

Abundant Transmission of Corona Virus Nsp2 RNA Topoisomerase I120F Mutants with Concurrence D614G Spike Protein Mutation in Australia

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ABSTRACT

We previously predicted Nsp2 Corona virus protein as RNA topoisomerase through amino acid homology among *Vibrio haemolytica* DNA topoisomerase IA/IV as well as DNA primase, DNA gyrase and bi-subunit *Trypanosoma brucei* DNA topoisomerase IB. Many DNA topoisomerase I/III have RNA topoisomerase activity and such ubiquitous enzymes are conserved and involved in the regulation of replication and transcription. We have checked here mutational profile of Nsp2 RNA topoisomerase analyzing >10000 orf1a 4405 amino acid length Corona virus polyprotein. Mutant proteins were selected by BLAST search having 99.84% sequence similarity and 181-818 aa portion Nsp2 protein (protein id. QIU82057) was analyzed using CLUSTAL Omega software. We found >30 different mutations where most changes were selected at Isoleucine and Alanine into Valine or Leucine into Phenylalanine pinpointing conserved nature of the Corona virus RNA topoisomerase. Major missense very abundant mutations were found at I120F (Isoleucine to Phenylalanine). Other important mutations were R27C, I198V, T85I, L410F, I559V and P583S. The I120F mutation was abundant in Australian isolates and its spread was seen in the Bangladesh and other countries like USA. We suggest that abundant I120F mutation of Nsp2 Topoisomerase may increase transmission of Corona virus by stabilizing RNA structure for efficient virus packaging. Interestingly, such mutations were found in association of D614G mutation of Spike protein, known to >70% increase infectivity. On the contrary, all P583S Nsp2 mutants analyzed had no concurrence D614G spike protein mutation. Many silent mutations (5-7) were detected by genome wide analysis but no N501Y Spike protein mutation. This is first report that predicts a link of greater Corona virus transmission with Nsp2 protein I120F mutation and may be important to discover new anti-viral drugs.

Keywords: Corona virus; Nsp2 protein; RNA topoisomerase; Point mutation; Multi-alignment; Higher transmission; Australian isolates

INTRODUCTION

The COVID-19 pandemic has caused more than 1.8 million deaths and over 50 million confirmed cases worldwide, according to the World Health Organization. Corona viruses are large single-stranded positive sense RNA viruses. There are 26 putative coding regions which cover about 98% of the 29.9 kb SARS-CoV-2 genome [1]. Approximately two-thirds of the genome at the 5' side encodes the two large non-structural polyproteins (orf1ab, 7096 aa and orf1, 4405 aa) in same reading frame and one-third at the 3' side encodes four structural proteins: spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N) [2,3]. Polyproteins are degraded into sixteen proteins like proteases (Nsp3 and Nsp5), RNA-dependent RNA polymerase (Nsp12), rRNA methyl transferase (Nsp13, Nsp16 etc.) and RNA topoisomerase

(Nsp2) [4,5]. We have suggested that peptide vaccine from RNA topoisomerase and rRNA methyl transferase may be useful as many mutations are accumulating in the mostly targeted Spike protein of Corona virus [6-8].

COVID-19 is related to SARS and MERS Corona viruses [9]. The mutation rate in the SARS Corona virus genome was estimated to be $0.80-2.38 \times 10^3$ nucleotide substitution per site per year which is in the same order of magnitude as other RNA viruses [10,11]. In other related infectious bronchitis virus, it was estimated to be $0.3-0.6 \times 10^{-2}$ per site per year [12]. Recent estimates suggest that circulating SARS-CoV-2 lineages accumulate nucleotide mutations at a rate of about 1-2 mutations per month [13]. The new variant strain, VUI-202012/01, may be up to 70% more transmissible and could increase the R number by 0.4. Mutations are common to the RNA virus and usually occur due to combination of natural drift

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and pressure from the human immune system [14].

