Biomedical Research and Therapy, 8(5):4367-4381

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Original Research

Immunological and mutational analysis of SARS-CoV-2 structural proteins from Asian countries

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ABSTRACT

Introduction: The emergence of a novel coronavirus, SARS-CoV-2, an etiologic agent of coronavirus disease (COVID-19), has led to a pandemic of global concern. Considering the huge number of morbidity and mortality worldwide, the World Health Organization declared, on 11th March 2020, the pandemic as an unprecedented public health crisis. The virus is a member of plus sense RNA viruses that can show a high rate of mutations. The ongoing multiple mutations in the structural proteins of coronavirus drive viral evolution, enabling them to evade the host immunity and rapidly acquire drug resistance. In the present study, we focused mainly on the prevalence of mutations in the four types of structural proteins- S (spike), E (envelope), M (membrane), and N (nucleocapsid)- that are required for the assembly of a complete virion particle. Further, we estimated the antigenicity and allergenicity of these structural proteins to design and develop a potentially good candidate vaccine against SARS-CoV-2. **Methods**: In the present *in silico* study, envelope protein was found to be highly antigenic, followed by the nucleocapsid, membrane, and spike proteins of SARS-CoV-2. **Results**: In this study, we detected 987 mutations from 729 sequences from Asia in October 2020, and compared them with China's first Wuhan isolate sequence as a reference. Spike protein showed the highest mutations with 807 point mutations among the four structural proteins, followed by nucleocapsid with 151 mutations, while envelope showed 19 mutations and membrane only 10 point mutations. **Conclusion**: Taken together, our study revealed that variations occurring in the structural protein of SARS-CoV-2 might be altering the viral structure and function, and that the envelope protein appears to be a promising vaccine candidate to curb coronavirus infections.

Key words: allergenicity, antigenicity, COVID-19, mutation, SARS-CoV-2, structural proteins, vaccine

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History

- Received: May 16, 2021
- Accepted: May 28, 2021
- Published: May 31, 2021

DOI : 10.15419/bmrat.v8i5.675

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INTRODUCTION

Human Coronavirus (SARS-CoV-2, Severe acute respiratory syndrome) is a positive-sense RNA virus. As an etiologic agent of coronavirus disease 2019 (COVID-19), the virus induces moderate to severe respiratory distress[1]. This pandemic originated from an animal market in Wuhan city of China[2]. The ripple effect of this contagious viral disease has created a humanitarian health crisis and has become an enormous challenge to the entire health systems across the globe.

SARS-CoV-2 is a member of the Coronaviridae family and Nidovirales order. The virus is considered the third zoonotic coronavirus (after SARS-CoV and MERS-CoV) and originated from bats. However, this novel coronavirus has been the only one having pandemic potential[3–6]. SARS-CoV-2, a beta coronavirus, is an enveloped single-stranded, positive-sense, non-segmented and genetically diverse RNA virus with the largest genome size among known RNA viruses (29,891 ase pair, encodes for approximately 9860 amino acids)[2,7,8]. The genome of SARS-CoV-2 encodes both structural proteins like spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as non-structural proteins ranging from NSP1 to NSP16.

RNA viruses, generally, show a drastically high rate of mutation, substantially higher than those of DNA viruses. Due to this high rate of mutation shown by SARS-CoV-2 over a short period, it has been observed that viruses exhibit genomic variability which enables them to modulate virulence properties in the host and subsequently evade the host immunity[9,10].

In the present research work, we detected 987 mutations from 729 sequences derived from Asia in in the October. Altogether spike showed the highest mutations with 807 point mutations among the four structural proteins, followed by nucleocapsid with 151 mutations. Envelope showed 19 mutations and membrane showed only 10 point mutations. The results of our study suggest that mutational analysis of this virus might be considered as a new approach to help understand its genomic variability. Similarly, using

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Cite this article : Kumar Jha D, Yashvardhini N, Kumar A. **Immunological and mutational analysis of SARS-CoV-2 structural proteins from Asian countries**. *Biomed. Res. Ther.;* 8(5):4367-4381.

the predictive tools of immunoinformatics approach, the antigenicity and allergenicity of the structural proteins of SARS-CoV-2 have been determined to develop efficacious antiviral therapeutics or vaccines against COVID-19.

METHODS

Data mining

The full-length protein sequences of SARS-CoV-2 structural proteins, *i.e.*, envelope protein, nucleocapsid phosphoprotein, surface glycoprotein and membrane glycoprotein, were retrieved from the NCBI virus database, as submitted from Asia in the month of October. There were 729 SARS-CoV-2 structural protein sequences submitted from Asia in the month of October, including sequences of 165 envelope proteins, 159 nucleocapsid phosphoproteins, 246 surface glycoproteins, and 159 membrane glycoproteins. A total of four reference sequences for envelope protein (YP_009724392), nucleocapsid phosphoprotein (YP_009724397), surface glycoprotein (YP_009724390), and membrane glycoprotein (YP_009724393) were also retrieved for mutational studies.

Multiple sequence alignment (MSA) and mutational identification

Multiple sequence alignment was performed using Clustal Omega online platform (http://www.clustal.org/) based on HMM profile seeded guide trees [11]. The envelope, nucleocapsid phosphoprotein, surface glycoprotein, and membrane glycoprotein were aligned with their respective reference sequences. The aligned files were viewed using Jalview (https://www.jalview.org/) to identify the point mutations occurring in different structural proteins with respect to the Wuhan type isolate.

Antigenicity and allergenicity evaluation

Vaxijen v2.0 server was used for the estimation of antigenicity of all the four structural proteins to study the capability of structural proteins to be used in vaccine production. This online server predicts antigens as per the auto cross-covariance (called ACC transformation) of the peptide sequences submitted to it [12]. A good vaccine needs to be non-allergenic to the host, hence the rationale for evaluating the allergenicity of these structural proteins, AllerTOP server was used, which predicts allergenicity based on size, flexibility, and other parameters [13].

