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GEOGRAPHICAL BASED GENOMIC AND PATHOGENIC STUDY OF THE SARS-COV-2

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ABSTRACT: The current pandemic COVID 19 caused by SARS-CoV-2 is very contagious and causes respiratory disease outbreaks. In a various genomic analysis, different strains of SARS-CoV-2 was found to infect different geographical locations of the world (India, USA, and Brazil) and also in different parts of India (Kerala, Surat, and Ahmedabad). Different genome sequences of SARS-CoV-2 isolated from different locations of the world and India are downloaded from the viral genome sequences database of the National Center for Biotechnology Information (NCBI), and comparative analysis is performed using various computational tools. Comparison of genome size, G+C content, and identified ORFs is performed for different sequences of SARS-CoV-2 isolated from different geographical locations of the world and also different cities/states of India using various bioinformatics tools. This study concludes that all the sequences isolated from different geographical locations are highly similar to the reference strain of Wuhan, with an identity percentage ranging from 99.56-99.98% and also with the same GC content of 37%. The difference in the identified ORFs of length >150 and a small difference in the identity percentages shows the presence of mutation between different strains of SARS-CoV-2.

INTRODUCTION: The coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). This outbreak started in Wuhan, China in December 2019. On 30 January 2020, the World Health Organization declared the outbreak as a Public Health Emergency of International concern. And, the COVID-19 was declared a pandemic on 11 March, 2020¹. Coronaviruses are named after their characteristic crown-shaped microscopic appearance². They are positive-sense single-stranded RNA viruses.

Taxonomically, they form part of the Coronaviridae sub-family (Coronaviridae family and Nidovirales order)^{3, 4}. The SARS-CoV-2 spreads primarily through droplets of saliva or discharge from the nose hence asked to cover nose and mouth while coughing or sneezing and also to maintain distance in public places⁵.

There is a difference in infection and death cases recorded from different geographical locations of the world and also from different states of India. Total cases reported till 4th September 2020 in Kerala and Gujarat (Surat and Ahmedabad) were 79,625 and 100,213, respectively. The death cases reported were 315 in Kerala and 3062 in Gujarat (Center for Systems Science and Engineering, 2020)⁷. Currently, the search for vaccines or treatments for COVID-19 is going on. To understand the root cause of differences in the infection and deaths rates, this study was planned

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to analyze the genomic and pathogenic similarities and differences between the strains of SARS-CoV-2 isolated from different geographical locations of the world and also from different parts of India.

METHODOLOGY: Different genome sequences from the viral genome sequences database of the National Center for Biotechnology Information (NCBI) and different comparative analyses were performed. For this analysis, we used the SARS-CoV-2 reference genome, NC_045512, isolated from China. We downloaded three more sequences of the SARS-CoV-2 isolated from different geographical locations *i.e.*, MT477885 isolated from India, MT886288 from USA, and MT 835383 from Brazil. For a further detailed study of SARS-CoV-2, sequences isolated from different parts of India were downloaded: MT050491 isolated from Kerala, MT806104 from Surat, and MT799970 from Ahmedabad.

Sequence Alignment: Samples were aligned to the reference genome in the nucleotide BLAST using Basic Local Alignment Search Tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to extract the differences between the genome variants and the reference genome.

The parameter was set to a highly similar sequence. The sequences of SARS-CoV-2 isolated from different geographical locations of the world and India was compared with the reference genome of SARS-CoV-2 to extract the differences and the similarities.

Comparison of the GC Content: The genomic GC content was calculated among all the sequences. The GC content was calculated by using GC content calculator of Biologics International Corp (BIC) (<https://www.biologicscorp.com/>

tools/). FASTA format sequence was entered in the box provided (in FASTA format). The window size was selected for 30 and the sequence was submitted to obtain the results. GC content is also called G+C ratio or GC-ratio and was calculated as a percentage. GC-content percentage was calculated as $\text{Count (G + C) / Count (A + T + G + C) * 100\%}$.

