

Genome Sequences of COVID-19 from Jordanian Patients in Comparison with the Global Pandemic Strains and the Transmission Route

Ihsan Mahasneh^{1*}, Mustafa Ennaji², Berjas Abumsimir²

¹Department of Applied Biology, Faculty of Science, University of Sharjah, Sharjah, UAE ²Virology Laboratories, Faculty of Sciences and Techniques Mohammedia, Hassan II University of Casablanca, Casablanca, Morocco Email: *imahasneh@sharjah.ac.ae, lvmqbetb.lab@gmail.com, berjas_last@yahoo.com

How to cite this paper: Mahasneh, I., Ennaji, M. and Abumsimir, B. (2021) Genome Sequences of COVID-19 from Jordanian Patients in Comparison with the Global Pandemic Strains and the Transmission Route. *Journal of Biosciences and Medicines*, **9**, 77-93.

https://doi.org/10.4236/jbm.2021.93009

Received: February 12, 2021 **Accepted:** March 26, 2021 **Published:** March 29, 2021

Copyright © 2021 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

http://creativecommons.org/licenses/by/4.0/

Abstract

In this research study, we reported a pioneer investigation on the phylogenetic profile of genomic sequences and the prevalence of COVID-19 strains in Jordan; in comparison with global strains, as part of concerted global efforts in combating the COVID-19 pandemic. A total of 28 genome sequences of the hCoV-19/Jordan strain were employed in this study. The phylogenetic analysis of the genome sequences of hCoV-19/Jordan strain, which was conducted on the databases of NCBI and GASAID revealed that the hCoV-19/ Jordan strain belongs to the G, V, and S clades with distribution coverage rates of 89%, 7% and 4%, respectively. Comparison of the hCoV-19/Jordan clades with global clades distribution profile showed evidently and conclusively, a unique pandemic profile of multiple geographical sources of COVID-19, imported into Jordan primarily through cryptic means from European countries (UK, Belgium, Sweden, Russia) and Morocco, which are entry points into Jordan. This is due to the reciprocal heavy air traffic route between Jordan and the capitals of these European countries, with Morocco being the primary entry point into Jordan. The results of this study represent a marked addition to investigations on the prevalence rate of the hCovid-19/Jordan strain in comparison to the global strains sequences, and provides a better understanding on comparative diagnosis technique for COVID-19 worldwide. Phylogenetic analysis of the top 100 and top 10 taxonomic trees revealed almost identical homology (99.98% - 100%) between hCoV-19/Jordan strain and the strains that broke out in Wuhan, the MT642226.1. Furthermore, it can be inferred that there was no marked change in the virus mutation that occurred during the period of this study taking into cognizance the fact that there was identical homology (99.98% - 100%) among the 28 sequences of hCoV-19/Jordan strains. Similarly, all sequences of the Jordanian strains of hCoV-19/Jordan

were compared to the England and American strains and showed identical homology of (99.98% - 100%).

Keywords

COVID-19 Phylogenetic, COVID-19 in Jordan, Coronavirus SARS-CoV-2, Viral Infection, COVID-19 Pandemic

1. Introduction

Since the World Health Organization (WHO) officially declared the outbreak of COVID-19 a pandemic on March 11, 2020, there has been a global growing interest in research targeted at combating the virus due to the surge in infection, as well as the prevalence rate. This has made it imperative and expedient for all probable preventative measures to be adopted in combating the global surge in infection pending the discovery of a vaccine for the virus. Currently, there are over 40 million confirmed cases globally as reported by WHO. The COVID-19 strain responsible for the current global pandemic was identified early this year as the β -coronavirus (CoV), which belongs to the *Coronaviridae* family and it has a single positive-strand RNA genome [1] [2] [3]. This strain-type is among several strains of coronaviruses that are pathogenic to humans; most are associated with mild clinical symptoms, with the exception of the Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS-CoV) and it was reported as a novel β -coronavirus two decades ago [4] [5] [6]. In 2012, the same strain-type was detected in Saudi Arabia, and since then, it has been known as the Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV) [7] [8]. It was later imported into South Korea, and caused about 2500 confirmed cases of infections [9] [10]. In late December, 2019, after global detection of the COVID-19 strain in many countries, the WHO has declared it as a pandemic on March 11, 2020.

A phylogenetic tree with the geographical mutation transmission of SARS-CoV-2 has previously been reported to have a genome size of about 30 kilobases, and encodes 10 genes of a single positive-stranded RNA (ribonucleic acid) [1] [2] [11]-[17]. Furthermore, a comprehensive study conducted by the World Health Organization [18] (employing 10,022 SARS-CoV-2 genomes from 68 different countries) showed how the SARS-CoV-2 genome has evolved over the years, spreading to different parts of the world. In total, 65,776 variants and 5775 distinct variants, comprised of the most frequent mutations causing the SARS-CoV-2 pandemic were detected by WHO. Additionally, 2969 missense mutations, 1965 synonymous mutations, and 484 mutations in non-coding regions were also discovered by WHO [1]. It was reported by [12] based on data from the public database of the Global Initiative on Sharing All Influenza Data (GISAID) that three major clades of SARS-CoV-2 exists, and have subsequently

been named as clade G (variant of the spike protein S-D614G), clade V (variant of the ORF3a coding protein NS3-G251), and clade S (variant ORF8-L84S).

Jordan and its Mediterranean crossing route, and global tourist attraction centers represent an important route for global spread of COVID-19. The spread of COVID-19 is most likely because of Jordan's heavy air traffic, which is induced by Jordan's route nexus with Europe and many north African countries. It is also attributed to Jordan's close border with Asian countries like China, Pakistan, Nepal, and gulf countries like Saudi, UAE and Bahrain. Up to date of this paper, the COVID-19 pandemic has spread progressively reaching 110,974,862 confirmed cases of COVID-19, including 2,460,792 deaths worldwide and showed a marked increase in infection in Jordan exceeding 364,000 confirmed cases. Therefore, this paper as the first study aimed at investigating the COVID-19 in Jordan as part of the global pandemic and in comparison to global strains, has a vital role for Jordan as well as other parts of the world for better understanding the COVID-19 pandemic across the world which will enhance the global understanding of the transmission routes as well as the mechanism of action to combating the its pandemic.

2. Materials and Methods

2.1. Jordan National SARS-CoV-2 Pandemic Surveillance Committee

After the first confirmed case of SARS-CoV-2 infection in Jordan, a National SARS-CoV-2 Pandemic Surveillance Committee was instituted with the National Order number (17) to oversee the SAR-CoV-2 surveillance and combat program.

2.2. Collection of Clinical Samples and Extraction of RNA Using RT-PCR

A total of 28 nasopharyngeal swab samples (26 from Amman & 2 from Irbid) were collected from symptomatic and high index of suspicion patients at the Biolab Diagnostic Laboratories, Amman, Jordan for SAR-CoV-2 investigation using the RT-PCR and RNA extraction technique (in line with the manufacturer's protocol). Quality control assurance measures for SAR-CoV-2 RNA purity were conducted in line with the extraction protocol. The genome of the 28 samples was later dispatched to the Scripps Research Institute (TSRI), La Jolla, California, USA for genome sequencing.

2.3. Sequencing of the Full Length Genome of Jordanian SAR-CoV-2 Strains

The (28) genome samples from Jordan (26 from Amman and 2 from Irbid) were subjected to complete genome sequencing in the Scripps Research Institute (TSRI), La Jolla, California, USA as previously described [11]. The resultant se-

quences were later deposited by the Scripps Research Institute (TSRI) into the databases of the NCBI GenBank (<u>http://www.ncbi.nlm.nih.gov/</u>) and Global Initiative on Sharing All Influenza Data (GISAID) (<u>http://www.gisaid.org/</u>) employing the tag hCoV-19/Jordan vis-à-vis each respective accession number (see **Table 1** for details of the accession numbers and other descriptions).

2.4. Using GISAID (Global Initiative on Sharing All Influenza) Public Domain Databank for Phylogenetic Comparison with COVID-19 Sequences of Global Strains

For comparison of our strains sequences of the COVID-19 with the genomic sequences of the global strains from different countries, we have used with acknowledgments the COVID-19 sequences available on the Public Domain Databank platform of GISAID and EpiFlu[™] database, as hosted by the Federal Republic of Germany; which provides us with rapid sharing of COVID-19 sequences enabling us for rapid and conclusive global comparison on the epidemic and pandemic of the COVID-19 (<u>http://www.gisaid.org/</u>).