Proteins are folded into highly complex 3-D shapes, depending on the interactions between different amino acids side chain in the same string [15]. Changing an amino acid of positively charged with one for a negatively charged one, will change the shape and active site. DNA topoisomerases are 2 types called type I and type II DNA topoisomerases which change linking number by 1 or 2 respectively [16]. Such enzymes have nuclease activity, swivel strand passing activity and ligation activity [17,18]. Some type I topoisomerases have shown with RNA topoisomerase activity [19]. We predicted a RNA topoisomerase activity in the Corona virus genome to resolve the knots of dsRNA to synthesis mRNAs and virus packaging of (+) sense ss-RNA [20]. We have shown unknown Nsp2 protein of Corona virus polyproteins has sequence similarities with *Vibrio haemolytica* DNA topoisomerase I/IV [4]. We also able to dissect the 5'-end ATPase, middle topoisomerase and 3'-end RNA-binding domains by similarity search with amino acid sequences of bi-subunit *Escherichia coli* DNA Gyrase and bi-subunit *Trypanoma brucei* DNA Topoisomerase IB where ATPase, DNA-binding and topoisomerase activities located in different subunits and domains [4,18]. Thus, we want to check the mutation motifs of Np2 protein from different countries and their abundance in the GenBank database to predict virus stability and infectivity. Such study with Corona virus spike protein gave monumental result where D614G mutation of spike protein showed abundant and had increase in infectivity due to its better interaction with ACE-2 receptor of human cells [21-23]. We found at least 26 different point mutations in the Nsp2 RNA topoisomerase and I120F mutants were abundant in the database. We also found most mutations of Nsp2 proteins associated with D614G spike protein mutation except Nsp2 P583S mutants [24].

MATERIALS AND METHODS

Identification of normal and mutant orf1a 4405 amino acid polyprotein amino acid sequence of Corona virus (COVID-19)

We selected Accession number QIU82057 China isolate as normal strain thinking such isolates originate in China first (Isolation date 22-01-2020). When we BLAST searched 648 aa Nsp2 sequence, >4000 sequences are 4405 amino acids with 100% identical and few hundred are 99.84% suggesting Nsp2 mutants [25]. To select more mutants from NCBI GenBank database, we write orf1a, corona virus and country name in the portal www.ncbi.nlm.nih.gov/protein and selected few 4405 aa length orf1a mutant polyprotein sequences. Those mutants were not abundant (1-8) and would take long time during BLAST. So we reduced the search algorithm parameter (at the 2/3 position of the NCBI portal) into 10. If 100% were mutants using such parameter as 10, then we increased algorithm into 100 to 250 to 5000 depending on 100% similarity outcome to trap all mutants [5].

Multi-alignment and selection of mutants and their spread in the different countries

We selected 638 amino acids length Nsp2 sequences and multi-align was performed by CLUSTAL-Omega software and from the mismatch of amino acid sequence we got mutant position [26,27]. We then selected phylogenetic relation and phylogenetic picture gave the cluster of normal verses mutants. Such study also showed the different countries in the same mutant cluster indicating the

spread from one country into another.

Detection of specific mutant population and spread into different countries

To check the mutant population in the GenBank database, we put different mutant Nsp2 amino acid sequence into NCBI BLAST for protein (www.ncbi.nlm.nih.gov/blast) and 100% similarity sequences are mutants and 99.84% are normal. We selected and opened all mutants amino acid sequences and checked their country origin of individual Accession number and date of collection collected. Most mutants protein id were given in the analysis so that anyone can check any orf1a sequence for further analysis. Further, RNA sequencing error might be happen and usually such sequence should be removed from database. Thus, those mutants appeared in many countries with high density and were sequenced by different reputed laboratories could be assigned as authentic.

Genome analysis and relation into spike protein D614G hyper-infectivity mutant

We first open orf1a amino acid sequence and then we selected individual accession number to find the spike protein sequence. From the downstream Corona virus sequence, we selected nucleotide sequences of Nsp2, S proteins or full length sequence. Then, we analyzed the sequences by Clustal-omega and Multalin softwares.

