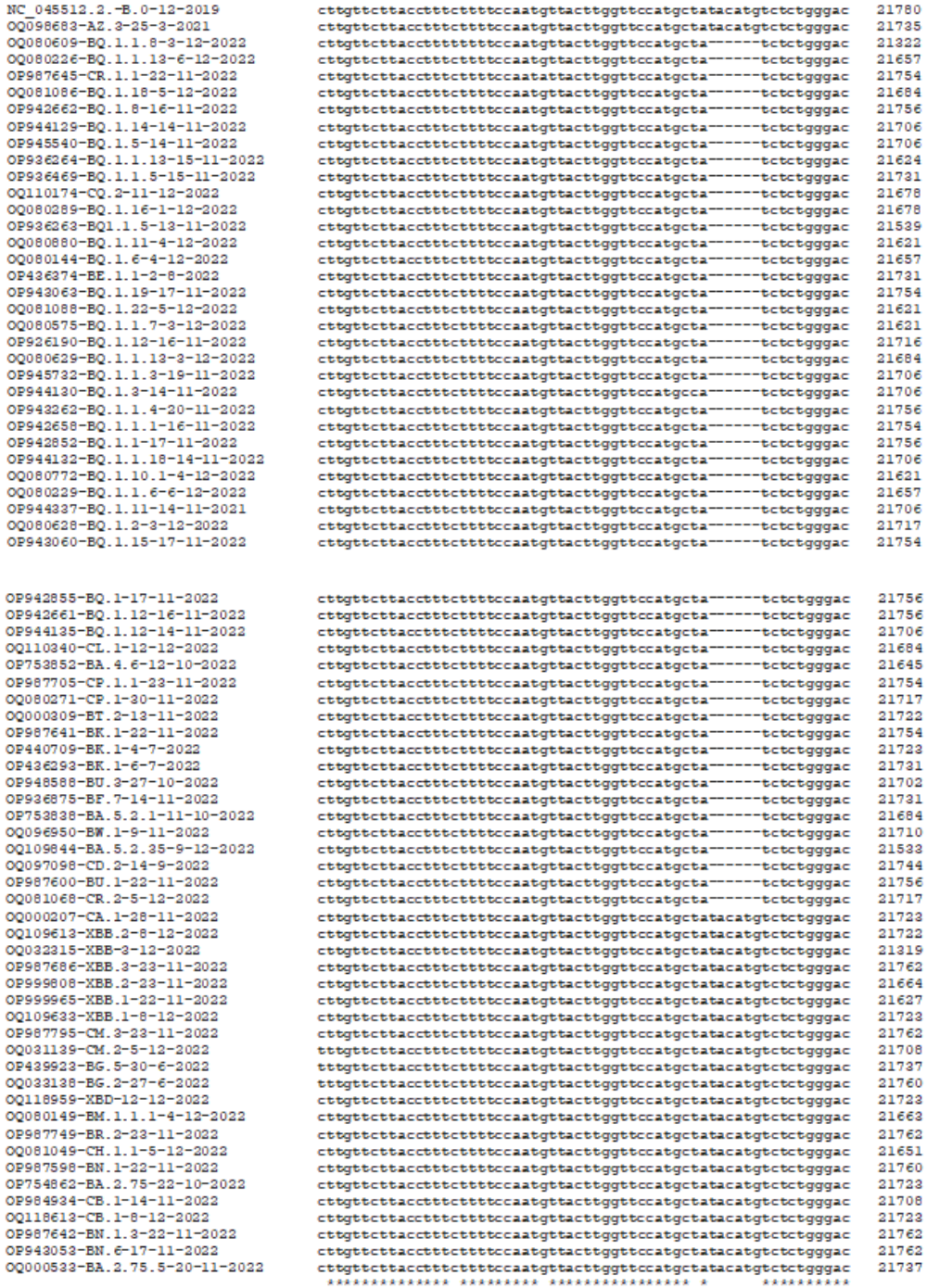

Figure 3: Multi-alignment of SARS-CoV-2 Omicron subvariants to demonstrate all BQ.1 subvariants had [69]HV deletion including BK, BW, CD, CR, CQ, and important BF.7 subvariants. But BG, BN, BR, CA, CB, CM, XBB, XBD are related to BA.2.75 subvariants and had no [69]HV deletion.