Sequence alignment was conducted using human coronavirus sequences registered in the Global Initiative on Sharing All Influenza Data (GISAID) and NCBI GenBank. Using the available sequences and the software on the Public Domain of the GISAID (<u>http://www.gisaid.org/</u>) and the NCBI

(https://blast.ncbi.nlm.nih.gov); the Phylogenetic trees were constructed employing for Neighbor Joining (NJ); Minimum Evaluation Analysis and the NJ-Taxonomic Distance Tree. The accession numbers of the 28 sequences of hCoV-19/Jordan were used to construct the phylogenetic tree in comparison with the closest sequences of the top 100 and top 10 taxonomic trees, and (99.98% - 100%) semblance to those sequences available on the NCBI

(https://blast.ncbi.nlm.nih.gov). In this comparative study, we selected a set of sequences from different parts of the world. Sequences were selected from Europe (3 from United Kingdom, 3 from Belgium, 2 from Sweden, and 3 from Russia), Asia (9 from Pakistan, 1 from Nepal, 9 from Bahrain, and 4 from Lebanon) and Africa (10 from Morocco) because of the geographical limitrophe of Jordan with these countries. Sequences were assembled and mapped to the SARS-CoV-2 that are available on the Public Domain of the GISAID (http://www.gisaid.org/) and the NCBI (https://blast.ncbi.nlm.nih.gov).

3. Results

The phylogenetic tree of the hCoV-19/Jordan sequences in comparison with selected global sequences is presented in **Figure 1** which shows almost identical homology (99.98% - 100%) with the top 100 and top 10 taxonomic trees. The results showed that the spreading profile in Jordan is composed of (5) geographical clusters vis-à-vis the origin of the pandemic. These include 2 different clusters similar to sequences from the UK, 1 cluster from Morocco and 2 clusters with local transmission designation. The neighbor joining (NJ) of distance-based **Table 1.** Sequences description for the Covid-2 of the genus Betacoronavirus and family Coronaviridae; during pandemic period of March 1st. and October 1st. 2020 as compared with selected global strains.

No	GeneBank/Sequence accession number	Submission date	Sequence length	Geographical location	
1	MW041156	2020-09-28	29,870		
2	MW031799	2020-09-23	29,903	Pakistan	
3	MW031800	2020-09-23	29,903	Pakistan	
4	MW031801	2020-09-23	29,903	Pakistan	
5	MW031802	2020-09-23	29,903	Pakistan	
6	MW031803	2020-09-23	29,903	Pakistan	
7	MT890462	2020-08-17	29,903	Russia: Moscow	
8	MT879619	2020-08-13	29,897	Pakistan	
9	MT873892	2020-08-11	29,821	United Kingdom	
10	MT811544	2020-07-29	29,409	Jordan: Amman	
11	MT811545	2020-07-29	29,409	Jordan: Amman	
12	MT811546	2020-07-29	29,409	Jordan: Amman	
13	MT811548	2020-07-29	29,409	Jordan: Amman	
14	MT811549	2020-07-29	29,409	Jordan: Amman	
15	MT811550	2020-07-29	29,409	Jordan: Amman	
16	MT811551	2020-07-29	29,409	Jordan: Amman	
17	MT811552	2020-07-29	29,412	Jordan: Amman	
18	MT811553	2020-07-29	29,409	Jordan: Amman	
19	MT811554	2020-07-29	29,409	Jordan: Amman	
20	MT811555	2020-07-29	29,409	Jordan: Amman	
21	MT811556	2020-07-29	29,409	Jordan: Amman	
22	MT811557	2020-07-29	29,409	Jordan: Amman	
23	MT811558	2020-07-29	29,409	Jordan: Amman	
24	MT811560	2020-07-29	29,409	Jordan: Irbid	
25	MT811561	2020-07-29	29,409	Jordan: Amman	
26	MT811562	2020-07-29	29,409	Jordan: Irbid	
27	MT811564	2020-07-29	29,409	Jordan: Amman	
28	MT811565	2020-07-29	29,409	Jordan: Amman	
29	MT811566	2020-07-29	29,406	Jordan: Amman	
30	MT811567	2020-07-29	29,409	Jordan: Amman	
31	MT811568	2020-07-29	29,409	Jordan: Amman	
32	MT811569	2020-07-29	29,409	Jordan: Amman	
33	MT801000	2020-07-27	29,854	Lebanon	
34	MT801001	2020-07-27	29,829	Lebanon	
35	MT801002	2020-07-27	29,865	Lebanon	
36	MT801003	2020-07-27	29,779	Lebanon	
37	MT789712	2020-07-22	29,856	Bahrain	

Continued				
38	MT745584	2020-07-13	29,860	Bahrain
39	MT745767	2020-07-13	29,879	Bahrain
40	MT745836	2020-07-13	29,879	Bahrain
41	MT745875	2020-07-13	29,800	Bahrain
42	MT747438	2020-07-13	29,782	Belgium
43	MT742782	2020-07-12	29,885	Bahrain
44	MT742783	2020-07-12	29,856	Bahrain
45	MT742784	2020-07-12	29,766	Bahrain
46	MT731285	2020-07-08	29,903	Morocco
47	MT731292	2020-07-08	29,903	Morocco
48	MT731327	2020-07-08	29,903	Morocco
49	MT731346	2020-07-08	29,867	Bahrain: Capital
50	MT731468	2020-07-08	29,903	Morocco
51	MT731673	2020-07-08	29,903	Morocco
52	MT731746	2020-07-08	29,903	Morocco
53	MT731764	2020-07-08	29,903	Morocco
54	MT733120	2020-07-08	29,903	Morocco
55	MT635445	2020-06-18	29,727	Russia: Moscow region
56	MT637143	2020-06-18	29,883	Russia: Moscow region
57	MT568645	2020-06-05	29,838	Morocco: Casablanca
58	MT513758	2020-06-03	29,875	Morocco
59	MT500122	2020-05-21	29,819	Pakistan: Karachi
60	MT262993	2020-03-31	29,836	Pakistan: KPK
61	MT240479	2020-03-25	29,836	Pakistan: Gilgit
62	MT093571	2020-02-21	29,886	Sweden
63	MT072688	2020-02-18	29,811	Nepal
64	MW031799	2020-09-23	29,903	Pakistan

phylogenetic analysis of the top 100 (**Figure 2**) and top 10 (**Figure 3**) taxonomic trees revealed almost identical homology between hCoV-19/Jordan strain and the strains that broke out in Wuhan, the MT642226.1.

The sequence description of the hCoV-19/Jordan strain in comparison with global sequences homology (99.98% - 100%) based on NCBI database filter results is presented in **Table 1**. All sequences of the hCoV-19/Jordan strain were complete, exceeding (29,400 bp), and example of the complete sequence of the hCoV-19/Jordan is given in **Figure 3**, which were therefore compared with similar complete global sequences. The clades and sub-clades descriptions of the genealogical lineage of the hCoV-19/Jordan strain are presented in **Table 2**; which shows that the hCoV-19/Jordan strain belongs to the G, V, and S clades with spreading frequencies of 89%, 7% and 4%, respectively.

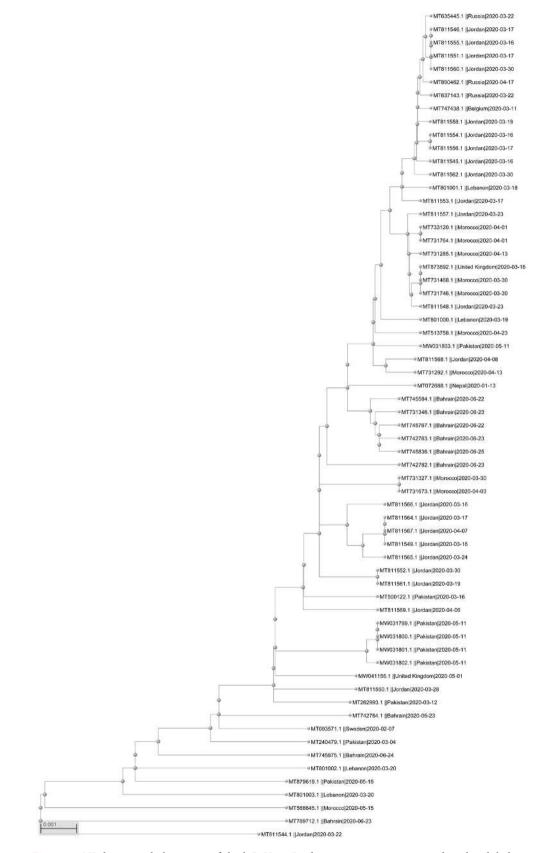


Figure 1. NJ-distance phylogenetic of the hCoV-19/Jordan sequences as compared to the global sequences.

