

Genesis of Recombinant XEC Variant and Comparable SWISS-Modelling of Spike of LB.1.7 and KP.3.1.1 Subvariants Coronaviruses

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Abstract

XEC variant was generated due to recombination between JN.1-lineages KP.3.3 and KS.1.1 subvariants. Multi-alignments among the XEC, KP.3.3 and KS.1.1 sequences at the NCBI Virus Portal suggested nt.21600-29900 originated from KP.3.3. Similarly, BLASTP-2 mutational differences between XEC/KP.3.3 and XEC/KS.1.1 genome confirmed the positions of XEC recombination. The sixty amino acids of N-terminus were generated from KS.1.1 as confirmed by BLASTP-2 analysis of spike proteins of XEC/KP.3.3 and XEC/KS.1.1. The ORF1ab polyprotein BLASTP-2 analysis suggested that AAs 2200-2300 region generated from KS.1.1 as T2283I mutation was only found in KP.3.3. While AAs 2100-2200 was originated from KP.3.3 as L2146F mutation was only found in KS.1.1. Interestingly, the A599T ORF1ab mutation in XEC was found but was not located in both KP.3.3 and KS.1.1. BLASTP analysis of 60 AAs of the mutated region suggested that FL.15 subvariant recombination between nt.450-650 might be occurred in XEC. The SWISS-Modelling suggested the PCov_GX spike (PDB: 7cn8.1) template gave the best fit 3-D model for XEC spike protein but JN.1 spike (PDB: 8y5j.1.A) template produced more compact 3-D trimeric spike and had 99.08% similarity as compare to 88.72% former. The bat spike (PDB: 8wly.1.B) template gave very open 3-D motif of LB.1.7 and KP.3.1.1 oligomeric spikes with protruding amino acids but no symmetry among three identical subunits. The parameters of SWISS-Modelling suggested that XEC variant spike oligomer is a more compact to interact with receptor explaining higher transmission rate and higher pathogenicity than parent JN.1 subvariants or precedent BA.2.86 variant.

Keywords: XEC; NCBI Virus; Amino acids; Polyprotein

Introduction

About seven million people were died due to corona virus infection worldwide between 2019-2024 [1,2]. Currently, six different coronavirus strains are known to infect humans. Such strains named as: HCoV-229E (229E), HCoV-OC43 (OC43), SARS-CoV (COVID-19), HCoV-NL63 (NL63), HCoV-HKU1 (HKU1), and MERS (MERS-CoV) [3,4]. COVID-19 has unique polyprotein (ORF1a, ORF1b), antigenic spike protein (S), and small regulatory proteins like orf3a, orf7a, orf8 and orf10 (Figure 1). The RNA polymerase (Nsp12), Proteases (Nsp3 and Nsp5), RNA topoisomerase (Nsp2), Capping RNA helicase (Nsp13), RNAases (Nsp14, Nsp15) and Methyltransferase (Nsp16) were

produced from the proteolysis of ORF1ab [5,6]. The COVID-19 first appeared in China during 2019 through the recombination among bat and pangolin corona viruses [7]. Vaccination and autoimmunity greatly controlled the alpha, beta and delta coronaviruses epidemic in during 2020-2022 but omicron coronaviruses (since November, 2021 and more than 149 countries) mild infections were still happening today [8-10]. Never-the-less patients with co morbidity and old age still are dying due to omicron BQ.1 (September, 2022), XBB.1.5 (and JN.1 (May 2024) sub variants infections. United States and United Kingdom are continuing their WGS sequence analysis from sample isolated from newly omicron coronavirus infected people. The great VOC of coronavirus classification were Alpha

with dominant N501Y spike mutation, Delta with dominant D614G, L452R, K444R, T478K, Y508H and P681R spike mutations including P1, P2, Beta and Gamma and few omicron variants that affected greatly between 2020-2023 [11,12]. While Omicron BA.1 followed by BA.2, BA.3 (only Africa), BA.4 and BA.5 affected with great specificity worldwide between 2022-2023 with lower severity, the recent XEC variant was reported as a new threat [13]. There are 21 common mutations between BA.1, BA.2, and BA.3 which are G142D, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K [14]. The XBB variant outbreaks was maximum which was made by recombination with two second-generation BA.2 lineages, BJ.1 (BA.2.10.1.1) and BA.2.75; and it contained a large number of antigenic receptor-binding domain mutations and was shown to be poorly neutralized by previous Omicron breakthrough antisera [15]. The major point mutations in the spike protein of JN.1 omicron coronavirus were demonstrated in (Table 1) Apart from that 17MPLF spike insertion is an important characteristic of JN.1 subvariants. Although, 7% JN.1 lineages had no such insertion as reported by Howard D et al contrary to Oppentrons P who suggested major JN.1 subvariants had no such insertion [16,17]. However, such insertion favoured the eight to nine amino acids deletions [24LPP, (31S), 69HV, 145Y, 211N and 483V]. We did not find yet the 31S deletion in XEC variant but in LB.1.7 subvariant, we detected 100% 31S deletion. The JN.1 variant and XEC variant both are threat as both dominant N501Y and D614G mutations accumulated enhancing transmission. Recently, Howard et al found about 5-7% omicron coronaviruses with no 17MPLF spike insertion but few European laboratories had submitted more JN.1 sequences without 17MPLF spike insertion. Further, NH2-terminas in JN.1 spike accumulated many mutations other than 315-525 AAs RBD region. Comparatively we found all omicron sub-lineages including BA.5, BQ.1.1, XBB.1.5 and JN.1 had less mutations in the 700-1269 AAs Carboxy-terminal region of spike [18]. Recombination among the coronavirus variants were seen from time to time and sometime such recombinant virus had higher transmission and pathogenicity (Table 2). The XEC coronavirus, a recombinant between KP.3.3 and KS.1.1, was first identified in Berlin in late June and has since grown rapidly across Europe, North America, and Asia, with the most significant increases observed in Germany and Denmark (September, 2024). The major symptoms are loss of sense of taste or smell, shortness of breath, runny nose, sore throat, congestion, diarrhea, fever or chills and fatigue. Scientists believe that the existing vaccinations targeting Omicron variants will likely continue to be effective against XEC. But exiting drugs like paxlovid (PLpro/Mpro proteases inhibitors) and remdesivir (Rd-RNA Polymerase inhibitor) were approved by FDA whereas