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).

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 NH2 terminal site (1-500 AAs) (figure-6E). Surprisingly, in XBB variant had no 69HV deletion in spike, but more curiously 142Y one AA deletion located in XBB.1 variant which we also located in BQ.1.8 (140Y deletion in BQ.1.8 and such position would be 145Y in Wuhan).

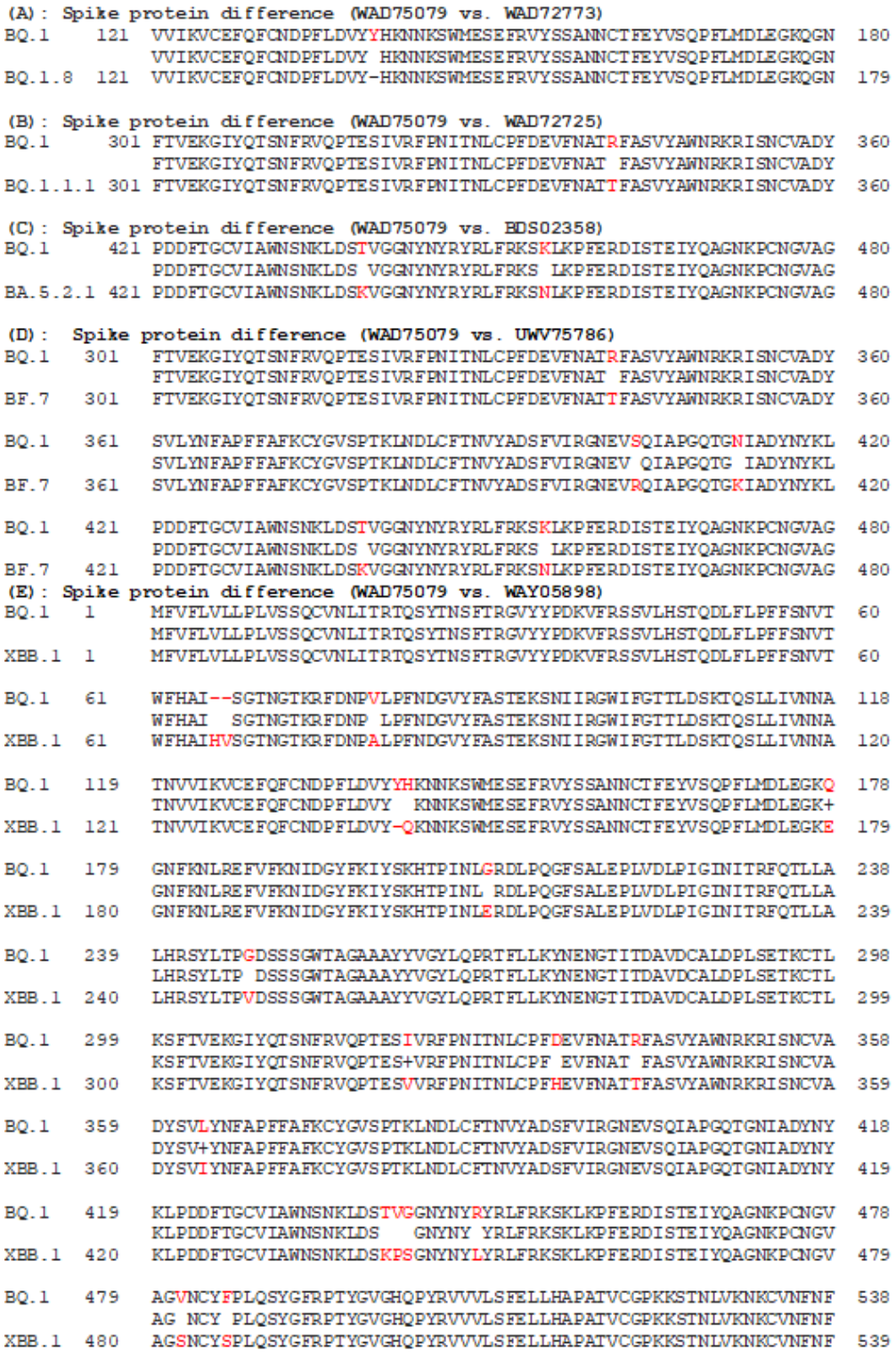

Figure 6: BLAST-2 homology to demonstrate the Spike protein differences in SARS-CoV-2 Omicron BQ.1 variant with BQ.1.8, BQ.1.1.1, BF.7 and XBB.1 subvariants. The alignment portions with AA difference only shown here in each case.