	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/JOR/SEARCH-0054-JOR/2020, complete g
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/JOR/SEARCH-0054-JOR/2020, complete g.,
· •	• Severe acute respiratory syndrome coronavirus 2 isolate NORW-EBA67 genome assembly, chromosome: 1
	Severe acute respiratory syndrome coronavirus 2 isolate NORW-EBD7D genome assembly, chromosome: 1
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CT-UW-651/2020, complete genome
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW213/2020, complete genome
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC860/2020, complete genome
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/MA-QDX-80/2020, complete genome
	Severe acute respiratory syndrome coronavirus 2 hCoV-19/Japan/P4-4/2020 RNA, complete genome
	viruses 4 leaves
	Viruses 5 leaves
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY-QDX-106/2020, complete genome
	viruses 7 leaves
	Severe acute respiratory syndrome coronavirus 2 hCoV-19/Japan/P4-2/2020 RNA, complete genome
	viruses 4 leaves
	viruses 5 leaves
	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/SAU/86327/2020, complete genome
	viruses 7 leaves
	viruses 29 leaves
10.000009	viruses 11 leaves
L 1	viruses 17 leaves

Figure 2. NJ-distance phylogenetic of the hCoV-19/Jordan sequences as compared to the top-100 hits of global sequences.

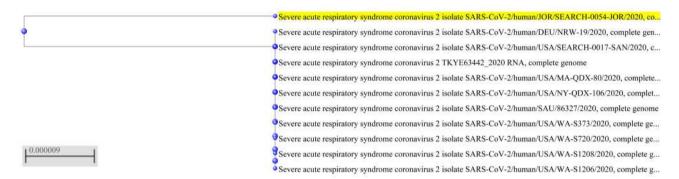


Figure 3. NJ-distance phylogenetic of the hCoV-19/Jordan sequences as compared to the top-10 hits of global sequences.

4. Discussion

The phylogenetic analysis of the hCoV-19/Jordan strain constructed from selected global genomes deposited into GISAID for comparison purposes revealed that the 28 genome sequences from Jordan employed in this study were compatible with the evolutionary tree of SARS-CoV-2, and had a complete genome length of 29,400 bp and homology of (99.98% - 100%) (Figure 1). This provides a conclusive evidence that the hCoV-19/Jordan is part of the global pandemic strains of SARS-CoV-2. The results of our study showed that the genome length and homology of the hCoV-19/Jordan strain are in consonance with those reported for the SARS-CoV-2 strains globally [1] [3] [11]. The neighbor joining phylogenetic analysis of the top 100 (Figure 2) and top 10 taxonomic trees (Figure 3) showed conclusively that the hCoV-19/Jordan strain had almost identical homology (99.98% - 100%) with the strains that broke out in Wuhan, the MT642226.1. This suggests that it is the most recent strain, and has a common ancestry with the strains transmitted and detected in Jordan. Similarly, all sequences of the Jordanian strains of hCoV-19/Jordan were compared to the England and American strains and showed identical homology of (99.98% -

No	Clade/ Lineage	Virus name	Accession ID	Collection date	Submission date	Length	Geographical region
1	G/B.1.1	hCoV-19/Jordan/SR-055/2020	EPI_ISL_430013	2020-04-02	2020-04-24	29,409	Asia/Jordan/Amman
2		hCoV-19/Jordan/SR-049/2020	EPI_ISL_430007	2020-03-19	2020-04-24	29,409	Asia/Jordan/Amman
3		hCoV-19/Jordan/SR-048/2020	EPI_ISL_430006	2020-03-23	2020-04-24	29,409	Asia/Jordan/Amman
4		hCoV-19/Jordan/SR-047/2020	EPI_ISL_430005	2020-03-26	2020-04-24	29,397	Asia/Jordan/Amman
5		hCoV-19/Jordan/SR-046/2020	EPI_ISL_430004	2020-03-17	2020-04-24	29,409	Asia/Jordan/Amman
6		hCoV-19/Jordan/SR-045/2020	EPI_ISL_430003	2020-03-16	2020-04-24	29,409	Asia/Jordan/Amman
7		hCoV-19/Jordan/SR-044/2020	EPI_ISL_430002	2020-03-16	2020-04-24	29,409	Asia/Jordan/Amman
8		hCoV-19/Jordan/SR-043/2020	EPI_ISL_430001	2020-03-17	2020-04-24	29,409	Asia/Jordan/Amman
9		hCoV-19/Jordan/SR-041/2020	EPI_ISL_429999	2020-03-17	2020-04-24	29,409	Asia/Jordan/Amman
10		hCoV-19/Jordan/SR-036/2020	EPI_ISL_429996	2020-03-23	2020-04-24	29,409	Asia/Jordan/Amman
11		hCoV-19/Jordan/SR-034/2020	EPI_ISL_429994	2020-03-17	2020-04-24	29,409	Asia/Jordan/Amman
12		hCoV-19/Jordan/SR-033/2020	EPI_ISL_429993	2020-03-16	2020-04-24	29,409	Asia/Jordan/Amman
13		hCoV-19/Jordan/SR-032/2020	EPI_ISL_429992	2020-03-22	2020-04-24	29,409	Asia/Jordan/Amman
14	G/B.1.1	hCoV-19/Jordan/SR-054/2020	EPI_ISL_430012	2020-03-30	2020-04-24	29,409	Asia/Jordan/Irbid
15	G/B.1.1	hCoV-19/Jordan/SR-052/2020	EPI_ISL_430009	2020-03-30	2020-04-24	29,409	Asia/Jordan/Irbid
16	GH/B.1.36	hCoV-19/Jordan/SR-0335/2020	EPI_ISL_450187	2020-04-08	2020-05-20	29,864	Asia/Jordan/Amman
17	B.2.1(O)	hCoV-19/Jordan/SR-084/2020	EPI_ISL_434516	2020-03-16	2020-04-29	29,406	Asia/Jordan/Amman
18		hCoV-19/Jordan/SR-057/2020	EPI_ISL_430015	2020-03-24	2020-04-24	29,409	Asia/Jordan/Amman
19		hCoV-19/Jordan/SR-056/2020	EPI_ISL_430014	2020-03-17	2020-04-24	29,409	Asia/Jordan/Amman
20		hCoV-19/Jordan/SR-053/2020	EPI_ISL_430011	2020-03-19	2020-04-24	29,409	Asia/Jordan/Amman
21		hCoV-19/Jordan/SR-051/2020	EPI_ISL_430008	2020-04-04	2020-04-24	29,409	Asia/Jordan/Amman
22		hCoV-19/Jordan/SR-042/2020	EPI_ISL_430000	2020-03-30	2020-04-24	29,412	Asia/Jordan/Amman
23		hCoV-19/Jordan/SR-039/2020	EPI_ISL_429998	2020-03-28	2020-04-24	29,409	Asia/Jordan/Amman
24		hCoV-19/Jordan/SR-037/2020	EPI_ISL_429997	2020-03-16	2020-04-24	29,409	Asia/Jordan/Amman
25		hCoV-19/Jordan/SR-035/2020	EPI_ISL_429995	2020-03-30	2020-04-24	29,412	Asia/Jordan/Amman
26	S/A	hCoV-19/Jordan/SR-0336/2020	EPI_ISL_450188	2020-04-06	2020-05-20	29,864	Asia/Jordan/Amman
27	V/B.2	hCoV-19/Jordan/SR-0338/2020	EPI_ISL_450189	2020-04-06	2020-05-20	29,883	Asia/Jordan/Amman
28	V/B.2	hCoV-19/Jordan/SR-0333/2020	EPI_ISL_450186	2020-04-07	2020-05-20	29,864	Asia/Jordan/Amman

Table 2. Characterization of the clades & genealogical-lineages of the COVID-19 strains clusters during pandemic in Jordan.

100%) (**Figure 2** and **Figure 3**) and as it can be seen from the example of the complete sequence of the hCoV-19/Jordan strain as given in **Figure 4**.

Comparison of the clades of hCoV-19/Jordan with that of SARS-CoV-2 showed that the most plausible transmission routes are European countries (UK, Belgium, Sweden, Russia) and Morocco, which are entry points into Jordan. This can be attributed to the fact that both the G and V clades reported in this study for hCoV-19/Jordan strain are similar to those reported earlier in these European countries [12] [19]. This further suggests that the strain was transmitted through the heavy air traffic transmission rout between EU countries-Moroc-co-Jordan) after the strains were transmitted from China to EU countries. This is

>MT811562.1:1-13203,13203-21290 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/JOR/SEARCH-0054-JOR/2020, complete genome

ATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTC GCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAA AGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTC ATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAG GCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGC TTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTA AAGTCATTTGACTTAGGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTA AACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACACTCGCTATGTCGA TAACAACTTCTGTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAA GCTTCATGCACTTTGTCCGAACAACTGGACTTTATTGACACTAAGAGGGGGTGTATACTGCTGCCGTGAAC ATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAAT TAAATTGGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCATA ATCAAGACTATTCAACCAAGGGTTGAAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCT ATCCAGTTGCGTCACCAAATGCAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGG TGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTGCGAATTTTGTGGCACTGAGAATTTGACT AAAGAAGGTGCCACTACTTGTGGTTACTTACCCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTC ACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAAAACCATTCT GCCTATTGGGTTCCACGTGCTAGCGCTAACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCG AAGGTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATATTGTTGGTGACTT TAAACTTAATGAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAAACT GTGAAAGGTTTGGATTATAAAGCATTCAAACAAATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAG GAAAAGCTAAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTGC ATCAGAGGCTGCTCGTGTTGTACGATCAATTTTCTCCCCGCACTCTTGAAACTGCTCAAAATTCTGTGCGT GTTTTACAGAAGGCCGCTATAACAATACTAGATGGAATTTCACAGTATTCACTGAGACTCATTGATGCTA TGATGTTCACATCTGATTTGGCTACTAACAATCTAGTTGTAATGGCCTACATTACAGGTGGTGTTGTTCA GTTGACTTCGCAGTGGCTAACTAACATCTTTGGCACTGTTTATGAAAAACTCAAACCCGTCCTTGATTGG CTTGAAGAGAAGTTTAAGGAAGGTGTAGAGTTTCTTAGAGACGGTTGGGAAATTGTTAAATTTATCTCAA CCTGTGCTTGTGAAATTGTCGGTGGACAAATTGTCACCTGTGCAAAGGAAATTAAGGAGAGTGTTCAGAC ATTCTTTAAGCTTGTAAATAAATTTTTGGCTTTGTGTGCTGACTCTATCATTATTGGTGGAGCTAAACTT AAAGCCTTGAATTTAGGTGAAACATTTGTCACGCACTCAAAGGGATTGTACAGAAAGTGTGTTAAATCCA GAGAAGAAACTGGCCTACTCATGCCTCTAAAAGCCCCCAAAAGAAATTATCTTCTTAGAGGGAGAAACACT TCCCACAGAAGTGTTAACAGAGGAAGTTGTCTTGAAAACTGGTGATTTACAACCATTAGAACAACCTACT AGTGAAGCTGTTGAAGCTCCATTGGTTGGTACACCAGTTTGTATTAACGGGCTTATGTTGCTCGAAATCA CGGTGCACCAACAAAGGTTACTTTTGGTGATGACACTGTGATAGAAGTGCAAGGTTACAAGAGTGTGAAT ATCACTTTTGAACTTGATGAAAGGATTGATAAAGTACTTAATGAGAAGTGCTCTGCCTATACAGTTGAAC TCGGTACAGAAGTAAATGAGTTCGCCTGTGTTGTGGCAGATGCTGTCATAAAAACTTTGCAACCAGTATC TGAATTACTTACACCACTGGGCATTGATTTAGATGAGTGGAGTATGGCTACATACTACTTATTTGATGAG TCTGGTGAGTTTAAATTGGCTTCACATATGTATTGTTCTTTTTACCCTCCAGATGAGGATGAAGAAGAAG GTGATTGTGAAGAAGAAGAGTTTGAGCCATCAACTCAATATGAGTATGGTACTGAAGATGATTACCAAGG TAAACCTTTGGAATTTGGTGCCACTTCTGCTGCTCTTCAACCTGAAGAAGAGCAAGAAGAAGAATTGGTTA GATGATGATAGTCAACAAACTGTTGGTCAACAAGACGGCAGTGAGGACAATCAGACAACTACTATTCAAA CAATTGTTGAGGTTCAACCTCAATTAGAGATGGAACTTACACCAGTTGTTCAGACTATTGAAGTGAATAG TTTTAGTGGTTATTTAAAACTTACTGACAATGTATACATTAAAAATGCAGACATTGTGGAAGAAGCTAAA AAGGTAAAACCAACAGTGGTTGTTAATGCAGCCAATGTTTACCTTAAACATGGAGGAGGTGTTGCAGGAG CCTTAAATAAGGCTACTAACAATGCCATGCAAGTTGAATCTGATGATTACATAGCTACTAATGGACCACT TAAAGTGGGTGGTAGTTGTGTTTTAAGCGGACACAATCTTGCTAAACACTGTCTTCATGTTGTCGGCCCA AATGTTAACAAAGGTGAAGACATTCAACTTCTTAAGAGTGCTTATGAAAATTTTAATCAGCACGAAGTTC TACTTGCACCATTATTATCAGCTGGTATTTTTGGTGCTGACCCTATACATTCTTTAAGAGTTTGTGTAGA TACTGTTCGCACAAATGTCTACTTAGCTGTCTTTGATAAAAATCTCTATGACAAACTTGTTTCAAGCTTT TTGGAAATGAAGAGTGAAAAGCAAGTTGAACAAAAGATCGCTGAGATTCCTAAAGAGGAAGTTAAGCCAT TTATAACTGAAAGTAAACCTTCAGTTGAACAGAGAAAACAAGATGATAAGAAAATCAAAGCTTGTGTTGA AGAAGTTACAACAACTCTGGAAGAAACTAAGTTCCTCACAGAAAACTTGTTACTTTATATTGACATTAAT

CATATATAGTGGGTGATGTTGTTCAAGAGGGTGTTTTAACTGCTGTGGTTATACCTACTAAAAAGGCTGG TGGCACTACTGAAATGCTAGCGAAAGCTTTGAGAAAAGTGCCAACAGACAATTATATAACCACTTACCCG GGTCAGGGTTTAAATGGTTACACTGTAGAGGAGGCAAAGACAGTGCTTAAAAAGTGTAAAAGTGCCTTTT ACATTCTACCATCTATTATCTCTAATGAGAAGCAAGAAATTCTTGGAACTGTTTCTTGGAATTTGCGAGA ACTATACAGCGTAAATATAAGGGTATTAAAATACAAGAGGGTGTGGTTGATTATGGTGCTAGATTTTACT TTTACACCAGTAAAACAACTGTAGCGTCACTTATCAACAACTTTAACGATCTAAATGAAACTCTTGTTAC AATGCCACTTGGCTATGTAACACATGGCTTAAATTTGGAAGAAGCTGCTCGGTATATGAGATCTCTCAAA GTGCCAGCTACAGTTTCTGTTTCTTCACCTGATGCTGTTACAGCGTATAATGGTTATCTTACTTCTTCTT CTAAAACACCTGAAGAACATTTTATTGAAACCATCTCACTTGCTGGTTCCTATAAAGATTGGTCCTATTC TGGACAATCTACACAACTAGGTATAGAATTTCTTAAGAGAGGTGATAAAAGTGTATATTACACTAGTAAT AAGTGAGGACTATTAAGGTGTTTACAACAGTAGACAACATTAACCTCCACACGCAAGTTGTGGACATGTC AATTCACATGAAGGTAAAACATTTTATGTTTTACCTAATGATGACACTCTACGTGTTGAGGCTTTTGAGT ACTACCACACACACTGATCCTAGTTTTCTGGGTAGGTACATGTCAGCATTAAATCACACTAAAAAGTGGAA ATACCCACAAGTTAATGGTTTAACTTCTATTAAATGGGCAGATAACAACTGTTATCTTGCCACTGCATTG TTAACACTCCAACAAATAGAGTTGAAGTTTAATCCACCTGCTCTACAAGATGCTTATTACAGAGCAAGGG CTGGTGAAGCTGCTAACTTTTGTGCACTTATCTTAGCCTACTGTAATAAGACAGTAGGTGAGTTAGGTGA TGTTAGAGAAACAATGAGTTACTTGTTTCAACATGCCAATTTAGATTCTTGCAAAAGAGTCTTGAACGTG GTGTGTAAAACTTGTGGACAACAGCAGACAACCCTTAAGGGTGTAGAAGCTGTTATGTACATGGGCACAC TTTCTTATGAACAATTTAAGAAAGGTGTTCAGATACCTTGTACGTGGTAAACAAGCTACAAAATATCT AGTACAACAGGAGTCACCTTTTGTTATGATGTCAGCACCACCTGCTCAGTATGAACTTAAGCATGGTACA TTTACTTGTGCTAGTGAGTACACTGGTAATTACCAGTGTGGTCACTATAAACATATAACTTCTAAAGAAA CTTTGTATTGCATAGACGGTGCTTTACTTACAAAGTCCTCAGAATACAAAGGTCCTATTACGGATGTTTT GAAATTGACCCTAAGTTGGACAATTATTATAAGAAAGACAATTCTTATTTCACAGAGCAACCAATTGATC TTGTACCAAACCAAACCAAACGCAAACGCAAGCTTCGATAATTTTAAGTTTGTATGTGATAATATCAAATT TGCTGATGATTTAAACCAGTTAAACTGGTTATAAGAAACCTGCTTCAAGAGAGCTTAAAGTTACATTTTTC CCTGACTTAAATGGTGATGTGGTGGCTATTGATTATAAACACTACACACCCTCTTTTAAGAAAGGAGCTA AATTGTTACATAAACCTATTGTTTGGCATGTTAACAATGCAACTAATAAAGCCACGTATAAACCAAATAC CTGGTGTATACGTTGTCTTTGGAGCACAAAACCAGTTGAAACATCAAATTCGTTTGATGTACTGAAGTCA GAGGACGCGCAGGGAATGGATAATCTTGCCTGCGAAGATCTAAAACCAGTCTCTGAAGAAGTAGTGGAAA ATCCTACCATACAGAAAGACGTTCTTGAGTGTAATGTGAAAACTACCGAAGTTGTAGGAGACATTATACT TAAACCAGCAAATAATAGTTTAAAAATTACAGAAGAGGTTGGCCACACAGATCTAATGGCTGCTTATGTA GACAATTCTAGTCTTACTATTAAGAAACCTAATGAATTATCTAGAGTATTAGGTTTGAAAACCCTTGCTA CTCATGGTTTAGCTGCTGTTAATAGTGTCCCTTGGGATACTATAGCTAATTATGCTAAGCCTTTTCTTAA CAAAGTTGTTAGTACAACTACTAACATAGTTACACGGTGTTTAAACCGTGTTTGTACTAATTATATGCCT TATTTCTTTACTTTATTGCTACAATTGTGTACTTTTACTAGAAGTACAAATTCTAGAATTAAAGCATCTA TGCCGACTACTATAGCAAAGAATACTGTTAAGAGTGTCGGTAAATTTTGTCTAGAGGCTTCATTTAATTA TTTGAAGTCACCTAATTTTTCTAAACTGATAAATATTATAATTTGGTTTTTACTATTAAGTGTTTGCCTA GGTTCTTTAATCTACTCAACCGCTGCTTTAGGTGTTTTAATGTCTAATTTAGGCATGCCTTCTTACTGTA CTGGTTACAGAGAGAGGCTATTTGAACTCTACTAATGTCACTATTGCAACCTACTGTACTGGTTCTATACC TTGTAGTGTTTGTCTTAGTGGTTTAGATTCTTTAGACACCTATCCTTCTTTAGAAACTATACAAATTACC ATTTCATCTTTTAAATGGGATTTAACTGCTTTTGGCTTAGTTGCAGAGTGGTTTTTGGCATATATTCTTT TCACTAGGTTTTTCTATGTACTTGGATTGGCTGCAATCATGCAATTGTTTTCAGCTATTTTGCAGTACA GTAATTCATCAACTTGTATGATGTGTTACAAACGTAATAGAGCAACAAGAGTCGAATGTACAACTATTGT TAATGGTGTTAGAAGGTCCTTTTATGTCTATGCTAATGGAGGTAAAGGCTTTTGCAAACTACACAATTGG AATTGTGTTAATTGTGATACATTCTGTGCTGGTAGTACATTTATTAGTGATGAAGTTGCGAGAGACTTGT CACTACAGTTTAAAAGACCAATAAATCCTACTGACCAGTCTTCTTACATCGTTGATAGTGTTACAGTGAA TTTGTTAACTTAGACAACCTGAGAGCTAATAACACTAAAGGTTCATTGCCTATTAATGTTATAGTTTTTG ATGGTAAATCAAAATGTGAAGAATCATCTGCAAAATCAGCGTCTGTTTACTACAGTCAGCTTATGTGTCA ACCTATACTGTTACTAGATCAGGCATTAGTGTCTGATGTTGGTGATAGTGCGGAAGTTGCAGTTAAAATG TTTGATGCTTACGTTAATACGTTTTCATCAACTTTTAACGTACCAATGGAAAAACTCAAAACACTAGTTG CAACTGCAGAAGCTGAACTTGCAAAGAATGTGTCCTTAGACAATGTCTTATCTACTTTTTCAGCAGC