Genome analysis to get nucleotide substitution and amino acid changes

We first download the genome sequence from Genbank database and compared with standard Corona virus genomic sequence with Accession no. MT079853. Two sequences were aligned by Multalin software and mismatch point noticed. To get the amino acid position and proper codon change in the alignment, we detected position of mutation and subtracted 225 nt UTR and then divided by 3 to get amino acid position. Then, we checked the corresponding position in the Nsp2 (say) in the ORF1a amino acid sequence and scanned the codon table to assure the amino acid [28]. If amino acid matched then we deducted 180 aa of Nsp1 amino acid to get the Nsp2 amino acid position. The position of sixteen Nsp non-structural proteins are known from the genome database. In case of structural N, M N, E proteins, corresponding nucleotide position was checked from database individual accession number to assure if it fall in that nucleotide sequence position and deducted nucleotides from the N-protein (say) start position and resulted nucleotide then divided by three to get N-protein amino acid position. Then, we checked the codon table to assign amino acid to fit normal verses mutant amino acid. Then, individual protein sequence (say N protein) was compared normal vs. mutant to assure correct interpretation and position by Seq-2 BLAST search. The normal genome RNA sequence Accession number MT079853 was taken as standard and normal orf1a protein amino acid sequence with protein id. QIU82057 was taken as standard.

RESULTS

We searched for Corona virus Nsp2 RNA topoisomerase mutants by multi-alignment and phylogenetic analyses (www.ncbi.nlm.nih.gov/blast). We searched orf1a, Covid-19 and name of country into NCBI portal (www.ncbi.nlm.nih.gov/protein) to get as many as sequences. Figure 1 showed the sequence of Nsp2

RNA topoisomerase and its position onto Corona virus 29.9 kb ss-RNA genome. The position of Spike protein at the 3'-end of the genome was also identified in Figure 1. Figure 2 showed the region of alignment of different Nsp2 proteins where maximum mutations were observed. The phylogenetic relation of mutants vs. normal Nsp2 proteins was shown in Figure 3 indicating about 1/3 of the Nsp2 proteins are mutants. We used China isolates from December 2019-January 2020 to get standard amino acid sequence of Nsp2 protein (Accession nos. YP_009725295, NC_045512, MT079853). Abundant mutations were observed as we predicted that Nsp2 RNA topoisomerase was conserved and such enzyme was involved in the regulation of Corona virus replication and transcription. So, we extended our studies to different countries, selecting NCBI GenBank database search using country name, orf1a and covid-19. When such Nsp2 mutants were analyzed, we found I120F Nsp2 mutant were abundant. Such multi-alignment portion was shown in Figure 4 and phylogenetic relation was shown in Figure 5 predicting that I120F mutants were very abundant in the database and thus hyper-infective similar to D614G (Aspartic acid vs. Glycine) mutant of spike (S) protein of Corona virus. It appears Australian Corona virus isolates were mostly I120F Nsp2 mutant and we selected more mutants from database. Analysis showed that Bangladesh Corona virus isolates were clustered with Australian isolates suggesting Corona virus spread between two countries (Figure 6). All the 26 mutations of Nsp2 protein was tabulated in Table 1.

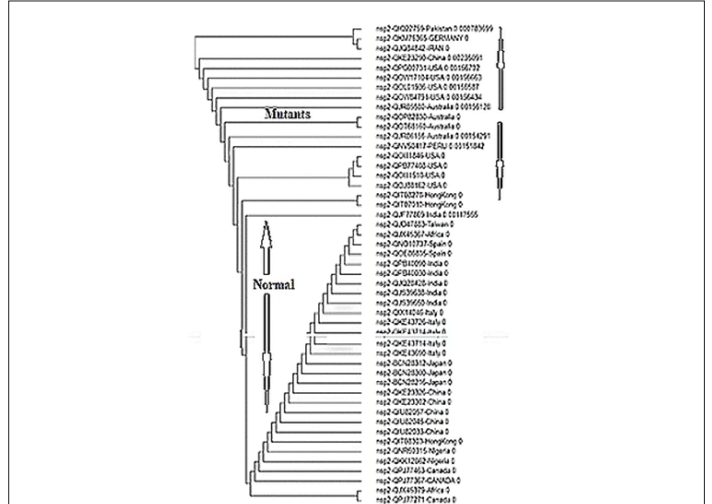


Figure 3: Phylogenetic selection of normal vs. mutant Nsp2 protein showing abundant mutation.

