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Covid19: Evolution, Genomics And Alternative Medicine

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Dedication

To mom and dad,

I dedicate my dissertation to the person who spent his entire life providing for me, educating me the best he could and brought me where I am today, to my beloved father who left early this year and is no longer here with me, to the person who deserves to get every single bit of credit my success has to offer, a work he so wanted to see, to Hemidi Larbi.

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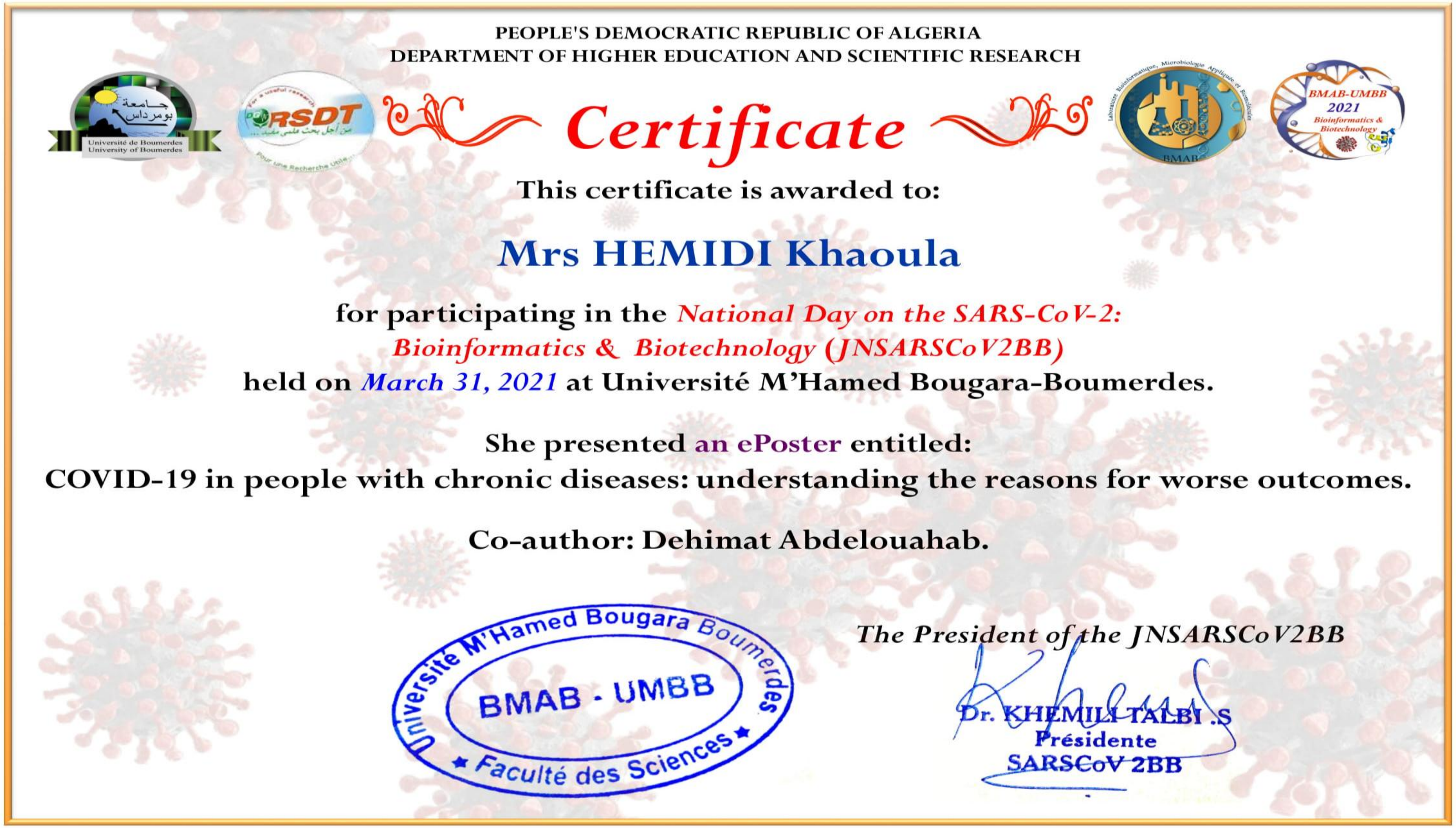

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Certificate

This certificate is awarded to:

Mrs HEMIDI Khaoula

for participating in the *National Day on the SARS-CoV-2: Bioinformatics & Biotechnology (JNSARSCoV2BB)* held on *March 31, 2021* at Université M'Hamed Bougara-Boumerdes.

She presented an ePoster entitled:
COVID-19 in people with chronic diseases: understanding the reasons for worse outcomes.

Co-author: Dehimat Abdelouahab.

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BMAB - UMBB
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The President of the JNSARSCoV2BB

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List of Abbreviations

ACE2: Angiotensin Converting Enzyme 2

ANGII: Angiotensin 2

AT1R: ANGII type 1 receptor

CDC: Centers for Disease Control and Prevention

ERGIC: Endoplasmic reticulum-Golgi intermediate compartment

FL: Fidelity Level

GMR: Gender mortality Rate

MERS: Middle East Respiratory Syndrome

RAS: Renin-Angiotensin System

RdRp: RNA-dependent RNA polymerase complex

RFC: RFC Relative Frequency of Citation

SARS: Severe acute Respiratory Syndrome

SIR: Susceptible Infected Recovered model

TMPRSS2: Transmembrane protease serine 2

UV: Use Value

WHO: World Health Organization.

Introduction

Introduction

The severe acute respiratory syndrome 2 (SARS-2) known as COVID-19 has left the world with a total of 177,108,695 confirmed cases including 3,840,223 deaths on a worldwide scale according to the World Health Organization (WHO), the numbers continue to increase and Algeria, no different from the rest, has joined this misfortunate event and is now with 134 840 confirmed cases with 3 605 deaths and counting (World Health Organization, 2021).

Although the country seems to be doing a way better job at maintaining the numbers within such low rates compared to other neighboring countries such as Morocco, Tunisia or even France, those are still significant amounts and the losses despite being manageable still need to be maximally reduced (World Health Organization, 2021).

The reason why this study was carried out is to not only to numeralize and set a visionary description based on datasets of the current Algerian and local (Biskra) situation within the pandemic but also to create a personalized strategy based on those digits in order to draw a firm line between presumptions such as the effect of factors like age, gender and temperature have on the virus, to determine whether the control measurements have been effective enough and if the tests chosen by the local hospital of Biskra Hakim Sadanne are efficacious and sensitive enough.

The work will touch a proportion of the population that is labeled as sensitive to a various amount of diseases which is the chronically ill people, curiosity as to how this virus affects such individuals motivated us to develop a survey and partake in a national seminar organized by the University of Boumerdes with this topic to enhance our case.

The influence left by multiple herbal treatments within the last two years couldn’t be neglected so the inclusion of such an important segment was more than necessary; in order to comprehend the use and the value of this alternative way of treatment another survey will be carried out along with a local interview with precise questions.

Additionally, the work will be preceded by a literature review in order to scan through the history of the virus and open the gate for comparison between previously seen human coronaviruses, moreover understanding the concept of functioning the new SARS-CoV-2 has adapted to spread and multiply its genome organization also the possible treatments and vaccines developed.

Literature Review

Chapter 1.

Evolution of Covid19: A Brief Historical Review

1.1. A Brief Historical Review

In the past two decades, a few signs of an upcoming pandemic have been signaled here and there, and it has been confirmed that by far, there are seven human coronaviruses responsible for infection amongst humans which are: 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (beta coronavirus that causes Middle East Respiratory Syndrome), SARS-CoV (beta coronavirus that causes severe acute respiratory syndrome) and at last SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19) according to the CDC (National Center for Immunization and Respiratory Diseases, 2020).

1.1.1. SARS 2003 Guangdong (Hong-Kong) -China-

1.1.1.1. Epidemiology

The first wave started on November of 2002, when on December 17th, 2002 a chef who worked at a restaurant in Shenzhen was diagnosed with atypical pneumonia. The chef was feeling unwell so he sought medical help, after contact with his family and medical staff, they were all infected and since he was a chef, he had daily interaction with a variety of live caged animals used as exotic game food (Zhong *et al*., 2003 ; Yang *et al*., 2020).

The second wave's index patient was noted to be a 46-year-old male seafood merchant and was admitted to the hospital of Zhongshan University on January 31st, 2003 and throughout his hospitalization has infected 30 medical staff, furthermore, 19 of his family members were also contaminated after close personal contact with the patient. One doctor who has been working in the hospital of Zhongshan happened to travel to Hong-Kong which is what led to the pandemic (Zhong *et al*., 2003).

By the end of the breakout, amongst the 32 countries were 8422 people infected with a death rate of 11% (919) whilst China alone held 5328 of these cases on a period of approximately eight months launching on November 2002 to June 2003 (Yang *et al*., 2020).

1.1.2. Middle East respiratory Syndrome (MERS)

1.1.2.2. Epidemiology

On the 20th of December there was a novel human coronavirus recovered from an adult male Saudi Arabian who died in June 2012 with severe respiratory syndrome and renal failure. And that was the 1st reported case of Middle East Respiratory Syndrome CoV 1 that has taken place in Jeddah, Saudi Arabia. The MERS has spread since then internationally to different countries including Qatar, Bahrain, Kuwait, Jordan, and Tunisia (Al-Osail and Al-Wazzah, 2017).

The MERS went on until 2015 WHO has confirmed that the virus officially reached a total of 26 countries and has 1621 confirmed cases and 584 deaths globally (Hui *et al*., 2020).

1.2. Severe Acute Respiratory Syndrome 2

1.2.1. Origin and Spread of COVID-19

In December of 2019, different health departments in Wuhan, a province of china have reported patients who suffered from severe pneumonia of unknown cause. It sounds like the exact thing that had happened with patients of SARS and MERS is being recreated with similar symptoms, seemingly among the 27 first alarming cases, most had a link to a wholesale seafood market that traded live animals, including poultry and wildlife. After being reported the WHO, on the 1st of January the virus has been identified as Coronavirus with 95% homology with bat coronavirus and 70% similarity with SARS-CoV (Singhal, 2020 ; Hu *et al*.; 2021).

The number of cases continued to increase alarmingly without any of the cases having contact with bats which put down to speculation the fact that was later confirmed possible: human-to-human transmission. With history repeating itself, the virus hitting in a festive Asian period, the Lunar new year made it easier for the spread to occur that later on spread to other countries which made the WHO in January 30 declare the virus as a public health emergency of international concern, naming it later on the 11th of February: COVID-19 (Singhal, 2020).

Chapter 2.

General Virology of SARS CoV-2

2.1. Taxonomy, Genomics and Mutations

2.1.1. Taxonomy and Virion Composition

2.1.1.1. Taxonomy

Coronaviruses are enveloped viruses with icosahedral symmetric particles of approximately 80 to 120 nm in diameter, they contain non-segmented, positive single-strand RNA, with the largest RNA genome (approximately 30 kb) (Weiss and Navas-Martin, 2005 ; Helmy *et al*., 2020).

Being one of the largest groups of viruses, it is subdivided into four main genra: Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Gammacoronavirus (γCoV), and Deltacoronavirus (δCoV) (Helmy *et al*., 2020).

According to NCBI genbank (2019) the current SARS- CoV-2 is classified as follows:

Clade: Riboviria

Kingdom: Orthornavirae

Phylum: Pisuviricota

Class: Pisoniviricetes

Order: Nidovirales

Suborder: Cornidovirineae

Family: Coronaviridae

Subfamily: Orthocoronavirinae

Genus: *Betacoronavirus*

2.1.1.2. Virion Morphology and Structural Proteins

The genome of the virus is complexed by a nucleocapsid protein (N) to form a helical capsid within the viral membrane. All the membranes of coronaviruses contain at least three viral proteins, the most prominent of all is the Spike glycoprotein (S) (previously named E2) type I glycoprotein that can form peplomers on the virion surface which has been deemed responsible for forming the binding of the virus to the host receptor, giving the virus its corona, or crown-like morphology under the electron microscope. The membrane (M) is the most abundant protein; it spans the membrane three times. The third protein is the (E) protein which is a small polypeptide, ranging from 8.4 to 12 kDa, highly hydrophobic, most data suggests that it is a transmembrane protein, one of the main functions this protein has is facilitating the assembly and release of the virus. A fifth protein is said to partake in the composition of the virion, the hemagglutinin-esterase (HE), present in a subset of betacoronavirus, and has the function of binding sialic acids on surface glycoproteins which is thought to enhance the S protein mediated cell-entry and virus spread (Weiss and Navas-Martin, 2005 ; Masters, 2006 ; Maier *et al*., 2015) **(Fig. 1)**.

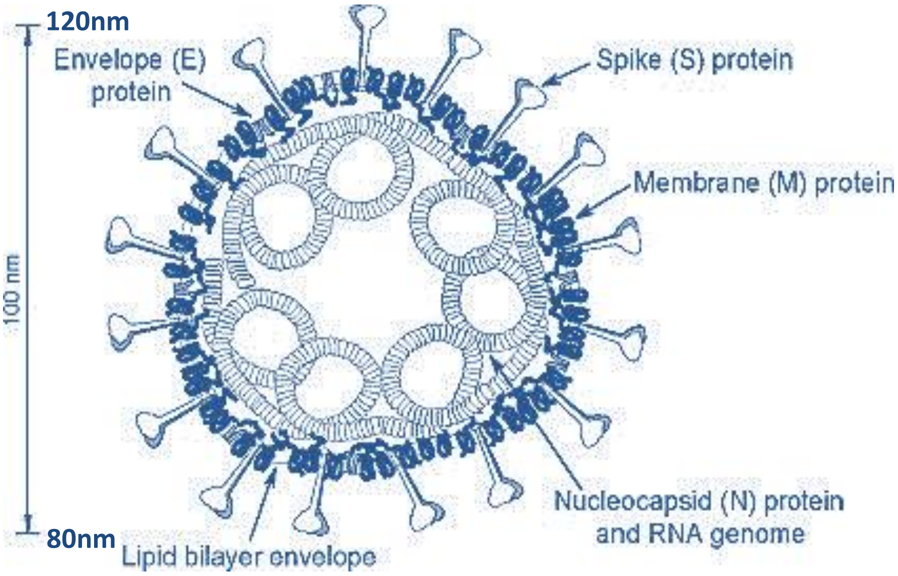


Figure 1. Schematic of the coronavirus virion, with the minimal set of structural proteins (Masters, 2006).

2.1.2. Genomics

SARS-CoV-2 has the 2nd largest known RNA genome with a size of 29,903 nucleotides and that's because it is a monopartite RNA virus, at the 5' and 3' ends the virus genome consists of two untranslated regions (UTRs) and 11 open reading frames (ORFs) those encode 27 proteins (Helmy *et al*., 2020) **(Fig. 2)**.

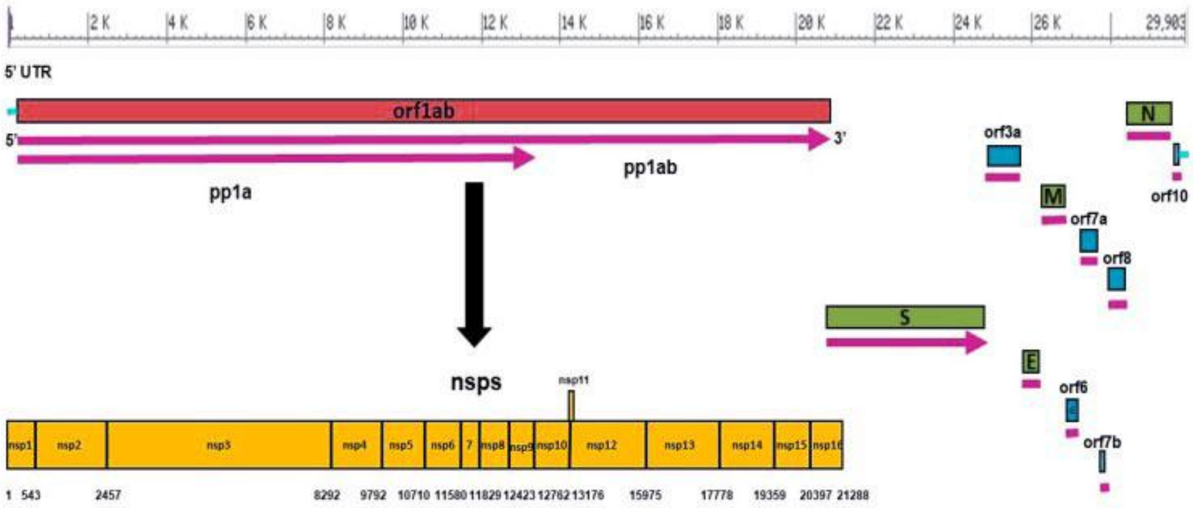


Figure 2. Genome organization of SARS CoV-2 and its encoded proteins (Helmy *et al.*, 2020).

2.1.3. Mutations of SARS-CoV-2 and their effects

In comparison to SARS-CoV, SARS-CoV-2 has a total of 348 mutations in nonstructural proteins (ORF1ab, 3a, 3b, 7a, 7b, 9b, and ORF14), 27 in S protein, and 5 in N protein, leaving us to the conclusion that E and M are highly conserved regions since no substitution was detected there (Helmy *et al.*, 2020).

2.2. Etiopathogenesis and Clinical Features

2.2.1. Symptoms and Clinical Features

SARS-CoV-2 infection is spread through droplets in the air that are produced by symptomatic patients in the midst of coughing or sneezing or asymptomatic patients that would later on develop symptoms (Hu *et al.*, 2021).

The increase of the number of cases later on confirmed human-to-human transmission. The symptoms resemble flu-like ones and may include: fever, fatigue, dry cough, sore throat, shortness of breath, headache, chest tightness, chest pain, and muscle pain. Additionally, loss of taste or smell and gastrointestinal symptoms like nausea, vomiting or diarrhea has also been reported by infected patients, all those symptoms differ with age as it's been proven that the infection within children and young proportions is mostly asymptomatic and briefer exposures to those patients is less likely to result in a transmission than an exposure to symptomatic patients (Dos Santos, 2020 ; Helmy *et al.*, 2020 ; Mahalmani *et al.*, 2020 ; Wiersinga *et al.*, 2020 ; Hu *et al.*, 2021).

Hu *et al*. (2021) affirm that the virus has an incubation period of 1 to 14 days, while it is most common for people to showcase signs of the disease on five days, and according to Wiersinga *et al*. (2020) approximately 97.5% of individuals who develop symptoms will do so within 11.5 days of infection.

2.2.2. Transmission of SARS-CoV-2

2.2.2.1. Animal to human transmission

Even though it was declared in the early pandemic that the infection is zoonotic, it isn’t clear yet if it is the case, the primary suspect in this case is the bat when on a whole genome level, Bat-CoV was 96% identical to COVID-19. Recent studies are also implying that the pangolin might be the missing link between the virus and humans since they provide a partial spike gene to COVID-19 (Rahman *et al*., 2020 ; Zhou *et al*., 2020).

2.2.2.2. Human to human transmission

A. Horizontal Transmission

This mode is currently the main transmission pathway of the virus. Airborne transmission is suspected to be the primary mode via aerosols that can be generated while doing daily activities such as talking, coughing, or sneezing by an infected subject. Thus it isn’t the only existing pathway for the virus, since gastrointestinal tract might also be regarded as a potent mode, on a third note, the body fluids and secretions aren’t much documented, aside from the respiratory secretions, less data is available on saliva, urine, semen and tears (Kaira *et al*., 2020 ; Rahman *et al*., 2020).

B. Vertical transmission

The COVID19 can indeed be transmitted from mother to child, and the first case was reported on Mach 2020 (Rahman *et al*., 2020).

2.3. Viral entry and reproduction cycle

2.3.1. Angiotensin converting enzyme 2 (ACE2)

The virus binds to a receptor named ACE2 that is expressed on numerous organs such as epithelial cells of lungs, intestine, kidney, and blood vessels that’s how it then gains entry into the host cell. The ACE2 is the renin-angiotensin system (RAS) main component and has an effective role of monitoring and maintaining fluid and salt balance along with blood pressure homeostasis, also the wide range expression of the ACE2 in several organs may explain the variety of symptoms that the patients get and the sites of infections (Cevik *et al*., 2020 ; Mahalmani *et al*., 2020 ; Trougakos *et al*., 2021).

When the SARS-CoV-2 suppresses the ACE2 which will be accompanied by an increase of Angiotensin 2 (ANGII), it will induce leakage of pulmonary blood vessels by the stimulation of ANGII type 1 receptor: AT1R. Therefore, an extensive viral infection with SARS-CoV-2 can provoke capillary leakage which if sustained may lead to viremia, local over-activation of the ACE/ANGII/AT1R signaling due to ACE2 diminishment, extensive inflammation and the so-called “cytokine storm”, after the last one occurs, the signals spread throughout the body and the ACE2 is overly expressed at this rate, and given the extensive expression of ACE2 that is now exaggerated after the pro-inflammatory cytokines in most human organs, the virus that might be circulating (in case of viremia) is now more susceptible to attack the vital organs such as kidneys and heart (Trougakos *et al*., 2021).

2.3.2. Virus attachment and penetration

Once the virus enters in contact with the cell, it can penetrate it in two ways: either via endosomes or plasma membrane fusion (**Fig. 3**), in both cases, the attachment occurs after the binding of the previously explained ACE2 and the S domain of SARS-CoV-2 receptors, it’s when virions can then either be taken into endosomes and spike proteins are activated by cathepsin L, which is more likely to provoke an immune response or, Transmembrane protease serine 2 (TMPRSS2) that is present on the surface of the host cell will clear the ACE2 and activate the receptor-attached S proteins, their activation will lead to some conformational changes that allow the virus then to penetrate the cell (Astuti et Ysrafil, 2020 ; Dos Santos, 2020).

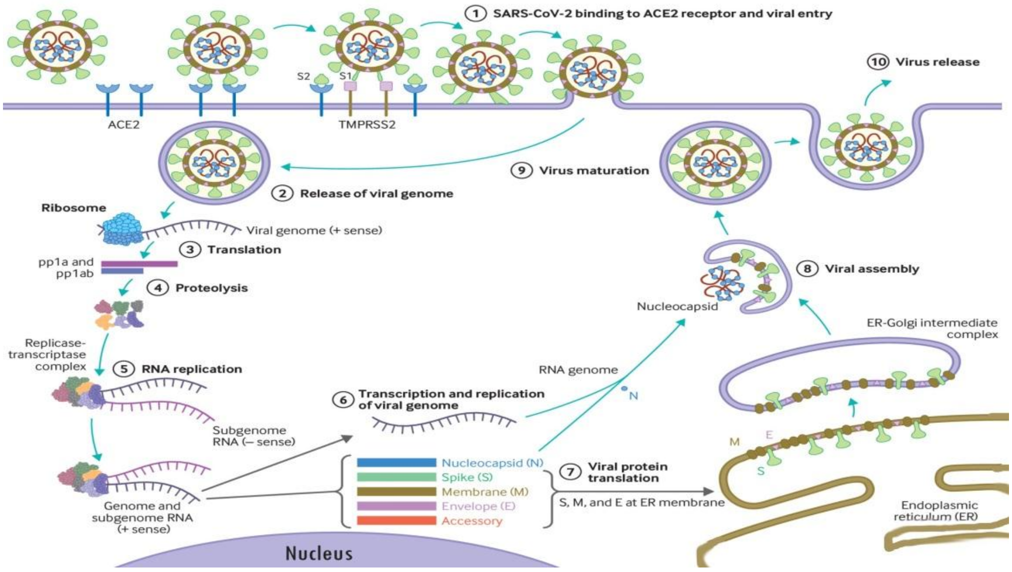


Figure 3. COVID 19 Replication cycle (Cevik *et al*., 2020)

(1) Virus binding to ACE2 which then leads to membrane fusion and viral entry (2). The virus uncoats (3) and its RNA replicates (4,5) to transcribe viral proteins (6,7). (8) is when the virus assembles and mature at (9) then leaves (10).

