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Detection of SARS-COV-2 and bacterial co-infections in patients with respiratory diseases in Babylon province

A Thesis

Submitted to the Council of College of Medicine-University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master in Science / Medical Microbiology

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بِسمِ اللهِ الرَحمِن الرَحِيم

﴿ وَمَا يَعْلَمُ تَأْوِيلَهُ إِلَّا اللَّهُ وَ الرَّاسِخُونَ فِي

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إِلَّا أُولُو الْأَلْبَابِ ﴾

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We certify that this thesis, entitled **'' Detection of (SARS-COV-2) and bacterial co-infections in respiratory disease in Babylon province ''** has been prepared under our supervision by **'' Noor Abbas Jawad Abdel Zahra'''** at college of medicine, university of Babylon, as a partial requirement for the degree of Master of Science in Medical Microbiology

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Dedication

I dedicate this thesis to….

To the strong and tender , who taught me to trust Allah, believe in hard work and the pursuit of my dreams ,My mother

To my dear father who taught me the meaning of persistence and that nothing is impossible in life with the strength of faith

To my great friends Maryam ,Heba , and Mervat, Ruqaya who supported and encouraged me all this work .

To the strength source and the loving partner of my life

My brother and sisters

Every science student who seeks to gain knowledge

To my uncle dr. Hashem Al -Mayali

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To everyone who taught me a letter

To everyone who supported me, even with a smile

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Summary

Sever acute respiratory syndrome - corona virus-2 responsible for ongoing pandemic worldwide and significantly high number of morbidity and mortality still recorded. SARS-COV-2 causes respiratory disease resulting from a life-threatening. The present study aims to determine the rate and sequencing of SARS-COV-2 infection among patients with respiratory symptoms in Babylon Governorate, among study population and investigating the correlation between SARS-COV-2 infection with bacterial co-infection.

Case control study included 50 patients with COVID-19 who were attended to Epidemiological Monitoring Unit at Emergency Department in Imam Al-Sadiq Teaching Hospital and Medical Marjan city in Babylon province , 28 were males and 22 were females, their ages range between 20-80 years old and compare with 50 apparently healthy individuals during the period from the September to the November 2021 , and confirmed by reverse transcriptase polymerase chain reaction. Nasopharyngeal swabs were collected from This study included all patients and was conducted directly on them. placed in tube contain 2ml of viral transport medium (VTM) then stored at -20 ºC until the time RNA extraction and reverse transcriptase polymerase chain reaction processing. Nasopharyngeal and oral pharyngeal swabs for bacterial infections diagnostic bacteria by vitik2 system to Gram-positive and Gram-negative these bacteria co-infection to COVID-19 .

The COVID-19 pandemic, which is happening all over the world, needs serious attention. COVID-19 initiated by an RNA type virus can cause more severe problems later on due to its ability to mutate. SARS-COV-2 genomes code for a ORF1a / ORF1ab polyproteins and four structural proteins widely studied as major drug targets. The genomes

also contain a variable number of open reading frames (ORFs) coding for accessory proteins that are not essential for virus replication, but appear to have a role in pathogenesis. This study was conducted on 50 nasopharyngeal swabs using RT-PCR and PCR for ORF1ab gene amplification after that, five samples were subjected the phylogenetic analysis. The current study results are confirmed by the ORF1ab analysis shows that the ORF1ab vary and mutated and have a number of mutation the could affect it similarity between countries in aimed of detection our local isolate were had a 100 identities with United State concluding that the extreme effect of virus transmission and it is mobile genetic holding.

Of fifty With a mean age of (20-80) years, COVID-19 patients were split into two groups: 28 (56%) males and 22 (44%) females. All of the individuals tested positive for bacterial infections in total, including, *Klebsiella pneumonia* (7) samples , *Streptococcus pneumonia* (5) , *Acinetobacter baumannii* (5) , Other bacteria are opportunistic and others are normal flora.

Distribution of study population according to gender show not significant difference at p value (0.54),according to age the current study revealed show significant difference at p value (0.080), the infection ratio of SARS-COV-2 appear significant difference at p value (0.000)**,** the chronic disease show significant difference at p value (0.001) ,odd Ratio for chronic disease (24.438) .

Summary .....

Correlation between age group and type of bacteria co-infection to SARS-COV-2 patient appear significant difference at p value (0.016), the infection ratio and type of bacteria show significant difference at p value (0.002),the correlation between chronic disease and type of bacteria show highly significant difference at p value (0.0001). Correlation between vaccinated and type of bacteria show significant difference at p value (0.0001*). Correlation between the type of bacteria and occupation appear significant difference at p value (0.001). Correlation infection ratio and SARS-COV2 infection (Nasopharyngeal swab, show significant difference at p value (0.000). Correlation specific treatment with antibiotic and type of bacteria, show significant at p value (0.000).