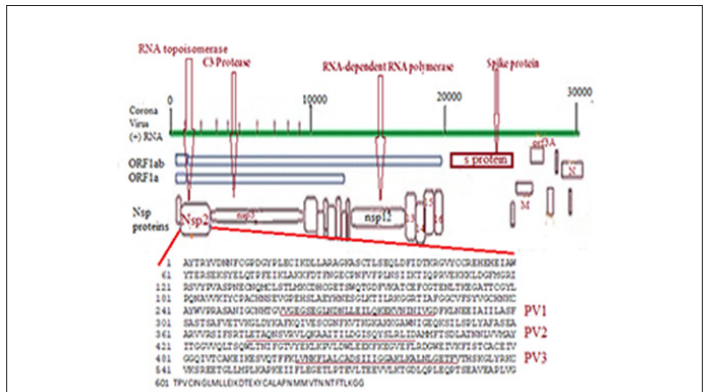


Figure 1: Structure of COVID-19 genome and amino acid sequence of Nsp2 RNA Topoisomerase.

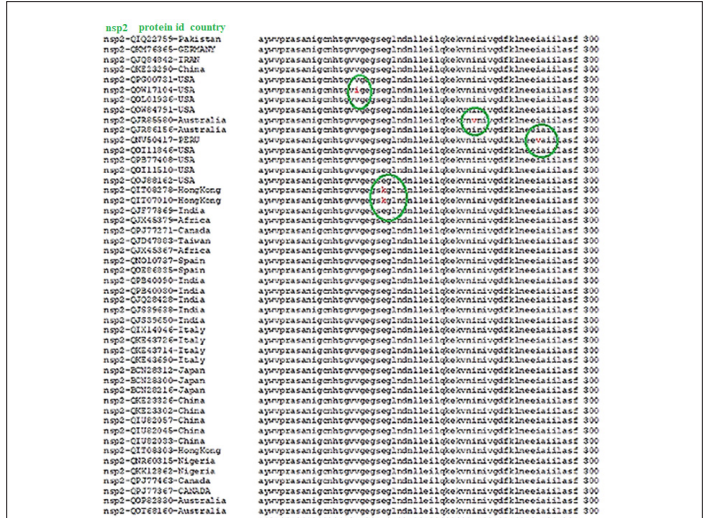


Figure 2: Multi-alignment of Nsp2 protein of different Corona virus isolates showing cluster of mutations between 240-300 amino acids.

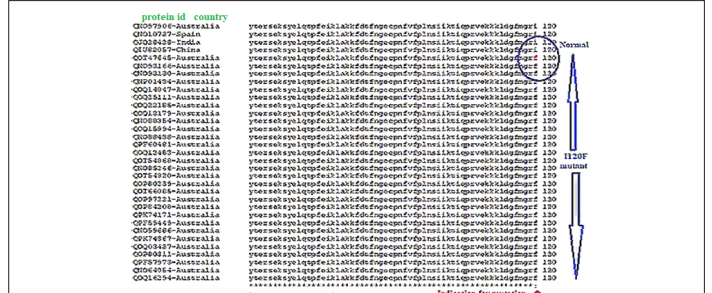


Figure 4: Multi-alignment of Nsp2 protein of Corona virus isolates showing I120F mutation and its high transmission into Australia.