2.3.3. Uncoating, Translation and Replication

When the virus penetrates the cell, and the viral shell dissembles to release the genomic material into the cytoplasm, following the Uncoating process, the first macromolecular synthetic event is the synthesis of an RNA-dependent RNA polymerase from the incoming viral genomic RNA, the genomic material will then become translated in the cytoplasm. The virus genomic RNA encodes nonstructural proteins (NS) 1a and 1b that cleaved to form functional NS proteins such as helicase or the RNA-dependent RNA polymerase complex (RdRp) (Cavanagh, 1997 ; Astuti and Ysrafil, 2020 ; Cevik *et al*., 2020 ; Dos Santos, 2020).

The sub-genomic proteins are translated into structural proteins such as S, M and E by ribosomes that are bound to endoplasmic reticulum and then moved to the Endoplasmic reticulum-Golgi intermediate compartment (ERGIC)**.** Simultaneously, the previously replicated genome joins the N protein to the nucleocapsid form and are assembled together to be moved afterwards into the ERGIC (Astuti and Ysrafil, 2020 ; Dos Santos, 2020).

2.3.4. Assembly and Release

At last, the virion compartments all meet and will be released through exocytosis (Dos Santos, 2020).

2.4. Vaccine and potential therapeutic options

2.4.1. Hydroxychloroquine (HCQ)

Being a less toxic derivative of Chloroquine (CQ) it has shown to reduce the viral load in infected patients by a lot, its combination with azithromycin has given better results than the Hydroxychloroquine (HCQ) alone, however the combination of these two remains questionable (Mahalmani *et al*., 2020). When HCQ and CQ were previously used against malaria and autoimmune disease, they were proven to possess the ability to inhibit the glycosylation of cellular receptors and interfere with virus–host receptor binding, yet no scientific consensus has been reached regarding their efficiency against SARS-CoV-2, although some studies showed their potential to inhibit the SARS infection *in vitro*, these are still insufficient (Mahalmani *et al*., 2020 ; Hu *et al*., 2021).

2.4.2. Vaccines

Traditionally vaccines undergo a long process that varies from 10 to 15 years in order to be finalized, all vaccines get tested until reaching the results desired, the developer is allowed to market the vaccine only if the risks are low and the outcomes are promising. Hence, the SARS-CoV-2 vaccine had to be prepared within a time-lapse of 12 to 24 months, clinical trials were reduced due to the need, and the necessity of production during the global pandemic made it possible to generate vaccines by 2021(WARP Speed) (Kashte *et al*., 2021).

A list of SARS-CoV-2 vaccines has been established (**Tab.1**).

Table 1. Examples of different types of SARS-CoV-2 vaccines.

Type of Vaccine and name		Vaccine/Producing laboratory/ Country	Target(T)/ Vector(V)/Molecule(M)/ Adjuvant(A)	Mechanism of action	level of efficacy Storage	Cost and dosage	Citation
Nucleic acid vaccine	**mRNA**	• mRNA-1273/Moderna/ USA • mRNA-BNT162b2/Pfizer and BioNTech/USA-Germany	• T: S protein of the virus/ V: lipid nanoparticle (LNP) • T: S protein/ V: Recombinant vesicular stomatitis viru-ΔG	mRNA uses the host cell to produce viral antigen that is recognized by B lymphocytes	• 94.1% at -20°C 6months/ 2-8°C 30days • 95% at -70°C 6months/ 2-8°C 5days	• $15 per 0.5ml • 19.50 per dose, 2 doses (21 days apart)	(Kyriakidis *et al.*, 2021 ; Lombardi *et al.*, 2021)
	DNA	INO-4800 / Inovio Pharmaceuticals/ USA	T: SARS-CoV-2 S protein/ vector plasmid pGX9501	Using ‘Cellectra’ to deliver small electric pulse to trigger T cell production.	95% Stable at room temperature(1 year)		(Smith *et al.*, 2020 ; Kyriakidis *et al.*, 2021)
Replication-defective viral vector vaccines	**AZD1222**	AstraZeneca and Oxford University/USA	M: DNA/ T: S protein/ V: chimpanzee adenovirus (ChAdOx1)	• DNA of adenovirus penetrates the host cell nucleus. • Without replicating the cell copies its mRNA and produces S protein that enhances T cell production.	82% at 2-8 °C	$4.00 per 0,5 ml 2 doses (28 days apart)	(Mullard, 2020; Kyriakidis *et al.*; 2021)

	Gam-COVID-Vac/ Sputnik V	Gamaleya Research Institute/Russian federation	M: DNA/ T: S protein / V: adenoviral vector serotype	Same as previous (AZD1222) + inducing strong humoral responses as well as CD4+ and CD8+ T cell activation	91% at 2-8°C for 3 months or -20°C for 3 years	• $10 per dose (0,5 ml) • 2 doses	(Kyriakidis *et al.*; 2021)
Inactivated pathogen vaccines	**BBIBP-CorV**	Beijing Institute of Biotechnology/ Sinopharm/ China	A: Aluminium hydroxide/T: S protein/ V: 19nCoV-CDC-Tan-HB02 inactivated strain by β-propiolactone	The inactivated Coronavirus strains penetrate the host cell and cause an immune response promoted by the adjuvant.	86% at 2-8°C	$29.75 per dose 2 doses	(Corum and Zimmer, 2021 ; Kyriakidis *et al.*; 2021)
Protein subunit vaccines	**NVX-CoV2373**	Novavax CZ AS/ Czech	M: Full-length recombinant SARS-CoV-2 S glycoprotein nanoparticle/ A: Saponin based Matrix M1/ V: baculovirus-Sf9 system	• The nanoparticles once injected cannot replicate. • Encounter antigen-presenting cells. • Causes the presentation to T cells therefore generating antibodies against the virus	86% at +2-8°C for 3 months or -20°C for 2 years	• $16 in the US per dose • 2 doses	(Kashte *et al.*, 2021 ; Kyriakidis *et al.*; 2021)

Experimental part

Chapter 3.
Materials and Methods

The emergence of the new virus has created an alarming crisis to the world until this day, thus why it’s been a greater challenge for a third world country like Algeria to adapt to the new measures imposed by this pandemic, it is due to the lack of local studies about how this virus has altered our lives and the impact it has left on our health that have motivated us to accomplish this study.

Table 2. List of main Software used in this study.

Software	Version	Features that were used	File formats used
Mega X	10.2.5	DNA/ Protein Alignment building Tree model detection Phylogenetic tree building.	.fas .txt .meg .mstx .nexus
SPSS (Statistical Package for the Social Sciences)	25	One way ANOVA test Test of homogeneity Chi square test	.spv .sav
FigTree	1.4.4	Phylogenetic tree visualization	.nexus .newick
Jalview	2.11.1.4	Converting files to fasta format	.clustal_num
Microsoft Excel	14.0.4760.1000 (32bits)	Different graphs design from data	.xlxs .cvs

3.1. Phylogenetic analysis for Coronavirus evolution over time

3.1.1. Data gathering

We started by running a research over the history of previously discovered coronaviruses of different species in order to establish a firm link to therefore trace the roots and origins of the virus. The data was collected from the platform NCBI's open access, annotated collection of all publicly available nucleotide sequences database GenBank.

Table 3. Accession numbers and basic information about the Genbank reference genomes used.

Name	Accession number	Host	Date of consultation
Camel 229E	KT253324.	Camels	09/06/2021
AlapacaCoV	DQ915164.2	Alpaca	10/06/2021
HCoV-229E	NC_002645.1	Human	8/06/2021
BtCoV/AT1A-F1	KT253272.1	Bat	12/06/2021
BtCoV/KW2E-F56	KT253271.1	Bat	30/05/2021
NL63	NC_005831	Human	12/06/2021
HKU1	NC_006577.2	Human	12/06/2021
MHV-A59	NC_001846.1	Mice	10/06/2021
HKU24	NC_026011.1	Rat	11/06/2021
HCoV- OC43	MW532108.1	Human	12/06/2021

BCoV-ENT	NC_003045.1	Bovine	11/06/2021

The software Mega X was used to treat the sequences since it possesses the interface that matches with the goal of our study and with the help of this tool we could accomplish:

- Visualizing the downloaded sequences under the format Fasta.
- Building a DNA (whole genome) and protein (selected coding sequences) alignment.
- Aligning multiple sequences of DNA and proteins.
- Building the best fitted phylogenetic tree model from the previously established alignment.

3.1.2 Data processing

Before the data was treated, the selection of the needed sequences of each genome was our first step as we picked the genome’s most prominent Coding Sequences (CDS) and conserved ones by order, in each virus the common CDS selected were the ones coding for the following protein successively: 1ab, 1a, S, E, M and N.

Those sequences were then saved connectively one to the other under FASTA format then visualized in Mega X after the following steps:

After opening the Mega X software, we started by building an alignment first (**Fig. 4)** and chose to retrieve the sequences from the already prepared FASTA file we downloaded.

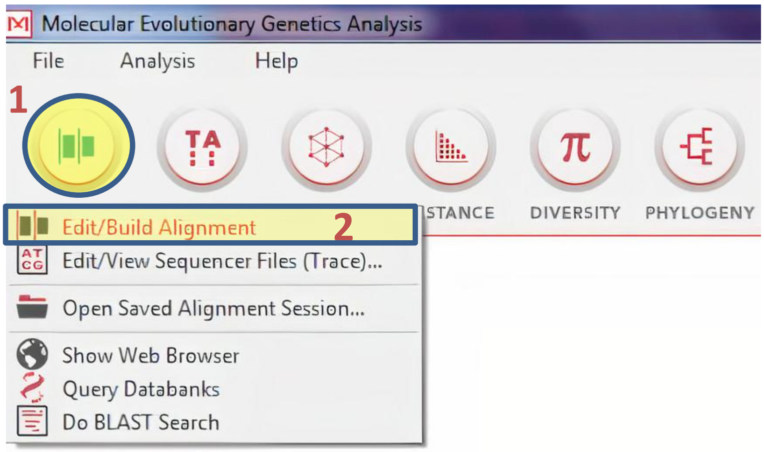
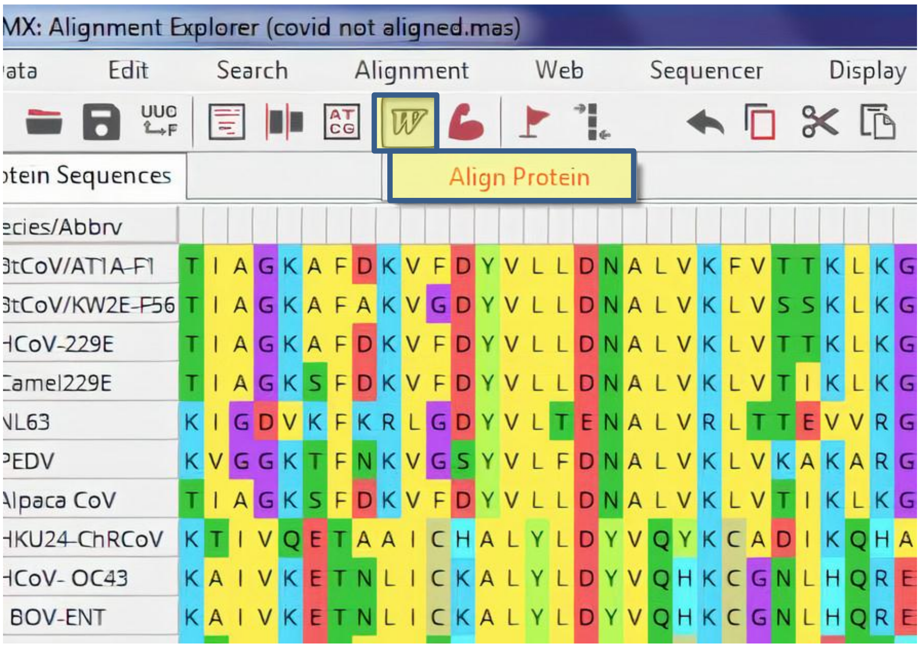


Figure 4. First step of building phylogenetic tree Mega X.
1: Align; 2: Edit/Build alignment

In order to align the obtained sequences, we chose the ClustalW algorithm (**Fig. 5).**



ecies/Abbrv	
tCoV/AT1A-F1	T I A G K A F D K V F D Y V L L D N A L V K F V T T K L K G
tCoV/KW2E-F56	T I A G K A F A K V G D Y V L L D N A L V K L V S S K L K G
CoV-229E	T I A G K A F D K V F D Y V L L D N A L V K L V T T K L K G
amel229E	T I A G K S F D K V F D Y V L L D N A L V K L V T I K L K G
L63	K I G D V K F K R L G D Y V L T E N A L V R L T T E V V R G
EDV	K V G G K T F N K V G S Y V L F D N A L V K L V K A K A R G
lpaca CoV	T I A G K S F D K V F D Y V L L D N A L V K L V T I K L K G
KU24-ChRCoV	K T I V Q E T A A I C H A L Y L D Y V Q Y K C A D I K Q H A
CoV- OC43	K A I V K E T N L I C K A L Y L D Y V Q H K C G N L H Q R E
BOV-ENT	K A I V K E T N L I C K A L Y L D Y V Q H K C G N L H Q R E

Figure 5. Representing the step of alignment of the proteins by ClustalW algorithm.

Once the alignment has taken place, the sequences had to be saved under FASTA and Mega format **(Fig. 6**).

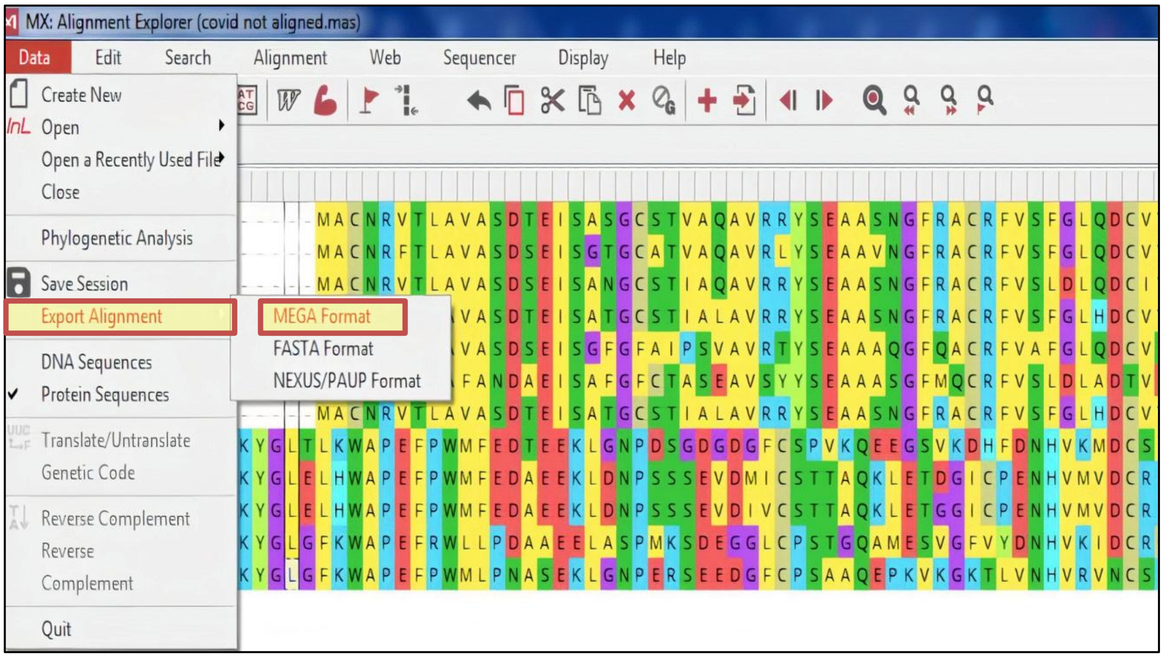


Figure 6. Saving the alignment under a Mega format.

Highlighting 70% of our saved Mega session after opening it was our next step before saving it again under a different name **(Fig. 7)**.

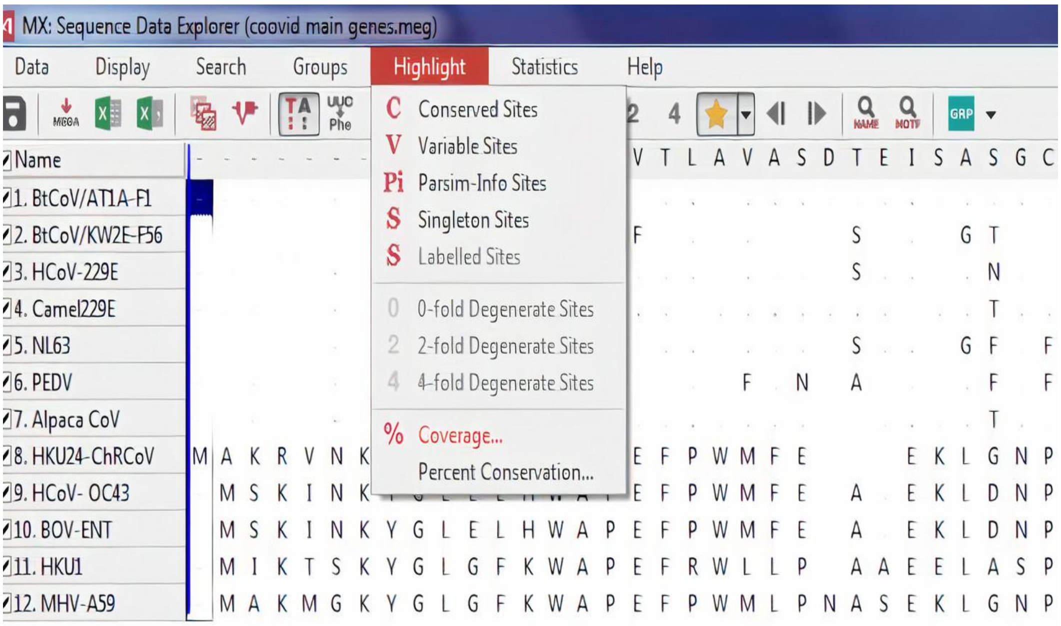
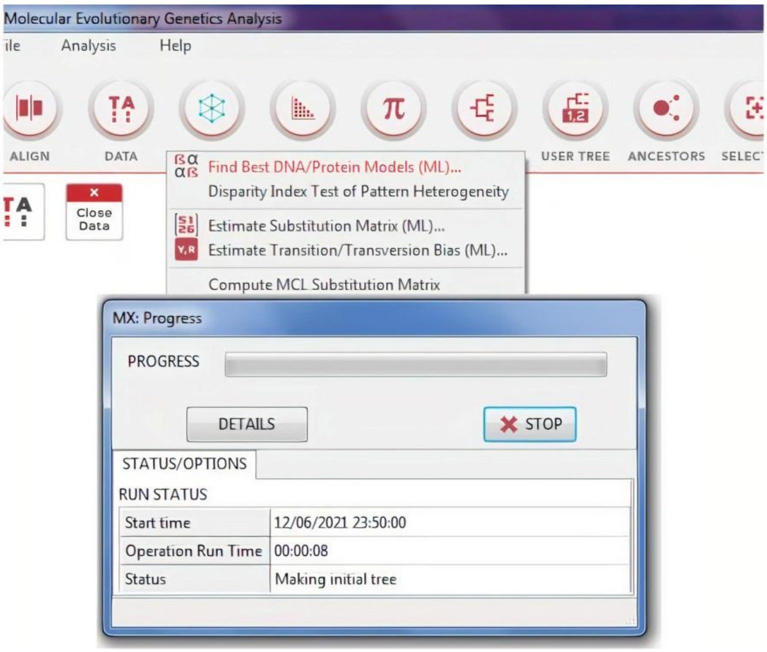


Figure 7. Selecting coverage of 70% from the Mega file.

Now that the file is treated, we use the 70% highlighted and saved Mega file to pick the best suitable tree, once that is calculated, we follow the parameters indicated in the results and the type of tree recommended in order to build the phylogenetic tree **(Fig. 8)**.

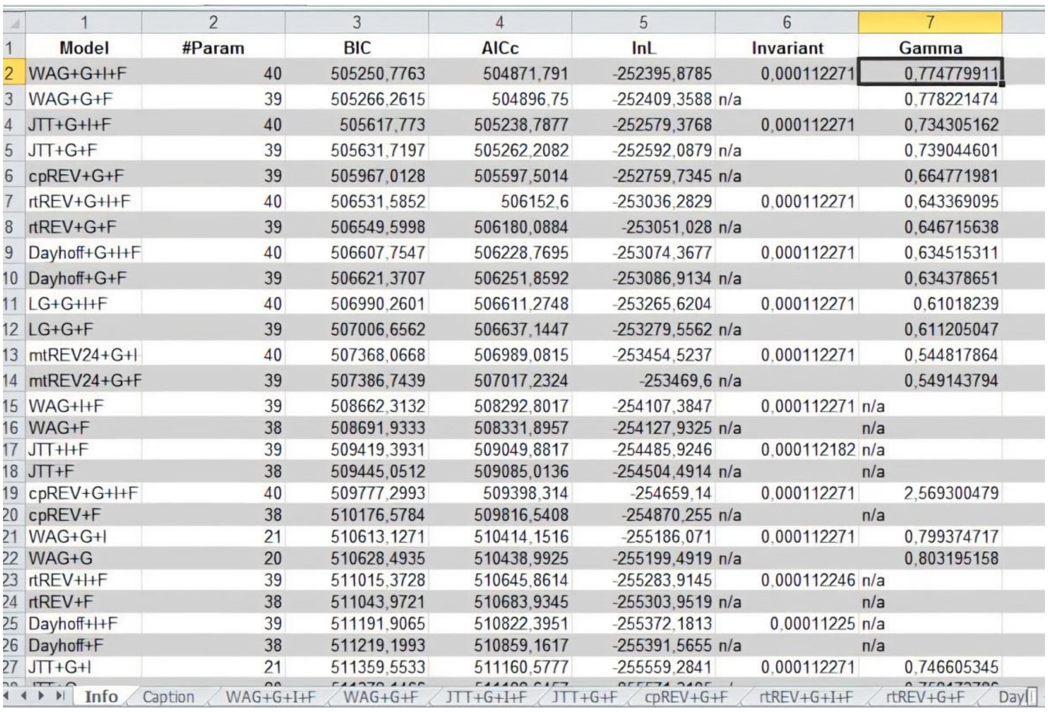


RUN STATUS

Start time	12/06/2021 23:50:00
Operation Run Time	00:00:08
Status	Making initial tree

Figure 8. Choosing the best tree model parameters.

The results could be saved in a Microsoft Excel file therefore won’t be lost **(Fig. 9)**.