TCGGCAAGGGTTTGTTGATTCAGATGTAGAAACTAAAGATGTTGTTGAATGTCTTAAATTGTCACATCAA TCTGACATAGAAGTTACTGGCGATAGTTGTAATAACTATATGCTCACCTATAACAAAGTTGAAAACATGA CACCCCGTGACCTTGGTGCTTGTATTGACTGTAGTGCGCGTCATATTAATGCGCAGGTAGCAAAAAGTCA CAACATTGCTTTGATATGGAACGTTAAAGATTTCATGTCATTGTCTGAACAACTACGAAAAACAAATACGT AGTGCTGCTAAAAAGAATAACTTACCTTTTAAGTTGACATGTGCAACTACTAGACAAGTTGTTAATGTTG ACTTGTGTTCCTTTTTGTTGCTGCTATTTTCTATTTAATAACACCTGTTCATGTCATGTCTAAACATACT GACTTTTCAAGTGAAATCATAGGATACAAGGCTATTGATGGTGGTGTCACTCGTGACATAGCATCTACAG ATACTTGTTTTGCTAACAAACATGCTGATTTTGACACATGGTTTAGCCAGCGTGGTGGTAGTTATACTAA TGACAAAGCTTGCCCATTGATTGCTGCAGTCATAACAAGAGAGTGGGTTTTGTCGTGCCTGGTTTGCCT GGCACGATATTACGCACAACTAATGGTGACTTTTTGCATTTCTTACCTAGAGTTTTTAGTGCAGTTGGTA ACATCTGTTACACACCATCAAAACTTATAGAGTACACTGACTTTGCAACATCAGCTTGTGTTTTGGCTGC TGAATGTACAATTTTTAAAGATGCTTCTGGTAAGCCAGTACCATATTGTTATGATACCAATGTACTAGAA TTCCTAACACCTACCTTGAAGGTTCTGTTAGAGTGGTAACAACTTTTGATTCTGAGTACTGTAGGCACGG CACTTGTGAAAGATCAGAAGCTGGTGTTTGTGTATCTACTAGTGGTAGATGGGTACTTAACAATGATTAT TAATTCAACCTATTGGTGCTTTGGACATATCAGCATCTATAGTAGCTGGTGGTATTGTAGCTATCGTAGT AACATGCCTTGCCTACTATTTATGAGGTTTAGAAGAGCTTTTGGTGAATACAGTCATGTAGTTGCCTTT AATACTTTACTATTCCTTATGTCATTCACTGTACTCTGTTTAACACCAGTTTACTCATTCTTACCTGGTG TTTATTCTGTTATTTACTTGTACTTGACATTTTATCTTACTAATGATGTTTCTTTTTAGCACATATTCA GTGGATGGTTATGTTCACACCTTTAGTACCTTTCTGGATAACAATTGCTTATATCATTTGTATTTCCACA AAGCATTTCTATTGGTTCTTTAGTAATTACCTAAAGAGACGTGTAGTCTTTAATGGTGTTTCCTTTAGTA GCTATTACCTCTTACGCAATATAATAGATACTTAGCTCTTTATAATAAGTACAAGTATTTTAGTGGAGCA ATGGATACAACTAGCTACAGAGAAGCTGCTTGTTGTCATCTCGCAAAGGCTCTCAATGACTTCAGTAACT CAGGTTCTGATGTTCTTTACCAACCACCACAAACCTCTATCACCTCAGCTGTTTTGCAGAGTGGTTTTAG AAAAATGGCATTCCCATCTGGTAAAGTTGAGGGTTGTATGGTACAAGTAACTTGTGGTACAACTACACTT AACGGTCTTTGGCTTGATGACGTAGTTTACTGTCCAAGACATGTGATCTGCACCTCTGAAGACATGCTTA ACCCTAATTATGAAGATTTACTCATTCGTAAGTCTAATCATAATTTCTTGGTACAGGCTGGTAATGTTCA ACTCAGGGTTATTGGACATTCTATGCAAAATTGTGTACTTAAGCTTAAGGTTGATACAGCCAATCCTAAG ACACCTAAGTATAAGTTTGTTCGCATTCAACCAGGACAGACTTTTTCAGTGTTAGCTTGTTACAATGGTT CACCATCTGGTGTTTACCAATGTGCTATGAGGCCCAATTTCACTATTAAGGGTTCATTCCTTAATGGTTC ATGTGGTAGTGTTGGTTTTAACATAGATTATGACTGTGTCTCTTTTTGTTACATGCACCATATGGAATTA CCAACTGGAGTTCATGCTGGCACAGACTTAGAAGGTAACTTTTATGGACCTTTTGTTGACAGGCAAACAG CACAAGCAGCTGGTACGGACACAACTATTACAGTTAATGTTTTAGCTTGGTTGTACGCTGCTGTTATAAA TGGAGACAGGTGGTTTCTCAATCGATTTACCACAACTCTTAATGACTTTAACCTTGTGGCTATGAAGTAC AATTATGAACCTCTAACACAAGACCATGTTGACATACTAGGACCTCTTTCTGCTCAAACTGGAATTGCCG TTTTAGATATGTGTGCTTCATTAAAAGAATTACTGCAAAATGGTATGAATGGACGTACCATATTGGGTAG TGCTTTATTAGAAGATGAATTTACACCTTTTGATGTTGTTAGACAATGCTCAGGTGTTACTTTCCAAAGT GCAGTGAAAAGAACAATCAAGGGTACACACCACTGGTTGTTACTCACAATTTTGACTTCACTTTTAGTTT TAGTCCAGAGTACTCAATGGTCTTTGTTCTTTTTTTGTATGAAAATGCCTTTTTACCTTTTGCTATGGG TTACCTTCTCTTGCCACTGTAGCTTATTTTAATATGGTCTATATGCCTGCTAGTTGGGTGATGCGTATTA AGCTGTAGTGTTACTAATCCTTATGACAGCAAGAACTGTGTGTATGATGGTGCTAGGAGAGTGTGGACA CTTATGAATGTCTTGACACTCGTTTATAAAGTTTATTATGGTAATGCTTTAGATCAAGCCATTTCCATGT **GGGCTCTTATAATCTCTGTTACTTCTAACTACTCAGGTGTAGTTACAACTGTCATGTTTTTGGCCAGAGG** TATTGTTTTATGTGTGTTGAGTATTGCCCTATTTTCTTCATAACTGGTAATACACTTCAGTGTATAATG CTAGTTTATTGTTTCTTAGGCTATTTTTGTACTTGTTACTTTGGCCTCTTTTGTTTACTCAACCGCTACT TTAGACTGACTCTTGGTGTTTATGATTACTTAGTTTCTACACAGGAGTTTAGATATAGAATTCACAGGG ACTACTCCCACCCAAGAATAGCATAGATGCCTTCAAACTCAACATTAAATTGTTGGGTGTTGGTGGCAAA CCTTGTATCAAAGTAGCCACTGTACAGTCTAAAATGTCAGATGTAAAGTGCACATCAGTAGTCTTACTCT CAGTTTTGCAACAACTCAGAGTAGAATCATCATCTAAATTGTGGGCTCAATGTGTCCAGTTACACAATGA CATTCTCTTAGCTAAAGATACTACTGAAAGCCTTTGAAAAAATGGTTTCACTACTTTCTGTTTTGCTTTCC ATGCAGGGTGCTGTAGACATAAACAAGCTTTGTGAAGAAATGCTGGACAACAGGGCAACCTTACAAGCTA TAGCCTCAGAGTTTAGTTCCCTTCCATCATATGCAGCTTTTGCTACTGCTCAAGAAGCTTATGAGCAGGC TGTTGCTAATGGTGATTCTGAAGTTGTTCTTAAAAAGTTGAAGAAGTCTTTGAATGTGGCTAAATCTGAA TTTGACCGTGATGCAGCCATGCAACGTAAGTTGGAAAAGATGGCTGATCAAGCTATGACCCAAATGTATA AACAGGCTAGATCTGAGGACAAGAGGGCAAAAGTTACTAGTGCTATGCAGACAATGCTTTTCACTATGCT TAGAAAGTTGGATAATGATGCACTCAACAACATTATCAACAATGCAAGAGATGGTTGTGTTCCCTTGAAC ATAATACCTCTTACAACAGCAGCCCAAACTAATGGTTGTCATACCAGACTATAACACATATAAAAATACGT GTGATGGTACAACATTTACTTATGCATCAGCATTGTGGGAAATCCAACAGGTTGTAGATGCAGATAGTAA AATTGTTCAACTTAGTGAAATTAGTATGGACAATTCACCTAATTTAGCATGGCCTCTTATTGTAACAGCT TTAAGGGCCAATTCTGCTGTCAAATTACAGAATAATGAGCTTAGTCCTGTTGCACTACGACAGATGTCTT GTGCTGCCGGTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACAACAAAGGG AGGTAGGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGGCTAGATTCCCTAAGAGTGAT GGAACTGGTACTATCTATACAGAACTGGAACCACCTTGTAGGTTTGTTACAGACACACCTAAAGGTCCTA AAGTGAAGTATTTATACTTTATTAAAGGATTAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAGC GTGTTAAGATGTTGTGTACACACACTGGTACTGGTCAGGCAATAACAGTTACACCGGAAGCCAATATGGA TCAAGAATCCTTTGGTGGTGCATCGTGTTGTCTGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAA GGATTTTGTGACTTAAAAGGTAAGTATGTACAAATACCTACAACTTGTGCTAATGACCCTGTGGGTTTTA CACTTAAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTATGGCTGTAGTTGTGATCAACTCCG CGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAACCGGGTTTGCGGTGTAAGTGCAGCCCGT CTTACACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTTTTGACATCTACAATGATAAAG TAGCTGGTTTTGCTAAATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAATTT AATTGATTCTTACTTGTAGTTAAGAGACACACACTTTCTCTAACTACCAACATGAAGAAACAATTTATAAT TTACTTAAGGATTGTCCAGCTGTTGCTAAACATGACTTCTTTAAGTTTAGAATAGACGGTGACATGGTAC CACATATATCACGTCAACGTCTTACTAAATACACAATGGCAGACCTCGTCTATGCTTTAAGGCATTTTGA TGAAGGTAATTGTGACACATTAAAAGAAATACTTGTCACATACAATTGTTGTGATGATGATTATTTCAAT AAAAAGGACTGGTATGATTTTGTAGAAAACCCAGATATATTACGCGTATACGCCAACTTAGGTGAACGTG TACGCCAAGCTTTGTTAAAAACAGTACAATTCTGTGATGCCATGCGAAATGCTGGTATTGTTGGTGTACT GACATTAGATAATCAAGATCTCAATGGTAACTGGTATGATTTCGGTGATTTCATACAAACCACGCCAGGT AGTGGAGTTCCTGTTGTAGATTCTTATTATTCATTGTTAATGCCTATATTAACCTTGACCAGGGCTTTAA