immune drugs tocilizumab (cytokine IL-6 antagonist) and baricitinib (Janus kinase inhibitor) might be useful. During March 2020 initial report suggested that human SARS-CoV-2 (COVID-19) might be originated from pangolins and later other report suggested that bat origin of human coronaviruses [19]. SARS-CoV-2's entire receptor-binding motif (RBM) was introduced through recombination with coronaviruses from pangolins, possibly a critical step in the evolution of SARS-CoV-2's ability to infect humans. The human seasonal coronaviruses all have recombination rates around 1×10^{-5} per site and year and satisfies a role of pathogenicity [20,21]. The highest subvariant was reported to be KP.3.1.1 (>50%) followed by KP.2.3, KP.3.3, LB.1.7 (>10%) and JN.1.11.1.2, JN.1.16.1 and KS.1.1 (<3%). The KP.3.1.1 had extra 31S deletion in addition to F456L, Q493E and V1104L spike point mutations that appeared in KP.3. The LB.1.7 had JN.1 mutations plus LB.1 specific Q183H, R346T and F456L pint mutations including new 31S deletion. The XEC spike had JN.1 mutations including T22N, F59S, F456L, Q493E and V1104L point mutations but no 31S spike deletion [22]. US statistics on coronavirus spread was reliable and whole genome sequencing by Howard D et al was highly satisfactory. As on 5th May 2022 confirmed Pangolin COVID-19 cases in India 37542 and in USA 999565 while GISAID cases (29TH July, 2022) in India 81017 and in USA 1431772. Thus, I preferred to study NCBI virus free database which at least clearly gave an idea of COVID-19 spread. The CDC estimated that the JN.1 variant accounted for 44% of cases in the US on 22th December 2023 and 62% of cases on 5th January 2024. The genesis of XEC variant was discussed and Swiss Model used to predict the spike protein structural differences with JN.1.

Methods

The sequences of XEC variant were obtained from SARS-CoV-2 database (NIH, USA). The multi-alignment of at least six sequences performed to show same structural integrity before Swiss-Modelling. The BLASTP performed to show spike protein homology between two amino acid sequences. CLUSTAL-Omega multi-alignments were performed to see protein or DNA homologies among different coronavirus genomes or spike proteins. Emboss transeq was used to convert nucleotide sequence to protein. Protein domains of SARS-CoV-2 was identified using Pfam, InterPro, and SMART protein domain annotation server. Clustal W and Mafft was used for multiple sequence alignments. The PDB was utilized to get the cryo-electron microscopy (cryo-EM) structure of the SARS-CoV-2 Omicron (BA.1) spike protein in complex with human ACE2 (refinement of RBD and ACE2; PDB ID: 7T9L). The structure of BA.1.1 and BA.2 was generated by homology modelling with SWISS-MODEL server with the BA.1 template (PDB: 7T9L) [23]. Similarly, the N-terminal domain (NTD) of spike protein was modelled using SWSS-

MODEL server: WT (PDB ID: 7L2C), BA.1 (PDB ID: 7TEI) and BA.2 (PDB ID: 7TO4) [24]. We compared Wuhan Bat origin (PDB: 8WLY). Wuhan pangoline origin (PDB: 7CN8) and JN.1 (PDB: 8Y5J).

Results

The JN.1 is class of omicron COVID-19 and spike amino acid differences with Wuhan has shown in (Table 1) while the XEC is a recombinant lineage of JN.1 lineages as has shown in (Figure 2). Multi-alignment is a good approach to locate the similarities among the different coronavirus genomes to locate the genesis of recombinant variant. In (Table 2) we have shown few recombinant coronaviruses. We multi-aligned many sequences (200) to check the variation among sequences including 17MPLF insertion in spike and 26-49nt deletion in the 3'-UTR. In (Figure 3A), we showed the NCBI SARS-CoV-2 portal analysis of XEC, KP.3.3 and KS.1.1 sequences indicating at least 3'-end (nt. 21700-29900) of XEC originated from KP.3.3. Next, we performed BLASTN-2 analysis between XEC/KP.3.3 and XEC/KS.1.1 to get mutational differences throughout the genome of KP.3.3 and KS.1.1. Such analysis was summarized in (Figure 3B) to confirm the genesis of XEC 3'-end from KP.3.3 (as many mutations at that region with XEC/KS.1.1) while middle part (nt.11000-21000) might be generated from KS.1.1. To verify the recombination point, we studied the BLASTP-2 analysis between spike, ORF1ab and N proteins of XEC/KP.3.3 and XEC/KS.1.1. The (Figure 4A) demonstrated the huge mutation in AAs 1-60 between XEC/KP.3.3 suggesting that part was obtained from KS.1.1. That means we narrowed the recombination point at the 3' end of XEC at the spike N-terminus. The (Figure 4B) confirmed the homology of KS.1.1 at spike NH2 terminus 60AAs but no similarity at the C-terminus. We also checked the BLASTP-2 between spike of KP.3.3 and KS.1.1 showing the said differences at the NH2-terminus (Figure 5). We also checked the BLASTP-2 of N-protein between KP.3.3/ KS.1.1 or XEC/KP.3.3 and XEC/KS.1.1 (Figure 6). In Figure 6A we showed the N-protein variation between KP.3.3/KS.1.1 which was also seen between XEC/KS.1.1 confirming 3'-end generated from KP.3.3 (Figure 6B). To note that there was 100% homology between BLASTP-2 of XEC/KP.3.3 (data not shown). To confirm the 5'-end junctions in XEC, we performed the multi-alignment of ORF1ab protein of XEC, KP.3.3 and KS.1.1 using MultAlin software (Figure 7). In Figure 7A, we showed an A599T mutation in XEC but not found in KP.3.3 and KS.1.1. The 60 AAs BLASTP search demonstrated many XEC sequences but few FL.15 sequences (WQR02769 and WOH26089) were obtained suggesting that such variant might be involved in the genesis of nsp2 protein (2nd protein of ORF1ab polyprotein). We got the protein id through BLASTP search and to get the sub variant status we had gone to SARS-CoV-2 portal at the respective