	1	2	3	4	5	6	7
1	Model	#Param	BIC	AICc	lnL	Invariant	Gamma
2	WAG+G+I+F	40	505250,7763	504871,791	-252395,8785	0,000112271	0,774779911
3	WAG+G+F	39	505266,2615	504896,75	-252409,3588	n/a	0,778221474
4	JTT+G+I+F	40	505617,773	505238,7877	-252579,3768	0,000112271	0,734305162
5	JTT+G+F	39	505631,7197	505262,2082	-252592,0879	n/a	0,739044601
6	cpREV+G+F	39	505967,0128	505597,5014	-252759,7345	n/a	0,664771981
7	rtREV+G+I+F	40	506531,5852	506152,6	-253036,2829	0,000112271	0,643369095
8	rtREV+G+F	39	506549,5998	506180,0884	-253051,028	n/a	0,646715638
9	Dayhoff+G+I+F	40	506607,7547	506228,7695	-253074,3677	0,000112271	0,634515311
10	Dayhoff+G+F	39	506621,3707	506251,8592	-253086,9134	n/a	0,634378651
11	LG+G+I+F	40	506990,2601	506611,2748	-253265,6204	0,000112271	0,61018239
12	LG+G+F	39	507006,6562	506637,1447	-253279,5562	n/a	0,611205047
13	mtREV24+G+I	40	507368,0668	506989,0815	-253454,5237	0,000112271	0,544817864
14	mtREV24+G+F	39	507386,7439	507017,2324	-253469,6	n/a	0,549143794
15	WAG+I+F	39	508662,3132	508292,8017	-254107,3847	0,000112271	n/a
16	WAG+F	38	508691,9333	508331,8957	-254127,9325	n/a	n/a
17	JTT+I+F	39	509419,3931	509049,8817	-254485,9246	0,000112182	n/a
18	JTT+F	38	509445,0512	509085,0136	-254504,4914	n/a	n/a
19	cpREV+G+I+F	40	509777,2993	509398,314	-254659,14	0,000112271	2,569300479
20	cpREV+F	38	510176,5784	509816,5408	-254870,255	n/a	n/a
21	WAG+G+I	21	510613,1271	510414,1516	-255186,071	0,000112271	0,799374717
22	WAG+G	20	510628,4935	510438,9925	-255199,4919	n/a	0,803195158
23	rtREV+I+F	39	511015,3728	510645,8614	-255283,9145	0,000112246	n/a
24	rtREV+F	38	511043,9721	510683,9345	-255303,9519	n/a	n/a
25	Dayhoff+I+F	39	511191,9065	510822,3951	-255372,1813	0,00011225	n/a
26	Dayhoff+F	38	511219,1993	510859,1617	-255391,5655	n/a	n/a
27	JTT+G+I	21	511359,5533	511160,5777	-255559,2841	0,000112271	0,746605345

Figure 9. Parameters for the best tree possible displayed on Excel.

The obtained results can then be invested in picking the necessary tree model matching to our data.

3.2. Phylogenetic analysis for SARS-CoV-2 in Algeria

3.2.1. Collect of Data

The collect of data here was aimed on the Algerian sequences that were obtained after gaining access to the COVID-19 database GISAID that is restricted for Covid-19 pandemic, the total number of sequence we obtained were 24 located in different parts of the country therefore we'll be listing the ones we used in our works as we previously did.

Table 4. Details of sequences used in this study.

Name	Accession number on GISAID	Date of submissions	Location	Genome length
hCoV-19/G0638_2264/2020	EPI_ISL_418241	02/03/2020	Algeria/Boufarik	1-29730
hCoV-19/G33030-8855/2020	EPI_ISL_766868	06/2020	Algeria/Alger	1-29782
hCoV-19/G35014-8856/2020	EPI_ISL_766869	09/06/2020	Algeria/El Oued	1-29782
hCoV-19/G37308-8864/2020	EPI_ISL_766875	17/06/2020	Algeria/Ourgla	1-29782
hCoV-19/G38599-8859/2020	EPI_ISL_766871	20/06/2020	Algeria/Laghouat	1-29782
hCoV-19/Algeria/G41498-8846	EPI_ISL_766861	29/06/2020	Alger/Alger	1-29768
hCoV-19/NT01/2020	EPI_ISL_419831	03/04/2020	Australia	1-29865
hCoV-19/QLD09/2020	EPI_ISL_414414	29/02/2020	Australia	1-29864
hCoV-19/VIC03/2020	EPI_ISL_416411	25/01/2020	Australia	1-29899
hCoV-19/CeMM0003/2020	EPI_ISL_419656	26/02/2020	Austria	1-29816
hCoV-19/BA-02291/2020	EPI_ISL_415159	29/02/2020	Belgium	1-29782
hCoV-19/NL-030447/2020	EPI_ISL_420314	04/03/2020	Belgium	1-29782
hCoV-19/BC_02421/2020	EPI_ISL_415581	01/03/2020	Canada	1-29782
hCoV-19/09c/2020	EPI_ISL_412116	09/02/2020	England	1-29885
hCoV-19/HDF-1465/2020	EPI_ISL_418218	21/02/2020	France	1-29777
hCoV-19/HDF-1684/2020	EPI_ISL_414626	29/02/2020	France	1-29884
hCoV-19/HDF-1795/2020	EPI_ISL_414627	02/03/2020	France	1-29861
hCoV-19/IDF-0373/2020	EPI_ISL_406597	23/01/2020	France	1-29809

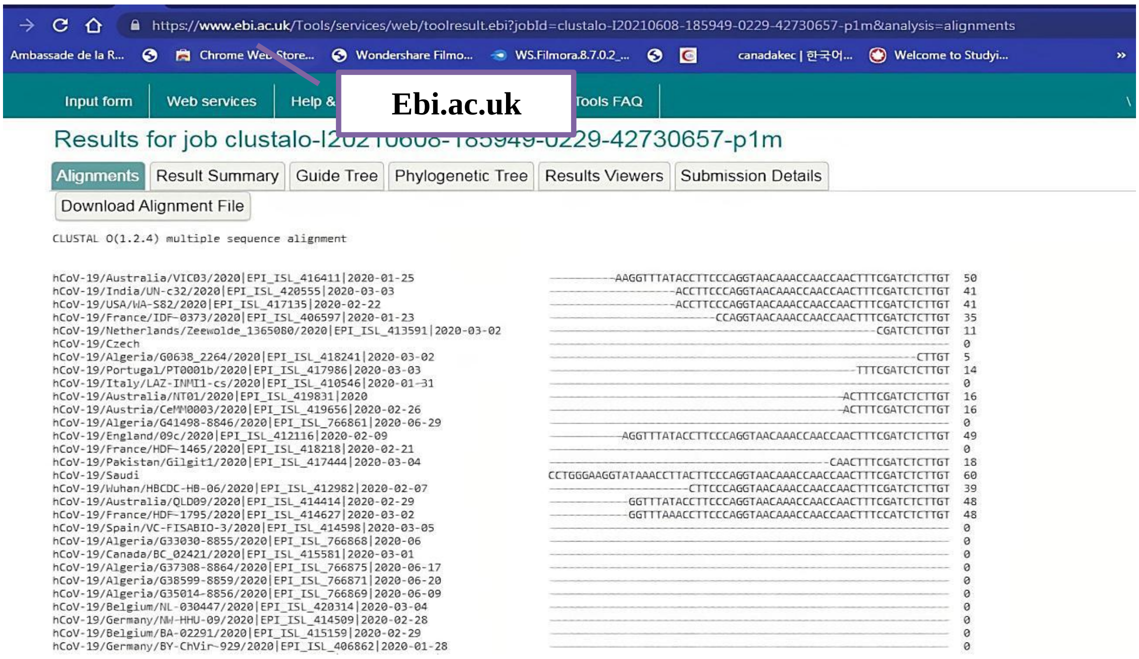
Name	Accession number on GISAID	Date of submissions	Location	Genome length
hCoV-19/BY-ChVir-929/2020	EPI_ISL_406862	28/01/2020	Germany	1-29782
hCoV-19/NW-HHU-09/2020	EPI_ISL_414509	28/02/2020	Germany	1-29782
hCoV-19/UN-c32/2020	EPI_ISL_420555	03/03/2020	India	1-29860
hCoV-19/LAZ-INMI1-cs/2020	EPI_ISL_410546	31/01/2020	Italy	1-29785
hCoV-19/LOM-ASST-CDG1/2020	EPI_ISL_412973	20/02/2020	Italy	1-29903
hCoV-19/Zeewolde_1365080/2020	EPI_ISL_413591	02/03/2020	Netherlands	1-29786
hCoV-19/Gilgit1/2020	EPI_ISL_417444	04/03/2020	Pakistan	1-29836
hCoV-19/PT0001b/2020	EPI_ISL_417986	03/03/2020	Portugal	1-29763
hCoV-19/SH0069/2020	EPI_ISL_416372	01/02/2020	Shanghai	1-29836
hCoV-19/AN-ISCIII-201272/2020	EPI_ISL_418243	28/02/2020	Spain	1-29781
hCoV-19/VC-FISABIO-3/2020	EPI_ISL_414598	05/03/2020	Spain	1-29782
hCoV-19/ZH-UZH-1000477377/2020	EPI_ISL_413020	27/02/2020	Switzerland	1-29864
hCoV-19/CA-CDPH-UC9/2020	EPI_ISL_413928	05/03/2020	USA	1-29885
hCoV-19/WA-S82/2020	EPI_ISL_417135	22/02/2020	USA	1-29857
hCoV-19/HBCDC-HB-06/2020	EPI_ISL_412982	07/02/2020	Wuhan	1-29838
hCoV-19/Wuhan/IPBCAMS-WH-01/2019	EPI_ISL_402123	24/12/2019	Wuhan	1-29899
hCoV-19/Wuhan/IVDC-HB-04/2020	EPI_ISL_402120	01/01/2020	Wuhan	1-29896

3.2.2. Data processing

Since we targeted this study to be more specific and detailed than the first one, we have settled on using the whole genome sequences instead of the coding ones only, which will make the relations established between each genome more specified.

For the previous matter, in order to gain time, we have used the European Molecular Biology Laboratory’s (EMBL) online platform Clustal Omega in order to obtain the alignment by simply inserting the Fasta file of the sequences we mentioned in Table 4 and that has been created by Mega X the same way as the one done for evolution and let the job run after choosing the type of molecules, in our case DNA.

The whole process took approximately five hours and the results were displayed as seen in (**Fig. 10**).



```
CLUSTAL O(1.2.4) multiple sequence alignment


hCoV-19/Australia/VIC03/2020|EPI_ISL_416411|2020-01-25                  ----------AAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 50
hCoV-19/India/UN-c32/2020|EPI_ISL_420555|2020-03-03                     -------------------ACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 41
hCoV-19/USA/WA-S82/2020|EPI_ISL_417135|2020-02-22                       -------------------ACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 41
hCoV-19/France/IDF-0373/2020|EPI_ISL_406597|2020-01-23                  -------------------------CCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 35
hCoV-19/Netherlands/Zeewolde_1365080/2020|EPI_ISL_413591|2020-03-02     -------------------------------------------------CGATCTCTTGT 11
hCoV-19/Czech                                                           ------------------------------------------------------------ 0
hCoV-19/Algeria/G0638_2264/2020|EPI_ISL_418241|2020-03-02               -------------------------------------------------------CTTGT 5
hCoV-19/Portugal/PT0001b/2020|EPI_ISL_417986|2020-03-03                 ----------------------------------------------TTTCGATCTCTTGT 14
hCoV-19/Italy/LAZ-INMI1-cs/2020|EPI_ISL_410546|2020-01-31               ------------------------------------------------------------ 0
hCoV-19/Australia/NT01/2020|EPI_ISL_419831|2020                         --------------------------------------------ACTTTCGATCTCTTGT 16
hCoV-19/Austria/CeMM0003/2020|EPI_ISL_419656|2020-02-26                 --------------------------------------------ACTTTCGATCTCTTGT 16
hCoV-19/Algeria/G41498-8846/2020|EPI_ISL_766861|2020-06-29              ------------------------------------------------------------ 0
hCoV-19/England/09c/2020|EPI_ISL_412116|2020-02-09                      -----------AGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 49
hCoV-19/France/HDF-1465/2020|EPI_ISL_418218|2020-02-21                  ------------------------------------------------------------ 0
hCoV-19/Pakistan/Gilgit1/2020|EPI_ISL_417444|2020-03-04                 ------------------------------------------CAACTTTCGATCTCTTGT 18
hCoV-19/Saudi                                                           CCTGGGAAGGTATAAACCTTACTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 60
hCoV-19/Wuhan/HBCDC-HB-06/2020|EPI_ISL_412982|2020-02-07                ---------------------CTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 39
hCoV-19/Australia/QLD09/2020|EPI_ISL_414414|2020-02-29                  ------------GGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGT 48
hCoV-19/France/HDF-1795/2020|EPI_ISL_414627|2020-03-02                  ------------GGTTTAAACCTTCCCAGGTAACAAACCAACCAACTTTCCATCTCTTGT 48
hCoV-19/Spain/VC-FISABIO-3/2020|EPI_ISL_414598|2020-03-05               ------------------------------------------------------------ 0
hCoV-19/Algeria/G33030-8855/2020|EPI_ISL_766868|2020-06                 ------------------------------------------------------------ 0
hCoV-19/Canada/BC_02421/2020|EPI_ISL_415581|2020-03-01                  ------------------------------------------------------------ 0
hCoV-19/Algeria/G37308-8864/2020|EPI_ISL_766875|2020-06-17              ------------------------------------------------------------ 0
hCoV-19/Algeria/G38599-8859/2020|EPI_ISL_766871|2020-06-20              ------------------------------------------------------------ 0
hCoV-19/Algeria/G35014-8856/2020|EPI_ISL_766869|2020-06-09              ------------------------------------------------------------ 0
hCoV-19/Belgium/NL-030447/2020|EPI_ISL_420314|2020-03-04                ------------------------------------------------------------ 0
hCoV-19/Germany/NW-HHU-09/2020|EPI_ISL_414509|2020-02-28                ------------------------------------------------------------ 0
hCoV-19/Belgium/BA-02291/2020|EPI_ISL_415159|2020-02-29                 ------------------------------------------------------------ 0
hCoV-19/Germany/BY-ChVir-929/2020|EPI_ISL_406862|2020-01-28             ------------------------------------------------------------ 0
```

Figure 10. Algeria and world seuqnces alignment result on Clustal Omega.

These sequences were then downloaded as Clustal_num format and converted to Fasta format using the software Jalview in order to be readable by Mega X, once that was accomplished, the same previous methods of building a tree were followed, the visualization of the tree of this part has been done by the software Figtree instead, after saving it from Mega X under Nexus format.

3.3. Hospital of Hakim Sadanne -Biskra-

3.3.1 Collect and Processing of Data

The hospital of Hakim Sadanne has agreed to share with us the data that has been collected throughout the pandemic within the last two years 2020 and 2021, the obtained data was since then stored in a Microsoft Excel file and classified according to the needed parameters.

The analysis of the information has been established by the use of various tests amid a few statistical (ANOVA 1, T-Test- Chi square) and epidemiological ones (Effective Reproduction number, Case Fatality Rate, Case Mortality Rate, Gender Mortality Rate…etc).

Before engaging, groups of ages were chosen according to the different age segments (Children, Teenagers, Adults and Elderly) and the equality in a group age proportion was taken upon the factor of different physiological factors that might influence the viral spread during different phases of life. When going through a statistical test, none of those proportions were used, instead a detailed entry for each individual was served.

3.3.1.1. Frequency estimation

A daily and monthly frequency was calculated within the admitted, healed and deceased patients throughout the year 2020 by counting the number of admitted, healed and deceased cases successively and organizing them by day and month. This was established in order to set the period in which the parameters chosen hit their highest and lowest.

3.3.1.2. Case Mortality and Fatality rates

Estimating the mortality and fatality rates allows us to establish realistic set between the total number of deaths from a number of patients belonging to a certain district of Biskra and the meaning that percentage holds to the totality of the population of that same district.

Cases fatality rate also known as case fatality risk is in epidemiology the proportion of people who die from a certain disease divided by the total amount of individuals who were diagnosed by the same disease during a certain period of time. Our study was carried along the year of 2020 and the case fatality rate has been calculated in total and individually for each month the resulting ratio was multiplied by 100 to yield a percentage. This parameter has been calculated for the reason of doing a scan over the districts of Biskra and monitoring the number of deceased individuals compared to the admitted ones in the hospital.

Mortality rate ratio differs from the fatality one since the proportion of deceased individuals is divided by the number of citizens in the study area.

3.3.1.3. Positive Sensitivity of SARS-CoV-2 tests used in the hospital of Hakim Sadanne –Biskra-

This test is ran for the sole purpose of validating the COVID-19 tests sensitivity. Test kits have different validities, this factor is measured by each test's sensitivity to either detect a positive or a negative result, the proportion of SARS-CoV-2 patients who had their diagnostic test indicate correctly as positive is called positive sensitivity, that was calculated by the number of people whom the test affirmed as positive divided by the number of people with the actual disease (Asai, 2021).

We multiplied all the results by 100 in order to obtain the ratio in percentages for a clearer visualization.

3.3.1.4. Gender Mortality Ratio (GMR)

In order to investigate the relationship between gender and the mortality already calculated before we chose to perform a Gender mortality ratio test that is simply calculated by dividing the male mortality rate on a female mortality rate, however our study went a little further by estimating the GMR for each age proportion individually.

3.3.1.5. Effective reproduction number R_T

The reproduction number is indeed an effective key in the epidemiological study of an infectious disease, it is defined according to Nishiura and Chowell (2009) as the actual average number of secondary cases per primary case at calendar time t where t>0, R(t) expresses the variation in a time-dependent variation that is to decline or increase adapting to the control measures taken by authorities against a viral spread. Hence, we used this specific indicative to assess the current dynamics of SARS-CoV-2 in the Wilaya of Biskra using Cori Anne's Susceptible Infected Recovered (SIR) model and that was presented in an .xls Excel file by the researcher.

In much simpler words, the effective number is the possible cases the initial case might generate (**Fig. 11**).

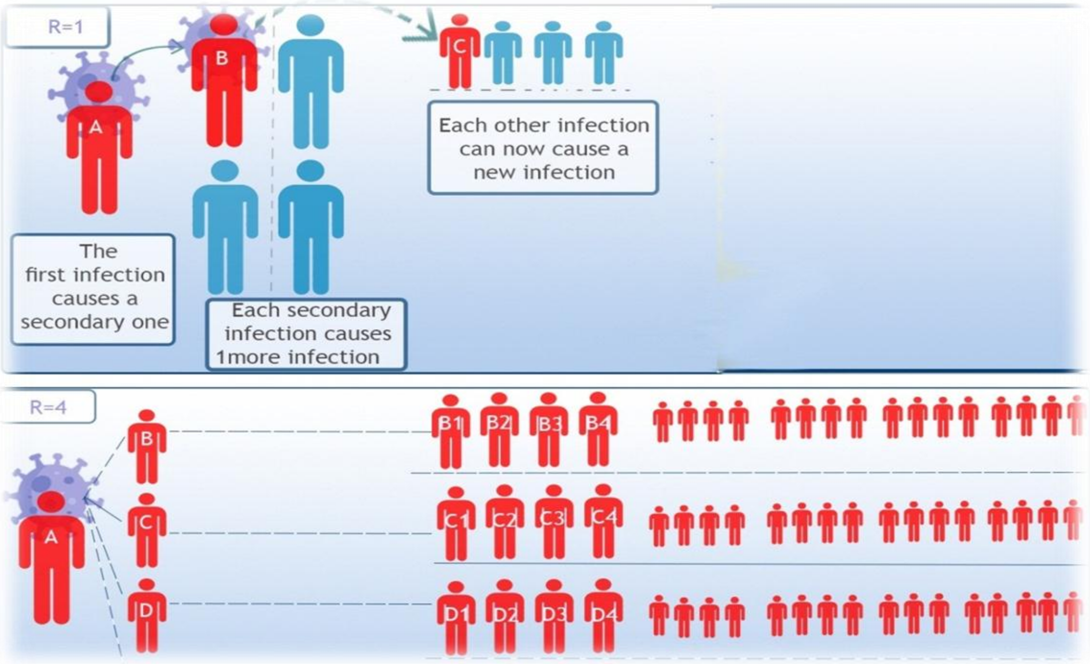
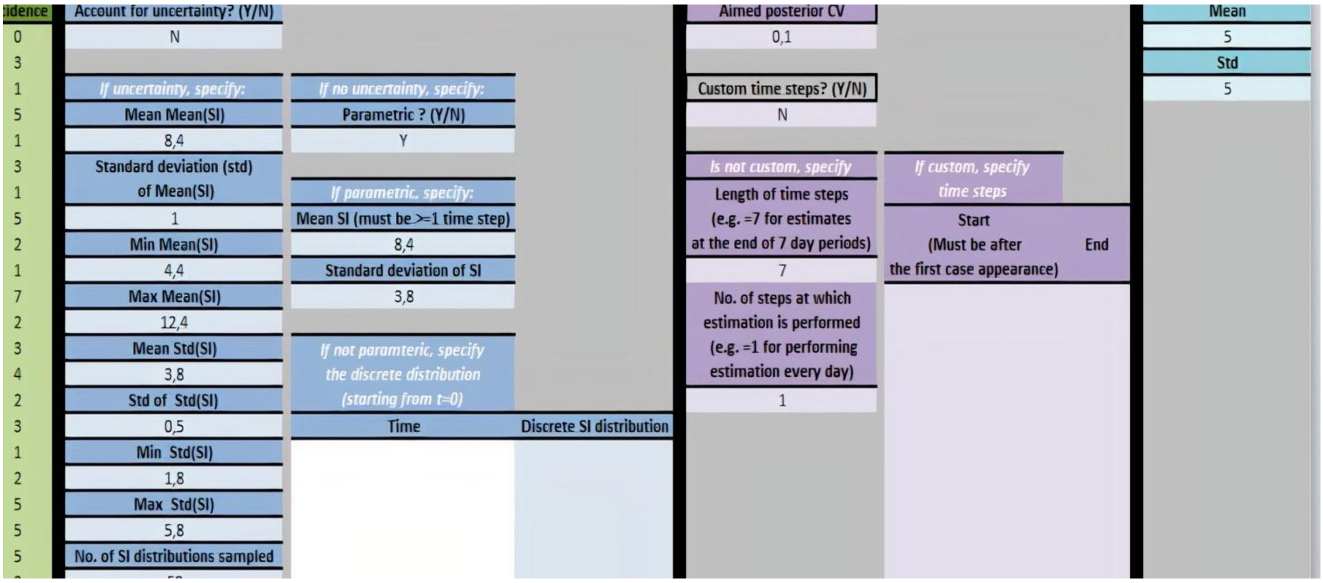


Figure 11. Explanatory figure of Effective Reproduction number R_t.

We used the frequency of daily cases as the incidence and studied the year 2020 for a time lapse of 236 days since the appearance of the first case in the hospital until the end of the year throughout different periods of the year. The aimed posterior Coefficient was lowered to 0,1 to get more accurate results meanwhile the rest of the parameters remained the same as in Cori's SIR model **(Fig. 12)**.


idence
0
3
1
5
1
3
1
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2
1
7
2
3
4
2
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2
5
5
5

Account for uncertainty? (Y/N)
N
If uncertainty, specify:
Mean Mean(SI)
8,4
Standard deviation (std) of Mean(SI)
1
Min Mean(SI)
4,4
Max Mean(SI)
12,4
Mean Std(SI)
3,8
Std of Std(SI)
0,5
Min Std(SI)
1,8
Max Std(SI)
5,8
No. of SI distributions sampled

If no uncertainty, specify:
Parametric ? (Y/N)
Y
If parametric, specify:
Mean SI (must be >=1 time step)
8,4
Standard deviation of SI
3,8
If not paramteric, specify the discrete distribution (starting from t=0)
Time
Discrete SI distribution

Aimed posterior CV
0,1
Custom time steps? (Y/N)
N
Is not custom, specify
Length of time steps (e.g. =7 for estimates at the end of 7 day periods)
7
No. of steps at which estimation is performed (e.g. =1 for performing estimation every day)
1

If custom, specify time steps
Start (Must be after the first case appearance)
End

Mean
5
Std
5

Figure 12. Parameters chosen to estimate the effective reproduction number in the SIR model.

3.3.1.6. Establishing the relationship between the factors: Age and Mortality by one way ANOVA test

The data that we dispose is detailed therefore the use of Analysis of variance (ANOVA) was the most suitable for our case, it was used to determine whether there are any statistically significant differences between the means of two independent (unrelated) groups which are age and mortality, a sample was chosen for the two months June and July of 2020 and the relationship was studied by the software SPSS for statistical analysis **(Fig. 13)**.