Table 1: Mutational location after Multiple Sequence Alignment of SARS-CoV-2 envelope protein sequence with position and sequence

Serial No.	Accession	Mutated sequence and position
1.	BCM16104	S68F
2.	BCM16116	S68F
3.	BCM16128	S68F
4.	BCM16176	S68F
5.	BCM16188	S68F
6.	BCM16200	S68F
7.	BCM16212	S68F
8.	BCM16140	S68F
9.	QOP57282	V75F
10.	QOP57300	V75F
11.	QOP57289	V75F
12.	QOP57280	V75F
13.	QOP57294	V75F
14.	QOS50800	V75F
15.	QOS50895	I46V
16.	QOS50728	V75F
17.	QOS50501	V75F
18.	QOU99241	I46V
19.	QOU99253	I46V

RESULTS

Mutational identification

A total of 729 structural protein sequences were retrieved from the NCBI virus database for spike glycoproteins, nucleocapsid phosphoproteins, envelope proteins, and membrane glycoproteins submitted from Asian countries in the month of October 2020, along with four references sequences. The size of the different reference structural proteins, *i.e.*, spikes glycoprotein, nucleocapsid phosphoprotein, envelope protein, and membrane glycoprotein being 1273, 419, 75, and 222 amino acids.

The sequences were viewed using Jalview after alignment to compare and detect the mutations among the Asian isolates with the Wuhan isolates with respect to structural proteins. Amongst the 729 sequences released from Asia, a total of 987 point mutations were detected in all four structural proteins (**Figure 1**). Among the 311 mutants, spike showed the highest mutations with 807 point mutations (**Table 3**), followed by nucleocapsid with 151 mutations (**Table 2**), while envelope showed 19 mutations (**Table 1**) and membrane showed only 10 point mutations (**Table 4**).

Table 2: Mutational location after Multiple Sequence Alignment of SARS-CoV-2 nucleocapsid phosphoprotein sequence with position and sequence

Serial No.	Accession	Mutated sequence and position
1.	QJF74875	R203K
2.	QJF74875	G204R
3.	QKM75385	R203K
4.	QKM75385	G204R
5.	QKM75397	R203K
6.	QKM75397	G204R
7.	QKM75409	R203K
8.	QKM75409	G204R
9.	QKM75421	R203K
10.	QKM75421	G204R
11.	QKM75433	R203K
12.	QKM75433	G204R
13.	QKM75445	P207L
14.	QKM75445	M210I
15.	QKM75505	R203K
16.	QKM75505	G204R
17.	QKM75505	D377G
18.	QKM75517	R203K
19.	QKM75517	G204R
20.	QKM75517	D377G
21.	QKM75529	R203K
22.	QKM75529	G204R
23.	QKM75529	D377G
24.	QKM75541	G204R
25.	QKM75541	D377G
26.	QKM75541	R203K
27.	QKM75552	R203K
28.	QKM75552	G204R
29.	QKM75552	D377G
30.	QKM75563	R203K
31.	QKM75563	G204R
32.	QKM75563	D377G
33.	QKM75575	R203K
34.	QKM75575	G204R
35.	QKM75587	R203K
36.	QKM75587	G204R
37.	QKM75599	R203K
38.	QKM75599	G204R
39.	QKM75647	R203K
40.	QKM75647	G204R
41.	QKM75659	R203K
42.	QKM75659	R204R
43.	QKM75683	R203K
44.	QKM75683	G204R
45.	QKM75695	R203K
46.	QKM75695	G204R
47.	QKQ30536	R203K
48.	QKQ30536	G204R
49.	QKQ30548	R40C
50.	QKQ30560	R203K
51.	QKQ30560	G204R
52.	QKQ30572	R203K
53.	QKQ30572	G204R
54.	QKQ30584	R203K
55.	QKQ30584	G204R
56.	QLA10246	R203K
57.	QLA10246	G204R
58.	QLA10270	R203K
59.	QLA10270	G204RR
60.	QLA10282	R203K
61.	QLA10282	G204R
62.	QLA10294	P383L
63.	QLA10294	R203K
64.	QLA10294	G204R
65.	QLA10306	R203K
66.	QLA10306	G204R
67.	QLA10318	G204R
68.	QLA10318	R203K
69.	QLA10330	G204R
70.	QLA10330	R203K
71.	QLA10342	G204R
72.	QLA10342	R203K
73.	QLA10354	R203K
74.	QLA10354	G204R
75.	QOI53600	P13L
76.	QOQ57020	S194L
77.	QOQ57032	S194L
78.	QOQ57044	S194L
79.	QOQ57056	S194L
80.	QOQ57068	S194L
81.	QOQ57092	M234I
82.	QOQ57104	S194L
83.	QOQ57116	S194L
84.	QOQ57129	S194L
85.	QOQ72552	S194L
86.	QOQ72564	S194L
87.	QOQ72576	S194L
88.	QOQ84803	S194L
89.	QOQ84834	S194L
90.	QOR63442	T205I
91.	QOR63454	S194L
92.	QOR63466	T205I
93.	QOR63514	A119S
94.	QOR63514	S194L
95.	QOR64241	S194L
96.	QOR64253	S194L
97.	QOS50459	P13L
98.	QOS50495	T91I

Continued on next page

Table 2 continued

Serial No.	Accession	Mutated sequence and position
99.	QOS50507	P13L
100.	QOS50519	P13L
101.	QOS50531	P13L
102.	QOS50590	P13L
103.	QOS50650	P13L
104.	QOS50674	P13L
105.	QOS50686	P13L
106.	QOS50686	D225Y
107.	QOS50722	P13L
108.	QOS50734	P13L
109.	QOS50746	P13L
110.	QOS50746	S413I
111.	QOS50758	S413I
112.	QOS50758	P13L
113.	QOS50770	P13L
114.	QOS50782	P13L
115.	QOS50818	P13L
116.	QOS50830	P13L
117.	QOS50853	P13L
118.	QOS50865	P13L
119.	QOS50889	Q9H
120.	QOS50889	P199S
121.	QOS50901	S202N
122.	QOS50924	S202N
123.	QOS50948	P13L
124.	QOS50960	P13L
125.	QOS50972	P13L
126.	QOS50996	P13L
127.	QOS51008	P13L
128.	QOS51020	P13L
129.	QOS51032	P13L
130.	QOS51068	R209I
131.	QOS51068	P367L
132.	QOS51080	R203K
133.	QOS51080	G204R
134.	QOS51092	P13L
135.	QOS51104	R203K
136.	QOS51104	G204R
137.	QOU99154	P14L
138.	QOU99201	Q9H
139.	QOU99201	P199S
140.	QOU99223	Q9H
141.	QOU99223	P199S
142.	QOU99247	S202N
143.	QOU99259	S202N
144.	QOU99270	Q9H
145.	QOU99270	P199S
146.	QOU99281	Q9H
147.	QOU99281	P199S
148.	QOU99292	Q9H
149.	QOU99292	P199S
150.	QOU99303	Q9H
151.	QOU99303	P199S