```
(A): ORF1ab protein AA difference (WAD75077 vs. WAY14400)
BQ.1    2041  CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEDVGHTDLMAAYV  2100
              CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITE+VGHTDLMAAYV
BQ.1.1 2041  CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV  2100

BQ.1    2161  LNRVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS  2220
              LNRVCTNYMPYF TLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS
BQ.1.1 2161  LNRVCTNYMPYFLTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS  2220

BQ.1    5581  DEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE  5640
              DEFSSNVA+YQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE
BQ.1.1 5581  DEFSSNVASYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE  5640

BQ.1    6001  AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDFSRVSA  6060
              AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLV VPTGYVDTPNNTDFSRVSA
BQ.1.1 6001  AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVVVPTGYVDTPNNTDFSRVSA  6060

(B): ORF1ab protein AA difference (WAD75077 vs. WAD72723)
BQ.1      2041  CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEDVGHTDLMAAYV  2100
                CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITE+VGHTDLMAAYV
BQ.1.1.1 2041  CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV  2100

BQ.1      5581  DEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE  5640
                DEFSSNVA+YQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE
BQ.1.1.1 5581  DEFSSNVASYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE  5640

BQ.1      6001  AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDFSRVSA  6060
                AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTG NLVAVPTGYVDTPNNTDFSRVSA
BQ.1.1.1 6001  AIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGANLVAVPTGYVDTPNNTDFSRVSA  6060

(C ) : ORF1ab protein AA difference (WAD75077 vs. UWV75784)
BQ.1  541    ARVVRSIFSRTLETAKNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAY  600
             ARVVRSIFSRTLETA+NSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAY
BF.7  541    ARVVRSIFSRTLETAQNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAY  600

BQ.1  2041   CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEDVGHTDLMAAYV  2100
             CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITE+VGHTDLMAAYV
BF.7  2041   CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV  2100

BQ.1  3781   CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQGLFPPKNSIDAFKLNIK  3840
             CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQGL PPKNSIDAFKLNIK
BF.7  3781   CFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQGLLPPKNSIDAFKLNIK  3840

BQ.1  4081   CDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALRANSAVKLQNNE  4140
             CDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNL WPLIVTALRANSAVKLQNNE
BF.7  4081   CDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLVWPLIVTALRANSAVKLQNNE  4140

BQ.1  4621   PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLKHDFTEERLKLFDRYFKYWD  4680
             PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLK+DFTEERLKLFDRYFKYWD
BF.7  4621   PVVDSYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWD  4680

BQ.1  5521   FEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVIPLSAPTLVPQEHYVRITGLYPTLNIS  5580
             FEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTV+PLSAPTLVPQEHYVRITGLYPTLNIS
BF.7  5521   FEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQEHYVRITGLYPTLNIS  5580
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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.

We knew that ^{143}VYY three AAs deletion was present in Omicron BA.1 variant and 145Y deletion also located in B.1.1.7 Alpha variant (accession nos. OQ204252, ON300077, OU225832) 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 ^{140}Y 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 ^{140}Y (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, ^{140}Y 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!.