CTGCAGAGTCACATGTTGACACTGACTTAACAAAGCCTTACATTAAGTGGGATTTGTTAAAATATGACTT CACGGAAGAGAGGTTAAAACTCTTTGACCGTTATTTTAAATATTGGGATCAGACATACCACCCAAATTGT GTTAACTGTTTGGATGACAGATGCATTCTGCATTGTGCAAACTTTAATGTTTTATTCTCTACAGTGTTCC CACTTACAAGTTTTGGACCACTAGTGAGAAAAATATTTGTTGATGGTGTTCCATTTGTAGTTTCAACTGG ATACCACTTCAGAGAGCTAGGTGTTGTACATAATCAGGATGTAAACTTACATAGCTCTAGACTTAGTTTT AAGGAATTACTTGTGTATGCTGCTGACCCTGCTATGCACGCTGCTTCTGGTAATCTATTACTAGATAAAC GCACTACGTGCTTTTCAGTAGCTGCACTTACTAACAATGTTGCTTTTCAAACTGTCAAACCCGGTAATTT CACTTCTTCTTTGCTCAGGATGGTAATGCTGCTATCAGCGATTATGACTACCATCGTTATAATCTACCAA CAATGTGTGATATCAGACAACTACTATTTGTAGTTGAAGTTGTTGATAAGTACTTTGATTGTTACGATGG TGGCTGTATTAATGCTAACCAAGTCATCGTCAACAACCTAGACAAATCAGCTGGTTTTCCATTTAATAAA TGGGGTAAGGCTAGACTTTATTATTGATTCAATGAGTTATGAGGATCAAGATGCACTTTTCGCATATACAA AACGTAATGTCATCCCTACTATAACTCAAATGAATCTTAAGTATGCCATTAGTGCAAAGAATAGAGCTCG CACCGTAGCTGGTGTCTCTATCTGTAGTACTATGACCAATAGACAGTTTCATCAAAAATTATTGAAATCA ATAGCCGCCACTAGAGGAGCTACTGTAGTAATTGGAACAAGCAAATTCTATGGTGGTTGGCACAACATGT TAAAAACTGTTTATAGTGATGTAGAAAAACCCTCACCTTATGGGTTGGGATTATCCTAAATGTGATAGAGC CATGCCTAACATGCTTAGAATTATGGCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGTAGCTTG TCACACCGTTTCTATAGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTT CACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAA CATTTGTCAAGCTGTCACGGCCAATGTTAATGCACTTTTATCTACTGATGGTAACAAAATTGCCGATAAG TATGTCCGCAATTTACAACACAGACTTTATGAGTGTCTCTATAGAAATAGAGATGTTGACACAGACTTTG TGAATGAGTTTTACGCATATTTGCGTAAACATTTCTCAATGATGATACTCTCTGACGATGCTGTTGTGTG TTTCAATAGCACTTATGCATCTCAAGGTCTAGTGGCTAGCATAAAGAACTTTAAGTCAGTTCTTTATTAT CAAAACAATGTTTTTATGTCTGAAGCAAAATGTTGGACTGAGACTGACCTTACTAAAGGACCTCATGAAT AAGAATCCTAGGGGCCGGCTGTTTTGTAGATGATATCGTAAAAACAGATGGTACACTTATGATTGAACGG TTCGTGTCTTTAGCTATAGATGCTTACCCACTTACTAAACATCCTAATCAGGAGTATGCTGATGTCTTTC ATTTGTACTTACAATACATAAGAAAGCTACATGATGAGTTAACAGGACACATGTTAGACATGTATTCTGT TATGCTTACTAATGATAACACTTCAAGGTATTGGGAACCTGAGTTTTATGAGGCTATGTACACACCGCAT ACAGTCTTACAGGCTGTTGGGGGCTTGTGTTTTGCAATTCACAGACTTCATTAAGATGTGGTGCTTGCA TACGTAGACCATTCTTATGTTGTAAATGCTGTTACGACCATGTCATAACATCACATAAATTAGTCTT GTCTGTTAATCCGTATGTTTGCAATGCTCCAGGTTGTGATGTCACAGATGTGACTCAACTTTACTTAGGA GGTATGAGCTATTATTGTAAATCACATAAACCACCCATTAGTTTTCCATTGTGTGCTAATGGACAAGTTT TTGGTTTATATAAAAATACATGTGTTGGTAGCGATAATGTTACTGACTTTAATGCAATTGCAACATGTGA CTGGACAAATGCTGGTGATTACATTTTAGCTAACACCTGTACTGAAAGACTCAAGCTTTTTGCAGCAGAA ACGCTCAAAGCTACTGAGGAGACATTTAAACTGTCTTATGGTATTGCTACTGTACGTGAAGTGCTGTCTG ACAGAGAATTACATCTTTCATGGGAAGTTGGTAAACCTAGACCACCTTAACCGAAATTATGTCTTTAC TGGTTATCGTGTAACTAAAAACAGTAAAGTACAAATAGGAGAGTACACCTTTGAAAAAGGTGACTATGGT GATGCTGTTGTTTACCGAGGTACAACAACTTACAAATTAAATGTTGGTGATTATTTTGTGCTGACATCAC ATACAGTAATGCCATTAAGTGCACCTACACTAGTGCCACAAGAGCACTATGTTAGAATTACTGGCTTATA CCCAACACTCAATATCTCAGATGAGTTTTCTAGCAATGTTGCAAAATTATCAAAAGGTTGGTATGCAAAAG TATTCTACACTCCAGGGACCACCTGGTACTGGTAAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACC CTTCTGCTCGCATAGTGTATACAGCTTGCTCTCATGCCGCTGTTGATGCACTATGTGAGAAGGCATTAAA ATATTTGCCTATAGATAAATGTAGTAGAATTATACCTGCACGTGCTCGTGTAGAGTGTTTTGATAAATTC AAAGTGAATTCAACATTAGAACAGTATGTCTTTTGTACTGTAAATGCATTGCCTGAGACGACAGCAGATA TAGTTGTCTTTGATGAAATTTCAATGGCCACAAATTATGATTTGAGTGTTGTCAATGCCAGATTACGTGC CTAGAACCAGAATATTTCAATTCAGTGTGTAGACTTATGAAAACTATAGGTCCAGACATGTTCCTCGGAA CTTGTCGGCGTTGTCCTGCTGAAATTGTTGACACTGTGAGTGCTTTGGTTTATGATAATAAGCTTAAAGC ACATAAAGACAAATCAGCTCAATGCTTTAAAAATGTTTTATAAGGGTGTTATCACGCATGATGTTTCATCT GCAATTAACAGGCCACAAATAGGCGTGGTAAGAGAATTCCTTACACGTAACCCTGCTTGGAGAAAAGCTG TCTTTATTTCACCTTATAATTCACAGAATGCTGTAGCCTCAAAGATTTTGGGACTACCAACTCAAACTGT TGATTCATCACAGGGCTCAGAATATGACTATGTCATATTCACTCAAACCACTGAAACAGCTCACTCTTGT AATGTAAACAGATTTAATGTTGCTATTACCAGAGCAAAAGTAGGCATACTTTGCATAATGTCTGATAGAG ACCTTTATGACAAGTTGCAATTTACAAGTCTTGAAATTCCACGTAGGAATGTGGCAACTTTACAAGCTGA AAATGTAACAGGACTCTTTAAAGATTGTAGTAAGGTAATCACTGGGTTACATCCTACACAGGCACCTACA CACCTCAGTGTTGACACTAAATTCAAAACTGAAGGTTTATGTGTTGACATACCTGGCATACCTAAGGACA TGACCTATAGAAGACTCATCTCTATGATGGGTTTTAAAATGAATTATCAAGTTAATGGTTACCCTAACAT GTTTATCACCCGCGAAGAAGCTATAAGACATGTACGTGCATGGATTGGCTTCGATGTCGAGGGGTGTCAT GCTACTAGAGAAGCTGTTGGTACCAATTTACCTTTACAGCTAGGTTTTTCTACAGGTGTTAACCTAGTTG CTGTACCTACAGGTTATGTTGATACACCTAATAATACAGATTTTTCCAGAGTTAGTGCTAAACCACCGCC TGGAGATCAATTTAAACACCTCATACCACTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTATAAAG ATTGTACAAATGTTAAGTGACACACTTAAAAATCTCTCTGACAGAGTCGTATTTGTCTTATGGGCACATG GCTTTGAGTTGACATCTATGAAGTATTTTGTGAAAATAGGACCTGAGCGCACCTGTTGTCTATGTGATAG ACGTGCCACATGCTTTTCCACTGCTTCAGACACTTATGCCTGTTGGCATCATTCTATTGGATTTGATTAC GTCTATAATCCGTTTATGATTGATGTTCAACAATGGGGGTTTTACAGGTAACCTACAAAGCAACCATGATC TGTATTGTCAAGTCCATGGTAATGCACATGTAGCTAGTTGTGATGCAATCATGACTAGGTGTCTAGCTGT CCACGAGTGCTTTGTTAAGCGTGTTGACTGGACTATTGAATATCCTATAATTGGTGATGAACTGAAGATT AATGCGGCTTGTAGAAAGGTTCAACACATGGTTGTTAAAGCTGCATTATTAGCAGACAAATTCCCAGTTC TTCACGACATTGGTAACCCTAAAGCTATTAAGTGTGTACCTCAAGCTGATGTAGAATGGAAGTTCTATGA TGCACAGCCTTGTAGTGACAAAGCTTATAAAATAGAAGAATTATTCTATTCTTATGCCACACATTCTGAC TAAACATGCATTCCACACCAGCTTTTGATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTC TATTACTCTGACAGTCCATGTGAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGTACCACTAA ACTTATAACCTCTGGAACACTTTTACAAGACTTCAGAGTTTAGAAAATGTGGCTTTTAATGTTGTAAATA AGGGACACTTTGATGGACAACAGGGTGAAGTACCAGTTTCTATCATTAATAACACTGTTTACACAAAAGT TGATGGTGTTGATGTAGAATTGTTTGAAAATAAAACAACATTACCTGTTAATGTAGCATTTGAGCTTTGG GCTAAGCGCAACATTAAACCAGTACCAGAGGTGAAAATACTCAATAATTTGGGTGTGGACATTGCTGCTA **GGTCAAGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTACAGAAGGTAGTGTTAAAAGGTTTAC** AACCATCTGTAGGTCCCAAACAAGCTAGTCTTAATGGAGTCACATTAATTGGAGAAGCCGTAAAAACACA AATTTACAAGAATTTAAACCCAGGAGTCAAATGGAAATTGATTTCTTAGAATTAGCTATGGATGAATTCA TTGAACGGTATAAATTAGAAGGCTATGCCTTCGAACATATCGTTTATGGAGATTTTAGTCATAGTCAGTT