Table 3: Mutational location after Multiple Sequence Alignment of SARS-CoV-2 surface glycoprotein sequence with position and sequence

S. No.	Accession	Mutated sequence and position
1.	QJF74843	V367F
2.	QJF74867	D614G
3.	QOI53592	M153I
4.	QMI57728	T95I
5.	QMI57728	N185K
6.	QOI53580	D614G
7.	QOR64233	D614G
8.	QOR64245	D614G
9.	QOQ57012	D614G
10.	QOQ57024	D614G
11.	QOQ57060	D614G
12.	QOR64233	A701T
13.	QOR64233	P812L
14.	QOR64245	P812L
15.	QOQ57012	P812L
16.	QOQ57060	P812L
17.	QOR64233	H1083Q
18.	QOR64245	H1083Q
19.	QOQ57012	H1083Q
20.	QOQ57024	H1083Q
21.	QOQ57072	D614G
22.	QOQ57084	D614G
23.	QOQ57096	D614G
24.	QOQ57108	D614G
25.	QOQ57121	D614G
26.	QOQ57108	A701T
27.	QOQ57072	A701T
28.	QOQ57096	P812L
29.	QOQ57121	P812L
30.	QOQ57096	H1083Q
31.	QOQ57121	H1083Q
32.	QOQ57108	H1083Q
33.	QOQ72544	L54F
34.	QOQ72556	L54F
35.	QOQ72568	L54F
36.	QOQ72544	D614G
37.	QOQ72556	D614G
38.	QOQ72568	D614G
39.	QOQ72580	D614G
40.	QOQ84795	D614G
41.	QOQ72544	A701T
42.	QOQ72556	P812L
43.	QOQ72568	P812L
44.	QOQ72580	P812L
45.	QOQ72544	H1083Q
46.	QOQ84826	D614G
47.	QOR63434	D614G
48.	QOR63446	D614G
49.	QOR63458	D614G
50.	QOR63470	D614G
51.	QOR63434	P812L
52.	QOR63470	P812L
53.	QOQ53335	S305T
54.	QOQ53335	C488R
55.	QOR63482	D614G
56.	QOQ57036	D614G
57.	QOQ57048	D614G
58.	QOR63506	D614G
59.	QOQ53335	D614G
60.	QOQ57048	A701T
61.	QOQ57036	P812L
62.	QOQ57048	P812L
63.	QOQ57036	H1083Q
64.	QOQ57048	H1083Q
65.	QOQ53339	F2L
66.	QOQ53339	V11I
67.	QOQ53339	S13R
68.	QOQ53339	Q14H
69.	QOQ53339	R34H
70.	QOQ53339	V42I
71.	QOQ53339	R44K
72.	QOQ53339	V47I
73.	QOQ53339	F59I
74.	QOQ53339	K77N
75.	QOQ53339	D111N
76.	QOQ53339	Q115H
77.	QOQ53339	A123T
78.	QOQ53339	N487I
79.	QOQ53339	V512L
80.	QOQ53339	A522P
81.	QOQ53339	A262T
82.	QOQ53339	Q677H
83.	QOQ53336	G199R
84.	QOQ53340	A262T
85.	QOQ53338	C301S
86.	QOQ53340	R328T
87.	QOQ53337	R457T
88.	QOQ53338	D614G
89.	QOQ53340	D614G
90.	QOQ53336	A684V
91.	QOQ53336	A688P
92.	QOQ53336	V705I
93.	QOQ53337	H1048Y
94.	QOQ53337	Q1180H
95.	QOQ53337	K1181Q
96.	QOQ53341	V11I
97.	QOL24227	V11I
98.	QOQ53341	R44K

Continued on next page

Table 3 continued

99.	QOQ53341	V47I
100.	QOL24227	K77N
101.	QOQ53341	K77N
102.	QOQ53341	K97N
103.	QOQ53341	D111N
104.	QOL24227	D111N
105.	QOL24227	R190K
106.	QOL24227	D198E
107.	QOL24225	E224K
108.	QOL24225	D228N
109.	QOL24226	E224K
110.	QOL24226	D228N
111.	QOL24227	A262T
112.	QOQ53341	Q271H
113.	QOQ53341	F275L
114.	QOL24228	V407L
115.	QOL24228	P412S
116.	QOL24227	D427H
117.	QOL24227	N440H
118.	QOL24227	Q474P
119.	QOL24228	D614G
120.	QOL24227	D614G
121.	QOQ53341	G669R
122.	QOQ53341	Q675R
123.	QOQ53341	Q677H
124.	QOL24227	S686I
125.	QOL24227	A688P
126.	QOL24225	K790Q
127.	QOL24226	K790Q
128.	QOL24225	R815K
129.	QOL24226	R815K
130.	QOL24225	D820N
131.	QOL24226	D820N
132.	QOL24225	D830N
133.	QOL24226	D830N
134.	QOL24228	P863H
135.	QOL24241	F2L
136.	QOL24241	V11I
137.	QOL24241	Q14H
138.	QOL24241	R34C
139.	QOL24241	Y37N
140.	QOL24241	V42I
141.	QOL24241	R44K
142.	QOL24241	F65I
143.	QOL78311	S94F
144.	QOL78311	T95P
145.	QOL24240	D111N
146.	QOL24240	A282T
147.	QOL78311	D568H
148.	QOL78311	D614G
149.	QOL24241	H655Y
150.	QOL24240	Q675RR
151.	QOL79057	S13N
152.	QOL79057	D40E
153.	QOL79057	V42L
154.	QOL79057	S161F
155.	QOL79057	S246N
156.	QOL79057	D614G
157.	QOL79058	D614G
158.	QOL79058	R1019K
159.	QOL79058	P1090L
160.	QOL79059	V11F
161.	QOL79059	R21K
162.	QOL79135	R21K
163.	QOL79135	A222V
164.	QOL79061	K529I
165.	QOL79059	D614G
166.	QOL79135	D614G
167.	QOL79061	E619K
168.	QOL79061	G652R
169.	QOL79061	Q677H
170.	QOL79061	Y695N
171.	QOL79061	V729A
172.	QOL79136	D614G
173.	QOL79137	V11I
174.	QOL79137	Q115H
175.	QOL79137	D614G
176.	QOL79137	P863H
177.	QOL79137	Q913H
178.	QOL79137	I934T
179.	QOL79333	C136W
180.	QOL79333	N137Y
181.	QOL79333	I203L
182.	QOL21485	H207P
183.	QOL20612	E224K
184.	QOL21486	E224K
185.	QOL21486	R237K
186.	QOL21486	F238V
187.	QOL21486	Q239P
188.	QOL20612	T240S
189.	QOL79332	A262T
190.	QOL79333	L252P
191.	QOL79332	D467N
192.	QOL21486	A475V
193.	QOL20612	Q506H
194.	QOL20612	V510E
195.	QOL20612	V512E
196.	QOL20612	D614G
197.	QOL21485	V511I
198.	QOL79333	D568H