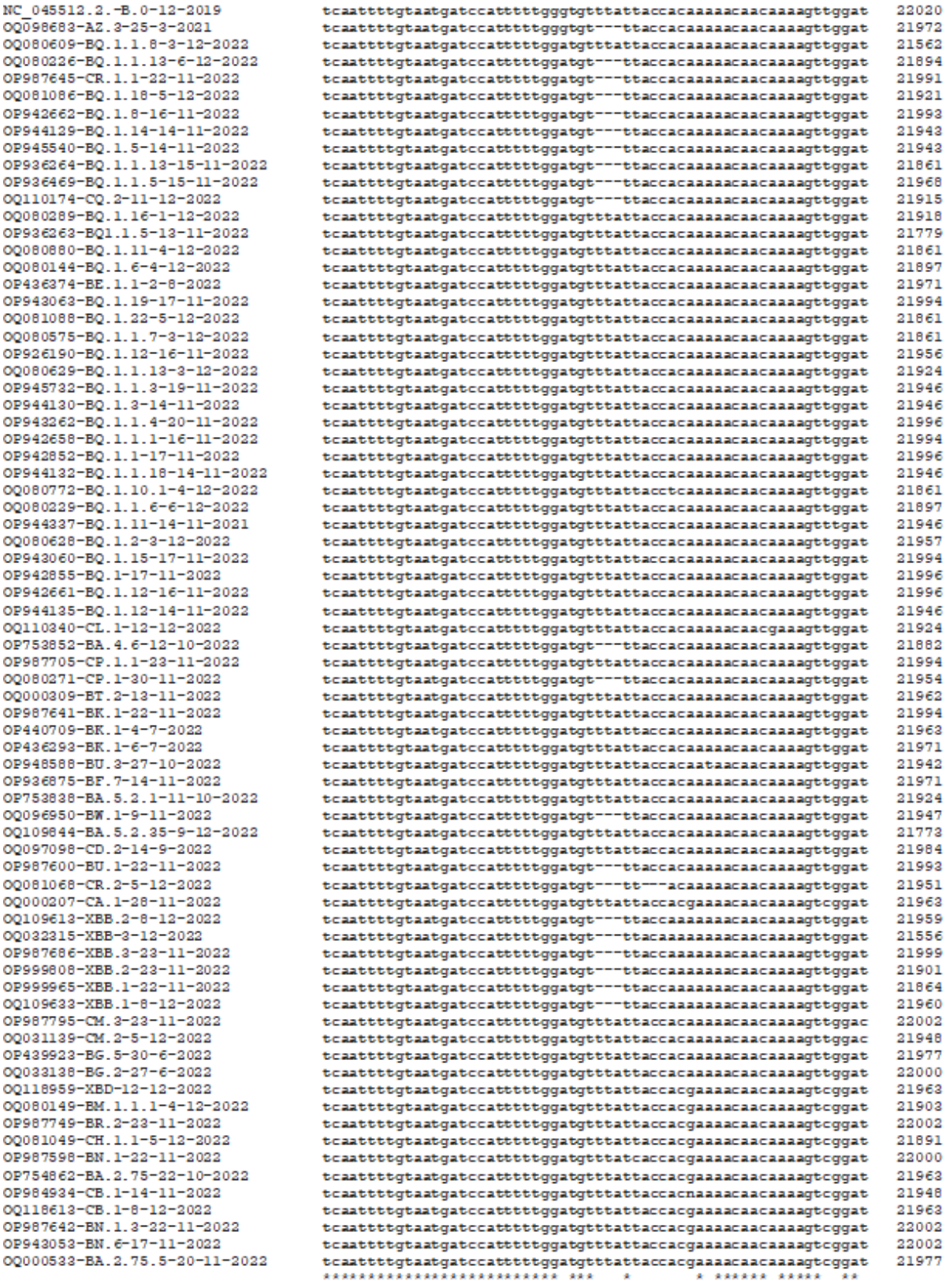

Figure 8: Multi-alignment of different SARS-CoV-2 subvariant genomes recently identified in NCBI database to demonstrate the 140Y deletion in spike protein of many BQ.1 sub-subvariants.