Identified ORFs (Length > 150 bps): The ORF finder is a program available at NCBI website. It identifies all the open reading frames or the possible protein-coding region in sequence. The Open Reading Frame, ORF tool of NCBI (<https://www.ncbi.nlm.nih.gov/orffinder/>) was used to identify all the ORFs present in different sequences of SARS-CoV-2 isolated from different geographical locations of the world and also from India. ORF finder searches for open reading frames (ORFs) in the DNA sequence entered. FASTA format sequence, which starts with a ‘>’ symbol followed by the sequence ID was entered in the box provided (in FASTA format). In the list of the genetic codes "standard" was selected with "AUG" as the start codon. Nested ORFs were ignored. The size of ORFs greater than 150 was selected. The ORF finder button was clicked to run the operation.

RESULTS AND DISCUSSION:

Sequence Comparison of SARS-Cov-2 (NC_045512.2) with Different Strains of SARS-Cov-2 Isolated from Different Geographical Locations - India, USA and Brazil: The reference genome of SARS-CoV-2 was aligned separately with different strains of SARS-CoV-2 isolated from different geographical locations, *i.e.*, India, USA, and Brazil. This comparison was carried out to confirm if different strains of SARS-CoV-2 were infecting the population of different countries as the death rates of these countries vary largely.

TABLE 1: SEQUENCE COMPARISON OF SARS-COV-2 (NC_045512.2) WITH DIFFERENT STRAINS OF SARS-COV-2 ISOLATED FROM DIFFERENT GEOGRAPHICAL LOCATIONS: INDIA, USA AND BRAZIL

Strains	GeneBank Accession Number	Country	Host	Year Isolated	Length (bp)	Query Cover (%)	E Value	Identify with Reference Sequence (%)
SARS-CoV-2	NC_045512.2	China	Homo sapians	2020	29903	100	0	100
SARS-CoV-2	MT477885	India	Homo sapians	2020	29899	99	0	99.98
SARS-CoV-2	MT886288	USA	Homo sapians	2020	29743	99	0	99.98
SARS-CoV-2	MT835383	Brazil	Homo sapians	2020	29858	99	0	99.98

All the three sequences were highly similar, with the reference sequence isolated from Wuhan, China. They all showed 99.98% identity with the reference sequence **Table 1**. A high homology was observed between the sequences but a very vast difference in the death cases reported by each country.

As of data till 4th September 2020, the death cases reported by China were 4,634; India was 68,598; USA was 191, 060 and Brazil was 124,729. Mohanty *et al.*, linked the difference in the death rates to factors such as a large proportion of the old population, obesity or existing illness, climate, and the genetic differences ⁸. The genetic difference of the different populations may have led to high immunity and weaker host-pathogen interaction of the individual ⁹.

Sequence Comparison of SARS-CoV-2 (NC_045512.2) with Different Strains of SARS-CoV-2 Isolated from Different Parts of India - Kerala, Surat and Ahmedabad: The reference

genome of SARS-CoV-2 was aligned separately with the strains isolated from Kerala, Surat, and Ahmedabad. The identity percentage of a strain isolated from Surat and Ahmedabad was highly similar to the reference strain resulted in 99.97% and 99.96%, respectively **Table 2**. And the strain isolated from Kerala resulted in an identity percentage of 99.56% lesser than the other two strains. From my Gov (<https://www.mygov.in/corona-data/covid19-statewide-status/>) total cases reported up to 4th September, 2020 in Kerala and Gujarat (Surat and Ahmedabad) were 79,625 and 100,213 respectively. The death cases reported were 315 in Kerala and 3062 in Gujarat (Center for Systems Science and Engineering, 2020) ^{7, 11} found that the SARS-CoV-2 strain infecting Gujarat is different from the rest of India. She showed two missense mutations in the SARA-CoV-2 strain of Gujarat. The first deleterious mutation, C28854T in nucleocapsid (N) gene, which leads to high mortality in Gujarat, and second G25563T, located in Orf3a plays crucial roles in viral pathogenesis ¹¹.