Continued on next page

Table 3 continued

199.	QOL79332	Q675R
200.	QOL20612	V826G
201.	QOL21485	V826G
202.	QOL20612	I844M
203.	QOL21486	I844F
204.	QOL21486	R847K
205.	QOL21486	D848N
206.	QOL21486	C851W
207.	QOL20612	R847K
208.	QOL20612	D848N
209.	QOL21535	T21I
210.	QOL21535	V42A
211.	QOL21535	V511E
212.	QOL21535	C525W
213.	QOL21535	K557R
214.	QOL21535	R567K
215.	QOL21535	N657I
216.	QOL21535	Q677H
217.	QOL21535	V722A
218.	QOL21535	D737H
219.	QOL21535	T768P
220.	QOL21535	G769E
221.	QOL21535	E780K
222.	QOL21535	V781F
223.	QOL21535	K790Q
224.	QOL21535	I834J
225.	QOL21536	E224K
226.	QOL21536	V826G
227.	QOL21536	Y837N
228.	QOL21536	R847K
229.	QOL21536	D848N
230.	QOL21536	C851W
231.	QOL21536	L858F
232.	QOL21681	D228N
233.	QOL21681	E780K
234.	QOL21681	D808N
235.	QOL21681	S816P
236.	QOL21681	D820N
237.	QOL21720	T22I
238.	QOL21720	V213G
239.	QOL21720	E224K
240.	QOL21720	C538W
241.	QOL21720	K825Q
242.	QOL10078	R402I
243.	QOL10078	V407L
244.	QOL10078	P412S
245.	QOL10078	D614G
246.	QOL10078	P863H
247.	QOJ86680	T22I
248.	QOK36756	Y160I
249.	QOK36756	N185I
250.	QOK36756	Y200N
251.	QOK36756	D228Y
252.	QOJ86685	L226I
253.	QOJ86685	V227M
254.	QOJ86685	D228I
255.	QOJ86685	L249S
256.	QOJ86685	T250F
257.	QOK36765	E224K
258.	QOK36765	T240S
259.	QOJ86685	A263S
260.	QOJ86684	A262T
261.	QOJ86684	D287Y
262.	QOJ86684	V289E
263.	QOK36765	A522T
264.	QOK36766	V511I
265.	QOJ86685	P464L
266.	QOJ86685	T500I
267.	QOJ86685	N501R
268.	QOJ86685	R509K
269.	QOJ86685	V511E
270.	QOJ86685	S514P
271.	QOJ86685	H519I
272.	QOJ86685	P521Q
273.	QOJ86685	A522P
274.	QOJ86685	T523P
275.	QOJ86685	P527T
276.	QOJ86685	D568N
277.	QOJ86685	D627N
278.	QOJ86684	E583A
279.	QOJ86684	D586E
280.	QOJ86684	T602K
281.	QOJ86684	N603K
282.	QOJ86684	N606Y
283.	QOJ86684	C617R
284.	QOJ86684	G652R
285.	QOJ86684	E661K
286.	QOJ86685	E661D
287.	QOJ86684	A672P
288.	QOK36766	Q675R
289.	QOK36766	N824K
290.	QOK36766	K825Q
291.	QOK36766	V826M
292.	QOK36765	R847K
293.	QOK36765	D848N
294.	QOK36765	P863H
295.	QOJ86680	P863H
296.	QOJ86680	T874P
297.	QOJ86680	L877M
298.	QOK36756	P863H

Continued on next page

Table 3 continued

299.	QOK36767	V11I
300.	QOK36767	R44K
301.	QOK36767	A684V
302.	QOK36767	V705I
303.	QOK51484	V11I
304.	QOK51484	K77N
305.	QOK51484	D111N
306.	QOK51484	Q115H
307.	QOK51484	V159L
308.	QOK51484	K187Q
309.	QOK51484	R190K
310.	QOK51484	K206N
311.	QOK51484	A262T
312.	QOK51484	D614G
313.	QOK51484	V620F
314.	QOK51484	G648R
315.	QOK51484	A706R
316.	QOL00258	V11I
317.	QOL00258	K77N
318.	QOL00258	D111N
319.	QOL00258	Q271H
320.	QOL00258	S443C
321.	QOL00258	G669R
322.	QOL00258	Q677H
323.	QOL00258	Q675R
324.	QOL00259	R44I
325.	QOL00259	K77N
326.	QOL00259	D111N
327.	QOL00259	R158L
328.	QOL00259	S162I
329.	QOL00259	E169Q
330.	QOL00259	Q173H
331.	QOL00259	Q183H
332.	QOL00259	D614G
333.	QOL00259	S686T
334.	QOL00259	E702Q
335.	QOL00259	V705I
336.	QOL00259	I714L
337.	QOL00263	V11I
338.	QOL00263	K77N
339.	QOL00263	Q115H
340.	QOL00263	H146P
341.	QOL00263	E1569D
342.	QOL00263	D614G
343.	QOL00263	S750T
344.	QOL00264	V11I
345.	QOL00264	V42I
346.	QOL00264	K77N
347.	QOL00264	D111N
348.	QOL00264	H207L
349.	QOL00264	A262T
350.	QOL00264	N487I
351.	QOL00264	V512L
352.	QOL00264	A522P
353.	QOL00264	G669R
354.	QOL00264	Q677H
355.	QOL00264	Q675R
356.	QOL00468	R44I
357.	QOL00468	K77N
358.	QOL00468	D111N
359.	QOL00468	R158L
360.	QOL00468	S162I
361.	QOL00468	E169Q
362.	QOL00468	Q173H
363.	QOL00468	V407L
364.	QOL00468	R408I
365.	QOL00468	A411T
366.	QOL00468	D614G
367.	QOL00468	D627N
368.	QOL00468	A653P
369.	QOL00468	N658H
370.	QOL00468	I664L
371.	QOL00469	V11I
372.	QOL00469	S13R
373.	QOL00469	T29Q
374.	QOL00469	R34H
375.	QOL00469	V42I
376.	QOL00469	R44K
377.	QOL00469	V47I
378.	QOL00469	F59I
379.	QOL00469	F65I
380.	QOL00469	K77N
381.	QOL00469	D111N
382.	QOL00469	S112T
383.	QOL00469	Q115H
384.	QOL00469	A123T
385.	QOL00469	A262T
386.	QOL00469	N487I
387.	QOL00469	V512L
388.	QOL00469	A522P
389.	QOL00469	E661D
390.	QOL00469	G669R
391.	QOL00469	A672P
392.	QOL00469	Q677H
393.	QOL00501	E224K
394.	QOL00501	I235V
395.	QOL00501	K814Q
396.	QOL00501	D830N
397.	QOL00501	P863H
398.	QOL00502	V11I