accession numbers and checked the variant status as FL.15 (accession numbers: PP042317 and OR707598). We also got the sequences from 2021 year (UBW36984 and UDA43273) and was hardly able to find the variant status at the portal (20000 pages were not possible to open). So, we check the spike protein through the BLASTP-2 search with Wuhan spike (YP_009724390) to confirm the 157FR deletion (protein id. UBW36986 and UDA43274) suggesting A599T mutation in ORF1ab of FL.15 might be obtained from Delta variant (data not shown). During ORF1ab proteins multi-alignment, we also got L2146F mutation in KS.1.1 (Figure 7B) while T2283I mutation in KP.3.3 (Figure 7C) to conclude the boundary of recombination at the 5'-end. So, it was concluded that from ORF1ab 2200 AAs to spike 70 AAs (nt. 6793-21691; accession number: PQ380322) was originated from KS.1.1 and then again part 650-2199 AAs (nt. 2143-6790) of ORF1ab was originated from KP.3.3. Critical point was our data that supported that 3'-end of XEC (nt.1-2142) was originated from FL.15 sequence. However, 3'-end had mutations as judged by BLASTN-2 search between XEC/FL.15 (Figure 8). The PQ380322 (XEC) vs OR707598 (FL.15) or PP042317 (FL.15) produced three or more mutations at the 3'-end of XEC sequence comparison as shown in Figure 9. Thus, a very narrow position in nsp2 gene (~nt.450-650) of XEC was obtained from FL.15 might be possible. Such region had no mutation between XEC/KP.3.3 (PQ380322 vs PQ206638) and XEC/KS.1.1 (PQ380322 vs PQ380361) (data not shown). We repeated the experiment with another XEC sequence (PQ386636) and FL.15 sequences (OR707598 and PP042317) producing same results. The important XEC point mutations and their penetration in the Database was shown in Table 3.

We next determined the 3-S structural differences between JN.1 and XEC as compared to Wuhan (Figure 10). The best fit trimeric 3D structure obtained with 7cn8.1.A template © and Model-1 with 8wly.1.B template and Model-4 with 8xut.1. A template made protruding amino acids (open complex). Next, we compared the 3D Swiss Modelling structures of spike protein of JN.1, XEC, KP.3.1.1 and LB.1.7 subvariants (Figure 11). We always found that template 7cn8.1.A was good even it had 88% similarity to JN.1 lineages while KP.3.1.1 gave more compact structures indicating its fitness to interact with ACE-2 receptor of lungs cells. Using 8y5j.1.A template with 99% similarity, the XEC coronavirus spike gave lower QMQE (0.63 vs. 0.66) but Clash score as compared to KP.3.1.1 subvariant (Table 4) while the MolProbity score was higher (1.30 vs. 1.27). We found that JN.1 spike had advantage trimeric structure due to lowest clash score using all templates (8wly.1.B=0.63; 7cn8.1.A=0.68 and 8y5j.1.B=0.48). The lowest MolProbity (1.21) and Clash score (0.45) in LB.1.7 indicated its spread at certain point but likely due to new mutations in spike such spread was diminished now. The higher stability of KP.3.1.1 with better transmission might be

occurred due to lower bad bonds (bad bonds=2) which was higher in JN.1 (bad bonds=7) including lower bad angles (172 vs 188). (Figure 12) showed the difference in the single subunit structure of Wuhan virus spike as compared to XEC and KP.3.1.1. It was found that XEC and KP.3.1.1 made a sword structure at the RBD domain and such structure was reported as the better fitness for spike of XEC and KP.3.1.1 sub variants to interact better with

ACE-2 receptor. Thus, the fitness of KP.3.1.1 and XEC depend on the acquisition new mutations in the spike. Mutational analysis suggested that spike Q180H and R187T mutations in KP.3.1.1 (accession number: PQ426785) had already occurred. However, 49nt deletion in the 3'-UTR surely deleterious for any coronavirus and we claimed that pre-death symptoms were initiated in coronavirus.

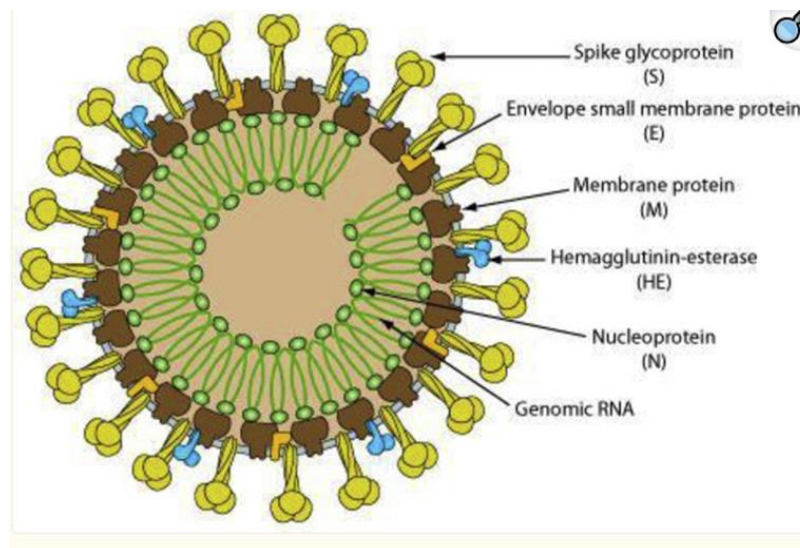


Figure 1: Structure of a 100nm omicron coronavirus (Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. *J Microbiol Immunol Infect.* 2021; 54(2): 159-163).