TABLE 2: SEQUENCE OF SARS-COV-2 (NC_045512.2) COMPARED WITH DIFFERENT STRAINS OF SARS-COV-2 ISOLATED FROM DIFFERENT PARTS OF INDIA: KERALA, SURAT AND AHMEDABAD

Strains	GeneBank Accession Number	Country	Host	Year Isolated	Length (bp)	Query Cover (%)	E Value	Identify with Reference Sequence (%)
SARS-CoV-2	NC_045512.2	China	Homo sapians	2020	29903	100	0	100
SARS-CoV-2	MT050491	Kerala	Homo sapians	2020	29850	95	0	99.56
SARS-CoV-2	MT806104	Surat	Homo sapians	2020	29800	99	0	99.97
SARS-CoV-2	MT799970	Ahmedabad	Homo sapians	2020	29800	99	0	99.96

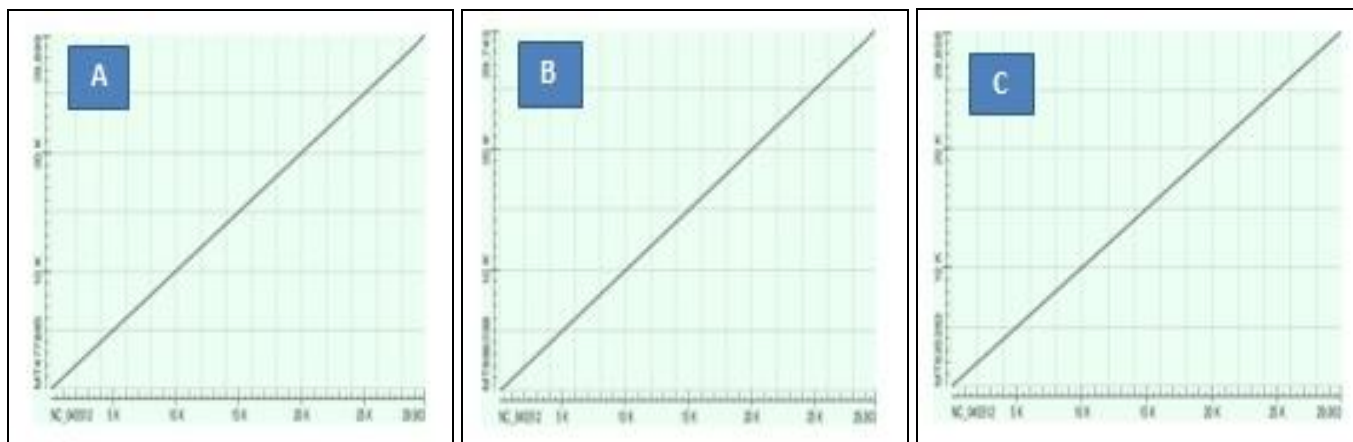


FIG. 1: DOT PLOT OF SEQUENCE ALIGNMENT A) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM INDIA (MT477885). B) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM USA (MT886288). C) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM BRAZIL (MT835383)