Continued on next page

Table 3 continued

399.	QOL00502	V47I
400.	QOL00502	R78K
401.	QOL00502	K77N
402.	QOL00502	D111N
403.	QOL00502	Q115H
404.	QOL00502	E156D
405.	QOL00502	R158P
406.	QOL00502	G648R
407.	QOL00502	G652R
408.	QOL00502	S758T
409.	QOL00502	V785L
410.	QOL00502	K814N
411.	QOL00502	K854N
412.	QOL00503	P9Q
413.	QOL00503	V11I
414.	QOL00503	S13R
415.	QOL00503	R102T
416.	QOL00503	L18P
417.	QOL00503	Y37N
418.	QOL00503	N74H
419.	QOL00503	G103R
420.	QOL00503	K77N
421.	QOL00503	R78K
422.	QOL00503	K97N
423.	QOL00503	A262T
424.	QOL00503	A653P
425.	QOL00503	N658H
426.	QOL00503	I664L
427.	QOL00503	R408T
428.	QOL00503	G413R
429.	QOL00503	D427N
430.	QOL00503	D614G
431.	QOL00503	D627N
432.	QOJ75919	V11I
433.	QOJ75919	L176F
434.	QOJ75919	L179P
435.	QOJ75919	A262T
436.	QOJ75919	Q498Y
437.	QOJ75919	P499T
438.	QOJ75919	T716P
439.	QOJ75919	I726F
440.	QOJ75920	S438C
441.	QOJ75920	N544T
442.	QOJ75920	H655Y
443.	QOJ75920	V705F
444.	QOJ75920	Q836H
445.	QOJ75921	R21K
446.	QOJ75921	R44K
447.	QOJ75921	D111N
448.	QOJ75921	S112P
449.	QOJ75921	A262T
450.	QOJ75921	T302A
451.	QOJ75921	D614G
452.	QOJ75922	A262T
453.	QOJ75922	N536K
454.	QOJ75922	T791P
455.	QOJ75922	K811N
456.	QOJ75922	T827A
457.	QOJ75923	N460I
458.	QOJ75923	K462N
459.	QOJ75923	P463L
460.	QOJ75923	I468L
461.	QOJ75923	G838D
462.	QOJ75923	G842A
463.	QOJ75923	D848A
464.	QOJ75924	E132V
465.	QOJ75924	V159I
466.	QOJ75924	S162N
467.	QOJ75924	E191D
468.	QOJ75924	I197T
469.	QOJ75924	Q173F
470.	QOJ75924	M177I
471.	QOJ75924	C1043G
472.	QOJ75924	G485S
473.	QOJ75924	L513F
474.	QOJ75924	D614G
475.	QOJ75924	G838A
476.	QOJ75924	T998P
477.	QOJ75924	Q1011H
478.	QOJ75924	A1015P
479.	QOJ75924	D178Y
480.	QOJ75924	V193L
481.	QOJ75924	K187N
482.	QOJ75924	N188H
483.	QOJ75924	R190I
484.	QOJ75924	F194L
485.	QOJ75925	Q564H
486.	QOJ75925	F565C
487.	QOJ75925	Q1180P
488.	QOJ75926	T22I
489.	QOJ75926	P225R
490.	QOJ75926	I231L
491.	QOJ75926	R237S
492.	QOJ75926	G667A
493.	QOJ75926	A672P
494.	QOJ75926	Q677H
495.	QOJ75926	L242P
496.	QOJ75926	R246S
497.	QOJ75926	A688T
498.	QOJ75927	F65Y