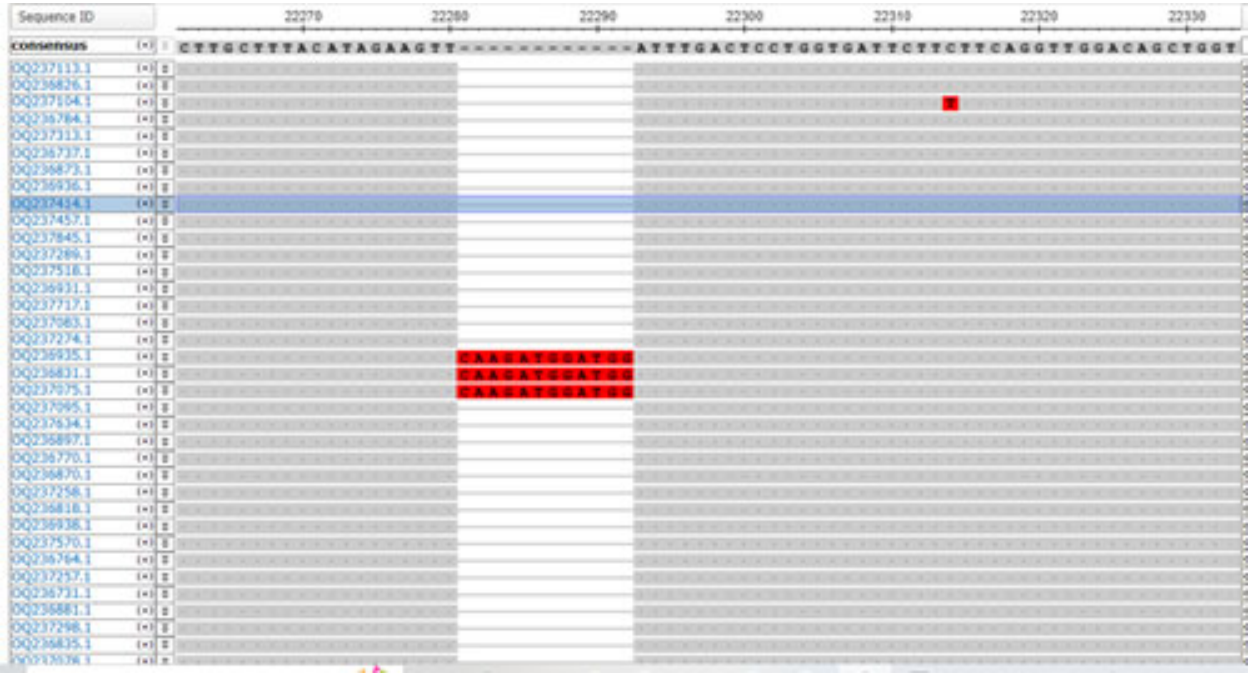

Figure 9: Detection of COVID-19 second insertion mutants in spike of Omicron BQ.1 subvariants. The selected BQ.1 variant sequences in the SARS-CoV-2 NCBI portal were aligned and scanned to insertion point and photographed.

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-10A). 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-10B). Interestingly, 245 sequences were obtained from California patients only and five from Florida, and Washington, Three from Arizona, two from Michigan and one each from Kansas, Colorado, Texas, Pennsylvania, New Mexico, Utah, Georgia, Nevada, District of Columbia and Ohio states of USA (figure-11A). 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.

There was 60 new RWMD spike insertion mutants were deposited in January, 2023 (figure-11C). 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. Further, we multi-aligned mutant genomes from thirteen US states to locate the SARS-CoV-2 spike RWMD insertion points demonstrating correct interpretation of our result (figure-11B). However, it appeared that major outbreak had occurred in California state of the USA and no such insertion mutant spread was found in the East (New York). After the preprint publication (Research Square, Springer-Nature), we further checked the status of RWMD spike insertion mutants in January, 2023 and found more 60 sequences addition (Total=271). Multi-alignment confirmed the spread in California with minor outbreaks in the Washington, Arizona, District of Columbia, Illinois and Florida states of USA (figure-11C). Further, we hardly found any such insertion in the BQ.1.1 sub-subvariant as well as BQ.1.1.X sub-subvariant as judged by multi-alignment and looking insertion junction (figure-12). We also found the spread of 249RWMD-mutant into Northern Ireland (figure.13A) and Germany (figure.13B). Interestingly, we also detected similar TLRA and SDA insertions in the spike but spread of such mutants were not observed (figure-14).

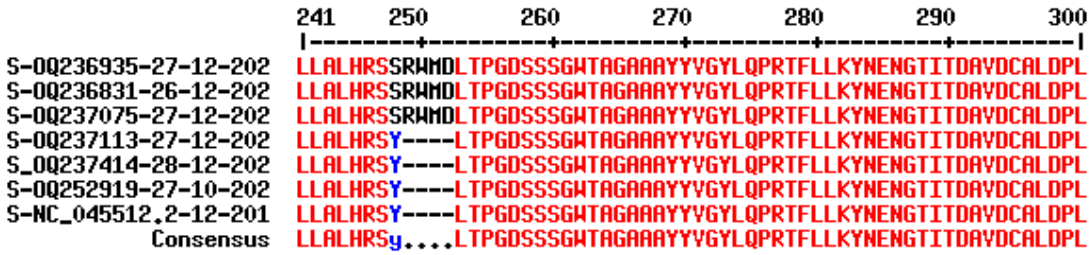

Figure 10: Multi-alignment of few Omicron BQ.1 spike protein sequence with or without four amino acids insertion as compared to Wuhan (NC_045512.2) and BA.5.2.1 (OQ252919).