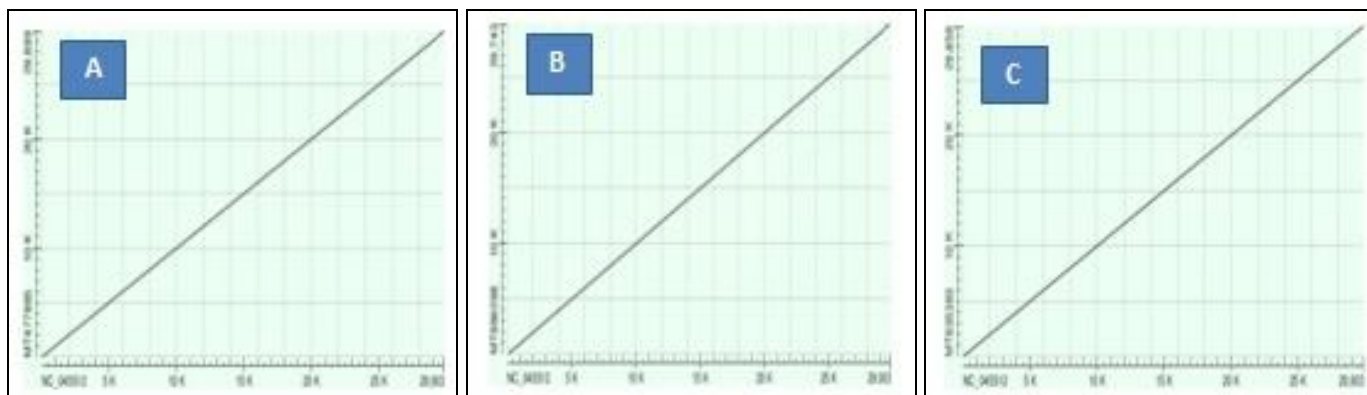


FIG. 2: DOT PLOT OF SEQUENCE ALIGNMENT A) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM KERALA (MT050491). B) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM SURAT (MT806104). C) SARS-COV-2 REFERENCE SEQUENCE (NC_045512.2) AND SARS-COV-2 ISOLATED FROM AHMEDABAD (MT799970)

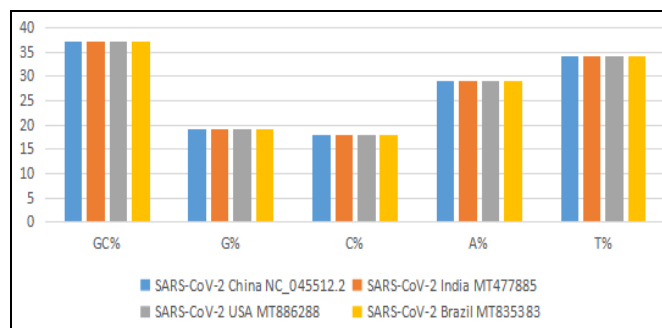
The same was also supported by CSIR-Institute of Genomics and Integrative Biology by representing strains of SARS-CoV-2 of Kerala and Gujarat in different clades *i.e.* B4 clade and A3 clade, respectively ¹² Rahila Sardar *et al.*, also found a high rate of mutations within India strains (Rahila Sardar, 2020). The difference in the identity percentage may also be due to the difference in the genome length of SARS-CoV-2 strains **Table 2**. The length of reference strain was 29903, while the genome length of a strain isolated from Kerala was 29850 giving low query value of 95%; thus giving low identity percentage.

The genome length of strains from Surat and Ahmedabad is 29800 with a query cover of 99%; resulting in a high identity percentage. The dot plots of SARS-CoV-2 strain isolated from Wuhan China (reference sequence) and SARS-CoV-2 isolated from India **Fig. 1A**, USA **Fig. 2B** and Brazil **Fig. 2C** showed a complete diagonal line concluding high similarity between the genomes. The same was concluded with their 99.98% identity percentage **Table 2**.

The dot plot of SARS-CoV-2 strain isolated from Wuhan China (reference sequence) and SARS-CoV-1 isolated from Kerala did not form a complete diagonal line concluding a low identity percentage of 99.56% **Table 2**. The insertions or deletions were observed as the breaks or discontinuities in the diagonal lines **Fig. 2A**. The dot plot of SARS-CoV-2 strain isolated from Wuhan China (reference sequence) and SARS-CoV-2 isolated from Surat **Fig. 2B** and Ahmedabad **Fig. 2C** showed a complete diagonal line hence are

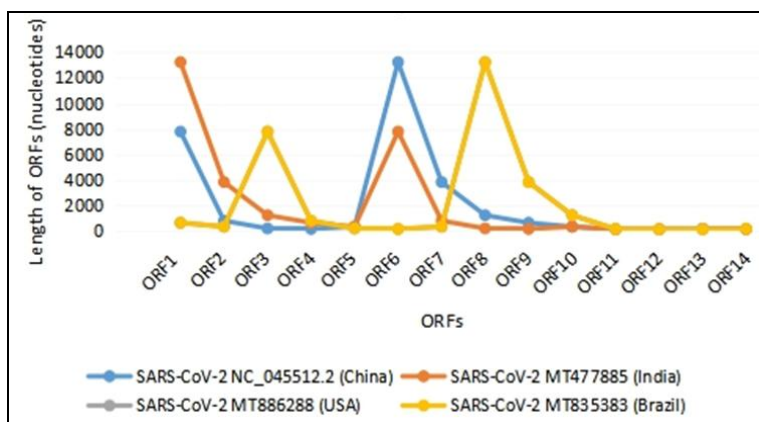
highly similar. The same was concluded with the identity percentage of 99.97% and 99.96% respectively **Table 2**.