Continued on next page

Table 3 continued

499.	QOJ75927	V120D
500.	QOJ75927	V127D
501.	QOJ75927	F133L
502.	QOJ75927	F135L
503.	QOJ75927	P139T
504.	QOJ75927	C166S
505.	QOJ75928	L244F
506.	QOJ75928	L533F
507.	QOJ75928	D809K
508.	QOJ75929	V11I
509.	QOJ75929	T22I
510.	QOJ75929	A262T
511.	QOJ75929	N30I
512.	QOJ75929	H66L
513.	QOJ75929	N487I
514.	QOJ75929	N74H
515.	QOJ75929	N641K
516.	QOJ75930	D614G
517.	QOJ75931	V11I
518.	QOJ75931	H245Q
519.	QOJ75931	S247N
520.	QOJ75931	D614G
521.	QOJ75931	R685G
522.	QOJ75932	D614G
523.	QOJ75932	W1102C
524.	QOJ75932	N1108K
525.	QOJ75932	T1116N
526.	QOJ75933	D614G
527.	QOJ75934	D614G
528.	QOJ75934	T827I
529.	QOJ75934	L828F
530.	QOJ75935	V11I
531.	QOJ75935	S13R
532.	QOJ75935	Q14H
533.	QOJ75935	D614G
534.	QOJ75935	R682P
535.	QOJ75935	S686N
536.	QOJ75935	E702K
537.	QOJ75935	N710T
538.	QOJ75935	I714T
539.	QOJ75936	F194V
540.	QOJ75936	D614G
541.	QOJ75936	F759S
542.	QOJ75936	N764T
543.	QOJ75936	E702K
544.	QOJ75936	V705F
545.	QOJ75936	T739S
546.	QOJ75936	I742L
547.	QOJ75936	T747P
548.	QOJ75936	G757R
549.	QOJ75937	L560P
550.	QOJ75937	D614G
551.	QOJ75938	G648R
552.	QOJ75938	E661D
553.	QOJ75938	G669R
554.	QOJ75938	A672P
555.	QOJ75938	Q677H
556.	QOJ75939	D614G
557.	QOJ75940	N440H
558.	QOJ75940	F486L
559.	QOJ75940	D614G
560.	QOJ75941	A262P
561.	QOJ75941	Y266N
562.	QOJ75941	D614G
563.	QOJ42687	V3D
564.	QOJ42687	L10I
565.	QOJ42687	V11I
566.	QOJ42687	Q14H
567.	QOJ42687	N544T
568.	QOJ42687	E702K
569.	QOJ42687	V705J
570.	QOJ42687	W64C
571.	QOJ42687	T385S
572.	QOJ42687	E619K
573.	QOJ42687	S686T
574.	QOJ42687	A688P
575.	QOJ42687	S689N
576.	QOJ42687	Y695N
577.	QOJ42687	S721T
578.	QOJ42687	E725K
579.	QOJ42687	L727P
580.	QOJ42687	V729A
581.	QOJ42687	M731K
582.	QOJ42687	T22I
583.	QOJ42687	Y28N
584.	QOJ42687	T29P
585.	QOJ42687	K41Q
586.	QOE90911	Y38C
587.	QOE90911	F86S
588.	QOE90911	T95I
589.	QOE90911	E96G
590.	QOE90911	D290N
591.	QOE90911	L296F
592.	QOE90911	L303V
593.	QOE90911	S151G
594.	QOE90911	N185K
595.	QOE90911	P272S
596.	QOE90911	A288P
597.	QOE90912	A222P
598.	QOE90912	V227I

Continued on next page

Table 3 continued

599.	QOE90912	K424N
600.	QOE90912	P863H
601.	QOE90912	K535S
602.	QOE90912	I1183F
603.	QOE90912	D1199Y
604.	QOE90914	D614G
605.	QOE90914	Y636F
606.	QOE90914	L650S
607.	QOE90914	P863H
608.	QOE90915	H656Y
609.	QOE83920	K77N
610.	QOE83920	A262T
611.	QOE83920	N542T
612.	QOE83920	D614G
613.	QOE83920	V620F
614.	QOE83972	P225T
615.	QOE83972	A262T
616.	QOE83972	N487I
617.	QOE83972	D614G
618.	QOE83972	V620I
619.	QOE83972	P863H
620.	QOE83973	Q115H
621.	QOE83973	E156D
622.	QOE83973	V171L
623.	QOE83973	K182N
624.	QOE83973	A262T
625.	QOE83973	D614G
626.	QOE83973	S758I
627.	QOE83973	P863H
628.	QOE83973	N925Y
629.	QOE83973	G932A
630.	QOE83973	T941P
631.	QOE83974	S13R
632.	QOE83974	L18P
633.	QOE83974	A262T
634.	QOE83974	P863H
635.	QOE83974	I931T
636.	QOE83974	G932A
637.	QOE83974	S943T
638.	QOE83974	Y37N
639.	QOE83974	I100T
640.	QOE83974	R408T
641.	QOE83974	D427N
642.	QOE83974	Q580H
643.	QOE83974	Y636F
644.	QOE83974	N641I
645.	QOE83974	G648R
646.	QOE83975	K535E
647.	QOE83975	H824N
648.	QOE83975	G842C
649.	QOE83975	V1177E
650.	QOE84134	I231L
651.	QOE84134	T240N
652.	QOE84134	N280D
653.	QOE84134	A288P
654.	QOE84134	T302A
655.	QOE84134	V736L
656.	QOE84134	N751H
657.	QOE84134	S816A
658.	QOE84134	D820N
659.	QOE84134	I788F
660.	QOE84134	K811N
661.	QOE84134	S813P
662.	QOE84134	K814Q
663.	QOE84221	F106L
664.	QOE84221	Q134P
665.	QOE84221	N148T
666.	QOE84221	A262T
667.	QOE84221	P863H
668.	QOE84221	V510E
669.	QOE84221	K535E
670.	QOE84221	N969H
671.	QOE84221	F486L
672.	QOE84221	N487I
673.	QOE84221	Q493P
674.	QOE84221	N501K
675.	QOE84222	A262T
676.	QOE84222	S459Y
677.	QOE84222	D614G
678.	QOE84223	P225S
679.	QOE84223	L229F
680.	QOE84223	I231L
681.	QOE84223	P863H
682.	QOE84223	K1181Q
683.	QOE84223	D820N
684.	QOE84223	L821R
685.	QOE84223	F823C
686.	QOE84223	R815K
687.	QOE84223	S816T
688.	QOE84223	I818F
689.	QOE84223	G798C
690.	QOE84223	K814M
691.	QOE84223	G838A
692.	QOE84223	G842R
693.	QOE84223	D843E
694.	QOE84223	V826L
695.	QOE84223	A829E
696.	QOE84223	D830H
697.	QOE84223	I834T
698.	QOE84224	D398N