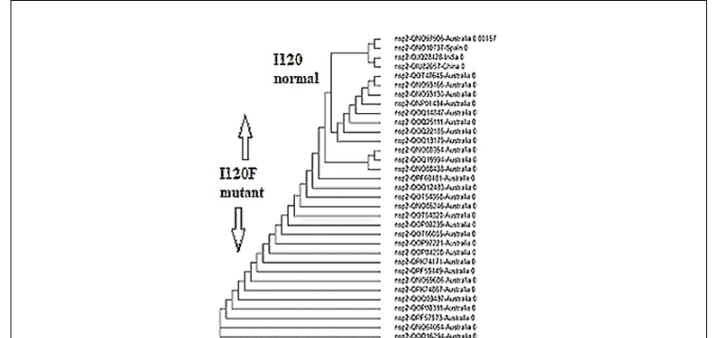


Figure 5: Phylogeny of I120F Corona virus Nsp2 protein mutant in Australian isolates between June-November collection dates.

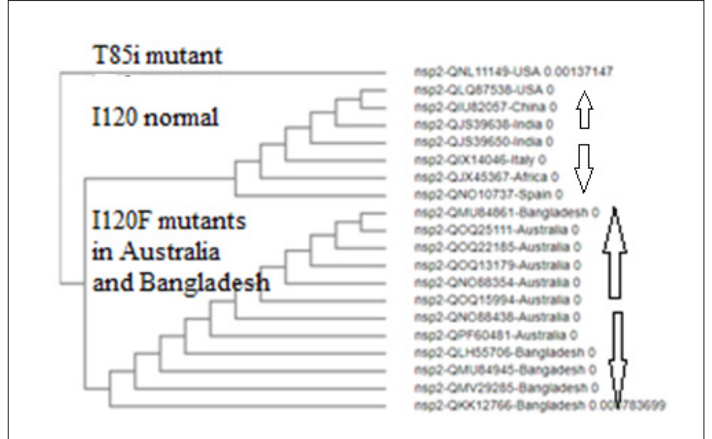


Figure 6: Phylogenetic relation of Nsp2 I120F mutant of Australian isolates and its spread to Bangladesh.

Table 1: Mutations and spread of Nsp2 RNA Topoisomerase of Corona virus.

Serial. No.	Mutation and amino acid position	Population of the nsp2 mutants	Accession number in the Genbank database	Nucleotide change in the Corona virus genome	Protein id ORF1a of Corona virus polyprotein	Origin of the Corona virus	Spike mutation at 614 amino acid
1	F10=L	8	MW420694	U>C	QQQ78828	USA	G614
2	L24=F	9	MW023471	U>C	QNU11135	USA	G614
3	R27=C	14	MW191505	C>U	QOU99215	India	D614*
4	T45=I	1	MW166221	C>U	QQQ72554	India	G614
5	T85=I	>80	MW309425	C>U	QPJ77271	Canada	G614
6	I120=F	>4000	MW183934	A>U	QOT47645	Australia	G614
7	A159=V	35	MW420612	C>U	QQQ79846	USA	G614
8	V198=I	12	MW81828	G>A	QOS50880	India	D614*
9	S248=N	1	MN994467	G>A	QHQ71962	USA	D614*
10	A249=V	2	MT883503	C>U	QNC69817	Serbia	G614
11	V259=I	1	MW197485	G>A	QOW17104	USA	G614
12	E264=K	5	MT846524	G>A	QMU92722*	USA	E614
13	I281=V	2	MT450961	A>G	QJR85580	Australia	D614*
14	I293=V	1	MW030272	A>G	QNV50417	Peru	G614
15	A294=V	1	MW077498	C>U	QOI11882	USA	G614
16	A302=V	4	MT929131	C>U	QNL12829*	USA	G614
17	A336=V	8	MW309470	C>U	QPJ77797	USA	G614
18	S366=P	7	MW420612	U>C	QQG77846	USA	G614
19	A375=T	1	MW065478	G>A	QOF21676	USA	G614
20	R380=H	12#	MW420694	G>A	QQG78828	USA	G614
21	I393=V	4	MW206450	A>G	QOW84791	USA	G614
22	L410=F	>30	MT928991	G>T	QNL11149	USA	G614
23	V425=I	1	MW280543	G>A	QPG00731	USA	G614
24	A476=V	4	MW280547	C>U	QPG00779	USA	G614
25	Q483=R	3	MW010238	A>G	QNT08568	USA	G614
26	V485=I	>30	MW077467	G>A	QOI11510	USA	G614
27	I559=V	>64	MT407655	A>G	QKE23290	China	D614*
28	P583=S	>64	MT407655	C>U	QKE23290	China	D614*
29	I605=V	1	MW115047	A>G	QOL01936	USA	G614
30	G638=S	1	MT093571	G>A	QIC53203	Sweden	D614*