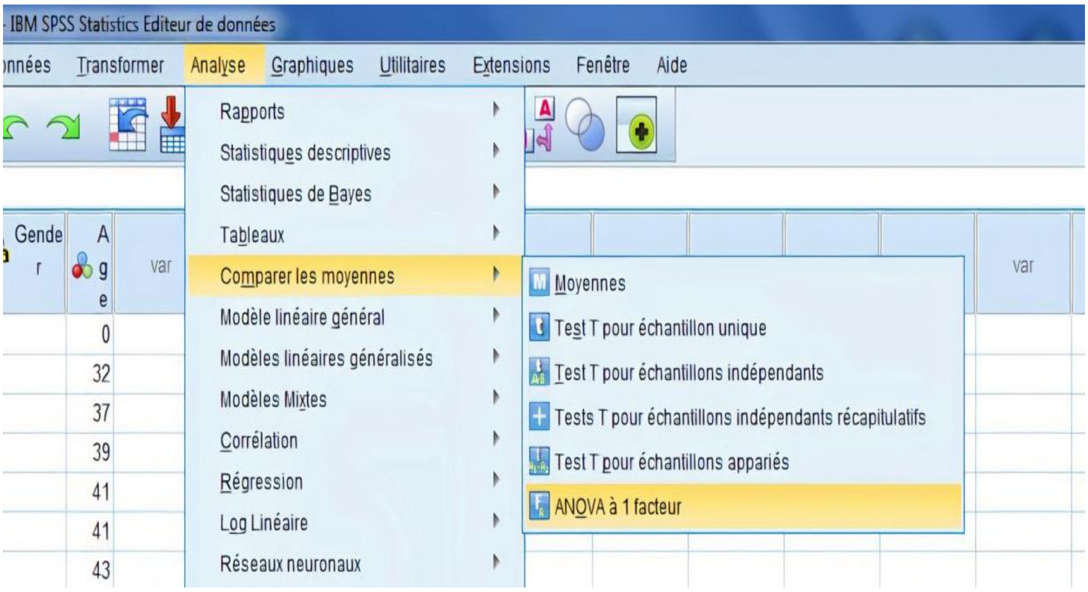


Figure 13. SPSS: One Way ANOVA test for Age and Mortality.

3.3.1.7. T-Test of homogeneity of independent samples for Sex and Mortality

The last test performed was the T-test to study the relationship between both genders Male and Female and the mortality rate of SARS-CoV-2 during the two months June and July of 2020 in the Wilaya of Biskra, the operation was also operated by the software SPSS.

3.4. Questionnaire survey on the effect of preexisting comorbidities on SARS-CoV-2 patients on a national level –Algeria-

After the participation in the SARS-CoV-2 day Webinar of the university of Bourmerdes on March 1st 2021 with this topic, it has brought to light the potential impact the virus could have on the chronically ill patients, without making any assumptions a detailed survey was lead on a national level among Algerian citizens, whilst a second more specific on was directed to the medical staff, generating over 300 and 20 responses successively.

The details of both questionnaires were analyzed by Microsoft Excel and represented graphically, furthermore, a comparison between the data obtained and China Wuhan hospital's data on comorbidities disposed on GitHub was carried out in order to support our argument and establish a more valid link also rationalize the results of comorbidities and COVID-19.

3.5. Questionnaire Survey and Interview on the use of Alternative Medicine in the Prevention and Treatment of SARS-CoV-2

3.5.1. The local interview (Biskra)

3.5.1.1. Area of study

Figure 14. Geographical localization of the study area -Biskra-race by Google Maps.

The approximate geographic location of Biskra is around 34° 51' 1.37" Northern latitude and 5° 43' 40.98" E longitude and at the time this study was established (April 19th 2021) the province of Biskra reported 1 352 cases of COVID 19 so far.

3.5.1.2. Herbalists in Algeria

The herbalists differ from one province to another and are usually males, their profession is acquired through inheritance from parents or a special training for a few years, and most of the herbalists consulted in this study are old, experienced male herbalists of ages varying between 39 to 67 years old of at least 7 years of experience.

3.5.1.3. Ethnopharmacological data collection

A questionnaire was prepared in order to gather data from herbalists concerning the anti Covid19 herbal use, the information are summarized in the table below (**Tab. 5**).

Table 5. Details about the ethnobotany interview in Biskra.

Herbalists	Total of 8
District	5 in Biskra, 1 in Ourlal, 2 in Toulga

All of our data was collected through interviews that were carried out face-to-face with the herbalists and that ranged from 10 to 20 minutes.

3.5.2. The international survey

In order to follow up with the results we could obtain from our interview, a questionnaire survey was launched online through the Google forms platform. Thus, comparing results obtained from the worldwide communities on their different herbal use against SARS-CoV-2, the frequency of the use and the reasons why those herbs were specifically used in each continent, a total of 99 response was generated and both results (interview and survey) have undergone a few test that are summarized in the table below (**Tab. 6**).

Table 6. Ethnopharmacological indices chosen.

Indices	Formula	Why was it used	Citation
Relative Frequency of Citation (RFC)	RFC= FC/N FC: Number of citation of a plant X N: Number of interviewed individuals	Reveals the local importance of each medicinal plant species used by the individuals of the area. Gives an indication of the medicinal species best known by the local inhabitants.	(Appiah *et al*., 2017)
Use Value	$UV = \sum U_i/N$ U: Number of use reports on X plant N: Number of interviewed individuals	Indicates highly used plants, most common ones, also indicates less known medically used plants. Estimated for comparison purposes between different plants.	(Houéhanou *et al*., 2016 ; Appiah *et al*., 2017)
Fidelity Level (FL)	$FL = I_P/I_U \times 100\%$ I_P: Number of informants who suggested the use of a plant X against a certain disease or symptom or part of the body I_U: Number of informants who listed the same X plant for any disease.	Indicates the plant's high or low efficacy in treating a certain disease	(Appiah *et al*., 2017)

Chapter 4.

Results and Discussion

4.1. CoV Genomic Origin Results

The tree was inspired by Forni *et al*. (2020) in which the same lineage viruses were picked ad processed except that the tree was preferred to be divided by lineage to simplify the understanding and bring focus on each lineage on its own (F**ig. 15**).

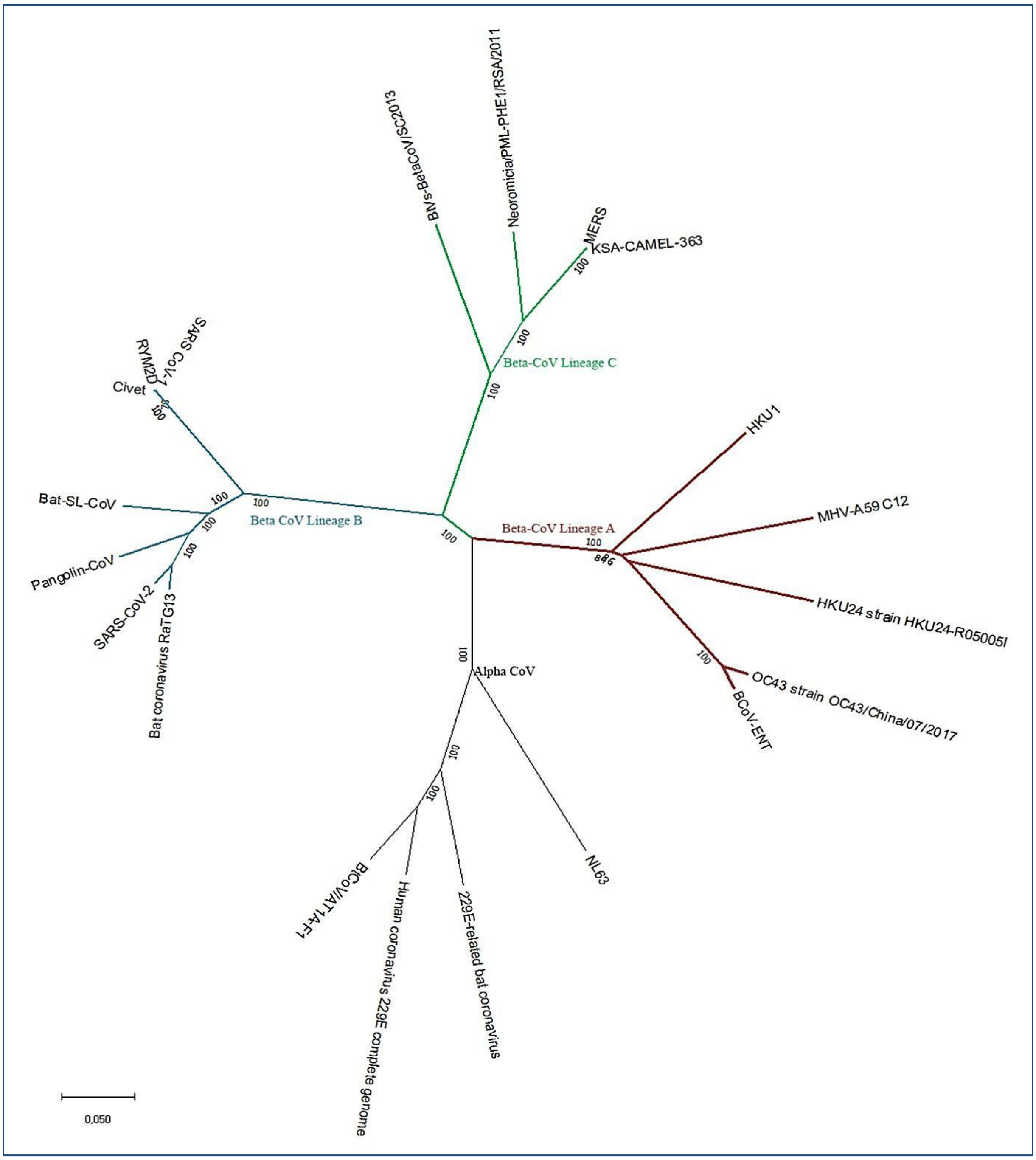


Figure 15. Mega X Phylogenetic tree result of all Coronaviruses Lineages.

4.1.1. Genomic Evolution of α-CoVs and β-CoVs (Lineage A)

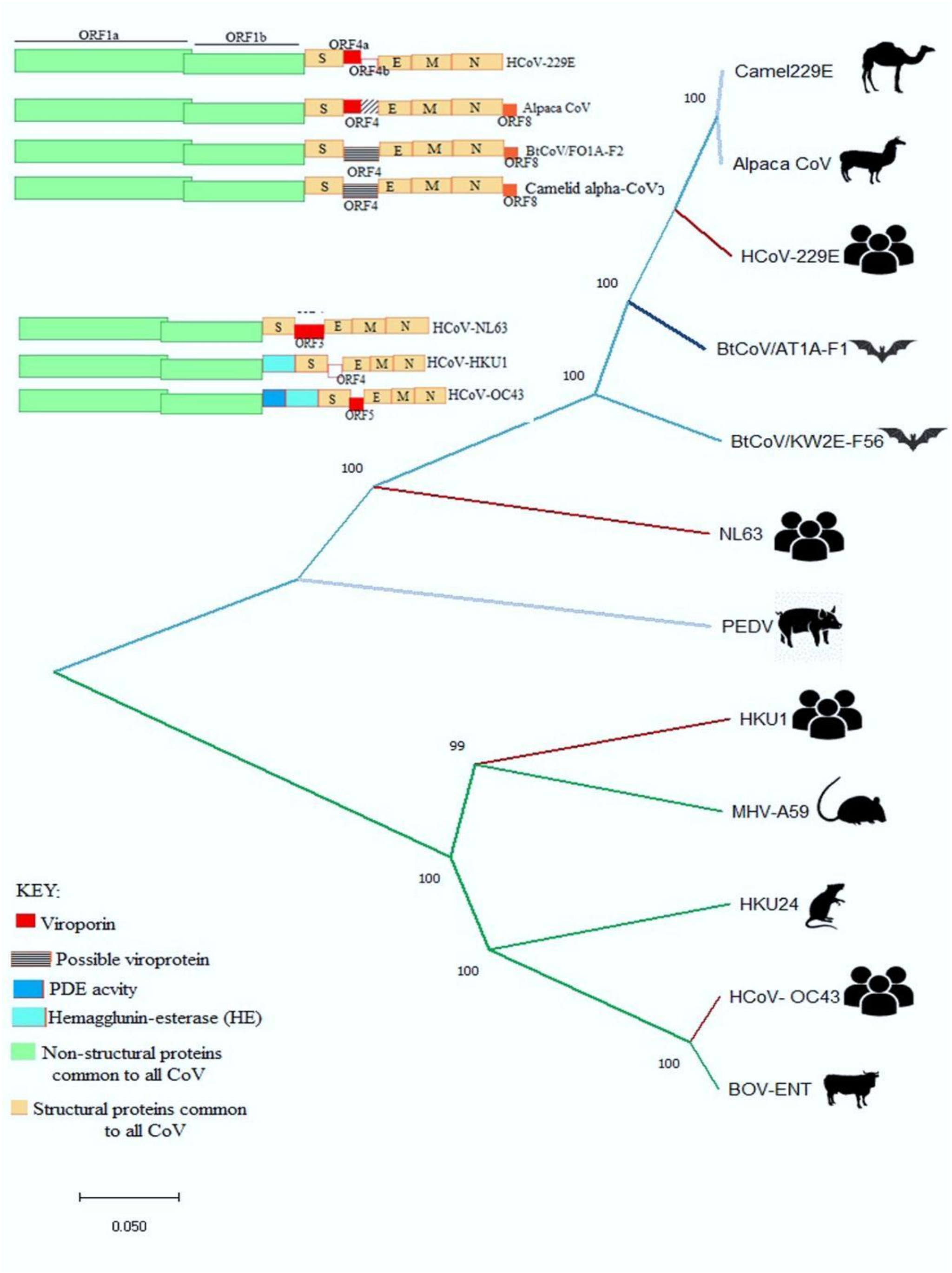


Figure 16. Result of the Mega X Evolutionary Phylogenetic tree of selected α-coronaviruses, β-coronaviruses (Lineage A) constructed with the main genome sequences.

The two α-coronavirus HCoV-229E in (Fig.14) shows that the node has derived from the BtCoV/KW2E-F56 clade of Ghana making it the ancestor of the 1960 human coronavirus, HCoV-229E, and while the distance of Alpaca and Camelid originating Coronaviruses is this same with the HCoV-229E, this one could have the camelids as a reservoir.

The NL63 was discovered in the Netherlands in 2003 and it causes in most cases a laryngotracheobronchitis according to Heike Hofmann *et al*. (2005). NL63 is pretty much similar to PEDV and that has been proved on Pyrc *et al*. (2006) that the M gene is actually more close to PEDV than to HCoV-229E which further more strengthens the argument of our result (**Fig. 16**).

The β-CoVs is constituted from three Lineages; HCoV- OC43 and HCoV-HKU1 are both part of the first one: Lineage A as stated by Kaka *et al*. (2020) in our results, the HCoV-OC43 in a sister clade with the cattle coronavirus BCoV-ENT generating a possibility of sharing the same reservoir, whilst both derive equally from the Chinese rat Coronavirus HKU24. The second virus of the lineage A is HKU1 that in the fig. 14 could hypothetically descend from rodents as it shares the same clade as MHV-59, they might also be their reservoir according to our tree.

The four coronaviruses we have just viewed are all human related viruses that show seasonally and not only they are widely dispersed around the world but they also represent a danger with their constant capability of mutating.

4.1.2. Results of the Genomic Evolution of β-coronaviruses (Lineage B and Lineage C)

This tree was drawn twice, once with only its protein sequences and again with the whole genome sequences to get more accurate results two trees was generated: a protein based tree (**Fig. 17**) and whole genome based tree (**Fig. 18**).

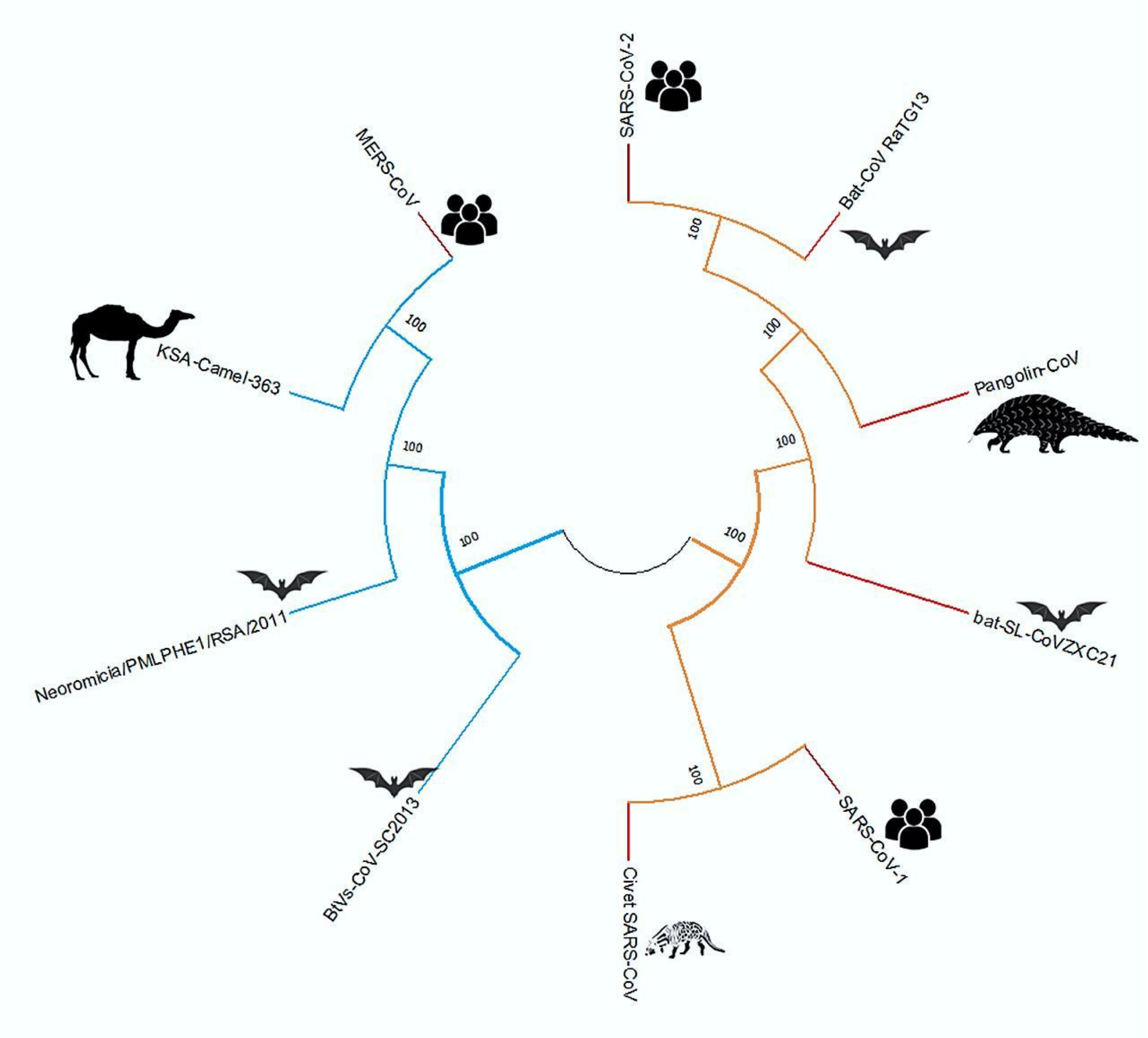


Figure 17. Result of Mega X Evolutionary Phylogenetic tree of selected β-coronaviruses (Lineage B and Lineage C) constructed with the genome sequences proteins ORF1a, ORF1b, S, E, M and N.

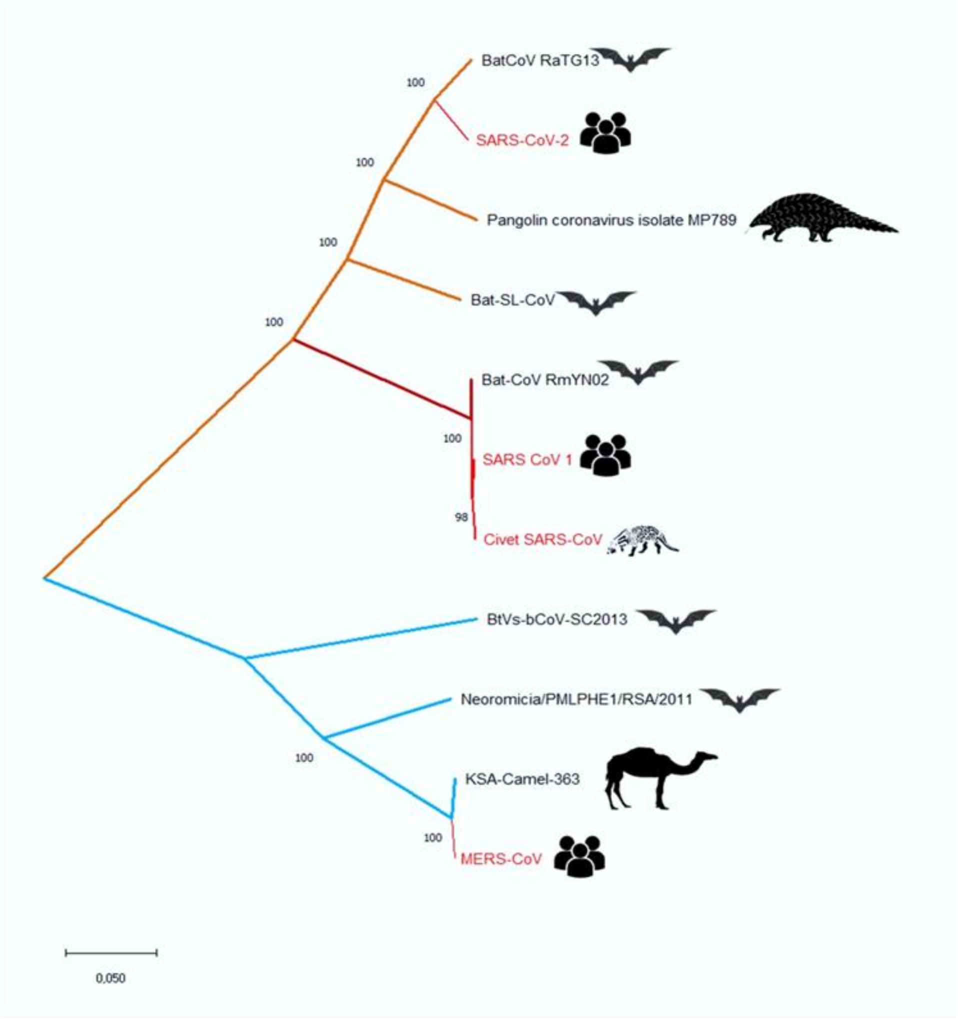


Figure 18. Result of Mega X Evolutionary history inferred using the Neighbor-Joining method of selected β-coronaviruses (Lineage B, C) constructed with the whole genome sequences.

The second lineage of the virus is the lineage B and it consists of two viruses we previously mentioned at the beginning of our works and are by now very familiar with: SARS-CoV-1 and SARS-CoV-2, while we have gone through historical review on the first one, both figures, 17 and 18 explain furthermore that it is strongly related to the Civet coronavirus which leads to the assumption of the animal being SARS-CoV-1 reservoir. The 2019 ongoing pandemic causal agent SARS-CoV-2 on the other hand has a strong bond with the zoonotic bat coronavirus Bat-CoV RaTG13, possibly being the origin of the pandemic while the reservoir is yet to differ from bats to the second closest related animal Pangolin which empowers further the previously made speculations (Title 2.2.1 of Chapter 2).

The MERS is a part of the third lineage of β-coronaviruses: Lineage C and it has a strong genetic similarity with KSA-Camel-363 since they belong to the same clade, suggesting the reservoir of the Middle east infection poossibly being the Camels (**Fig. 18**).

4.2. Evolutionary Phylogenetic and Phylogeographic Analysis results of Algerian Seqeunces

With the sequences obtained from GISAID, an evolution phylogenetic tree of Algeria was established (**Fig. 19**).