Continued on next page

Table 3 continued

699.	QOE84224	K535G
700.	QOE84224	E702K
701.	QOE84224	V705F
702.	QOE84224	E725K
703.	QOE84224	T736P
704.	QOE84224	D775N
705.	QOE84224	P863H
706.	QOE84224	Y837N
707.	QOE84224	A879P
708.	QOE84224	E918Q
709.	QOU99158	D614V
710.	QOU99170	D614V
711.	QOU99296	P1263L
712.	QOS50441	K77M
713.	QOS50451	A701S
714.	QOS50594	D614G
715.	QOS50606	D614G
716.	QOS50630	D614G
717.	QOS50654	D614G
718.	QOS50654	D936H
719.	QOS50678	D627P
720.	QOS50726	K77M
721.	QOS50750	I624T
722.	QOS50834	F135L
723.	QOS50845	M731I
724.	QOS50952	K77M
725.	QOS50964	K77M
726.	QOS50928	D614G
727.	QOS50928	K1045N
728.	QOS50988	K77M
729.	QOS51000	K77M
730.	QOS51012	K77M
731.	QOS51024	K77M
732.	QOS51036	K77M
733.	QOS51024	D88H
734.	QOS50988	D1084Y
735.	QOS51000	D1084Y
736.	QOS51012	D1084Y
737.	QOS51024	D1084Y
738.	QOS51036	D1084Y
739.	QOS51060	D614G
740.	QOS51072	D614G
741.	QOS51096	D614G
742.	QOS51108	D614G
743.	QOS51120	D614G
744.	QKM75377	D614G
745.	QKM75389	D614G
746.	QKM75401	D614G
747.	QKM75413	D614G
748.	QKM75425	D614G
749.	QKM75377	T547I
750.	QKM75413	V213L
751.	QKM75473	I569S
752.	QKM75437	D614G
753.	QKM75449	D614G
754.	QKM75461	D614G
755.	QKM75473	D614G
756.	QKM75485	D614G
757.	QKM75485	F797C
758.	QKM75485	G799S
759.	QKM75497	D614G
760.	QKM75509	D614G
761.	QKM75521	D614G
762.	QKM75533	D614G
763.	QKM75545	D614G
764.	QKM75556	D614G
765.	QKM75567	D614G
766.	QKM75579	D614G
767.	QKM75591	D614G
768.	QKM75603	D614G
769.	QKM75579	H146Q
770.	QKM75591	H146Q
771.	QKM75556	Y680F
772.	QKM75567	G1167V
773.	QKM75615	D614G
774.	QKM75627	D614G
775.	QKM75639	D614G
776.	QKM75651	D614G
777.	QKM75663	D614G
778.	QKM75639	N211Y
779.	QKM75651	N211Y
780.	QKM75627	G769V
781.	QKM75675	D614G
782.	QKM75687	D614G
783.	QKQ30528	D614G
784.	QKQ30540	D614G
785.	QKQ30552	D614G
786.	QKQ30528	G142V
787.	QKM75687	N211Y
788.	QKQ30552	N211Y
789.	QKQ30552	T236S
790.	QKM75675	S256L
791.	QKM75675	V1264M
792.	QKQ30564	D614G
793.	QKQ30576	D614G
794.	QLA10238	D614G
795.	QLA10250	D614G
796.	QLA10262	D614G
797.	QKQ30576	N211V
798.	QLA10274	D614G

Continued on next page

Table 3 continued

799.	QLA10286	D614G
800.	QLA10298	D614G
801.	QLA10310	D614G
802.	QLA10322	D614G
803.	QLA10298	G75V
804.	QLA10322	V1068F
805.	QLA10334	D614G
806.	QLA10346	D614G
807.	QLA10346	H146Q

Table 4: Mutational location after Multiple Sequence Alignment of SARS-CoV-2 membrane glycoprotein sequence with position and sequence

Sl. No.	Accession	Mutated sequence and position
1.	QOR64248	S214R
2.	QOQ57027	S214R
3.	QOQ57075	S214R
4.	QOQ72547	S214R
5.	QOQ84829	S214R
6.	QOQ57051	S214R
7.	QKM75606	A2V
8.	QKM75618	A2V
9.	QKQ30543	A2V
10.	QLA10253	A2V

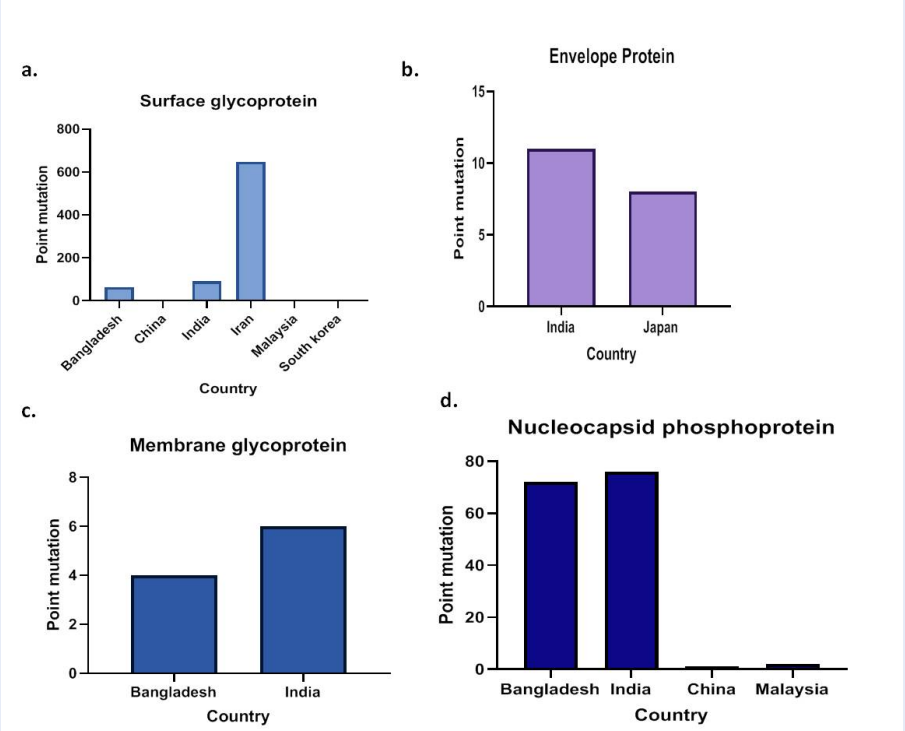

Figure 1: **Showing the total number of mutations occurring in the structural proteins. a**. Surface glycoprotein, **b**. Envelope protein, **c**. Membrane glycoprotein and, **d**. Nucleocapsid phosphoprotein.

Table 5: Showing the antigenicity and allergenicity of different structural protein of SARS-CoV-2

Structural protein	Antigenicity	Allergenicity
Surface glycoprotein	0.4696	Non-allergen
Envelope protein	0.6025	Non-allergen
Nucleocapsid phosphoprotein	0.5059	Non-allergen
Membrane glycoprotein	0.5102	Non-allergen

Assessment of antigenicity and allergenicity

VaxiJen v2.0 server was used to predict the antigenicity of all four structural proteins of SARS-CoV-2. A peptide to be used in vaccine production must bind with the B-cell and T-cell receptors and enhance the cell's immune response. This estimation indicated that envelope protein was the most antigenic one among the four with an antigenicity of 0.6025, followed by membrane glycoprotein, having antigenicity of 0.5102. In contrast, the antigenicity of nucleocapsid phosphoprotein ranked third with a value of 0.5059, and surface glycoprotein was the least antigenic with antigenicity of 0.4696 as shown in **Table 5**. The allergenicity of these proteins was also estimated to discern whether these antigenic peptides were allergenic or not. A good vaccine should be non-allergenic to the host, and it should not produce any IgE-mediated immune responses in the host. The allergenicity analysis revealed that all four structural proteins were non-allergenic to the host and could be used as potent vaccine targets.