Note: * means normal amino acid at 614 position. # means some partial sequences have mutant allele but not included here.

We searched individual mutant Nsp2 amino acid sequence by BLAST to get the population density of individual mutant (Table 1, Column 3). We found that I120F Nsp2 mutant were very abundant (>4000 mutants) with respect to other mutant strains. Then, we checked if spike protein D614G mutation was occurred with Nsp2 I120F mutation. Our first such analysis was shown in Figure 7 indicating abundant D614G mutation in our Nsp2 I120F isolates. Such analysis detected Spike protein S477N mutation in four Australian isolates with Accession nos. MW183334, MW153105, MW156105 and MT971831. We also found T22I Spike mutation in MT582454 Germany isolate (protein id. QKM76366) with no D614 mutation and two V3G and L5F extra mutations in Canada isolate (protein id. QPJ77464) with D614G mutation (data not shown). Further, to confirm our hypothesis of higher transmission of I120F mutants, we searched more mutants from Australia (accession nos. MW154848, MW154818, MT971202, MT969734, MW184478, MW156737, MT971875, MT970805, MT970517, MW153100, MW185536, MW15981, MW156921, MW185678, QW156991, MT972745, MW157103, MW184445,

MW185110, MW277558, MW154653, MT971287, MW154664, MW321131, MW184624, MW185253, MW184329). Similarly, we found few United States isolates with I120F mutants (Accession nos. MW075791, MT811489, MW056129, MW276575) and one Bangladesh isolates with I120F mutant. All I120F mutants from Australia, USA and Bangladesh discussed above had D614G mutation in the spike protein of Corona virus. Further we extended our search to prove the Australian I120F Nsp2 mutants transmitted into Bangladesh. For that we used genomic mutant sequence (901 gggtgaaaa gaaaaagctt gatggcttta tggtagatt tcgatctgtc tatccagttg) for BLASTn and received >4500 I120F mutant sequences referring ORF1ab 7096 aa large polyprotein. Although most sequences were isolated in the Australia, we identified 16 Bangladesh I10F Nsp2 mutants with D614 spike protein mutation together to prove that I120F Nsp2 mutants indeed spread into Bangladesh. The Accession numbers of the Bangladesh double mutant isolates were: MT576639, MT846005, MT731734, MT576641, MT539159, MT664171, MT847211, MT661278, MT856455, MT676414, MT581426, MT667351, MT846011, MT731735, MT578017, and

detected few extra mutations (S704L and M740I; Accession no. MW276622) and (Q321Z; Accession no. MW276643) in the spike protein. Thus, we clearly demonstrated a relation of deadly D614G Spike protein and Nsp2 I120F mutant with Corona virus spread worldwide [23].

Kailany, et al. have reported orf1ab mutations (A1176V, L3606F, E3764D, Y5720C, P6083L) of Corona virus but we found accession numbers (LC522972 and LC522972) were removed from the NCBI database reflecting some error in the sequencing [30]. When Nsp2 protein of rest four sequences were analyzed, we found end amino acid of Nsp2 protein was changed from Glycine to Serine (Figure 12). Kailany, et al. also reported many L84S mutation in the orf8 gene whose function remains elusive (see, accession nos. LC522973, LC522974, LC522975, MT066175, MT188339, MN975262, MN994467).