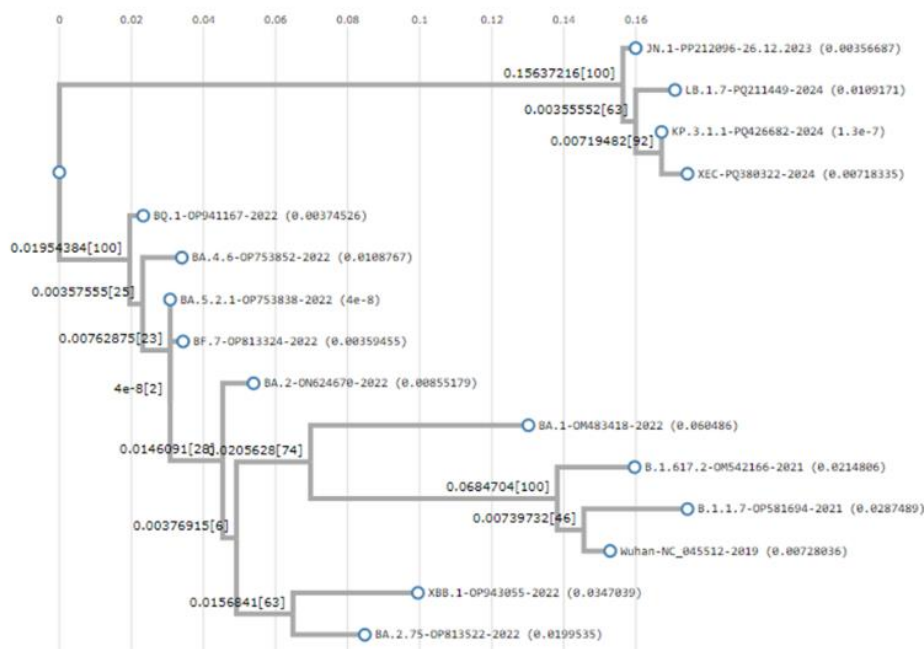


Figure 2: Phylogenetic tree of COVID-19 variants showing distances and bootstrap values. Multi-alignment showed that C-terminal 155 amino acids (NTF VSG NCD VVI GIV NNT VYD PLQ LEL DSF KEE LDK YFK NHT SPD VDL GDI SGI NAS VVN IQK EID RLN EVA KNL NES LID LQE LGK YEY YIK WPW YIW LGF IAG LIA IVM VTI MLC CMT SCC SCL KGC CSC GSC CKF DED DSE PVL KGV KLH YT-COOH) were identical in all subvariants analyzed here. The JN.1 is a distinct class and XEC plus KP.3.1.1 aligned being very similar with 31S deletion in the spike.

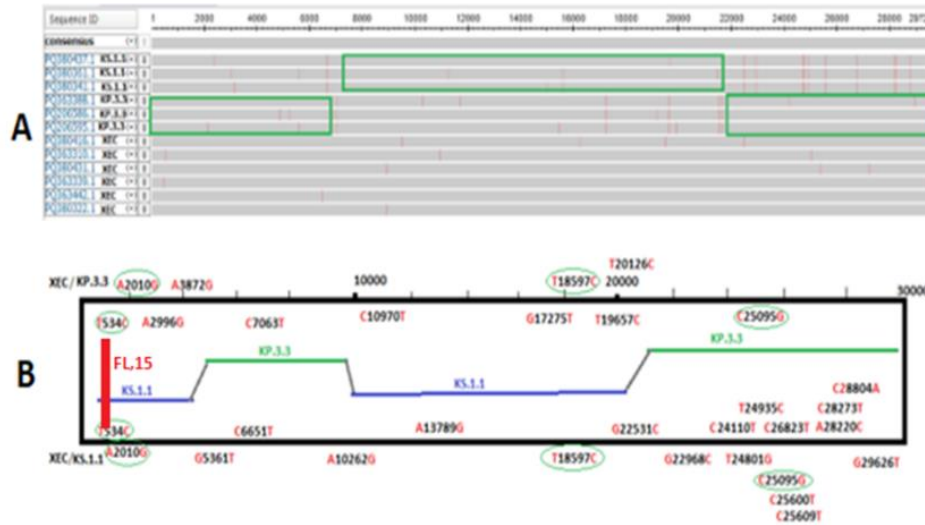


Figure 3: Determination of KP.3.3 and KS.1.1 regions during the generation of XEC variant. Fig.2A denoted by SARS-CoV-2 portal multi-alignment demonstrating the mutations points by red lines. The KP.3.3 3'-end matched with XEC but many red lines with KS.1.1. Fig.2B made by observing the BLASTN-2 mutations between XEC/KP.3.3 and XEC/KS.1.1 genomes. The less mutations suggested the regions of XEC genome formation. Green line indicated KP.3.3 regions and blue line denoted the KS.1.1 regions in XEC genome.

(A) Spike difference between XEC and KP.3.3

XEC	1	MEVFLVLLPLVSSOCVMPLENLITTNOSYNSFTGRVYYPDKVFRSSVLHLDLFLPFS	60
KP.3.3	1	MEVFLVLLPLVSSOCVMPLENLITTIOSYNSFTGRVYYPDKVFRSSVLHLDLFLPFP	60

XHC86854 1141 LDSFKEELDKYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEVAKNLSLIDLQOLG 1200

XG236098 1141 LDSFKEELDKYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEVAKNLSLIDLQELG 1200

(B) Spike differences between XEC and KS.1.1

XEC	301	KSFVEKGIYQISNFRVQPTESIVRFNVTNLCPFHEVFENATRFASVYAWNRTRISNCVA	360
KS.1.1	301	KSFVEKGIYQISNFRVQPTESIVRFNVTNLCPFHEVFENATRFASVYAWNRTRISNCVA	360

XHC86854 481 GPNCYFPFLSYGFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN 540

XG236110 481 GPNCYFPFLSYGFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN 540

Query 1081 GKSHFPRGCVFVSNHTWFLTORNFYEPQIITDNTFVSGNCDVWIGIVNNTVYDPLQLE 1140

Splice 1081 GKSHFPRGCVFVSNHTWFLTORNFYEPQIITDNTFVSGNCDVWIGIVNNTVYDPLQLE 1140

Query 1141 LDSFKEELDKYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEVAKNLSLIDLQOLG 1200

Splice 1141 LDSFKEELDKYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEVAKNLSLIDLQELG 1200

Figure 4: BLASTP-2 sequence similarities of spike between XEC/KP.3.3 variants and XEC/KS.1.1 variants. As 60 amino acids had the maximum differences with XEC with KP.3.3, such region was obtained from KS.1.1. So, recombination was occurred after 60AAs of spike of KP.3.3 and KS.1.1.