DISCUSSION

On 11^{th} March 2020, the WHO announced COVID-19 as a global pandemic due to its rapid global spread to numerous countries, and declared it as a serious threat to public health across the world [14]. Therefore, antiviral therapeutics or candidate vaccines were imminently necessary to curb SARS-CoV-2 infections. The virus primarily caused acute to severe respiratory illness and pneumonia in humans. The symptoms of COVID-19 began within two days, and could continue up to 14 days.

The emergence of a huge number of novel mutations in the genome of SARS-CoV-2 may interrupt ongoing vaccine development and trial strategies in different parts of the world. Regular monitoring of mutations has been crucial in tracking and tracing the circulation of this virus among individuals and in different geographical locations. SARS-CoV-2 (Wuhan-Hu-1), complete genome sequence, was deposited in the NCBI Gene bank [15] in January 2020.

RNA viruses, including SARS-CoV-2, exhibit a high frequency of novel point mutations which are supposedly beneficial for the viruses to adapt and evolve in changing climatic conditions, thereby enhancing their potential transmission worldwide [16,17]. The mutation is considered as the essential natural selection phenomenon and most often select for those traits of the virus that are a pre-requisite for survival in the highly dynamic host environment. These characteristic features of viruses (*e.g.* SARS-CoV-2) could complicate the ongoing efforts of researchers to combat this contagious disease because the high frequency of viral mutations induces drug resistance and rapid immune evasion [18,19].

The results of our study revealed the presence of a total of 987 mutations from 729 sequences from Asia countries in October. Spike glycoproteins were found to be highly mutagenic amongst the structural proteins, followed by nucleocapsid (151 mutations). Meanwhile, envelope protein showed 19 mutations and only 10 point mutations were found in the membrane proteins. Furthermore, we estimated the antigenicity and allergenicity of these structural proteins to design and develop potentially potent candidate vaccines against SARS-CoV-2. Among the structural proteins, envelope (E) was found to be highly antigenic and least allergenic, followed by nucleocapsid (N), membrane (M), and spike (S) protein of SARS-CoV-2. Antigenicity and allergenicity are the essential criteria to develop efficacious antiviral therapeutics or vaccines against COVID-19.

The mutations cause alterations in the structural proteins of the SARS-CoV-2 virus and, therefore, help in evading the host immunity. The occurrence of the high frequency of mutations creates a barrier in the development of antiviral drugs. Our results shown in this article, here presents a snap shot picture of an enormously changing situation due to SARS-CoV-2.

CONCLUSIONS

In the present study, the occurrence of novel mutations in the different structural proteins of the SARS-CoV-2 virus provides further insight into the identification and magnitude of virulence properties of virus strains, from a large repertoire of strains. Our findings might be useful in the development of effective therapeutic strategies against all types of SARS-CoV-2 strains.

ABBREVIATIONS

COVID-19: coronavirus disease 2019

E: envelope

M: membrane

N: nucleocapsid

S: spike

SARS: Severe acute respiratory syndrome

ACKNOWLEDGMENTS

Not applicable

AUTHOR'S CONTRIBUTIONS

All authors read and approved the final manuscript.

FUNDING

None

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565–74. PMID: 32007145. Available from: 10.1016/S0140-6736(20)30251-8.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–33. PMID: 31978945. Available from: 10.1056/NEJMoa2001017.
3. Zhang T, Wu Q, Zhang Z. Pangolin homology associated with 2019-nCoV. BioRxiv. 2020;Available from: 10.1101/2020.02.19.950253.
4. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci USA. 2005;102(39):14040–5. PMID: 16169905. Available from: 10.1073/pnas.0506735102.
5. Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, Burbelo PD. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio. 2014;5(2):e00884–14. PMID: 24570370. Available from: 10.1128/mBio.01002-14.
6. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003;302(5643):276–8. PMID: 12958366. Available from: 10.1126/science.1087139.
7. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Napoli RD. Features, Evaluation, and Treatment of Coronavirus (COVID-19). https://wwwncbinlmnihgov/books/NBK554776/. 2020;.
8. Barnard DL. Coronaviruses: molecular and cellular biology. Future Virol. 2008;3(2):119–23. PMID: 32218805. Available from: 10.2217/17460794.3.2.119.
9. Ogando NS, Ferron F, Decroly E, Canard B, Posthuma CC, Snijder EJ. The Curious Case of the Nidovirus Exoribonuclease: Its Role in RNA Synthesis and Replication Fidelity. Front Microbiol. 2019;10:1813. PMID: 31440227. Available from: 10.3389/fmicb.2019.01813.
10. Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS Pathog. 2010;6(5):e1000896. PMID: 20463816. Available from: 10.1371/journal.ppat.1000896.
11. Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res. 2019;47:636–41. PMID: 30976793. Available from: 10.1093/nar/gkz268.
12. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007;8(1):4. PMID: 17207271. Available from: 10.1186/1471-2105-8-4.
13. Dimitrov I, Flower DR, Doytchinova I. AllerTOPserver for in silico prediction of allergens. BMC Bioinformatics. 2013;14:4. PMID: 23735058. Available from: 10.1186/1471-2105-14-S6-S4.
14. Organization WH. Coronavirus Disease 2019 Situation Report 51. WHO Bull; 2020.
15. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265–9. PMID: 32015508. Available from: 10.1038/s41586-020-2008-3.
16. Daniele M, Federico MG. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Front Microbio; 2020.
17. Domingo E, Holland JJ. RNA virus mutations and fitness for survival. Annu Rev Microbiol. 1997;51(1):151–78. PMID: 9343347. Available from: 10.1146/annurev.micro.51.1.151.
18. Irwin KK, Renzette N, Kowalik TF, Jensen JD. Antiviral drug resistance as an adaptive process. Virus Evolution. 2016;2(1):vew014. Available from: 10.1093/ve/vew014.
19. Yashvardhini N, Kumar A, Jha DK. Immunoinformatics Identification of B- and T-Cell Epitopes in the RNA-Dependent RNA Polymerase of SARS-CoV-2. Canadian J Infec Diseases Med Microbiol. 2021;2021:6627141. Available from: 10.1155/2021/6627141.