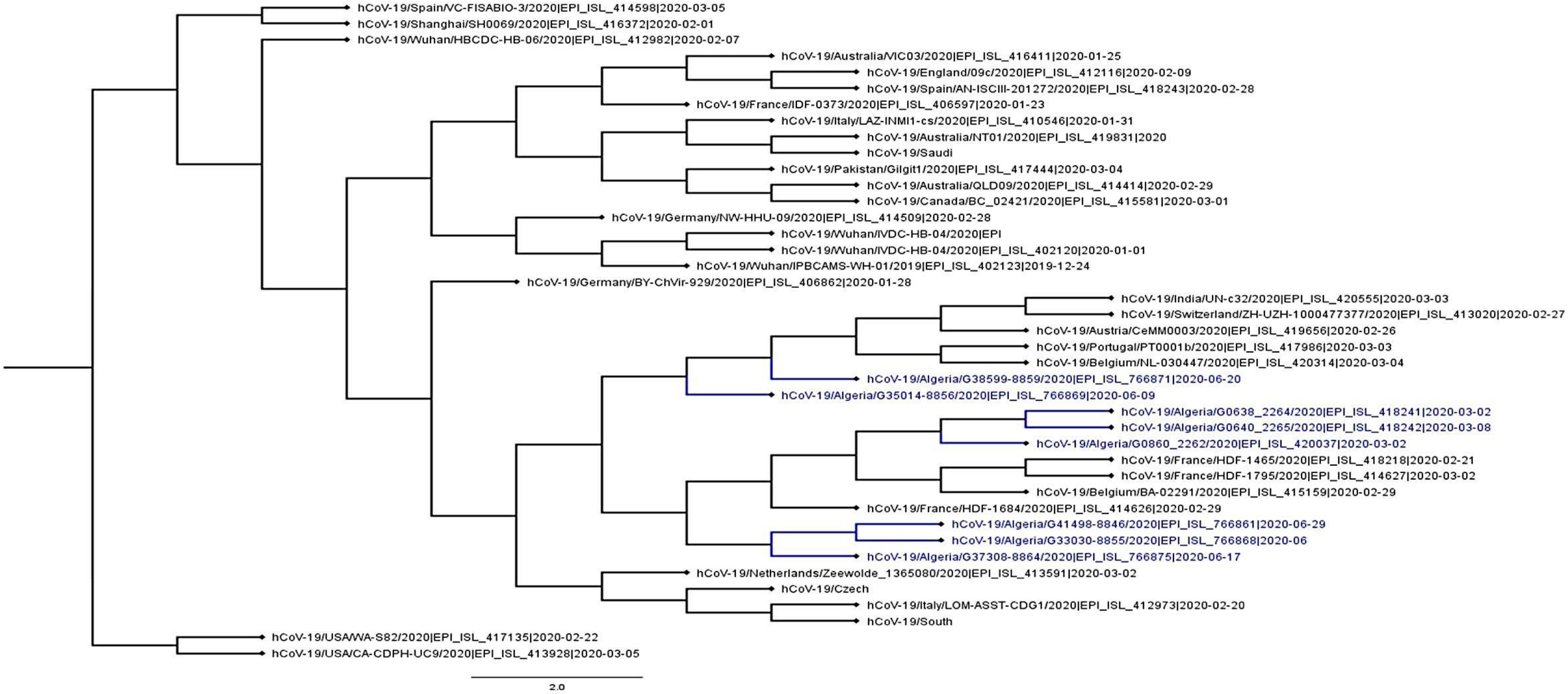


Figure 19. Phylogenetic analysis results for SARS-CoV-2 in Algeria launched by Mega X and visualized by FigTree of four different regions: Boufarik, Alger, Ouargla and Laghouat.

The phylogenetic tree has given us an idea about the distribution of Algeria's virus sources, likewise, the phylogenetic analysis confirmed China as the origin of the pandemic but further more investigation leads to notice that the virus went a long journey before making it to the lands of Algeria, the closest ancestral sequences to most of the Algerian ones are the European ones (**Fig. 19**).

Indeed we can only assume that the virus spread further due to international travel from China to different parts of the world and the resemblance and high level of similarity implies that France was the gate that allowed the first virus penetration onto the lands, to further explain the matter, a map was established (**Fig. 20**).

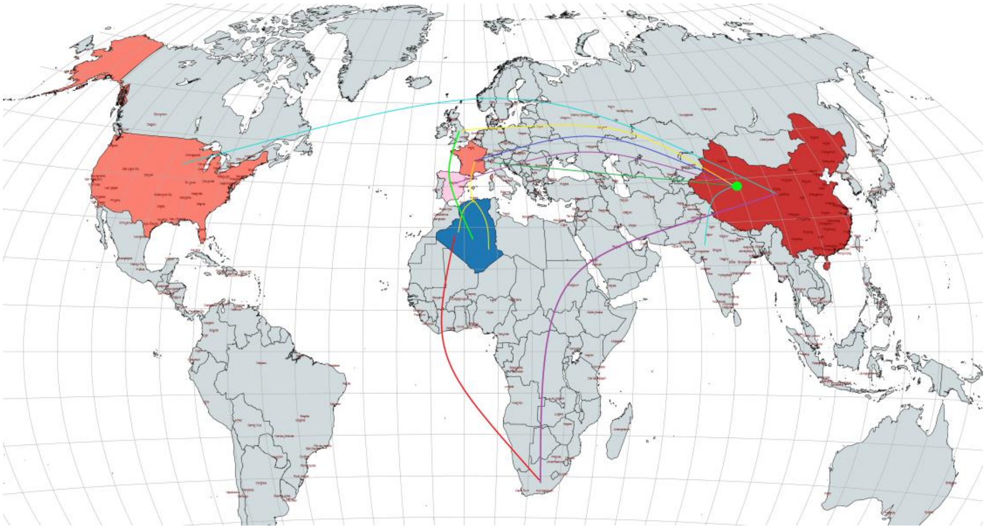


Figure 20. World map showing the virus pathway from China to Algeria.

The sequence of the 30 year old female from Ouargla also exhibits a certain level of similarity with not only the French, Italian roots but might be generated from South Africa either.

Due to the long distance it can be seen that indeed the Algerian sequences are far from having any level of reliability with the United States of America in this case.

4.3. Biskra's Hospital Hakim Sadanne results

4.3.1. Frequency of cases and deaths in the Wilaya of Biskra

This index was estimated by cumulative frequencies of admitted patients during each day of every month aligned with deceased ones (**Fig. 21**).

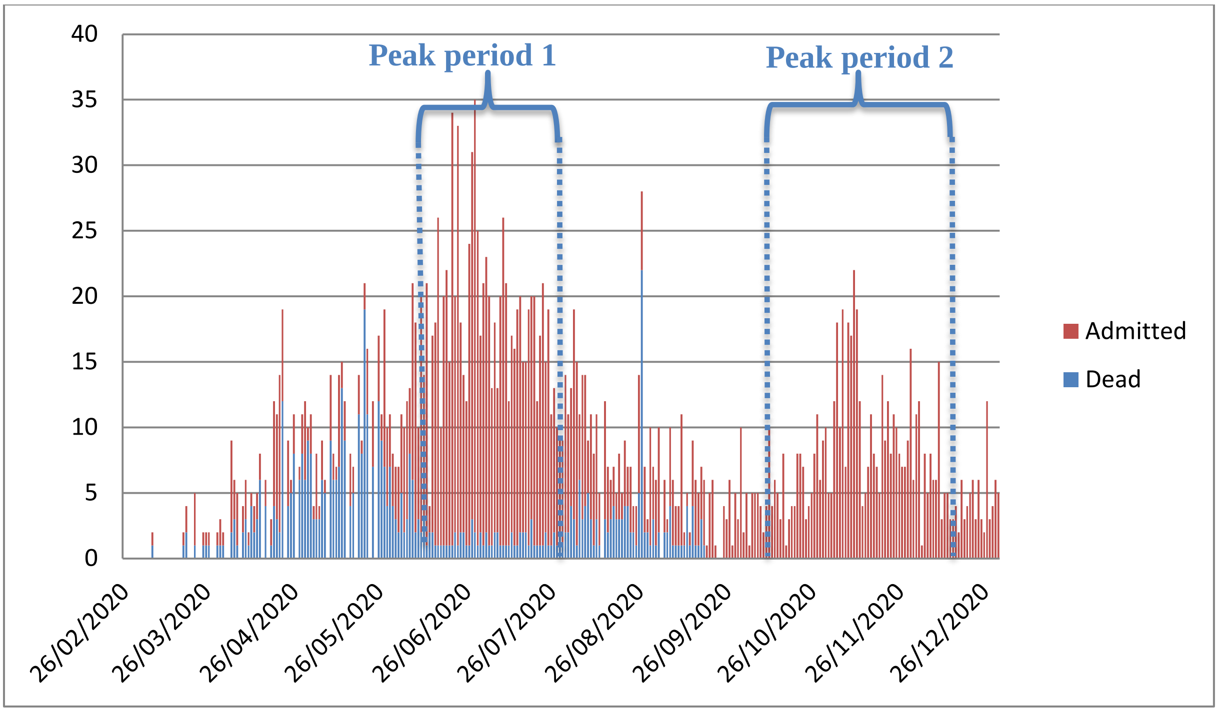


Figure 21. An overview of frequency of daily cases and deaths over the year of 2020 in the Wilaya of Biskra.

The results show the presence of two major peaks that fall on two periods of time: The June and July period and October to November period.

4.3.2. Frequency of admitted patients according to Gender and Age in the hospital of Hakim Sadanne –Biskra-

The next graph was chosen for the reason of highlighting the link there is between each age proportion and its gender, it only focuses on the number of admitted patients (**Fig. 22**).

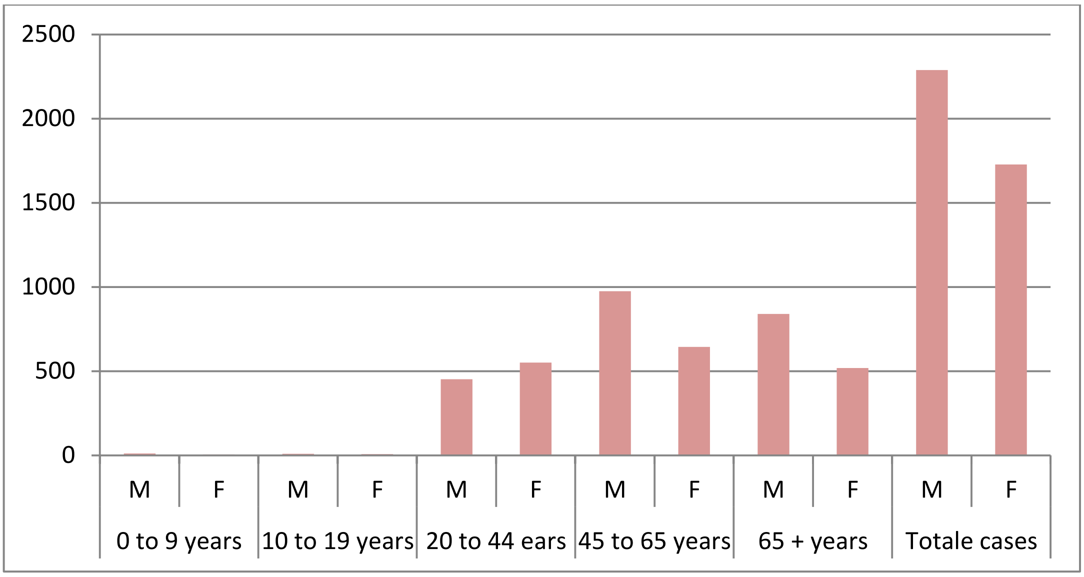


Figure 22. Results of admitted males and females by age and gender in 2020 in the Hospital of Hakim Sadanne-Biskra-.

The first emerging case of SARS-CoV-2 in the Wilaya of Biskra was confirmed on February 26th ,2020, from that day on the cases have been on a gradual increase as the citizens have struggled to keep the situation under control during some months of the year.

In terms of age categories of admitted patients in the hospital, the data has showed us interesting diversified results in which:

- The age proportions from 0 to 19 years old were presented with a total of 35 cases.
- The category of males and females ranging from 20 to 44 years old were admitted with a percentage of 19,80% and 31,90%, respectively.
- The males whose age ranged from 45 to 65 years old had an admission percentage of 42,57% meanwhile the females of the same age group displayed a percentage of 37,29%.
- The oldest aged category had percentages of a total of 36, 71% and 30,05% for males and females respectively (Appendix 01: Table 1).

The results clearly displayed an age and gender dispersion when it came to the amount of patients who caught the virus, the younger proportion varying between 1 and 19 years old proved to be less affected than the older proportion we have in the samples which leads us to assume that patients above 45 years old are more susceptible to catch the infection than those underneath 44 years old.

Gender wise, the males numerically surpassed the females starting from ages of 45 and above; the infection indeed varies in our data according to the genders of the beholders in the Wilaya of Biskra (**Fig. 22**).

4.3.3. Male verses Female admission rate by months within the year 2020

The previously displayed results in Figure 22 have been detailed according to the most affected proportion of age, the first two figures are for 45 to 65 males (**Fig. 23**) 65 and above males (**Fig. 24**).

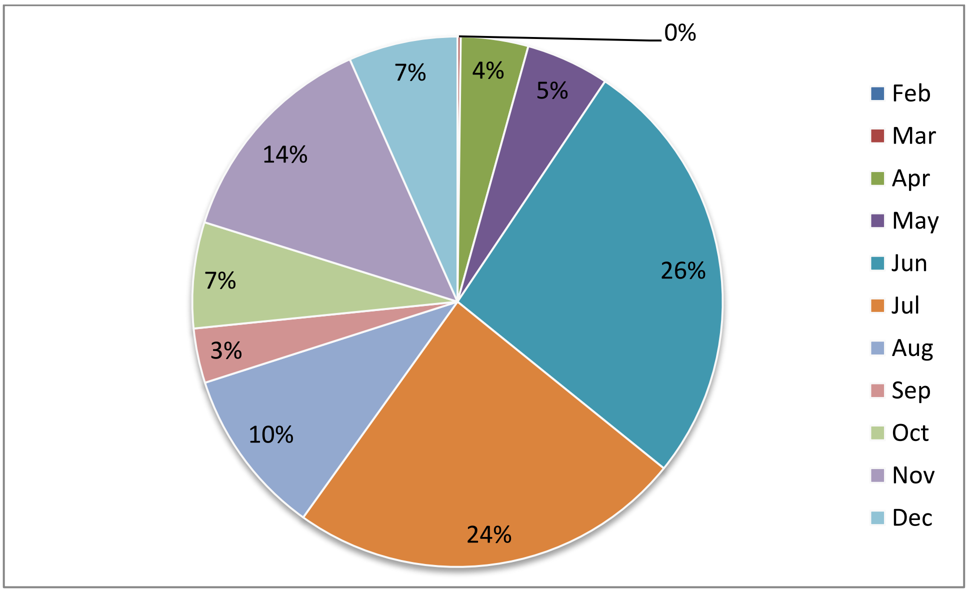


Figure 23. Most admitted proportion of age (45 to 65 years old) within males by months during the year of 2020.

According to the displayed results, the most affected proportion of age within the male gender had its peak during the month of June with a 26% of admission rate and the lowest during the three months March, September and May with percentages of 0,1%, 3% and 4% respectively.

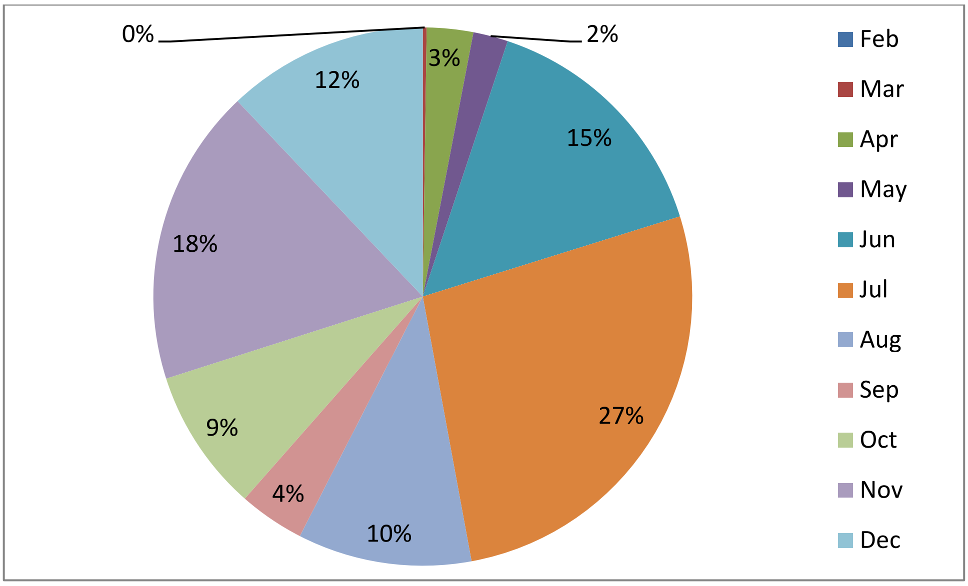


Figure 24. Second most admitted proportion of age (65+) within females by months during the year of 2020 in the hospital of Hakim Sadanne Biskra.

Comparing the two results from figure 23 with those of figure 24, it is quite obvious that starting from February until the month of August the males who are aged above 65 years old have the upper hand in the number of admissions, while after August, during the months f September October, November and December, the first proportion of males age from 45 to 65 years old is the one that climbs up rates with the highest percentages of admission (Appendix02: Table 2)

The upcoming graph contains results of both females ranging from 45+ **(Fig. 25)**.

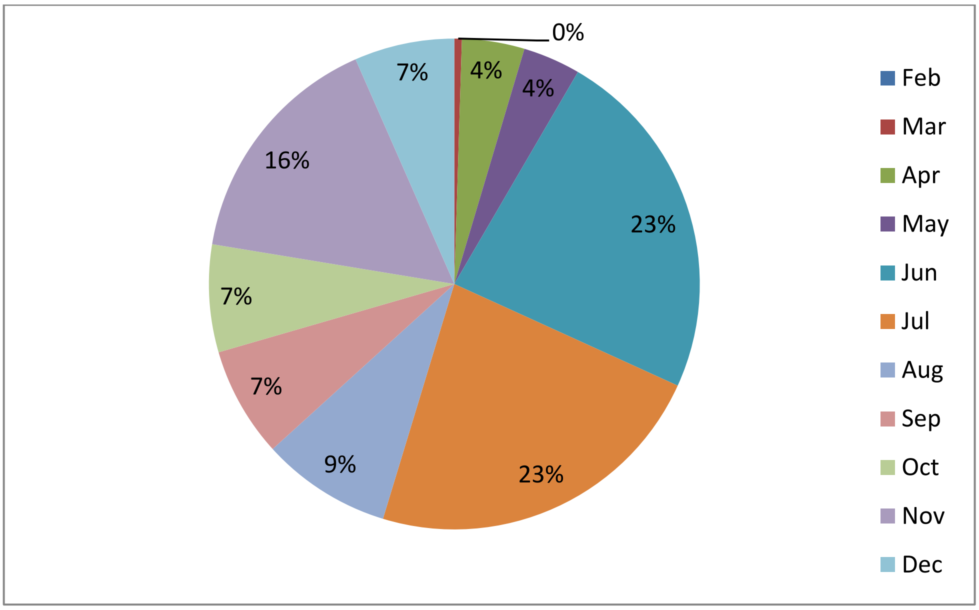


Figure 25. Most admitted proportion of age (45+) within females by months during the year of 2020 in the hospital of Hakim Sadanne Biskra.

In terms of females highest admission rated category of age, the results were as the following:

- During months of June and July of 2020 the highest percentage of admission was recorded within females by 23% for both months.
- Meanwhile the month March, May and April of 2020 showcased the lowest level of admission with percentages of 0,2% and 4% respectively (Appendix 02: Table 3)

The difference created between males and females during both age categories could be due to the following factors:

- **Gender**

The reason why one gender is more vulnerable than another in our findings could be due to certain factors:

- **Genetics**

Correlating our findings with the fact that the ACE2 spoken about previously (Section 3 of Chapter 2) is according to Bwire (2020) more expressed in the male genes than the female ones is the reason why the our study could have such high levels of admission within the first gender compared to the second.

- **Immunology**

Differences between the male and female immune system could explain the divergent risks, while a study carried by Karnam *et al*. (2012) showed that the inhibitory CD200 receptor (CD200R) has a primordial role in balancing the immune system during a viral infection, the lack of signaling in the CD200 enhances the immune response within females which means the higher the signaling the more strength to endure infection the organism possesses.

The concept of hormones coming side to side by immune response has also been looked through and the fact that estrogen receptors blocking has been proved to increase the infection within SARS-CoV-2 female mice according to Bwire (2020) is a further more explanation of our results.

- **Age**

When aging, the immune system changes in two major ways and embraces two terms known as: immunosenescence and inflammaging, relating those two with our results might explain why older people are more susceptible of catching the virus than the younger community.

The male proportion ranging from 45 to 65 is indeed more susceptible to the infection because of the two factors: Age and gender, with the first one being part of older category and the second one belonging to the male gender, this specific portion we have picked is the most exposed to danger out of all.

On the other hand, within the same group of age, what we found logical to explain why the males from 45 to 65 years old had a higher admission percentage within the first half of the year comparing to those above 65 years old has to do with two factors:

- The fear factor: During the first half of the year elderly were severely made aware of the danger the virus holds therefore they strictly followed the instructions and out of fear, since they are well aware they’re the most vulnerable proportion of society, they stayed indoor most of the time.

- Social status: the males above 65 years old are almost if not all retired so they didn’t have to get out as often as the males from 45 to 65 years old.

The first factor had a double effect on the males above 65 years old, it minimized the possibility of interaction with the outside world directly which made them a little less susceptible to catch the virus but it also negatively influenced their immune system, since while staying indoors for a period of time their stress levels increase as well as their vitamin D lacks and if not taken into seriously, such insignificant matter will have a crucial effect on building the results that we have gotten during the second half of the year.

The second factor hid the most vulnerable proportion and put to line the second most vulnerable proportion which is men from 45 to 65 years old, with those have the second most weak immune system, their chances go higher the higher their interaction outdoor is.

The second half flipped the results, since while keeping in mind the first factors, the fear was overpowered by the acclimation therefore, the strong belief of fatality and infection of the virus faded, the Algerian government also, specifically at this time of the year came out with orders of softening the curfew measures and lifting the travel ban on 29 Wilaya on August, as well as bringing back a few activities such as coffee shops that have a primordial influence on older males as leisure as well as re-opening Mosques, which increased the potential of elderly men exposition to the virus, thus, higher admission rates.

4.3.4. Case Mortality rates in the hospital of Hakim Sadanne-Biskra-

This factor has been calculated by:

$$\frac{\text{The number of dead patients in a month}}{\text{The number of admitted patients in the same month}}$$ **(Fig. 26)**.