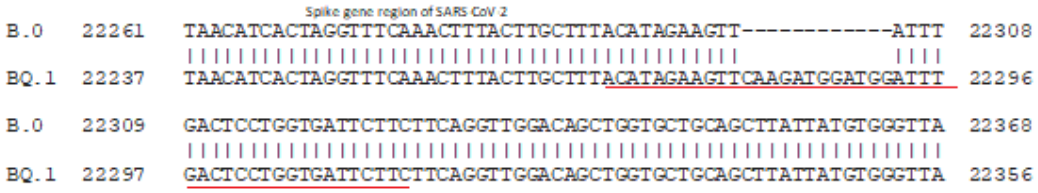

Figure 11: BLAST-2 homology between NC_045512.2 Wuhan virus and BQ.1 insertion mutant to find an oligonucleotide (red underline) at the insertion boundary for BLAST-N search to get related insertion BQ.1 mutants.

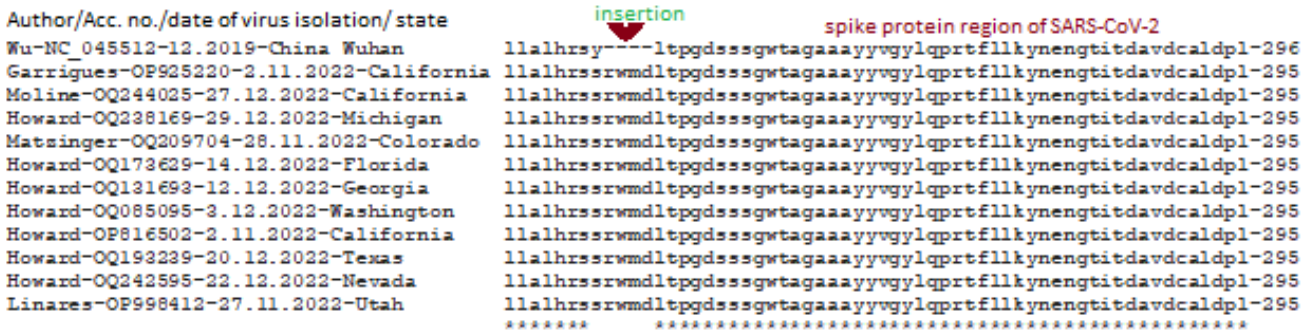

Figure 12: Multi-alignment of spike proteins from RWMD insertion mutants of Omicron SARS-CoV-2 isolated from the different US states and sequenced in the different laboratories as compared to Wuhan virus.

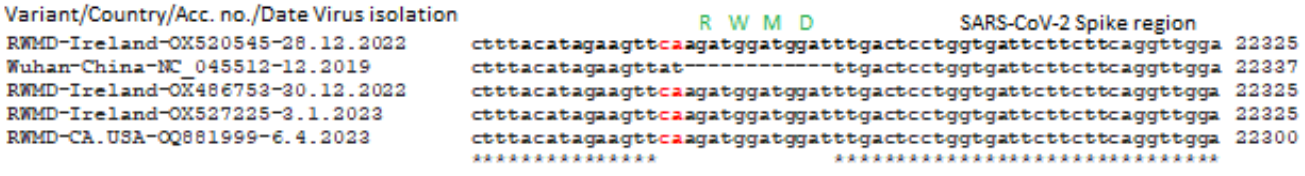

Figure 13A: Spread of RWMD-mutant into Northern Ireland. COVID-19 sequence data submitted from Europe as monopartite i.e. no protein expression data and hence full-length sequences were aligned.