SPICE	708T	CKHNBEGALASGICIMHMLIOBIMLAEBOIILLDMILASGICDAAIGIAMIAADBTGTE	7140
QuesA	708T	CKHNBEGALASGICIMHMLIOBIMLAEBOIILLDMILASGICDAAIGIAMIAADBTGTE	7140
SPICE	48T	SBMSALBTGASGEBLAGACHOBKAAAGSEETHTNBYFLACSBKKSLITAKIKSAMIEM	240
QuesA	48T	SBMSALBTGASGEBLAGACHOBKAAAGSEETHTNBYFLACSBKKSLITAKIKSAMIEM	240
B03#2143	30T	KZLIAEKCIAOISIMLAEBOBESIALBEMALITCSBHEALWIFLBAALUMNIBYISCAU	360
B03#2143	30T	KZLIAEKCIAOISIMLAEBOBESIALBEMALITCSBHEALWIFLBAALUMNIBYISCAU	360
K2'T'T	J	NELAGATTGTA280CAMBEMITILLI0SALISBEGALABDKABE2ATHTFLODGTGBE	60
Kb'3'3	J	NELAGATTGTA280CAMBEMITILLI0SALISBEGALABDKABE2ATHTFLODGTGBE	60

Figure 5: Spike amino acid differences between KP.3.3 and KS.1.1 sub variants. Parts of the alignment with mutations only were shown here.

(A): Amino acid differences of N-protein between KP.3.3 and KS.1.1 omicron variants.

KP.3.3	1	MSDNGPQNQRNALRITFGGSPDS	TGSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	60
		MSDNGPQNQRNALRITFGGSPDS	GSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	
KS.1.1	1	MSDNGPQNQRNALRITFGGSPDS	TGSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	60
PQ345742	181	SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	240
		SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	
PQ345743	181	SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	240

(B): Amino acid differences of N-protein between XEC and KS.1.1 omicron variants.

XEC	1	MSDNGPQNQRNALRITFGGSPDS	TGSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	60
		MSDNGPQNQRNALRITFGGSPDS	GSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	
KS.1.1	1	MSDNGPQNQRNALRITFGGSPDS	TGSNQNGGARSQRRPQGLPNNTASWFTALTQHGKED	60
XHC86862	181	SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	240
		SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	
XGZ36118	181	SRSSSRNRSSRNSTPGSSK	TSPARMAGNGGDAALALLLLDRLNKLESQMSGKGQQQQG	240

Figure 6: Amino acid differences between N-protein of KP.3.3/KS.1.1, XEC/KP.3.3 and XEC/KS.1.1 variants as demonstrated by BLASTP-2. The XEC/KP.3.3 N-protein had no mutation (data not shown) confirming again that 3'-end of XEC variant was originated from KP.3.3 sub variant.

	541	550	560	570	580	590	600
XEC-PQ416229-XHN3178	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMXY
XEC-PQ416242-XHN3193	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ416274-XHN3231	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ416304-XHN3267	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ380322-XHG9508	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ416291-XHN3252	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ416338-XHN3308	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ363310-XHC8685	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ363442-XHC8843	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ380416-XHG9621	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
XEC-PQ416166-XHN3102	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMXY
KP.3.3-PQ206638-XE02	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KP.3.3-PQ345742-XG23	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KP.3.3-PQ363388-XHC8	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KP.3.3-PQ363390-XHC8	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KP.3.3-PQ206586-XE02	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
XEC-PQ416267-XHN3223	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVHTY
KS.1.1-PQ380361-XHG9	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KS.1.1-PQ380341-XHG9	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
KS.1.1-PQ345743-XG23	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY
Consensus	ARVVRISF	SRTLETAQNSV	RVLQKARIT	ILDGISQYSL	RLLIDAHMFT	SDLATNNL	VVMAY

Figure 7A: Multi-alignment of ORF1ab polyprotein of XEC, KP.3.3 and KS.1.1 variants to find a portion of sequence may be obtained from FL.15 sub variants.

Consensus	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
K2*T*T-b0342143-XE53	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
K2*T*T-b038034T-XHE3	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
K2*T*T-b038032T-XHE3	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE5E1-XHN3553	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
Kb*3*3-b050E28E-XE05	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
Kb*3*3-b03E3330-XHC8	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
Kb*3*3-b03E3388-XHC8	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
Kb*3*3-b0342145-XE53	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
Kb*3*3-b050E28E-XE05	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE2E-XHN3T05	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b03804TE-XHE3E5T	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b03E3445-XHC8843	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b03E33T0-XHC8E82	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE338-XHN3308	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE53T-XHN3525	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b0380355-XHE3208	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE304-XHN35E1	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE534-XHN353T	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE545-XHN3T33	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
XEC-b04TE553-XHN3T18	DI22G I I K K B I E G 2 K A G E G K I G U I N E G U A I 2 A B M I I U I A U K B E I K A A 2 I I I I A I B C
	ST0T STT0 STS0 ST30 ST40 ST20 ST00

Figure 7B: Multi-alignment as in figure-5 to demonstrate that part of XEC sequence (ORF1ab AAs at the L2146F boundary) obtained from KP.3.3 sub variant.

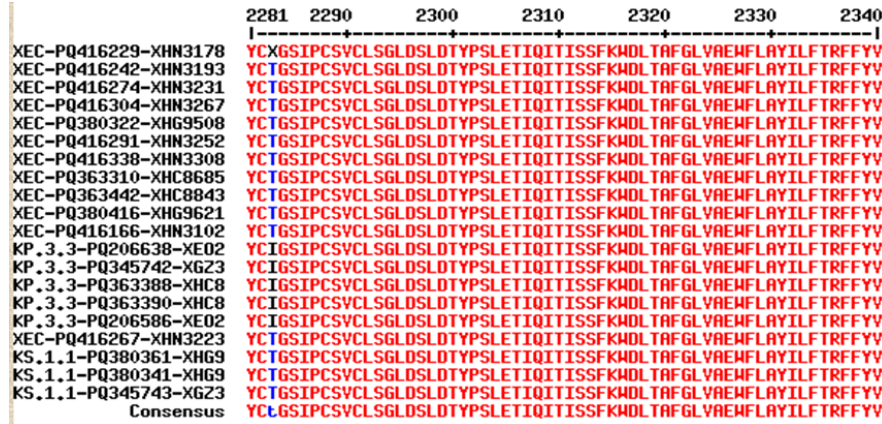


Figure 7C: Multi-alignment as in figure-5 to demonstrate that part of XEC sequence (ORF1ab AAs at the T2283I mutation boundary) obtained from KS.1.1 sub variant