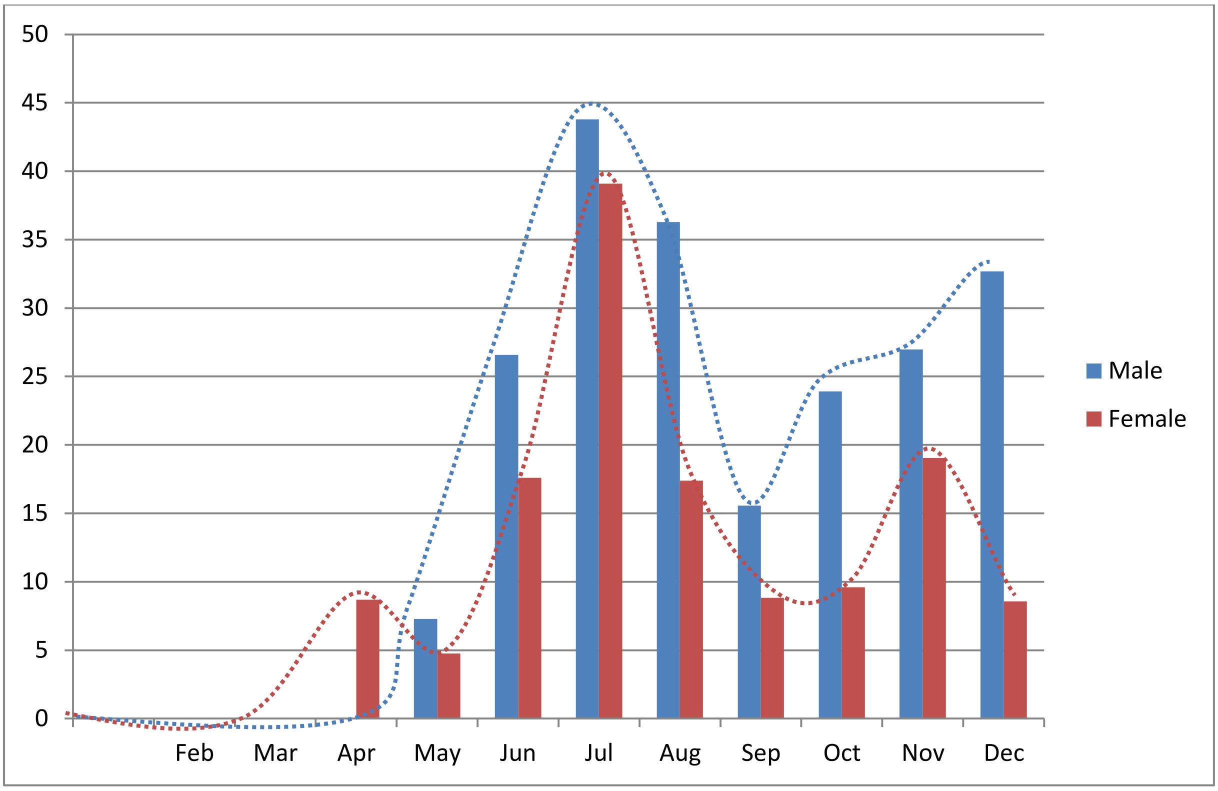


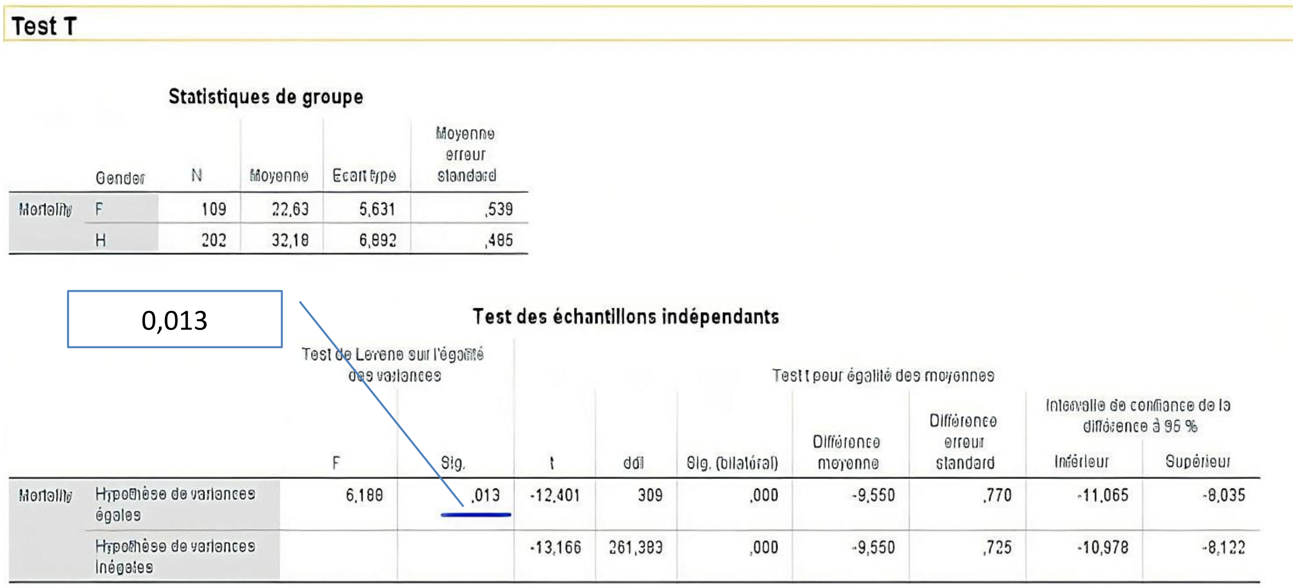
Figure 26. Case Mortality rate of SARS-CoV-2 among Males and Females in the Hospital of Hakim Sadanne Biskra along the year 2020.

The results showed that the mortality rates of females were higher than the male's one during the month of April only, while the male mortality rate was surpassing the female one for the rest of the year. (Appendix 03: Table 4).

4.3.5. Statistical Analysis of Mortality rates, Gender, Age and Temperature

Two tests were ran in order to study the influence of these three factors on mortality rates previously calculated separately, the first one is an independent samples test of homogeneity for gender (**Fig. 27**) the second one is a one way ANOVA run twice for Age and Temperature (**Tab. 7**) (**Tab. 8**).

4.3.5.1. Test of homogeneity of independents samples (T-Test)



Test T

Statistiques de groupe

	Gender	N	Moyenne	Ecart type	Moyenne erreur standard
Mortality	F	109	22,63	5,631	,539
	H	202	32,18	6,892	,485

0,013

Test des échantillons indépendants

		Test de Levene sur l'égalité des variances		Test t pour égalité des moyennes						
									Intervalle de confiance de la différence à 95 %	
		F	Sig.	t	ddl	Sig. (bilatéral)	Différence moyenne	Différence erreur standard	Inférieur	Supérieur
Mortality	Hypothèse de variances égales	6,188	,013	-12,401	309	,000	-9,550	,770	-11,065	-8,035
	Hypothèse de variances inégales			-13,166	261,383	,000	-9,550	,725	-10,978	-8,122

Figure 27. Results of Test of independent samples of gender and mortality.

To read the results, there are to hypothesis to put down:

- Gender has no relationship/effect on mortality rates.
- Gender has a relationship/effect on mortality rates.

The first hypothesis is going to be the null hypothesis in which H_0: $\mu_1=\mu_2$, the second hypothesis is the alternative one where: H_1: $\mu_1 \neq \mu_2$.

The risk has been determined at $\alpha = 0.05$, our results showed that P-value is at: 0,013 and comparing the two: P-value < α.

(Full table results available in Appendix 04: Table 5)

With the P-value greater than the risk α, the null hypothesis is rejected therefore, our results are significant and the alternative hypothesis is correct, so gender has an impact on the mortality rate indeed.

4.3.5.2. Test of One Way Analysis Of Variance for Age and Mortality (ANOVA)

Table 7. Results of one way ANOVA by SPSS

ANOVA

Mortality

	Somme des carrés	ddl	Carré moyen	F	Sig.
Intergroupes	12553,615	63	199,264	7,158	,000
Intragroupes	6875,677	247	27,837		
Total	19429,292	310			

The two hypotheses generated for the ANOVA test are:

- H_0: Age is non-significant to mortality rates
- H_1: Age is significant to mortality rate

P-value equal 0,0001 in this case, the results were held after a descriptive analysis and Levene's statistic is calculated to determine whether our groups have equal variances(Results in Appendix 05: Table 6).

A statistically significant difference was found between our groups as proven by our one-way ANOVA (F= 7,158, $p < 0,0001$) with p-value highly significant therefore our means are not equal and H_0 is rejected in the favor of H_1, therefore, age indeed has an effect on mortality rates.

4.3.5.3. One way ANOVA test for temperature and mortality

Table 8. Results of one way test ANOVA for temperature effect on mortality in the hospital of Hakim Sadanne Biskra.

	Somme des carrés	ddl	Carré moyen	F	Sig.
Intergroupes	106539,722	6	17756,620	3,886	,219
Intragroupes	9138,500	2	4569,250		
Total	115678,222	8			

Two hypotheses were generated from the test:

1. H_0: Null hypothesis in which temperature has no significant effect on mortality
2. H_1: Alternative hypothesis in which temperature has a significant effect on mortality.

P-value is equal to 0,219 which is significantly above the set α-value of (0,05).

Since our P-value is greater than the α-value in this case (0,219>0,05) we fail to reject the null hypothesis which means there is no significant effect the temperature has on the mortality caused by the virus.

Our results are in parallel with those of Jamshidi *et al*. (2020) in which they state that weather alone is not a strong enough factor to influence the course of Covid19. Thus, going back on our results in the figure 26 the death cases visually seem to be higher during the summer months (June and July) than in the winter ones (January, February and December) , the logical explanation could be that not only during the winter months of 2020 were the cases and deaths low because the pandemic was barely starting in Biskra, but also during summer months the activity of the citizens increased with summer vacations, seasonal weddings and celebrations that are all relative factors that push forward the argument as to why the relevance can’t be found when it comes down to weather.

4.3.6. Gender mortality rates GMR

We could get the graph (**Fig. 28**) by the rule: $\frac{\text{Total Mortality within Males}}{\text{Total Mortality within Females}}$

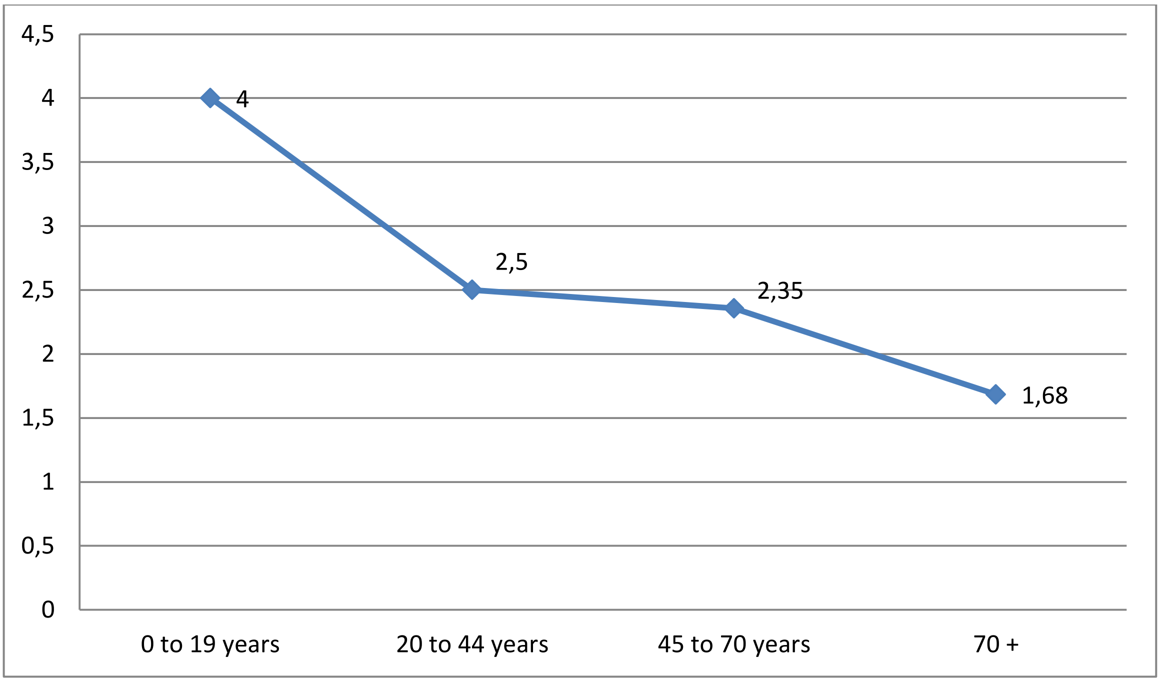


Figure 28. Gender mortality rate due to COVID 19 in Biskra.

The difference is that both male’s and female’s mortality rates were used to estimate this parameter, the SARS-CoV-2 has shown a stronger gender-age correlation and it could be explained by:

- A high GMR in the category of 0 to 19 years old is due to the low death rate of females of that age which enlarges the gap between the females and males of that same category

- A decreasing GMR is due to the aging factor influencing the female category which shrinks the gap between both genders mortality.

Different factors could participate in the impact of GMR including ones mentioned in this chapter previously (Title 4.3.3) but one is to stand out: habits; such as smoking that is very common within male individuals that makes it a critical factor of the large gap between both young males and females.

4.3.6. Fatality and Mortality rates in the Wilaya of Biskra by districts

The fatality was estimated by the law: $\frac{\text{Number of deceased patients in an area}}{\text{Number of citizens in that area}}$ and was used to compare with mortality rate since they're estimated by the rule:

$\frac{\text{Number of deceased patients}}{\text{Number of total admitted patients}}$ **(Fig. 29)**.

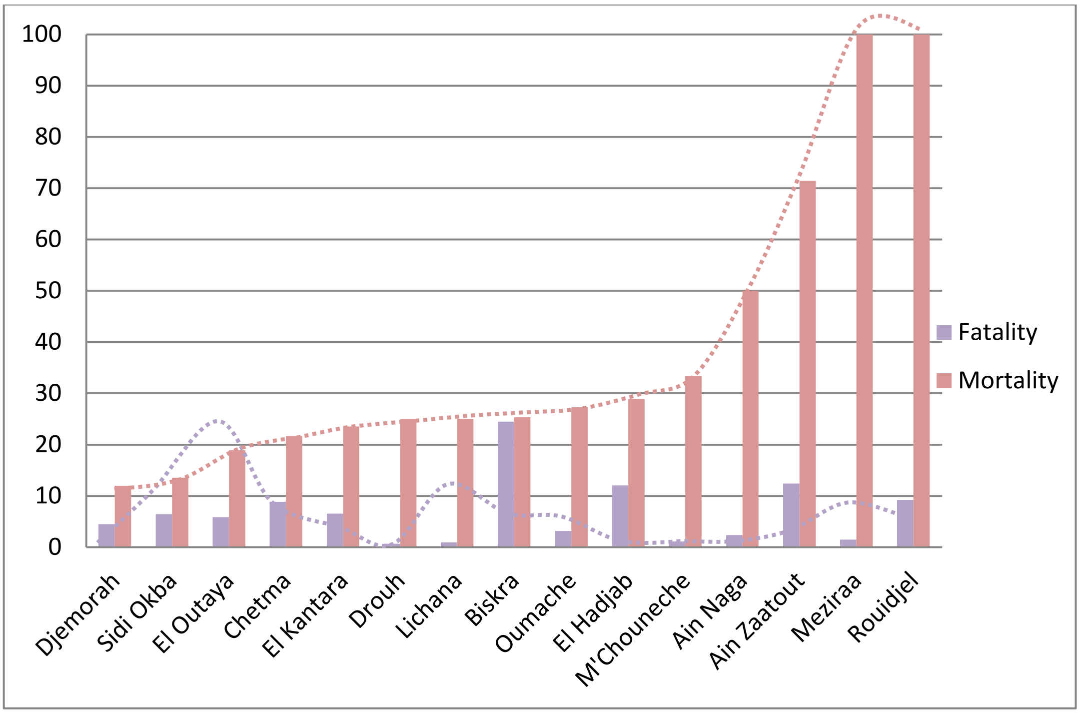


Figure 29. Fatality and Mortality rates of SARS-CoV-2 in Biskra according to Hakim Sadanne hospital by district during the year of 2020.

According to our results, patients from El Hadjab, Ain Zaatout and Biskra who have been admitted to Hakim Sadanne have the highest percentage of fatality with 12,01% 12,41% and 24,45% respectively. Which directly implies thee relevance of the dead cases in regard of the population, this means that those results are having an impact on the number of citizens especially the one of Biskra.

Meanwhile patients from Drouh and Lichana recorded nearly a non-existent very low percentage of fatality with percentages of 0,68% and 0,94% respectively, which means that these dead cases are irrelevant when put in front of the total number of citizens in those areas.

When it's about mortality, places like Ain Naga, Ain Zaatout, Meziraa and Rouidjel had high successive percentages of 50%, 71,43%, 100%, 100% respectively which means all or most of the patients from that are who have been hospitalized in Hakim Sadanne have passed away. While in places that recorded a high fatality rate such as Biskra the mortality was low by a percentage of 25% only.

Despite the results belonging to the same Wilaya, the difference of the localization has a greater influence than the one we see, our fatality and mortality rates differ for multiple reasons that can’t all be brought down and discussed therefore an estimation of the age mean has been established to better understand the relationship these two hold with their location.

The places that only have an admission of one person and death of that same patient in mortality have been neglected which means: Rouidjel, Mziraa will not be considered significant only during correlation with age mean.

The highest age mean of age 66,90 was recorded in Ain Zaatout while the lowest was in Sidi Okba with 49,93 while this isn’t the only factor that influences the results, it’s a crucial one and with our previous results of the strong relationship between age and mortality(Tittle 3.4.5.2) , the high age mean indeed makes Ain Zaatout the location with the highest mortality rate and makes Sidi Okba the second lowest location with mortality rate. (Detailed percentages of mortality and fatality in Appendix 06: Table 7).

4.3.7. SARS-CoV-2 tests used on Hakim Sadanne patients and their sensitivity-Biskra-

The total number of each test used was estimated (**Fig. 31**) and the sensitivity as measured by the rule: $\frac{\text{Number of results the test showed positive}}{\text{Number of actually positive people}}$ in order to tell which test is the most accurate (**Fig. 32**).

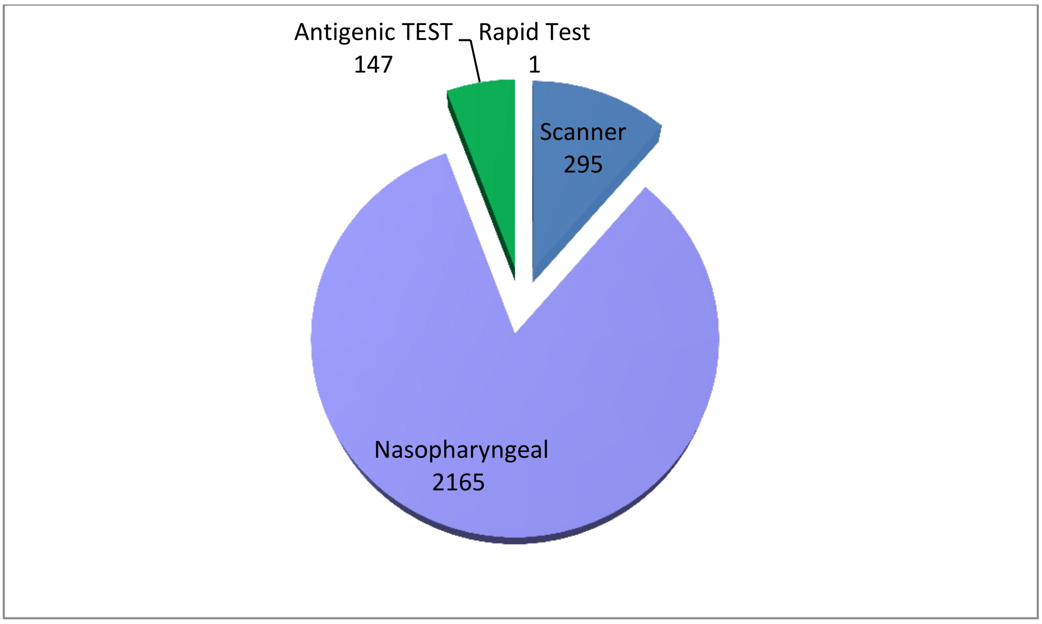


Figure 30. Proportions of different SARS-CoV-2 tests used in the hospital of Hakim Sadanne-Biskra-

In terms of the number of tests performed on the hospital's patients during the last two years, the results showed:

- Four different types of tests were held across the course of two years
- The first and most used test is the Nasopharyngeal (NSP) one with a total of: 83%
- The most used test more the year 2021 is the antigenic test with a percentage of 6% out of the other tests.

The least used test is the rapid test and it has only been used once.

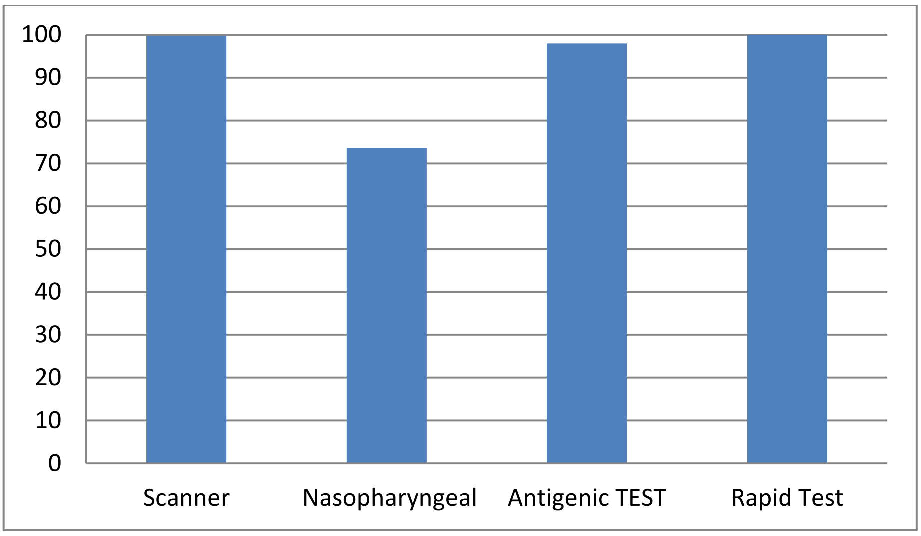


Figure 31. Tests positive sensitivity estimated in the hospital of Hakim Sadanne Biskra, 2020-2021.

When it comes to accuracy these tests displayed the following results:

- The scanner test and rapid test with 100% positive sensitivity.
- The antigenic test displayed a percentage of 98% accuracy.
- The nasopharyngeal test has 73% accuracy.
- The rapid test results were neglected due to only 1 test being performed.

The tests sensitivity doesn't have to do with the number of tests being performed although that might increase a negligible error value, but it's rather about the test's ability to detect a true positive/negative result out of the actual positive or negative patients.

The use of a NSP test is required, thus the possibility of having false negatives/positives is present, the requirement to enhance the test's accuracy is the combination with another test, in a study performed by Fang *et al*. (2020), it has been proven that a CT scanner test is more sensitive to the RT-PCR one, therefore, an advised compilation of the two tests is preferable. Hence a serological test can also be empowered by another test's simultaneous use.

Although the hospital of Biskra Hakim Sadanne is performing tests according to their availability, for a better disease monitoring, it is advised to seek for the more sensitive tests and recombine them.

4.3.8. Effective reproduction number

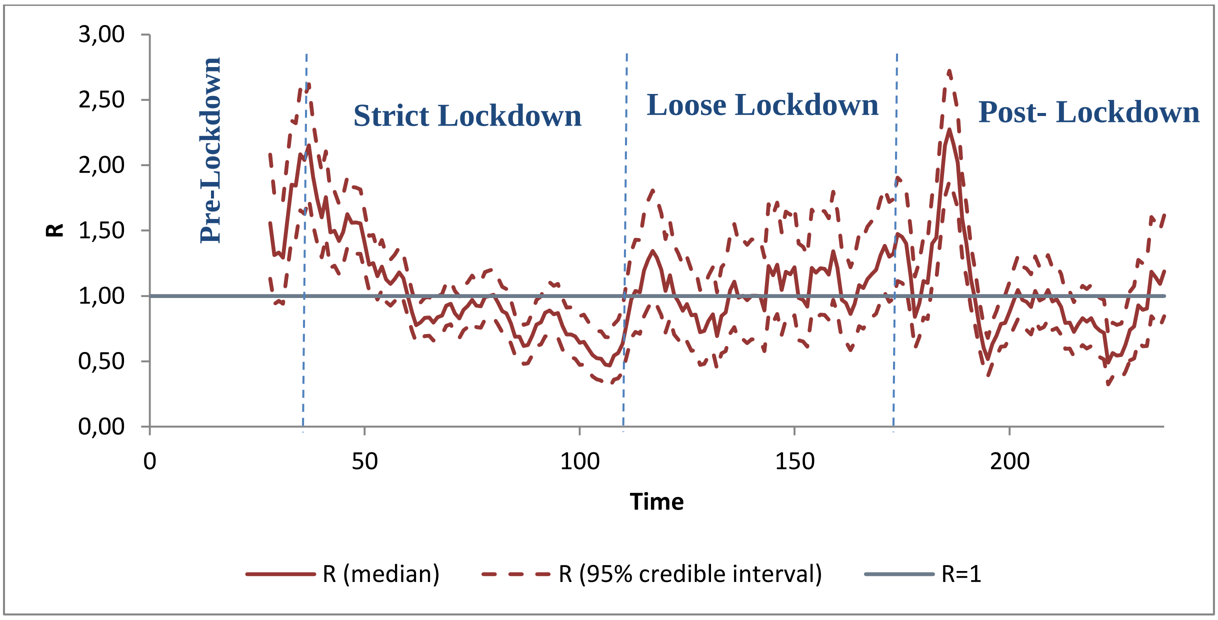


Figure 32. R_T averaged over time periods in Biskra (posterior median and 95%credible intervals)

The results displayed on the figure 31 come out with the following:

- Two main peaks one on point 37 which is compatible with the second week of June and the second one on point 187 which is on the first week of November.
- The maximum value R_t has reached was: 2,7.
- The least value R_t has reached was 0, 53.