Comparison of GC Content: The GC content of SARS-CoV-2 from all the four geographical locations- China, India, USA and Brazil showed the same GC content of 37% **Graph 1**. Also, the GC content of all the SARS-CoV-2 strains isolated from different parts of India showed the same GC content of 37% **Graph 1**. Hence, the coding regions of the core genome could be the same in all the strains of SARS-CoV-2 isolated from different geographical locations.



GRAPH 1: GC CONTENT TO DIFFERENT GENOMES OF SARS-COV-2 ISOLATED FROM DIFFERENT GEOGRAPHICAL LOCATION

Identified ORFs (Length > 150 bps): Comparing the length of different ORFs of various strains of SARS-CoV-2 isolated from different geographical locations *i.e.*, China, India, USA, and Brazil **Table 3** it can be concluded that the strains isolated from China (NC_045512.2) and India (MT477885) are similar to each other with similar lengths. But, India strain (MT477885) was highly assorted from Brazil (MT835383) and USA (MT886288) strains.



GRAPH 2: TOTAL ORFs AND LENGTH OF ORFs WAS COMPARED WITH DIFFERENT STAINS OF SARS-COV2 ISOLATED FROM INDIA, USA, BRAZIL

TABLE 3: TOTAL ORFS AND LENGTH OF ORFS WAS COMPARED WITH DIFFERENT STRAINS OF SARS-COV-2 ISOLATED FROM INDIA, USA AND BRAZIL

Strain	GeneBank Accession Number (Country)	Total ORFs (>150)	Length of ORFs (Nucleotides)													
			OR F1	O RF 2	ORF 3	O RF 4	OR F 5	ORF 6	ORF 7	ORF 8	ORF 9	ORF 10	ORF 11	ORF 12	ORF 13	ORF 14
SARS-CoV-2	NC_045512.2 (China)	14	7788	82	228	18	366	13218	3849	1260	669	366	156	162	192	177
	MT477885 (India)	14	1321	38	1260	66	366	7788	828	228	186	366	156	162	192	177
	MT886288 (USA)	13	669	36	7788	82	228	186	366	13218	3849	1260	177	162	192	
	MT835383 (Brazil)	14	669	36	7788	82	228	186	366	13218	3849	1260	177	156	162	192

ORFs of length greater than 150bps that begin with a start codon AUG and end with stop codons UAA, UAG or UGA were detected in different sequences of SARS-CoV-2 (Takahiko Koyama, 2020). USA and Brazil have the highest number of COVID-19 cases and high death rates (WHO, 2019). Observing the obtained result **Graph 2**, the same highly virulent strain of SARS-CoV-2 infects the population of the USA and Brazil. B Korber *et al.*, tracked Spike (S) real-time protein mutation in SARS-CoV-2. In his study, he found that the strains spreading so quickly in Europe and the U.S. have a mutated S “spike” protein that makes it about 10 times more infectious than the strain that was originally identified in Asia. This mutation does not make the virus more deadly, but it makes it more contagious. The original strain in China is D614, while the one found in North America is dubbed G614¹⁵. The Indian microbiologist Rup Lal with his team, studied the complete genomes of 95 strains of SARS-CoV-2 reported that the strain found in India almost matches with the sequence of

SARS-CoV-2 found in Wuhan, but it is less virulent¹⁶.