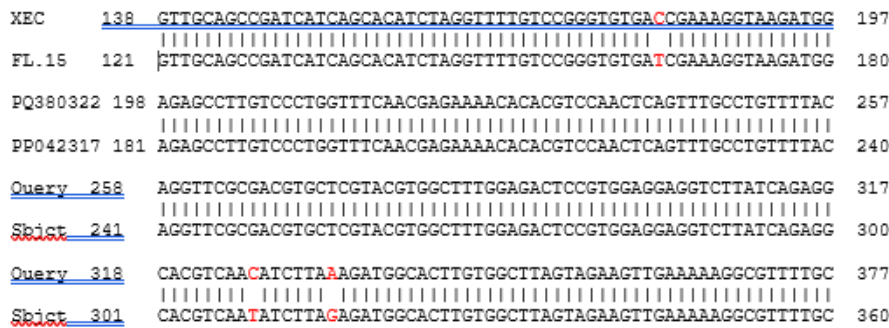


Figure 8: BLASTN-2 demonstration between XEC (PQ380322) and FL.15 (PP042317) that 3'-end was generated from KP.1.1 or KS.1.1 but not from FL.15 as few mutations were seen. Note that such region did not contain any mutation between BLASTN-2 of XEC/KP.3.3 or XEC/KS.1.1 (PQ380322 vs PQ206638 or PQ380322 vs PQ380361).

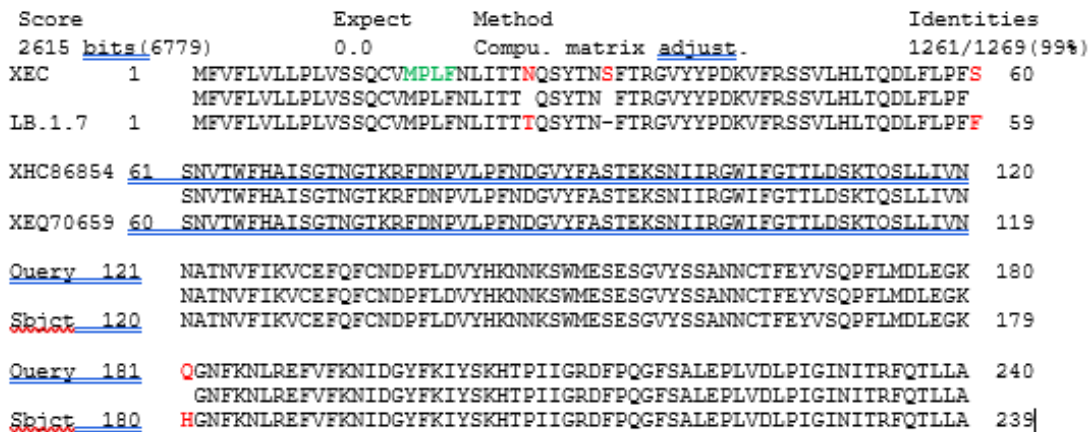


Figure 9: The BLASTP-2 similarities between spike of XEC variant and LB.1.7 sub variant. The only NH2-terminus position was shown here. The both sequences contained 17MPLF spike insertion and 24LPP and 69HV deletions. The 31S deletion was not found with XEC variant and might be seen later. The N26T, S60F, Q181H point mutations might be significant and R343T, E489Q, L1100V and Q1198E mutations were not shown here. The spike of LB.1.7 variants with accession numbers PQ211287, PQ211379, PQ211386, PQ211432, PQ211449, PQ211461, PQ211608, PQ211628, PQ211731, PQ211740 and PQ211834 had similar spike sequences.

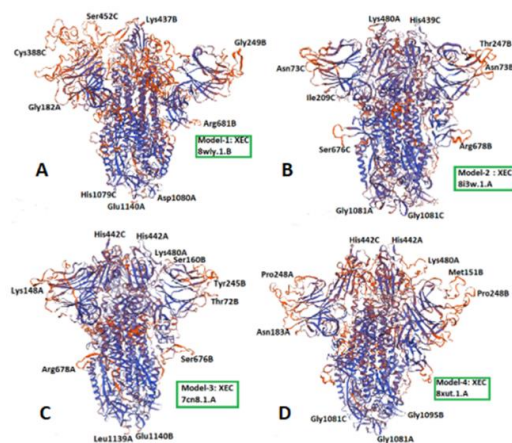


Figure 10: Swiss-Modelling of XEC variant spike with four templates. The best fit oligomeric 3D structure obtained with 7cn8.1.A template © and Model-1 with 8wly.1.B template and Model-4 with 8wly.1.A template made protruding amino acids (open complex). The same three subunits were denoted by A, B and C with few amino acid positions were described.

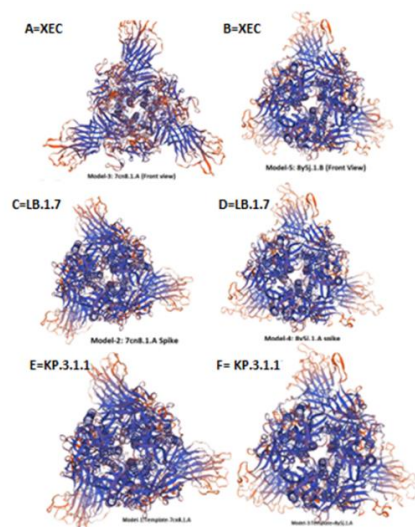


Figure 11: Swiss-Modelling of XEC spike (Front view) and compared with LB.1.7 spike and KP.3.1.1 spike using best fit 7cn8.1.A template and 8y5j.1.A template. The best symmetrical oligomeric 3D spike structure was found with 7cn8.1.A template and XEC spike but very comparable.

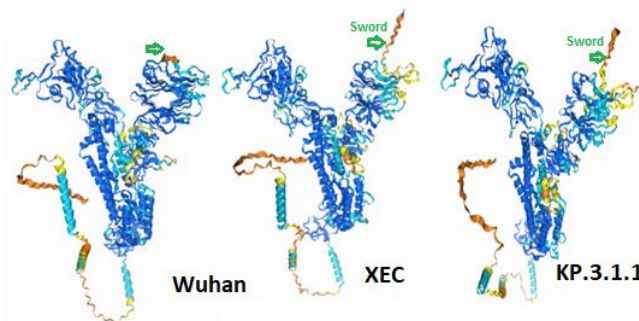


Figure 12: Open complex of single subunit coronavirus spike of Wuhan (B.0), XEC and KP.3.1.1. The XEC and KP.3.1.1 had some sword conformation at the RDB (see arrow) useful for better binding to lungs cells. Such up conformation in XEC and KP.3.1.1 favors ACE-2 binding (Zhao et al., 2022).