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List of Abbreviations

Abbreviation	Meaning
ACE2	**Angiotensin-converting enzyme 2**
ACI	**acute cardiac injury**
ADE	**antibody-dependent enhancement**
AGEs.	**Advanced glycation end products**
ARDS	**Acute respiratory distress syndrome**
ARs	**Acute Radiation Syndrome ,**
AUC	**area under the curve**
BMI	**Body Mass Index**
BP	**Blood pressure**
Bp	**Base pair**
Bpm	**Beats per minute**
C. pneumonia	***Chlamydia pneumoniae***
CAP	**community-acquired pneumonia**
CBC	**Complete blood count**
CD4	**Cluster of differentiation 4**
CD8	**Cluster of differentiation 8**
CDC	**Centers for disease control**
cDNA	**Complementary DNA**
CFR	**Case fatality rates**
cKp	**classic *Klebsiella pneumoniae***
CNS	**Central nervous system**

COVID-19	**Corona virus disease 2019**
CQ	**Chloroquine**
CRS	**Cytokine release syndrome**
CS	**Corticosteroids**
CSSE	**Center for Systems Science and Engineering**
CT	**Computed tomography**
Ct	**cycle threshold**
CVC	**Central venous catheter**
CVD	**Cardiovascular disease**
CY5	**Sulfo-Cyanine5**
d NTPs	**Nucleoside triphosphates**
DAD	**Diffuse alveolar damge**
DCs	**Dendritic cell**
DIFAT	**Direct immunofluorescence Antibodies technique**
E	**Envelope protein**
EDTA	**Ethylenediaminetetraacetic acid**
ER	**Endoplasmic Reticulum**
ERGIC	**Endoplasmic reticulum- Golgi intermediate compartment**
Eth.Br	**Ethidium bromide**
FAM	**Fluorescein amidites**
GCSF	**Granulocyte colony-stimulating factor**
GGO	**Ground –glass –opacity**
GM-CSF	**Granulocyte-macrophage colony-stimulating factor**

HAdV	**Human Adenovirus C**
HCoV	**Human coronaviruses**
HCOV 229E	**human coronavirus 229E (named after a student specimen coded 229E)**
HCOV- OC43	**human coronavirus named OC43 (OC for organ culture).**
HCoV-HKU1	**Human coronaviruses Hong Kong University 1**
HCoV-NL63	**Human corona virus –Netherlands**
HCQ	**Hydroxychloroquine**
HCWs	**Healthcare workers**
HEX	**Hexachloro-fluorescein**
HEPA	**High Efficiency Particulate Air**
HRP	**Horseradish peroxidase**
HRV	**Human rhinoviruses**
hvKp	**high virulence *Klebsiella pneumoniae***
ICTV	**International Committee on Taxonomy of Viruses**
ICU	**Intensive Care Unit**
ICU	**intensive care unit**
IFN	**Interferon**
Ig	**Immunoglobulin**
IL	**Interleukin**
ILC3	**Group 3 innate lymphoid cells**
IP-10	**Induced protein-10**
K	**Potassium**
k Da	**Kilo Dalton**

kb	**Kilo base**
L.pneumophila	***Legionella pneumophila***
M	**Membrane protein**
M. pneumonia	**Mycoplasma pneumonia**
MCP-1	**Monocyte chemo attractant protein 1**
MDR	**multidrug-resistant**
MEGA6	**Molecular Evolutionary Genetics Analysis software version 6**
MERS-CoV	**Middle East respiratory syndrome**
mRNA	**Messenger RNA**
MRSA	**Methicillin-resistant Staphylococcus aureus**
N	**Nucleocapsid**
Na	**Sodium**
NAAT	**Nucleic acid amplification tests**
NCBI	**National Center of Biotechnology Information**
NF-Kβ	**Nuclear factor kappa B**
NK	**Natural killer**
Nm	**Nano meter**
Nsp	**Nonstructural protein**
OD	**Optical density**
ORFs	**Open reading frames**
PICC	**peripherally inserted central catheter**
Pp	**Polyproteins**
RAAS	**Renin-Angiotensin Aldosterone**

RBD	**Receptor binding domain**
RNA	**Ribonucleic acid**
ROC	**Receiving Operator Characteristic**
ROX	**6-Carboxyl-X-Rhodamine**
RT-qPCR	**Real time-polymerase chain reaction**
S	**Spike protein**
SARS COV	**sever acute respiratory syndrome coronavirus**
SAS	**Statistical Analysis System**
Spo2	**Peripheral Oxygen Saturation**
ssRNA	**Single strand Ribonucleic acid**
T h-17	**T helper-17**
TCZ	**Tocilizumab**
TGF-β	**Transforming growth factor beta**
Th1	**T Helper 1 Cells**
Th2	**T Helper 2 Cells**
TMB	**Tri Methyl Borate**
TMPRSS2	**Trans membrane protease serine 2**
TNF-α	**Tumor necrosis factor alpha**
UVB	**Ultraviolet B**
VAP	**ventilator-associated pneumonia**
VRE	**Vancomycin-resistant enterococci**
VTM	**Viral transport medium**
WHO	**World Health Organization**
Ml	**microliter**

Chapter One

Introduction and Literatures Review

1.1.Introduction

The Coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since its first recorded case in the city of Wuhan, China in December 2019. According to the COVID-19 Dashboard on August 31st, 2020 by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University, over 25 million people in more than 200 countries have been infected and killed more than 840,000(Hopkins ., 2020).