The effective reproduction number increased most after the authorities decided to lighten the burden on merchants and business owners by re-opening and allowing the activities to take place again on the 26th April, once the lockdown was set again after Ramadhan, the R_T decreased significantly underneath 1 for a period of more than two months.

The effective started to increase after the announcement of lifting the traffic ban and re-authorizing public transportation of July 14th and August 03rd successively, and then went on the second peak that happened to be in synchronization with the start of the new academic year, with workers and students taking their seats back and the movement coming back to the Wilaya of Biskra, the local authorities have lost hang of the control for a while.

With the number of susceptible patients on decrease, the R_t values are depending on the amount of control and awareness authorities and citizens of Biskra can showcase and impose.

4.4. Questionnaire survey on chronic diseases and COVID-19

4.4.1. Algerian survey results

After obtaining responses from different levels, they were combined into one graph in order to study impact of COVID-19 on patients with chronic diseases (**Fig. 34**).

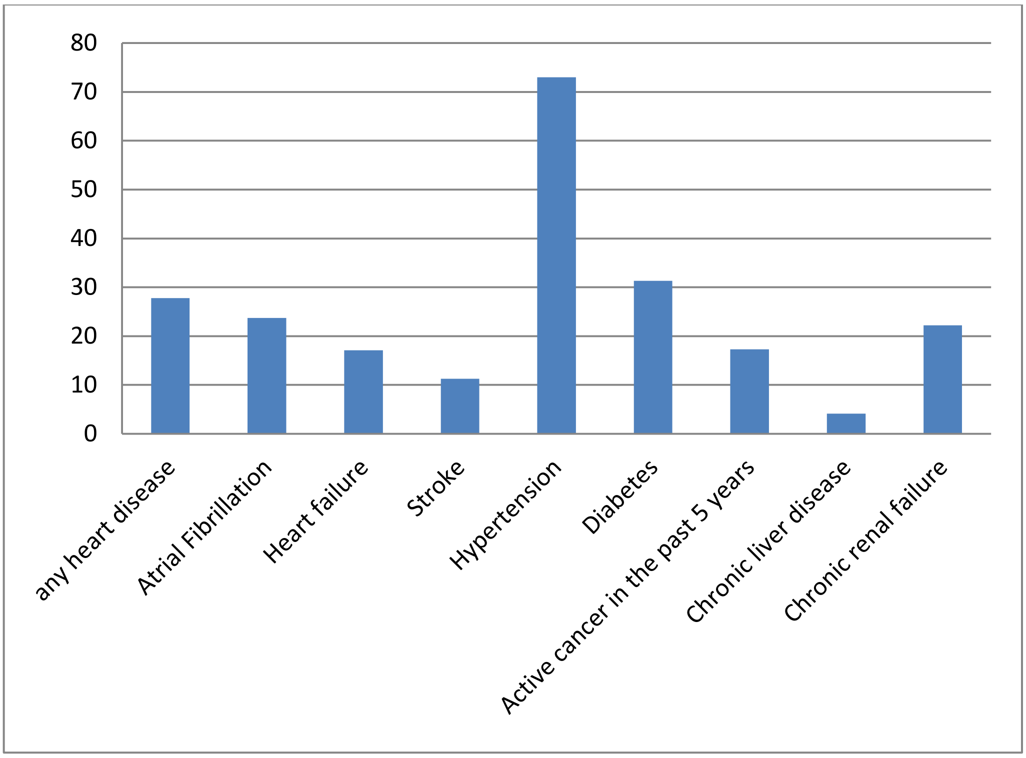


Figure 33. Chronic disease relative number of patients who got admitted with SARS-CoV-2 to the hospital in Algeria.

The survey responses both combined together to get a maximum accuracy of answers gave the following results:

- Hypertension ill individuals are the ones the catch the virus more than others with a percentage of 73%.
- The second most common comorbidity to catch SARS-CoV-2 is diabetes with a percentage of 31,3%.
- The least present comorbidity in our results is the Chronic liver disease with a total of 4,1% only.

4.4.2. Chi Square test to find the before and after effect of COVID-19 people with pre-existing comorbidities with

The results of the Chi-Square test performed on Algerian survey data show:

Table 9. Chi square test results on Algerian Comorbidity survey data

	Valeur	ddl	Signification asymptotique (bilatérale)
khi-carré de Pearson	16,584[a]	6	,011
Rapport de vraisemblance	18,675	6	,005
N d'observationsvalides	215		

The results as previous tests make us generate two hypotheses:

• The null hypothesis: H_0: The Covid19 has no significant effect after infecting people with comorbidities

• The alternative hypothesis H_1: The Covdi19 has a significant effect after infecting people with comorbidities.

The p-value read equals to 0,011.

4.4.3. China results on comorbidities

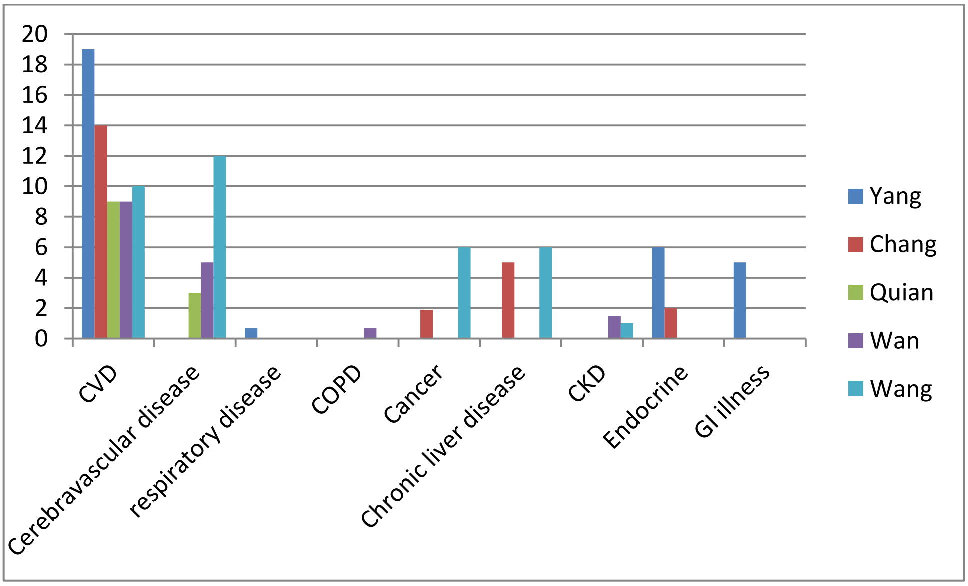


Figure 34. Results from multiple China hospitals of COVID-19 patients with pre-existing comorbidities from GitHub (Hanlon *et al.*, 2020).
CVD: Cardiovascular disease; COPD: chronic obstructive pulmonary disease; CKD: Chronic kidney Disease.

In terms of pre-existing comorbidities in China’s Covid19 patients, the results were as following:

- Patients suffering from cardiovascular diseases (CVD) are the ones with the highest level of admission in most cases, reaching levels of: 19 %, 14%, 9%, 9%, and 10% in Yang, Chang, Quian, Wan and Wang’s study’s respectively.

- In Wang’s work, cerebrovascular admitted patients surpassed the rest with a level of 12%.

- Chronic obstructive pulmonary disease are the least frequently admitted patients with a percentage of 0,7% in Wan’s results.

As we have stated in our seminar abstract , Hence, the association between the renin-angiotensin aldosterone system’s (RAAS) master regulator ACE2 and susceptibility to COVID19 has disadvantages such as uncontrolled hypertension and cardiac function deterioration (Hemidi and Dehimat, 2021).

COVID 19 attaches with RAS master regulator ACE2 that’s expressed mainly in the heart and lungs. The binding of SARS-CoV-2 to ACE2 can result in alteration of ACE2 signaling pathways, leading to acute myocardial and lung injury. Pre-existing cardiovascular disease increases the risk of death in patients with COVID-19, whereas COVID-19 itself can also induce myocardial injury, arrhythmia and acute coronary syndrome (Hemidi and Dehimat, 2021).

Thus, the virus will only affect the organs in which the ACE2 is expressed the most, the higher the expression, the more risk and danger on the patient there is. Therefore lungs and heart deceased patients come on the front line. In a previous report by Guo *et al*. (2020) 35% of the patients with SARS-CoV infection, the SARS-CoV genome was positively detected in the heart. Speculations were raised for the fact that the virus directly damages the cardiomyocytes, which is something SARS-CoV-2 is also capable of, since the two share the same mechanism.

Patients who suffer from diabetes have already damaged pancreatic cysts, and the virus make the matters worse, since it binds to ACE2 receptors, which, are also expressed in pancreatic tissue and β-cells in particular that will result in an acute loss of insulin secretory capacity along with a stress condition and the cytokine storm could lead to a rapid metabolic deterioration with development of diabetic ketoacidosis or hyperglycaemic hyperosmolar syndrome. (Hemidi and Dehimat, 2021)

A brief explanatory figure to sum up the whole pre-existing comorbidities concept (**Fig. 36**).

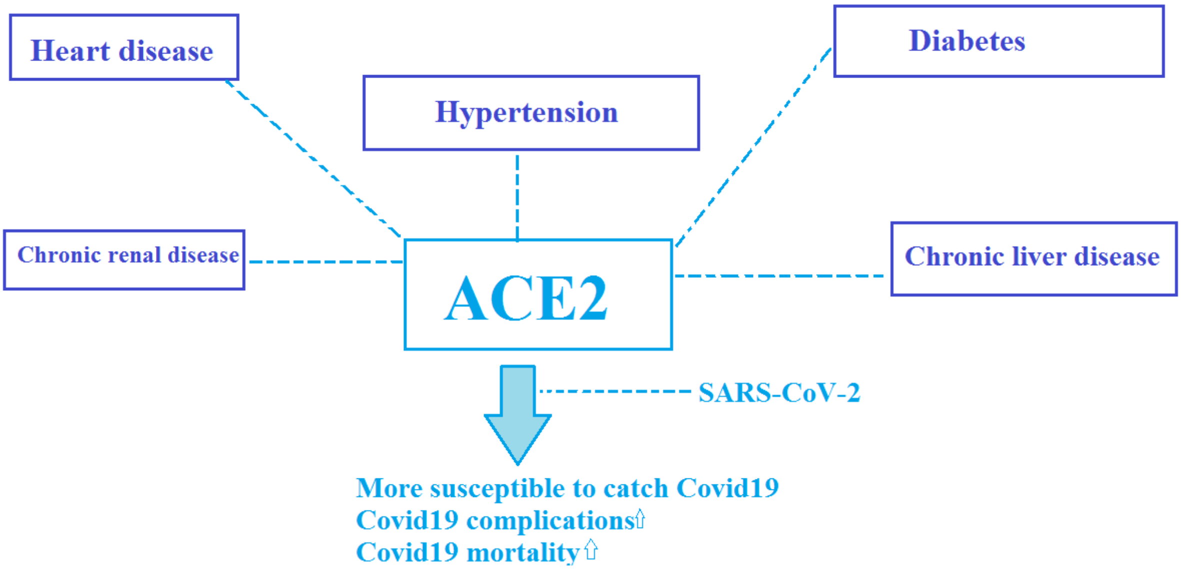


Figure 35. Schematization of comorbidities effect on SARS-CoV-2.

The Chi-square proved a p-value smaller than the standard α-value, as we previously have done, the null hypothesis is rejected and only the alternative one is accepted, therefore, the virus has indeed been proven to have an effect on the patients who suffer from pre-existing comorbidities after infecting them.

Those results are only logical with our explanation about the alteration of ACE2 tissues in multiple organs, in fact within Hakim Sadanne hospital of Biskra, healthy patients with no previous history of comorbidities have developed a type of complexity in some organs after being severely affected by SARS-CoV-2.

4.5. Questionnaire survey and interview on the use of alternative medicine to combat SARS-CoV-2

4.5.1. Frequency of citation

4.5.1.1. Frequency of citation in Biskra interview

This index is estimated by the rule: $\frac{\text{Number of citation of a plant X}}{\text{Number of informants interviewed}}$ mainly in order to find out the importance of medicinal plants locally.

A total of six plants belonging to four families were reported in our study area: Biskra (**Fig. 37**).

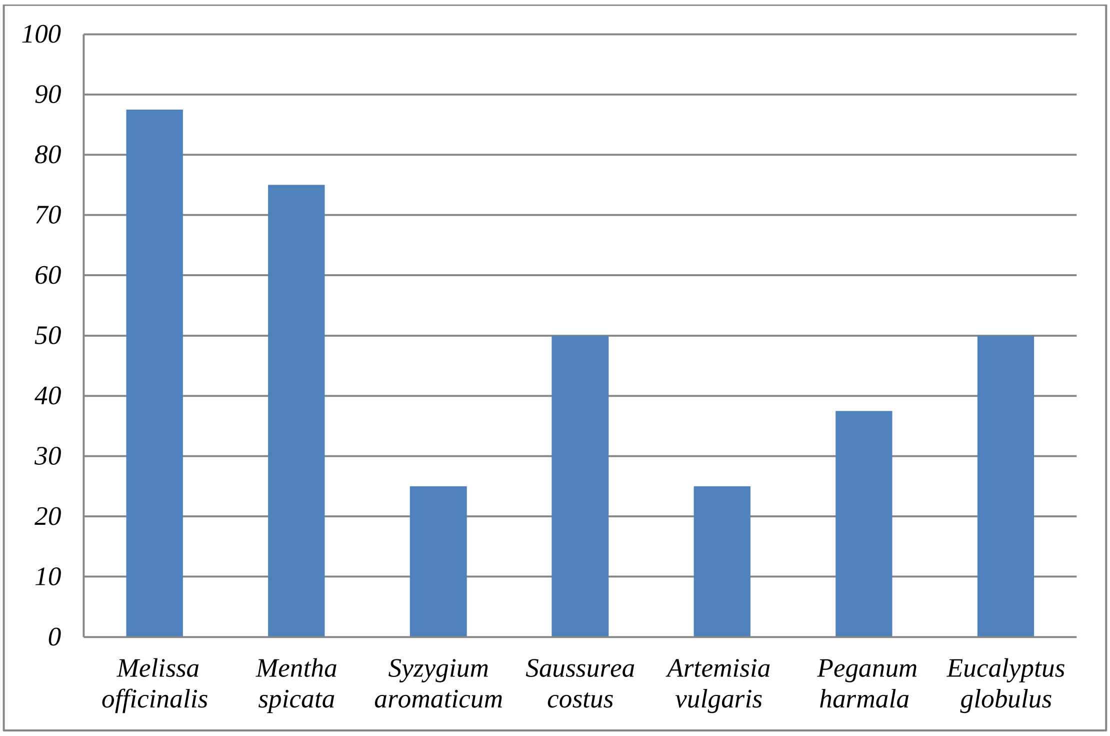


Figure 36. Frequency of citation of different plants mentioned by herbalists in Biskra.

In terms of frequency of citation, the results showed the following:

- *Melissa officinalis* as the most mentioned herb sold and used by consumers during the period of the pandemic with a percentage of 87,5%.
- Half of the herbalists mentioned *Eucalyptus globulus* (50%).
- *Syzygium aromaticum* and *Artemisia vulgaris* as the two least mentioned herbs with a total of 25% per each (Appendix 07: Table 8).

4.5.1.2. Frequency of citation in International survey results

Results of international survey were processed and the frequency of citation was estimated using the mentioned method in the previous title (**Fig. 40**).

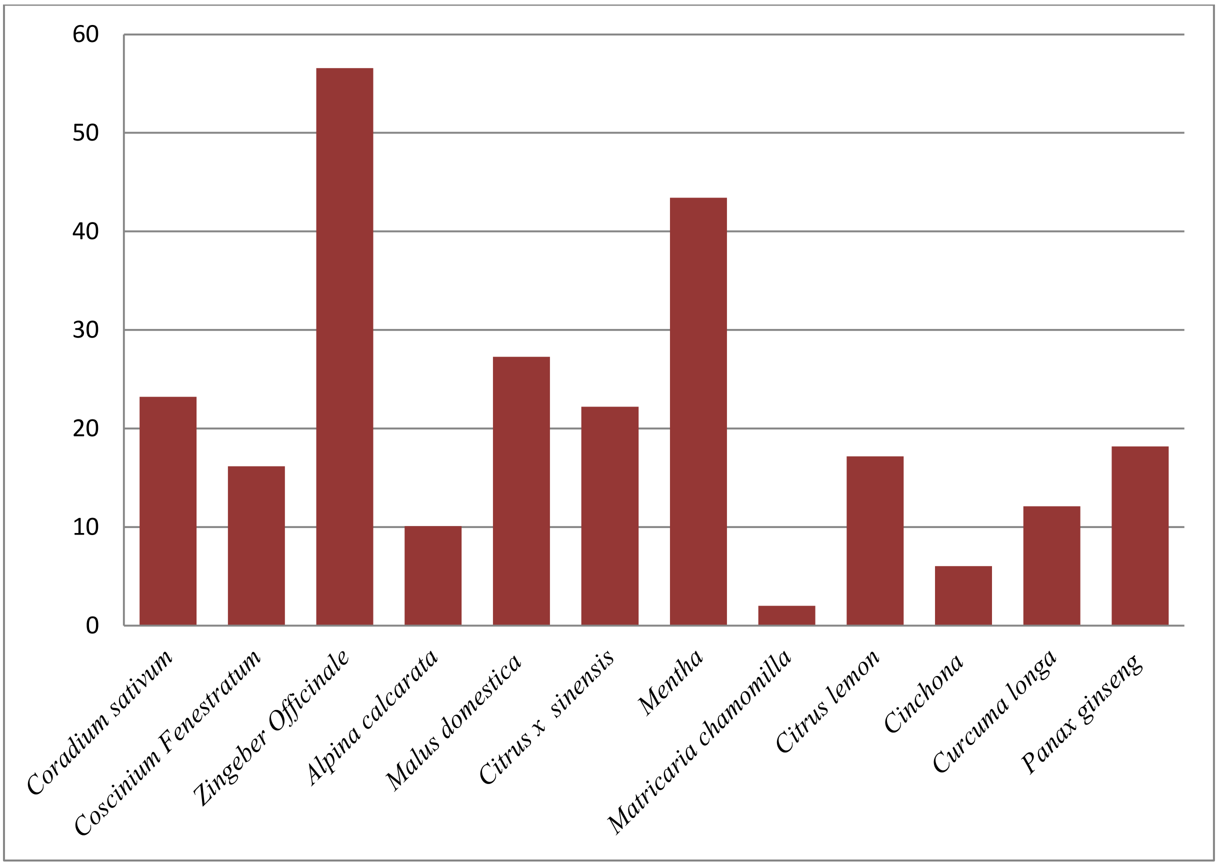


Figure 37. Frequency of citation of worldwide used plants in the questionnaire survey.

The results display that:

- The most cited plant in the survey was *Zingeber officinale* that is commonly known as ginger with a percentage of 56,56%.
- Followed by *Menth*a with a 43,43% level of citation,
- The least mentioned species *was Matricaria chamomilla* commonly known as chamomile with only 2% citation frequency.

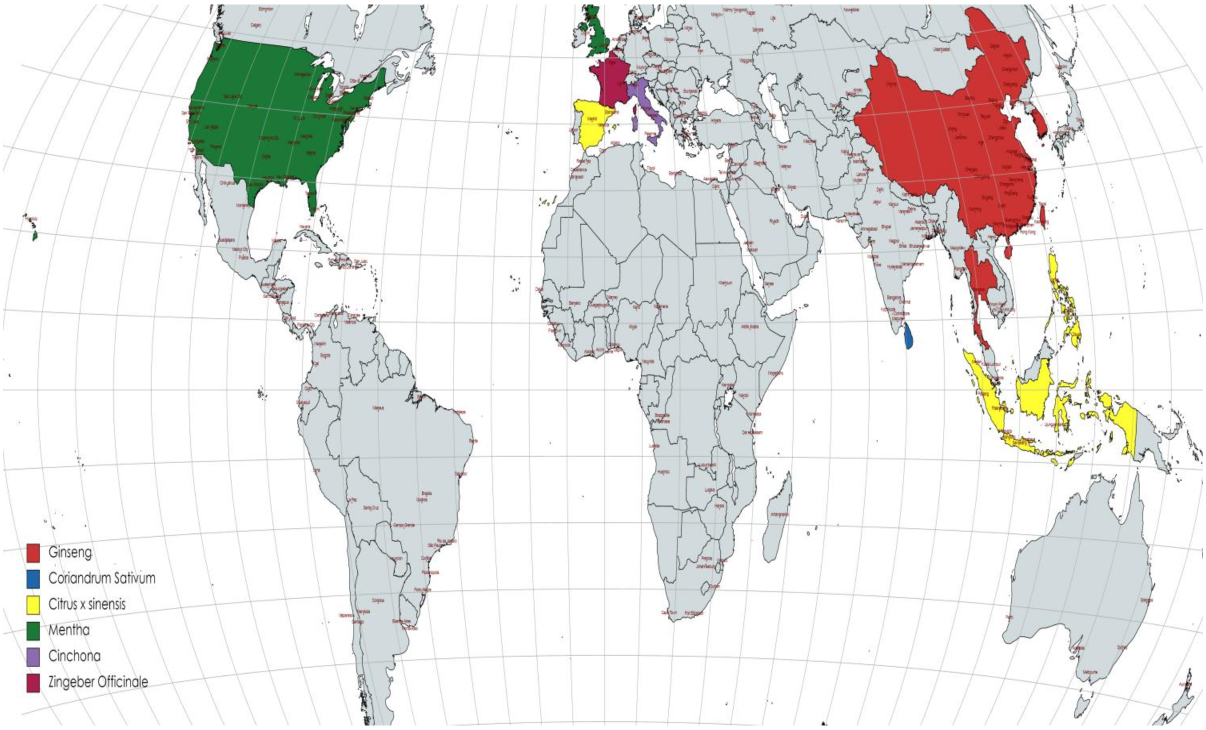


Figure 38. Survey's Mentioned plants by their geographical localization.

The plants with a high RFC are both locally and internationally plants that are very common in the area, let's take *Artemisia vulgaris* as an example; the species enjoys dry and warm weather, which is what the area of Biskra offers. Some species are used due to the traditional backgrounds they have within the community for being well known to treat common colds and strengthen the immune system.

While in the international community, if we take *Panax ginseng* as an example, the plant is thought to have therapeutic and curative properties and that old belief that has started long ago continues to thrive when researchers like Lee and Rhee (2021) have works put out of the beneficial anti-viral effects of this plant.

The RFC then depends on the population's thinking and old traditions or habits, and on the availability of a certain plant in the area and its abundance.

Table 10. List of plant species used to treat COVID-19 in Biskra, Algeria.