Table 1: Spike protein amino acid point mutations in Omicron JN.1 coronavirus (protein id. WUY16791) as BLASTP-2 compared with Wuhan spike.

P O S	1 9	2 1	2 7	5 0	1 2 7	1 4 2	1 5 7	1 5 8	1 7 7	2 1 2	2 1 3	2 1 6	2 4 5	2 6 4	3 3 2	3 3 9	3 5 6	3 7 1	3 7 3	3 7 5	3 7 6	4 0 3	4 0 5	4 0 8	4 1 7	4 4 0	4 4 5	4 4 6	4 5 0
B J P O S	T I 4 5 2	R T 4 5 5	A S 4 6 0	S L 4 7 7	V F 4 8 1	G D 4 8 4	F S 4 8 6	R G 4 8 6	M I 4 9 8	L I 5 0 1	V G 5 0 5	L F 6 1 4	H N 6 2 1	A D 6 5 5	I V 6 7 8	G T 7 6 4	K F 7 9 6	S P 9 3 9	S F 9 4 0	S A 9 6 9	T K 1 1 4	R N 1 1 3	D S 1 1 1	R N 1 1 1	K H 1 1 1	N V 1 1 1	G S 1 1 1	N D 1 1 1	
P O S	4 5 2	4 5 5	4 6 0	4 7 7	4 7 8	4 8 1	4 8 4	4 8 6	4 9 8	5 0 1	5 0 5	6 1 4	6 2 1	6 5 5	6 7 8	6 6 1	7 7 4	7 9 6	9 3 9	9 4 0	9 6 9	1 1 4 3							
B J P O S	L W S K N	L S S K N	N K N K N	S K N K N	T K N K N	N K N K N	E K N K N	F K N K N	Q P N K N	N Y N K N	Y H N K N	D G N K N	P S N K N	H Y N K N	N R N K N	P K N K N	N Y N K N	S F N K N	Q H N K N	N K N K N	P L N K N								

Table 2: Characteristics of few recombinant corona viruses (2019-2024)

No	Variant	Recombinant variants	Accession numbers of important corona viruses that had gone recombination	Actual name of the coronaviruses
1	XAM	AY.122 + BA.2	AY.122=OQ119975/OQ119980 BA.2=OP633365/OQ030587/OQ028416	BA.2=B.1.1.529.2 Delta=AY.122
2	XAJ	BA.2.12.1 + BA.4	BA.2.12.1=OQ098129/OQ098131/ON386383 BA.4=OP825918	BA.2=B.1.1.529.2 BA.4=B.1.1.529.4
3	XBC	BA.2 + B.1.617.2	BA.2=OM901219 B.1.617.2=OM265438/OP599775/OK465723	BA.2=B.1.1.529.2 Delta=B.1.617.2
4	XBF	BA.5.2 + CJ.1	BA.5.2=OP936468/OQ097528/ON999606 CJ.1=	CJ.1=BA.2.75.3.1.1.1.1 BA.5.2=B.1.1.529.5.2
5	XDQ	BA.2.86.1 + FL.15.1.1	FL.1=OR252031 FL.15=OR251957	FL.1=XBB.1.9.1.1 FL.15=XBB.1.9.1.15
6	XBB	BJ.1 + BM.1.1.1	XBB=OQ080149/OX394639 BA.2.75=OP699966 BM.1.1.1=OQ253574	BJ.1->BA.2.10.1 BM.1.1.1-> BA.2.75
7	XEC	KP.3.3 + KS.1.1	XEC=PQ313310/PQ380322 KS.1.1=PQ380361/PQ345743 KP.3.3=PQ380374/PQ206638	KS.1.1=JN.1.13.1.1.1 KP.3.3=JN.1.11.1.3.3

Table 3: Penetration of KP.3.2, KS.1.1 and XEC amino acid point mutations in GenBank database

Protein id	BLASTP sequence (First 60 amino acids of spike protein)	Penetration (GenBank)
1. XEQ88390	Mfvflvllplvssqcvmplfnlitttqsytnsftrgvyyppdkvfrssvlhltqdlflpfs-60	650
2. XEQ89525	Mfafvlvllplvssqcvmplfnlitttqsytnsftrgvyyppdkvfrssvlhltqdlflpfs-60	1
3. XGE36110-KS.1.1	Mfvflvllplvssqcvmplfnlitttqsytnsftrgvyyppdkvfrssvlhltqdlflpfs-60	130
4. XGE36098-KP.3.3	Mfvflvllplvssqcvmplfnlitttqsytnsftrgvyyppdkvfrssvlhltqdlflpff-60	>5000
5. XGD20832-K1081R	Grahfpregvfvsngthwfltrqnfypqiittdntfvsngcdvvgivnntvydplqlle-1140	>2050

6.	XGZ36098-KP.3.3-Q489E	Gpncyfplesygfrptygvghqpyrvvvlselfhapatvcgpkstnlvknkcvnfnfn-540	>4000
7.	V1100L-A1082S	Chdgkshfpregvfvsngthwfltrnfyepqiittdnfsvsgncdvvigivnntvydpl-1138	20
8.	A599T XHN31935	arvvrsifsrletaqnsrvrlqkaaitldgisqysrlidammftsdatnnlvvmty-600	495

Table 4: Parameters of SWISS-MODELLING of XEC variant Spike Protein of SARS-CoV-2