Figure 13B: Spread of RWMD-mutant into Germany. COVID-19 sequence data submitted from Europe as monopartite i.e. no protein expression data and hence full-length sequences were aligned.

Figure 14: COVID-19 RWMD locus in spike has TLRA and SDA insertions located in few omicron variants.

4. 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 [40-42]? 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 [43-45]. 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 271 (211+60) 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'). After the submission of data to preprint server, we got more 60 mutant viruses that were isolated in January, 2023 (figure-11C).

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 [41]. 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 [46]. The BQ.1.1 and BQ.1.1.1 had unique R341T mutation but surprisingly 249RWMD insertion yet was not found in 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 subvariants disproportionally (figure-8). Further, RWMD spike BQ.1 insertion mutant was not detected in the East zone of the United States (figure-11B and figure-11C).

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-like full length 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 [47-49].

During the review process, we found the RWMD-BQ.1 insertion mutant was increased into 448 sequences in SARS-CoV-2 NCBI database (dated 20.8.2023). All mutants had 24LPP and 69HV deletion relating BA.5 lineage except one accession number OQ431559 had no 69HV deletion implying BA.2 lineage. Analysis suggested the sequence was not related to BA.2, BA.2.75 and XBB.1.5. Then, we BlastN searched nt. 20041-29733 of OQ431559 sequence and found no 100% similar sequence and two 99.87% similarity sequences (accession nos. OQ444557/OQ116164) were taken for analysis. The OQ444557 sequence was deposited in the database on 16.2.2023 and the virus was isolated from Texas on 28.1.2023. We found the virus belonged to BA.2.10.1 although it had no RWMD insertion (found on page 489 on dated 29.3.2023, SARS-CoV-2 Database, Sequence deposit date-16.2.2023). The result suggested that RWMD insertion was also occurred in BA.2.10.1 lineage which originally recombined with BA.2.75 to produce more infectious virus XBB.1 variant. Such data was very interesting because XBB.1.5 variant was now 90% population of the total corona virus spreading worldwide.

Multi-alignment of Omicron BQ.1 RWMD-mutant spike proteins suggested few new mutations. The P39H mutation appeared in OQ516415 isolate dated 10.2.2023 from California. The I316T mutation in OQ590911 dated 15.2.2023 isolate and in OQ510734 dated 12.2.2023 isolate also from California. The S71F mutation in OQ580300 isolate of dated 11.2.2023 from Illionis and the K1185N mutation appeared in OQ590365 dated 12.2.2023 from Nevada. A [140]Y ([145]Y in Wuhan) deletion also prominent in OQ327425 isolate dated 4.1.2023 from Florida. Such information will help to track the spread of any new mutant with time and origin.

Multi-alignment of few RWMD mutant ORF1ab proteins also identified new mutations. As for example, an RNA Topoisomerase (nsp2) G327V mutation (accession no. OQ631891), three RNA-dependent RNA Polymerase mutations (nsp12): T4474I (accession no. OQ610794) as well as H4662Y and G5060S (accession

no. OQ661136) and RNA helicase-capping methyltransferase (nsp13) I5554M mutation might be important. The L3606F mutation with ^{82}GHVMV deletion in nsp1 found in accession number OQ619196 dated 28.2.2023 isolate from Washington. The T4126A mutation also identified (accession nos. OQ650020 and OQ654379) in California State and L890F mutation in accession number OQ691870 from Oregon State where as A6911S mutation in accession number OQ610794 from Michigan State. Thus, mutation, deletion and insertion were detected in SARS-CoV-2 since 2020. Presently Omicron viruses (XBB.1.5; XBB.1.16; BQ.1.1.1) got 30nt deletion in the 3'-UTR but ^{69}HV deletion and N501Y dominant mutation of spike was carried into Omicron from B.1.1.7 lineages including D614G dominant spike mutation. Fortunately, notorious B.1.1.7 and B.1.617.2 lineages were not found due to herd immunity. However, new Omicron virus lineages like EG.5.1.3, FL.1.5.1, GN.1.1, XBB.1.5.100, GK.1.1 and Fu.1.1 may cause new epidemic in the future.

5. 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. Interestingly, in this month no ^{249}RWMD-mutant was detected in the NCBI Virus database.

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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.

Ethical Issues

No human and animal were used in this study.

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