CONCLUSION: SARS-CoV-2 strains of China and India have highly similar. But, ORF length was different from USA and Brazil. However, in the same way, length and identity percentage of the Kerala strain of SARS-CoV-2 was different from the reference sequence of china. From this outcome, we speculate, various factors causing the difference in reported death cases could be a result of mutation that has been observed in the sequence alignment data. Other possible factors could be large proportion of the old population, obesity or existing illness, climate, and genetic differences. An incomplete diagonal line in the dot plot of SARS-CoV-2 (Kerala) indicated insertion or deletion in the sequence. The GC content of SARS-CoV-2 from different geographical locations of the world and also from different parts of India showed the same GC content of 37%. The ORF comparison study further revealed that the SARS-CoV-2 strains

are completely similar in the USA and Brazil. This could be linked with the high virulence capacity of the strain, causing a high number of COVID-19 cases and deaths in these countries. The SARS-CoV-2 reference strains of India are most similar to the reference strain isolated from Wuhan while the death rate of India is still very less. This provides insight for further studies on linking the low COVID-19 death rate in India with the underlying immunity. Based on this speculation, still more research work is required in a genomic, pathogenic, and immunological approach based on geographical location of the world, and in India, it must identify the exact solution; hence people can get rid of the pandemic situation.

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REFERENCES:

1. WHO, Coronavirus disease (COVID-19) pandemic. [Online] Available at: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019> [Accessed 23 May 2020].
2. Goldsmith CS, Tatti KM and Ksiazek TG: Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis* 2004; 10(2): 320-26.
3. Auewarakul P: Composition bias and genome polarity of RNA viruses. *Virus Res* 2005; 109(1): 33-37.
4. Coronaviridae. ICTV, 2020. [Online] Available at: talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae.
5. Andersen KG, Rambaut A, Lipkin WI, Holmes EC and Garry RF: The proximal origin of SARS-CoV-2. *Nat Med* 2020; 26(4): 450-52.
6. Kumar D, Malviya R and Sharma KP: Corona virus: a review of COVID-19. *EJMO* 2020; 4(1): 8-25.
7. Center for Systems Science and Engineering (CSSE), 2019. "COVID-19: Novel coronavirus." Data repository. [Online] Available at: <https://github.com/CSSEGISandData/COVID-19>.
8. Mohanty SK, Satapathy A and Naidu MM: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19) - anatomic pathology perspective on current knowledge. *Diagn Pathol* 2020; 15: 103.
9. Sharma G and Mehra NK: Indian population could have intrinsic immunity to resist COVID-19 challenge: International Union of Immunological Sciences (<https://www.immunopaedia.org.za/breaking-news/indian-population-could-have-intrinsic-immunity-to-resist-covid-19-challenge/>) 2020.
10. Santos AM: Covid-19 state wise status. [Online] Available at: <https://www.mygov.in/corona-data/covid19-statewise-status/> 2020.
11. Joshi M, Puvar A, Kumar D, Ansari A, Pandya M, Raval J and Patel Z: Genomic variations in SARS-CoV-2 genomes from Gujarat: Underlying role of variants in disease epidemiology. *BioRxiv* 2020; 07-10: 197095.
12. Kumar P, Pandey R, Sharma P, Dhar MS and Vivekanand A and Uppili B: Integrated genomic view of SARS-CoV-2 in India. *BioRxiv* 2020; 6(4): 128751.
13. Koyama T, Platt D and Parida L: Variant analysis of SARS-CoV-2 genomes. *Bulletin of the World Health Organization* 2020; 98: 495-04.
14. WHO, World Health Organisation. [Online] Available at: [https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-\(mers-cov\)](https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov)), 2019.
15. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Foley B, Giorgi EE, Bhattacharya T, Parker MD, Partridge DG, Evans CM and de Silva TI: Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2. *BioRxiv* 2020; 04(29): 069054.
16. Lal R, Kumar R, Verma H, Singhvi N, Sood U and Gupta V: Comparative genomic analysis of rapidly evolving SARS-CoV-2. Reveals Mosaic Pattern of Phylogeographical Distribution Systems 2020; 5(4): 00505-2.

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