Family of the plant	**Species and vernacular name**	**Parts involved/ Mode of preparation**	**Bioactive compounds**	**Effect of this plant (bioactivity)**
Asteraceae	*Artemisia vulgaris* ; Shih شيح	Leaf Infusion	Eudesmane-type sesquiterpene, morin, luteolin, triterpenes, coumarin, flavonoids, eriodyctol (Nigam et al., 2019).	Antioxidant, hepatoprotective, antispasmolytic,and antiviral against SARS-CoV-2 (Ekiert *et al*., 2020 ; Kshirsagar and Rao, 2021).
	Saussurea costus ; Al Quist Al hindi القسط الهندي	The root Ground and infused in water	Terpenes, flavonoids, anthraquinones, alkaloids, tannins, inulin, sesquiterpene lactones (Bagheri *et al*., 2018).	Anti-ulcer activity, Antiangiogenic activity, Plant growth regulation, antimicrobial (Zhao *et al*., 2017).
Lamiaceae	*Mentha spicata* ; Na’na’ نعناع	Leaf Infusion	Carvone , limonene and dihydrocarveol (Ay Kee *et al*., 2017 ; Ali-Shtayeh *et al*., 2019).	Antioxidant activity, anti-inflammatory, antimicrobial and sedative (Ali-Shtayeh et al., 2019).
	Melissa officinalis ; Na’na’ soufi النعناع الصوفي	Leaf Infusion	Terpenes α-Pinene cis-p-Meth-2 en-7-ol 2-Pinen-4-one Nerol acetate Phenolic compounds: Patchoulene 1R-α-Pinene Isogeraniol Geraniol (Miraj *et al*., 2017).	Anti-oxidant activity, digestive, carminative, antispasmodic, sedative, analgesic, tonic, and diuretic properties (Miraj *et al*., 2017).

	Syzygium aromaticum ; Tib الطيب	Flowser Maceration	2-Phenyl Ethanol, Benzyl Alcohol, Eugenol, Chavicol, 4-Hydroxy, 3-Methoxy-Benzeneacetic Acid, Hexadecanoic Acid (Chaachouay *et al*. 2021).	Anti-inflammatory, Antimicrobial, Antioxidant (Chaachouay et al. 2021).
Myrtaceae	*Eucalyptus globulus ;* Kalitous الكاليتوس	Leaf Infusion	Myricetin, linalool, urosolic acid, apigenin (Chaachouay et al. 2021).	SARS-CoV, antiviral, hepatitis B and C virus (Chaachouay et al. 2021).

4.5.1.3. Use value (UV)

Use value was estimated by following the rule of: $\frac{\text{Number of use reports on X plant}}{\text{Number of interviewed individuals}}$ (**Fig. 38**).

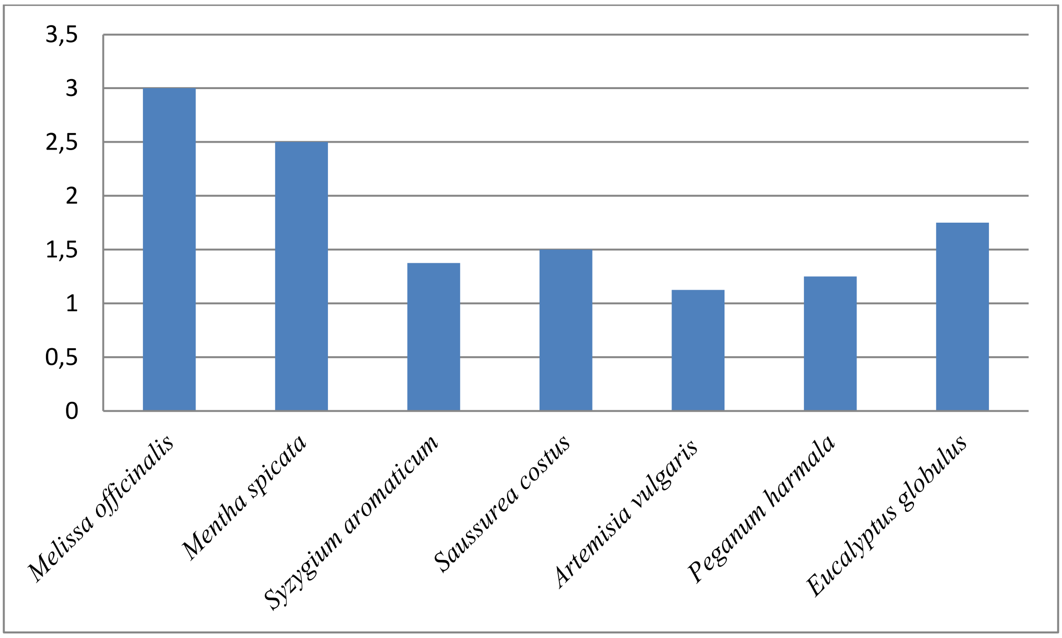


Figure 39. Use value of different plants mentioned by Biskra's herbalists.

The use value of the plants mentioned by herbalists gave the following results:

- *Melissa officinalis* followed by *Mentha spicata* had the highest use value of 3 and 2,5 respectively.

- *Saussurea costus*, *Syzygium aromaticum* and *Peganum harmala* have use values of 1,5 ;1,3 and 1,2 respectively.
- A*rtemisia vulgaris* is the species that own the least use value with a 1,12 (Appendix 07: Table 9).

The relative importance of species in reference to more use reports cited by the interviewees is what the use value reflects, when a plant has a high UV such as *Melissa officinalis* then it means that it has high use reports, now the low UV of a certain plant does not condemn the plant to be useless, discredits it or decreases its importance, we have to keep in mind that those numbers are reflected by the common knowledge of the individuals of Biskra and their use is subjective in most cases.

Regardless, not to contradict the importance of species with both high UV and RFC since these show high healing potential such as *Melissa officinalis* who classifies as one, the species molecules specifically the two phytoconstituents, Luteolin-7-glucoside-3′-glucuronide and Melitric acid-A in Prasanth *et al*. (2021) molecular study were proven to have an inhibiting effect against the SARS-CoV-2.

4.5.1.4. Fidelity level (FL)

The factor was estimated according to the rule:

$$\frac{\text{Number of informants mentioning plant X against a certain disease or symptom}}{\text{Number of informants listing the same plant X against any disease or symptom}}$$ (**Fig. 39**).

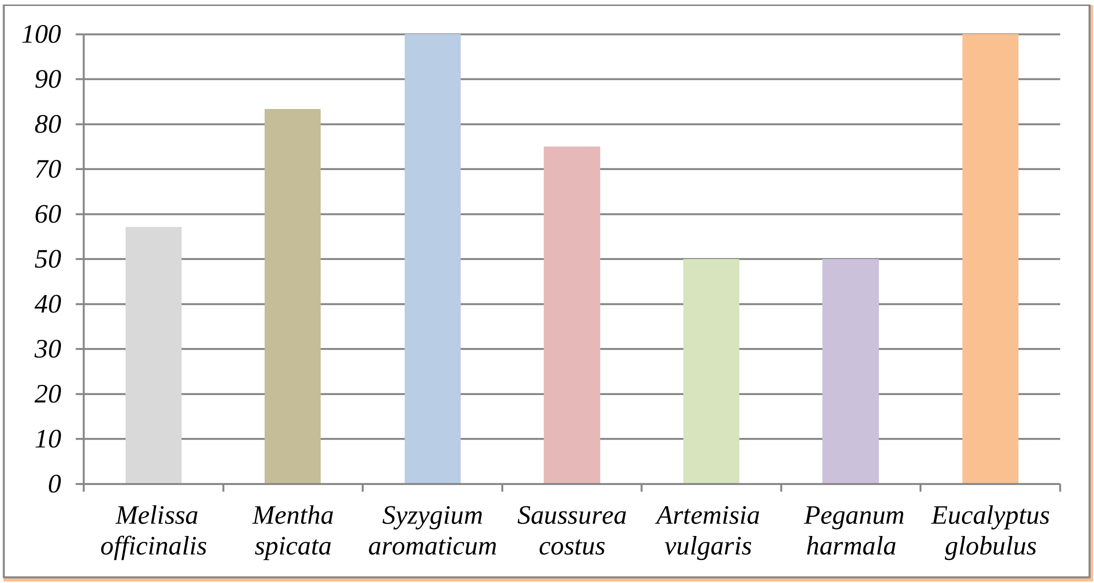


Figure 40. Fidelity level of mentioned plants in curing Covid19 in Biskra.

The results showed that both species *Eucalyptus globulus* and *Syzgium aromaticum* have reached fidelity levels of 100%.

The least SARS-CoV-2 curative plants mentioned were *Artimisia vulgaris* and *Peganum harmala* with fidelity levels of only 50% both (Appendix 08: Table 11).

This approach is specifically employed to make out the difference between the common use of a certain species against whatever diseases or uses that are close to the actual disease gone against, and between the specific use of this certain species against Covid19 itself. For instance *Syzygium aromaticum* is a species that has a lower UV and RFC but has a high FL which makes it the perfect example for our explanation; the individuals that use this plant do not use it to fight cold like symptoms, to heal fever or boost immunity but to specifically fight Covid19, unlike *Melissa officinalis* which is used for a wide range of reports and healing from the virus is one of them, which will lower it FL.

Again, the difference in use of each plant is strongly relied a community of indigenous people's strong belief that it has the beneficial element to cure whatever illness they're facing, this is why some irrational values might be spotted and in order to value that factor, the pharmacological treatment a SARS-CoV-2 patient is bound to respect has been broken down from the pharmacy "Lounis" (**Fig. 41**).

Figure 41. SARS-CoV-2 Medical treatment prescribed by doctors in Biskra.

The treatment prescribed is: Orokal 200mg, Zomax 500mg, Zinc, Vitamin C, Lovenox 0,4 ml- 0,8 ml, Doliprane 500mg.

This is when we can create a correlation between these treatments, since Orokal is an antimicrobial treatment with Cefixime, used mainly to treat infections caused by bacteria such as bronchitis (infection of the airway tubes leading to the lungs) (Quintiliani, 1996). This property is present within species of Asteracea (*Saussurea costus*) that has an efficient antimicrobial effect especially against respiratory bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Othman, 2013).

According to Butler et al. (2021) the Zomax 500mg works with azithromycin as an ant-inflammatory and antiviral agent increasingly used in the period of COVID-19 even within the United Kingdom, a property indigenous people saw in the use of *Melissa officinalis* and in *Syzygium aromaticum* for their pre-existing antiviral activity indeed against viruses such as herpes virus 1 and 2 (Cortés-Rojas *et al.*, 2014) aside from their antimicrobial effect mentioned in Table 11.

People in the area of Biskra aren’t really aware that by consuming Artimisia they obtain very high Zn amounts than any other vegetable they consume (Wang *et al*., 2020).

We can conclude that the consumption of herbs within the area of Biskra is not fully on a blind scale, not to discredit the inhabitants of the area, although the belief of a plant possessing a stronger curative effect than another is solely built on presumptions and has to do with the presence of that herb in the area or the influence one individual has on the rest of the community in convincing them of its efficacy but most of these plants are slowly proven to be helpful in either fighting the virus or just minimizing the interval of COVID-19 outcomes, which is thought to be beneficial for the consumer.

Conclusion

Conclusion

The SARS-CoV-2 is putting at stake the life and finance of the whole human race in general and Algeria, specifically Biskra, who has suffered these last two years from this virus and it is still in the continuous combat alongside with the rest of the world in order to get out of this pandemic with the least damages.

The virus is still thriving and no one is safe when it comes to its unexpected variations, thus it isn't the world's first pandemic, this will remain a black hole in the world's history, and the fatal outcomes will stain in the lives of many.

The first part of the study ended with a full sight of the Algerian sequences position next to the world's, coming mostly from France and less frequently from other Europe countries, the matter was quite obvious when the most common flights and travels are made through French airlines in most cases.

At the end of the second part, the data of the Hospital Hakim Sadanne in Biskra was thoroughly analyzed allowing us to figure out that the factors gender, age and mortality are all related and that the impact of the weather on the last one was irrelevant. It also turned out that the control measurements have a great impact on the effective reproduction number which translates to having a stronger impact on cases incidence and frequency. It has also been figured out that in order to have a more accurate test result it is preferable to combine more than one type of test such as Scanner and PCR or Scanner and antigenic tests.

Meanwhile the third part proved that people suffering from pre-existing comorbidities are not only more likely to catch the virus but they also have risks of worse complications after infection, that chronically ill people are indeed more vulnerable to the virus and that specifically individuals who suffer from Cardiovascular diseases, hypertension and diabetes are the most risk-exposed patients.

At last, the ethnopharmacological study made room for potential COVID-19 inhibitors and that local people are very keen on using alternative treatments than pharmaceutical ones because of their strong belief that these ones are not only less harmful and have less side effects and toxicity than the normal treatment but are also more efficient, also, those plants had a better availability than the medical treatment during the period when authorities were struggling to meet the need of the demands and were overwhelmed by the number of cases.

Perspectives and recommendations

A possibility of diving further into SARS-CoV-2 molecules and finding ways of inhibiting the virus via bioinformatics is now open due to the use of software for docking and employing the work into discovering a potential molecule to inhibit virus. The idea of studying the effect the vaccine has emerged since vaccination has already started in the corners of Algeria.

This work has opened gates for debates due to the valuable possible rules to follow:

✓ For authorities:

- The strictness of the lockdown has to be maintained and rushed decisions of loosening curfew and cancelling a lockdown ought to be studied before applied.
- The two highlighted periods that align with the festive season (weddings, summer vacation, beach season…etc.) and the new academic year need more focus and better control management.
- Bringing awareness to citizens and imposing strict measurements for people to follow for a safer season can control the peaks within summer.

✓ For citizens:

- Respecting the curfew time, distance measurements, sanitizing oneself and wearing a mask is the least each individual is ought to do during the pandemic.

The pandemic brought some positive points that should be enhanced:

- The powerful use of technology and substitution of presential meetings with software and applications for online use therefore it’s a must to improve the internet services and encourage local developers.
- Opening new horizons for new job opportunities by employing more people into delivery services, online apps creation, maintenance and online services.

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Appendix

Appendix 01

Table 1. Percentages of admitted patients of different age proportions of males and females in the hospital of Hakim Sadanne Biskra

From 0 to 9 years		From 10 to 19		From 20 to 44 years		From 45 to 65 years		Above 65 years	
M	F	M	F	M	F	M	F	M	F
0,52	0,34	0,43	0,40	19,79	31,90	42,56	37,29	36,71	30,05

Appendix 02

Table 2. Percentage results of the most admitted proportion of age (45-65) and 65+ within males by months during the year of 2020 in the hospital of Hakim Sadanne Biskra.

Month	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**
% 45- 65	0,23201856	2,78422274	2,08816705	15,0812065	26,9141531	10,4408353
% 65+	0,19569472	4,10958904	5,08806262	26,4187867	24,0704501	10,1761252
Month	**Sep**	**Oct**	**Nov**	**Dec**		
% 45- 65	3,94431555	8,58468677	17,8654292	12,0649652		
% 65+	3,32681018	6,45792564	13,5029354	6,65362035		

Table 3. Percentage results of the most admitted proportion of age within females (45+) by months during the year of 2020 in the hospital of Hakim Sadanne Biskra.

Mar	**Apr**	**May**	**Jun**	**Jul**	**Aug**
0,49423394	4,11861614	3,78912685	23,3937397	22,8995058	8,56672158
Sep	**Oct**	**Nov**	**Dec**		
7,24876442	7,08401977	15,815486	6,58978583		

Appendix 03

Table 4. Percentage results of the Mortality rate of SARS-CoV-2 among males and females in the hospital of Hakim Sadanne Biskra along the year 2020.

Month	**Mortality rates**	
	M	F
Feb	0	0
Mar	0	0
Apr	2,22222222	8,695652174
May	7,27272727	4,761904762
Jun	26,5682657	17,59259259
Jul	43,7931034	39,08045977
Aug	36,2831858	17,39130435
Sep	15,5555556	8,823529412
Oct	23,9130435	9,589041096
Nov	26,9662921	19,04761905
Dec	32,6732673	8,571428571

Appendix 04

Table 5. Results of the Test of independent samples SPSS.

		Test de Levene sur l'égalité des variances		Test t pour égalité des moyennes						
									Intervalle de confiance de la différence à 95 %	
		F	Sig.	t	ddl	Sig. (bilatéral)	Différence moyenne	Différence erreur standard	Inférieur	Supérieur
Mortality	Hypothèse de variances égales	6,188	,013	-12,401	309	,000	-9,550	,770	-11,065	-8,035
	Hypothèse de variances inégales			-13,166	261,383	,000	-9,550	,725	-10,978	-8,122

Appendix 05

Table 6. Homogeneity test of variances.

		Statistique de Levene	ddl1	ddl2	Sig.
Mortality	Basé sur la moyenne	6,065	52	247	,000
	Basé sur la médiane	,876	52	247	,712
	Basé sur la médiane avec ddl ajusté	,876	52	137,438	,703
	Basé sur la moyenne tronquée	4,939	52	247	,000

Appendix 06

Table 7. Fatality and Mortality percentages in the Wilaya of Biskra by district during 2020.

	Fatality	Mortality
Ain Naga	2,33436007	50
Ain Zaatout	12,4090305	71,4285714
Biskra	24,4524105	25,3547777
Chetma	8,85343309	21,6666667
Djemorah	4,4678479	12
Drouh	0,68103331	25
El Hadjab	12,0185207	28,8888889
El Kantara	6,561655	23,5294118
El Outaya	5,86752941	18,9189189
Lichana	0,94938596	25
M'Chouneche	1,10277938	33,3333333
Meziraa	1,46500936	100
Oumache	3,19180734	27,2727273
Rouidjel	9,21901673	100
Sidi Okba	6,42618064	13,5294118

Appendix 07

Table 8. Frequency of citation of different herbs used against Covid19 mentioned by herbalists in Biskra

Herb	*Melissa officinalis*	*Mentha spicata*	*Syzygium aromaticum*	*Saussurea costus*	*Artemisia vulgaris*	*Peganum harmala*	*Eucalyptus globulus*
Frequency of citation	87,5%	75%	25%	50%	25%	37,5%	50%

Table 9. Use value of the different mentioned plants in Biskra.

Herb	1	2	3	4	5	6	7	8	Use value
Melissa officinalis	0,375	0,625	0,25	0,625	0,25	0,375	0,5		3
Mentha spicata	0,375	0,5	0,375	0,625		0,125	0,5		2,5
Syzygium aromaticum	0,625				0,75				1,375
Saussurea costus		0,375		0,375	0,25	0,5			1,5
Artemisia vulgaris	0,5		0,625						1,125
Peganum harmala				0,25		0,375		0,625	1,25
Eucalyptus globulus	0,375		0,25		0,625	0,5			1,75

Glossary

Cardiomyocytes: The cells that make up the heart muscles, they are also known as myocardiocytes.

Cytokine storm: A severe immune reaction in which the body releases too many cytokines into the blood too quickly, it can be as a result of infection, autoimmune disease or just another illness.

Etiopathogenesis: Reasons and ways of how a disease is developed.

Ethnopharmacy: Science that link research on medicinal plants with socio-cultural studies and could possibly serve in developing new drugs.

Exocytosis: The vesicle fuses with the plasma membrane and releases its contents outside the cell.

Immunosenescence: Changes in the function of the immune system within elderly.

Inflammaging: A chronic systemic inflammation that occurs during the process of aging.

Operation Warp Speed: Accelerating the production of a vaccine before knowing whether it’s working or not, first WARP speed happened in 1955 with polio virus.

Uncoating: A tightly regulated process in which the virus capsid shell dissembles after the viral entry.

Viremia: The presence of infectious virus in the circulation.

ملخص

ظهور الوباء الجديد فيروس كورونا المستجد أثر على سيرورة الوضع في العديد من الدول و الجزائر لم تكن بمنأى عما يحدث, لهذا السبب تأخذ هذه الدراسة على عاتقها مسؤولية اجراء مسح شامل لبيانات ميدانية سجلتها ولاية بسكرة على مستوى المستشفى حكيم سعدان بمجموع 2108 حالة خلال سنة 2020 من بينها 931 وفاة, اجريت من خلالها اختبارات لربط عوامل كالحرارة, العمر و الجنس بمعدل الوافيات و التي أثبت من خلالها ان العاملين الاخيرين هما فقط من يمتلكا تأثير على الوافيات. ركزت الدراسة على ادراج وسائل المعلوماتية الحيوية و اسنادها للحصول شجرة النشوء والتطور للفيروس من خلال الحصول على تسلسل كامل لجينوم الفيروس لعينات جزائرية و البحث عن أقرب مصدر محتمل لها الذي تبين انه فرنسا. حققت الدراسة بعد وضع استبيانات عبر الانترنت و اخرى ميدانية وصلت الى ما يفوق 400 شخص من بينها 99 أجنبي و 300 جزائري الى استنتاجات هامة فيما يخص ارتباط الأمراض المزمنة المسبقة الوجود باكتساب العدوى و التي توصلنا من خلالها ان الاشخاص الذين يعانون من داء السكري و امراض القلب و ضغط الدم هم أقل الاشخاص حصانة, و أن اللجوء للطب البديل للتداوي من الكوفيد19 يرتبط بثقافة المحنطة و شعبية النبتة خلال فترة الوباء ففي ولاية بسكرة الزعتر, الطيب و القسط الهندي كانوا اكثر الاعشاب استهلاكا من طرف سكان المنطقة.

الكلمات المفتاحية: فيروس, كوفيد19,الجزائر, بسكرة, شجرة النشوء و التطور, امراض مزمنة, تداوي بالأعشاب, حكيم سعدان.

Abstract

The emergence of the SARS-CoV-2 pandemic has affected the situation in many countries including Algeria. That's why this study takes responsibility for conducting a full scan of field collected data recorded in the state of Biskra at the hospital Hakim Sadanne with a total of 2,108 cases in 2020, including 931 deaths, through which, several statistical tests were conducted to link factors such as heat, age and sex with mortality rate. The study focused on the use of bioinformatics means in order to build a neighbor-joining phylogenetic tree of the virus by obtaining complete sequences of the virus genome of Algerian samples through GISAID and searching for their nearest possible source that turned out to be from France. After developing online and in-field questionnaires reaching more than 400 people, including 99 foreigners and 300 Algerians, the study reached important conclusions regarding the association of pre-existing chronic diseases with the infection in which we found that people with diabetes, cardiovascular heart disease and blood pressure are the least immune to the virus. The resort to alternative medicine to treat COVID19 has been a great alternative to medical treatment within Biskra.

Keywords: SARS- CoV-2, COVID19, Biskra, Hakim Sadanne, Phylogenetic tree, chronic disease, alternative medicine.

Résumé

L’émergence de la pandémie de SARS-CoV-2 a affecté la situation dans de nombreux pays dont l’Algérie. C’est pourquoi cette étude prend la responsabilité de mener une analyse complète des données collectées sur le terrain au niveau de la Wilaya de Biskra à l’hôpital Hakim Sadanne avec un total de 2 108 cas en 2020, dont 931 décès, à travers lesquels plusieurs tests statistiques ont été menés pour lier des facteurs tels que la chaleur, l’âge et le sexe avec le taux de mortalité. L’étude s’est concentrée sur l’utilisation de moyens bioinformatiques afin de construire un arbre phylogénétique du virus en obtenant des séquences complètes du génome du virus d’échantillons algériens et en recherchant l’origine de provenance la plus proche possible qui s’est avérée être la France. Après avoir développé des questionnaires en ligne et sur le terrain touchant plus de 400 personnes, dont 99 étrangers et 300 Algériens, l’étude est parvenue à des conclusions importantes concernant l’association des maladies chroniques préexistantes avec l’infection dans laquelle nous avons constaté que les personnes atteintes de diabète, maladies cardiaques cardiovasculaires et pression artérielle sont les moins immunisées contre le virus. Le recours à la médecine alternative pour traiter COVID19 a été une excellente alternative au traitement médical au sein de Biskra.

Mots clés : COVID19, SARS-CoV-2, Biskra, Arbre phylogénétique, maladies chroniques, médecine alternative.