RNA topoisomerase is an important enzyme of COVID-19 and such research must be augmented [32]. We checked 26 mutants of Nsp2 enzyme but only six mutants with moderate spread would be important and I120F hyper-spread mutant was a concern. High rate mutation accumulation over short time periods have been reported previously in studies of immune-deficient or immune-suppressed patients who are chronically infected with SARS-CoV-2 [27]. Corona virus B.1.1.7 lineage N501Y mutation increased ACE2 receptor affinity to enter into human lung cells [21]. N501Y has been associated with increased infectivity and virulence in a mouse model but data limited in the GenBank. The N501Y mutation co-occurs with several mutations in the N, ORF1a, ORF8 proteins and in S protein 501Y Variant 2, but such \pm complete genome data were not deposited to GenBank yet [33,34]. A222V Spike mutation is also emerging in B.1.177 lineage and last month its number increased to >7000 in the UK (https://www.cogconsortium.uk/wp-content/uploads/2020/12/Report-1_COG-UK_20-December-2020_SARS-CoV-2-Mutations_final_updated2.pdf). Thus, our data is worthy to predict role I120F mutation of Nsp2 RNA topoisomerase emerging in the Australia [35]. We could not find much I120F mutant in Indian isolates but we should carefully perform RNA sequencing to predict such transmission which may be occurring in the Bangladesh. Recently, many vaccines for Corona virus have developed and vaccination started in Europe and America [36]. We have shown some similarity of Nsp2 RNA topoisomerase to ribosomal L1 protein and with other non-structural proteins like Nsp13 (L6 and L9) capping mRNA methyl transferase with RNA helicase activity. These suggested such Corona virus proteins may assemble into host ribosome affecting protein synthesis and oxidative phosphorylation [6,37]. Recently, nanotechnology and gene medicine have been given priority to challenge Corona virus spread with 1.7 millions death worldwide [38]. Integrative mutant analyses of SAR-COV-2 genomes from different countries may give clues for host-virus interaction, pathogenesis and development of better therapeutics [14,15,39]. Truly, vaccination research has given priority and vaccination against Corona virus has started [1,36,40,41]. We have proposed three peptide vaccine candidates from Nsp2 RNA topoisomerase [258-285 aa; 372-401 aa and 501-531 aa regions] (Figure 1 underlined sequence). E264K mutation may affect vaccine efficacy in PV1 peptide, similarly A375I mutation may increase vaccine efficacy in PV2 peptide. While no mutation in the PV3 peptide was noticed (NH₂-LVNKFLALCADSIIGGAKLKLNLGETFV-CO₂H) suggesting a good peptide vaccine candidate with many hydrophobic amino acids and glycosylation signals [4]. Corona

virus pathogenesis often associated with multidrug-resistant bacterial infection. We have shown that phyto-extracts from Cassia fistula and Suregada multiflora bark and root efficiently inhibited different MDR-bacteria isolated from Ganga River, Chicken and Milk as well as human subject [42-44]. We demand action of different phyto-chemicals should be tested on the replication and transcription of Corona virus.

Recently COVID-19 mutation database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/scov2_snp) has listed more mutations but such mutations have no role in corona virus spread. The new Nsp2 mutations reported are: P17S, E19D, T44S, G47S, E53D, Y61F, P91S, R107S, V126F, P129L, G265S, D268N, L274F, A360T, V364L, R370H, P371I, T436I, K454E, L462F, V485I, E493K, Q496H, K500N, K534R, G548C, P568L, T569R, P585S, P597S, K618N, P624S and T634I etc.

CONCLUSION

We have detected 30 RNA topoisomerase mutants of corona virus and we think I120F mutants have role in higher transmission. Interestingly D614G spike protein and P4715L RdRP mutations have abundant association whereas nsp4 S2874L mutation has some association in corona virus spread. Such epidemiological analysis is important to choose peptide vaccine candidate and may help for new drug design.

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CONFLICT OF INTEREST

The Author has no conflict of interest.

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