Model-1: Parameters of Swiss-Modelling between XEC spike protein (red) and compared with JN.1 (green), LB.1.7 (Blue) and KP.3.1.1 (yellow) variants using template 8wly.1.B (Bat origin)								
Protein id	QMQE	QSQE	Identity	MolProbity score	Clash score	Ramchandran favoured	Bad bonds	Bad angles
XDF23570	0.69	0.99	74.64%	1.67	1.11	88.42%	1	214
XHC86854	0.68	0.99	74.49%	1.61	0.63	87.51%	0	219
XEQ70659	0.68	0.99	74.63%	1.75	1.69	88.44%	2	244
XHP24317	0.63	0.99	74.27%	1.69	1.19	88.27%		
Model-2: Parameters of Swiss-Modelling between JN.1, XEC and LB.1.7 spike protein variants using template 7cn8.1.A (Pangoline origin)								
Protein id	QMQE	QSQE	Identity	MolProbity score	Clash score	Ramchandran favoured	Bad bonds	Bad angles
XDF23570	0.68	0.97	88.90%	1.45	0.90	94.90%	0	125
XHC86854	0.68	0.97	88.74%	1.37	0.67	95.02%	5	110
XEQ70659	0.68	0.97	88.72%	1.48	0.94	94.66%	0	151
XHP24317	0.69	0.96	88.23%	1.44	0.84	94.9%	0	126
Spike protein Swiss Modelling parameters of XEC variant omicron corona virus using 8y5j.1.B template								
Protein id	QMQE	QSQE	Identity	MolPrabity score	Clash score	Ramchandran favoured	Bad bonds	Bad angles
XHC86854	0.63	0.92	99.00%	1.30	0.48	90.13%	7	188
XEQ70659	0.66	0.93	99.08%	1.21	0.45	89.90%	1	181
XHP24317	0.66	0.92	99.07%	1.27	0.68	90.06%	2	172

Discussion

The XEC variant was originated from KP.3.3 and KS.1.1 JN.1 lineages but we showed that FL.15 subvariant was also involved in the process [25-27]. In otherwards, spike N-terminal amino acid sequence from KS.1.1 made the XEC virus more unique. So far spike amino acids 315-510 (RBD domain) have been implicated in ACE-2 binding. With our model, we have seen the modulation of surface interacting spike amino acids in omicron variants [28]. Indicated universal mutation G142D and common mutation Q146H and R158G as well as A83V to A83F, V90L to V90Y in the NTD domain and T346R/ T346I in the RBD domain of spike of JN.1 variant would possibly contribute to the ongoing evolution of viral lineages, and their overall fitness and

adaptability [29]. Coronavirus replicative RNA recombination may be involved in the entire genome [30]. Non-replicative RNA recombination of an animal plus-strand RNA virus might be possible in the absence of efficient translation of viral proteins and solely mediated by cellular recombination proteins [31]. Certainly, RNA virus populations are diverse due to a variety of factors, including lack of proofreading of the viral RNA-dependent RNA polymerase (RdRP) [32]. There are 11 shared common mutations G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, and N501Y in RBD Omicron sub-variants that may contribute significantly to change the host spectrum of SARS-CoV-2 in immune evasion and potential transmission. The BA.1 variant has 18 unique mutations A67V, H69del, V70del, T95I, V143del, Y144del, Y145del,

N211I, L212V, V213R, ins214EP, R216E, S371L, G446S, G496S, T547K, N856K, and L981F. Whereas, BA.2 has 10 unique mutations T19I, L24del, P25del, P26del, A27S, V213G, S371F, T376A, D405N, and R408S. The Omicron sub-variants (BA.1.1, BA.2 and BA.3) are likely more transmissible than omicron (BA.1) and Delta. This may facilitate RBD interaction with the negatively charged ACE2, hence enhancing the ACE2 receptor's affinity [33]. Kumar et al also predicted that the T95I, N211I, and V213R mutations to be deleterious in BA.1 while Y505H, N786K to be deleterious both BA.1 and BA.2 for ACE-2 interactions. Parsons and Acharya recently reported that omicron trimeric 3-D spike structure was more compact to interact with ACE-2 receptor. They have not used the 3-D JN.1 or XEC spike proteins and have compared the 3-D structure with PDB IDs; Alpha, PDB: 7R13; Beta, PDB: 7LYL; Delta B.1.617.2, PDB: 7TOU; D614G, PDB: 7KDK; BA.1, PDB: 7TL1; BA.2, PDB: 7UB6. Important question if the three RBD domains are down or one RBD is up to interact first with ACE-2 receptor as postulated with recent JN.1 omicron viruses. According to deep mutational scanning, ELISA, and SPR analysis, some of these mutations increased affinity for ACE-2 (N440K, S477N, T478K, G496S, Q498R, N501Y) while others decreased ACE-2 affinity (R346K, K417N, G496S, E484A, G496S, Y505H) [34]. We postulated His442, Pro482, Lys480, Lys478 might be involving amino acids to first interact with ACE-2 receptors. The 31S deletion decreases nAb titers of LB.1, KP.2.3 and KP.3.1.1 in bivalent mRNA-vaccinated healthcare workers and causes escape of antibodies in BA.2.86/JN.1-wave convalescent sera [12]. The JN.1 subvariants, including LB.1, KP.2.3, and KP.3.1.1, are completely resistant to neutralization by S309, one of the most broadly neutralizing antibodies have tested. The Q493E mutation in KP.3.1.1 was thought to be important for recent higher infectivity of KP.3.1.1 similar to L455S and F456L enhanced the ACE-2 binding. Mutations within CD8+ epitopes in N protein (M322I and L331F), M protein (L90F) and the spike protein (L270F) and Nsp3 T504P were reported within minority variants during the course of acute infections, resulting in loss of epitope-specific responses and pathogenesis [10]. The M-protein I82T and N-protein R203M/R203K mutations were also reported worldwide [35]. We found D3H, A63T and A104V mutation in the M-protein of JN.1 variants. Thus, convergent evolution in JN.1 subvariants with R346, L455, and F456 mutations in the RBD of spike has further exacerbated immune escape, underscoring an urgent need for updated vaccine development during 2024 [36]. Interestingly, Paxlovid drug was quite satisfactory reducing the symptoms of omicron coronaviruses [37,38]. Surely, XEC and KP.3.1.1 should be studied more carefully using whole genome sequencing and new vaccine development with modified spike must be continued.

Conclusion

We concluded and reviewed the genesis of XEC variant and KP.3.1.1 variant that had favorable compact 3-D conformation for higher transmission. The main differences XEC variant had no 31S deletion in spike while most KP.3.1.1 and LB.1.7 variants had 31S spike deletion. The important 17MPLF spike insertion was found in most (>95%) omicron JN.1 lineages. The 49nt deletion in the 3'-UTR of JN.1-related variants is minimum (<1%) and few scientists are not sequencing that region as getting conflict data.

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