AGGTGGTTTACATCTACTGATTGGACTAGCTAAACGTTTTAAGGAATCACCTTTTGAATTAGAAGATTTT **GTTCTGTTATTGATTTATTACTTGATGATTTTGTTGAAATAATAAAATCCCAAGATTTATCTGTAGTTTC** TAAGGTTGTCAAAGTGACTATTGACTATACAGAAATTTCATTTATGCTTTGGTGTAAAGATGGCCATGTA GAAACATTTTACCCAAAATTACAATCTAGTCAAGCGTGGCAACCGGGTGTTGCTATGCCTAATCTTTACA AAATGCAAAGAATGCTATTAGAAAAGTGTGACCTTCAAAATTATGGTGATAGTGCAACATTACCTAAAGG CATAATGATGAATGTCGCAAAATATACTCAACTGTGTCAATATTTAAACACATTAACATTAGCTGTACCC TATAATATGAGAGTTATACATTTTGGTGCTGGTTCTGATAAAGGAGTTGCACCAGGTACAGCTGTTTTAA GACAGTGGTTGCCTACGGGTACGCTGCTGTCGATTCAGATCTTAATGACTTTGTCTCTGATGCAGATTC AACTTTGATTGGTGATTGTGCAACTGTACATACAGCTAATAAATGGGATCTCATTATTAGTGATATGTAC GACCCTAAGACTAAAAATGTTACAAAAGAAAATGACTCTAAAGAGGGTTTTTTCACTTACATTTGTGGGT TTATACAACAAAAGCTAGCTCTTGGAGGTTCCGTGGCTATAAAGATAACAGAACATTCTTGGAATGCTGA TCTTTATAAGCTCATGGGACACTTCGCATGGTGGACAGCCTTTGTTACTAATGTGAATGCGTCATCATCT CAAATTACATATTTTGGAGGAATACAAATCCAATTCAGTTGTCTTCCTATTCTTTATTTGACATGAGTAA ATTTCCCCTTAAATTAAGGGGTACTGCTGTTATGTCTTTAAAAGAAGGTCAAATCAATGATATGATTTTA TCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAAACAACAGAGTTGTTATTTCTAGTGATGTTCTTG TTAACAACTAA

Figure 4. Example of the complete sequence of the hCoV-19/Jordan.

in consonance with previous reports that stated that clade G apparently originated from Shanghai before it was imported into European countries [17] [20] [21].

5. Conclusion

The results of this study show conclusively a unique pandemic profile of multiple geographical sources of COVID-19 importation into Jordan primarily through UK, Belgium, Sweden, Russia and Morocco, which are entry points into Jordan. This is also due to the reciprocal heavy air traffic route between Jordan and European capitals. It can be inferred based on the identical homology (99.98% - 100%) of the 28 sequences of hCoV-19/Jordan strain that there was no marked change in the virus mutation that occurred during the period of this study.

Acknowledgements

The authors would like to acknowledge the generous support of Professor Kristian Andersen (Scripps Research Institute, TSRI, La Jolla, CA, USA) and his joint-permission with the Biolab Diagnostic Laboratory, Jordan (personal communication) to use the SARS-CoV-2 sequences from Jordan in this paper. The authors also would like to acknowledge the use of the data and the bioinformatics software available on the Public Domains of both NCBI GeneBank

(<u>http://www.ncbi.nlm.nih.gov/</u>) and the Global Initiative on Sharing All Influenza Data (GISAID) (<u>http://www.gisaid.org/</u>). The authors would like to acknowledge the efforts of the Ministry of Health (<u>http://www.moh.gov.jo</u>), Government of Jordan for the financial support and the valuable efforts on administrating the National Surveillances Program (as set by the National orders numbers; 17 - 20) including the samples collections of the SARS-CoV-2).

Data Availability

All the 28 sequences of the hCoV-19/Jordan used in this paper have been used as available on the public Domains in both NCBI GeneBank

(<u>http://www.ncbi.nlm.nih.gov/</u>) and the Global Initiative on Sharing All Influenza Data (GISAID) (<u>http://www.gisaid.org/</u>) under the name of hCoV-19/ Jordan as per each respective accession number. All clinical data of the patients, epidemiological details as well as sampling surveillances were maintained in the National record of the Ministry of Health of Jordan.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- Koyama, T., Platt, D. and Parida, L. (2020) Variant Analysis of SARS-CoV-2 Genomes. *Bulletin of the World Health Organization*, 98, 495-504. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200410-sitrep-81-covid-19 https://doi.org/10.2471/BLT.20.253591
- [2] Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., Chen, J., Meng, Y., Wang, J., Lin, Y., Yuan, J., Xie, Z., Ma, J., Liu, W.J., Wang, D., Xu, W., Holmes, E.C., Gao, G.F., Wu, G., Chen, W., Shi, W. and Tan, W. (2020) Genomic Characterization and Epidemiology of 2019 Novel Coronavirus: Implications for Virus Origins and Receptor Binding. *The Lancet*, **395**, 565-574. https://doi.org/10.1016/S0140-6736(20)30251-8
- [3] Zhou, P., Yang, X., Wang, X., et al. (2020) A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin. Nature, 579, 270-273. https://doi.org/10.1038/s41586-020-2012-7
- [4] Cavanagh, D. (2007) Coronavirus Avian Infectious Bronchitis Virus. Veterinary Research, 38, 281-297. https://doi.org/10.1051/vetres:2006055
- Ismail, M.M., Tang, A.Y. and Saif, Y.M. (2003) Pathogenicity of Turkey Coronavirus in Turkeys and Chickens. *Avian Diseases*, 47, 515-522. https://doi.org/10.1637/5917
- [6] Moira, C.-Y. and Xu, R.H. (2003) SARS: Epidemiology. *Respirology*, 8, S9-S14. <u>https://doi.org/10.1046/j.1440-1843.2003.00518.x</u>
- [7] Corman, V.M., Müller, M.A., Costabel, U., Timm, J., Binger, T., Meyer, B., et al. (2012) Assays for Laboratory Confirmation of Novel Human Coronavirus (hCoV-EMC) Infections. *Eurosurveillance*, 17, 20334. https://doi.org/10.2807/ese.17.49.20334-en
- [8] Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D. and Fouchier, R.A. (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *The New England Journal of Medicine*, **367**, 1814-1820. https://doi.org/10.1056/NEJMoa1211721
- [9] Lee, J., Chowell, G. and Jung, E. (2016) A Dynamic Compartmental Model for the Middle East Respiratory Syndrome Outbreak in the Republic of Korea: A Retrospective Analysis on Control Interventions and Superspreading Events. *Journal of*

Theoretical Biology, 408, 118-126. https://doi.org/10.1016/j.jtbi.2016.08.009

- [10] Chung, E.H., Kim, Y.-J., Chung, E.H., Kim, D.-W., et al. (2017) The Clinical and Virological Features of the First Imported Case Causing MERS-CoV Outbreak in South Korea, 2015. BMC Infectious Diseases, 17, Article No. 498. https://doi.org/10.1186/s12879-017-2576-5
- Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. and Garry, R.F. (2020) The Proximal Origin of SARS-CoV-2. *Nature Medicine*, 26, 450-452. <u>http://www.nature.com/naturemedicine</u> <u>https://doi.org/10.1038/s41591-020-0820-9</u>
- [12] Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K.W., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L., Mulders, D.G.J.C., Haagmans, B.L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M., Peiris, M., Goossens, H., Reusken, C., Koopmans, M.P.G. and Drosten, C. (2020) Detection of 2019 Novel Coronavirus (2019-nCoV) by Real-Time RT-PCR. *Eurosurveillance*, **25**, Article ID: 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
- [13] Forster, P., Forster, L., Renfrew, C. and Forster, M. (2020) Phylogenetic Network Analysis of SARS-CoV-2 Genomes. *Proceedings of the National Academy of Sciences* of the United States of America, 117, 9241-9243. https://doi.org/10.1073/pnas.2004999117
- [14] Guan, Q., Sadykov, M., Mfrrej, S., Hala, S., Naeem, R., Nugmanova, R., Al-Omari, A., Salih, S., Al-Mutair, A., Carr, M.J., Hall, W.W., Arold, S.T. and Pain, A. (2020) A Genetic Barcode of SARS-CoV-2 for Monitoring Global Distribution of Different Clades during the COVID-19 Pandemic. *International Journal of Infectious Diseas*es, 100, 216-223. <u>https://doi.org/10.1016/j.ijid.2020.08.052</u>
- [15] Gu, W., Miller, S. and Chiu, C.Y. (2019) Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. *Annual Review of Pathology: Mechanisms of Disease*, 14, 319-338. https://doi.org/10.1146/annurev-pathmechdis-012418-012751
- [16] Mercatelli, D. and Giorgi, F.M. (2020) Geographic and Genomic Distribution of SARS-CoV-2 Mutations. *Frontiers in Microbiology*, **11**, 1-13. <u>https://doi.org/10.3389/fmicb.2020.01800</u>
- [17] Zehender, G., Lai, A., Bergna, A., Meroni, L., Riva, A., Balotta, C., Tarkowski, M., Gabrieli, A., Bernacchia, D., Rusconi, S., Rizzardini, G., Antinori, S. and Galli, M. (2020) Genomic Characterization and Phylogenetic Analysis of SARS-COV-2 in Italy. *Journal of Medical Virology*. https://doi.org/10.1101/2020.03.15.20032870
- [18] World Health Organization (WHO) (2020) Coronavirus Disease 2019 (COVID-19) Situation Report—81. <u>https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200410-sitrep-81-covid-19</u>
- [19] European Centre for Disease Control and Prevention (ECDC) (2020) COVID-19 Situation update Worldwide, as of 10 April 2020.
 https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases
- [20] Bartolini, B., Rueca, M., Gruber, C., Messina, F., Carletti, F., Giombini, E., et al. (2020) SARS-CoV-2 Phylogenetic Analysis, Lazio Region, Italy, February-March 2020. Emerging Infectious Diseases, 26, 1842-1845. https://doi.org/10.3201/eid2608.201525
- [21] Giovanetti, M., Angeletti, S., Benvenuto, D. and Ciccozzi, M. (2020) A Doubt of Multiple Introduction of SARS-CoV-2 in Italy: A Preliminary Overview. *Journal of Medical Virology*, 92, 1634-1636. https://doi.org/10.1002/jmv.25773