SARS-CoV-2 infection causes asymptomatic and mild diseases more than severe pneumonia. Severe cases may develop acute respiratory distress syndrome (ARDS) and death with an average mortality rate of 6% (range 1–14.4%) (Sohrabi *et al* .,2020).

Sever acute respiratory syndrome - corona virus-2 is an enveloped, non-segmented, single-stranded, positive sense RNA virus (+ssRNA). The genome size ranges from (27 to 32 kb) a cap structure at the 5′ end followed by a reader sequence of about 70 bases, several open reading frames (ORFs) coding various proteins, and a non-translated region including a poly-A sequence at the 3′ end (Khailany *et al.*, 2020).

The main transmission route of SARS-CoV-2 through direct, indirect, or close contact with infected people through saliva and respiratory secretions or their respiratory droplets, which are expelled during coughing , sneezing , talks or singing (Pung *et al.*, 2020).

Sever acute respiratory syndrome - corona virus-2 RNA has also been detected in other biological samples, including the urine and feces of some patients (Pan *et al*., 2020).

The COVID-19 infection mainly presents flu-like symptoms such as cough, fever, fatigue and myalgia. Patients may initially present with diarrhea and nausea a few days before developing a fever. This suggests that fever, is dominant but not the premier symptom of infection. A small number of patient have headache or hemoptysis (Wang *et al*., 2020a).

The clinical presentation of SARS-CoV-2 start within 14 days of exposure; however, in most cases symptoms present after about 5 days and symptom onset is within 11 days in 97.5% of individuals (Lauer *et al*., 2020). Co-infections and super infections are common in respiratory viral infections (Paget and Trottein., 2019).

According to the laboratory, clinical and epidemiological studies, secondary or bacterial co-infections with other viruses can significantly increase the mortality rate in patients infected with viral infections (Metzgerand Sun., 2013).

Aim of study:

The study aims to investigate molecular characterization of COVID- 19 isolate associated with respiratory bacterial pathogen in Babylon province hospitals.

Objectives and Study Plan

1-Infected Group

Nasopharyngeal swabs infect with COVID 19 associated with respiratory bacterial infections will be taken from patients and checking by RT-PCR , vitk2 system for (viral and bacterial) infections respectively through prevalence period of the disease.

2-control group

1.2. Literature Review

1.2.1.History of Severe Acute Respiratory Syndrome -2 SARS-COV-2

Initially referred to as 2019 novel coronavirus, the virus has now been designated severe acute respiratory syndrome coronavirus-2 (Wu *et al* .,2020).

As 4 February 2021, there have been 103.989.900 confirmed cases of COVID-19, including 2.260.259 deaths, according to world health organization (WHO) reports (WHO., 2020).

The earliest ones studied were from human patients with the common cold, which were later named human coronavirus 229E and human coronavirus OC43 (Cyranoski *et al*., 2020).

Other human coronaviruses have since been identified, including SARSCoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and SARS-CoV-2 in 2019. Most of these have involved serious respiratory tract infections(Lau *et al*., 2020).

In December 2019, the capital of the Chinese province Hubei, Wuhan city, witnessed an outbreak of “pneumonia of unknown source” attributed to a newly identified culprit: a novel coronavirus(Purcell and Charles., 2019).

COVID-19 has been confirmed to present a higher risk of occurrence in older men with comorbidities (Chen *et al*., 2019).

1.2.2. SARS-COV-2 Classification and Structure

Coronaviruses (CoVs) are spherical and approximately 125 nm in diameter with club-shape spikes projecting from the surface of the virus giving the appearance of a solar corona, prompting the name, coronaviruses. Within the envelope is the helically symmetrical nucleocapsids, which is actually uncommon among positive-sense RNA viruses (Neuman *et al*., 2009).

Coves are classified under the order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae (Figure1-1). With genome sizes ranging from 26 to 32 kilobases (kb) in length, CoVs have the largest genome for RNA viruses. Based on genetic and antigenic criteria, CoVs have been organized into four groups: alpha coronavirus (α-CoV), beta coronavirus (β-CoV), gamma coronavirus (γ-CoV) and delta coronavirus (d-CoV) (woo *et al*., 2012).

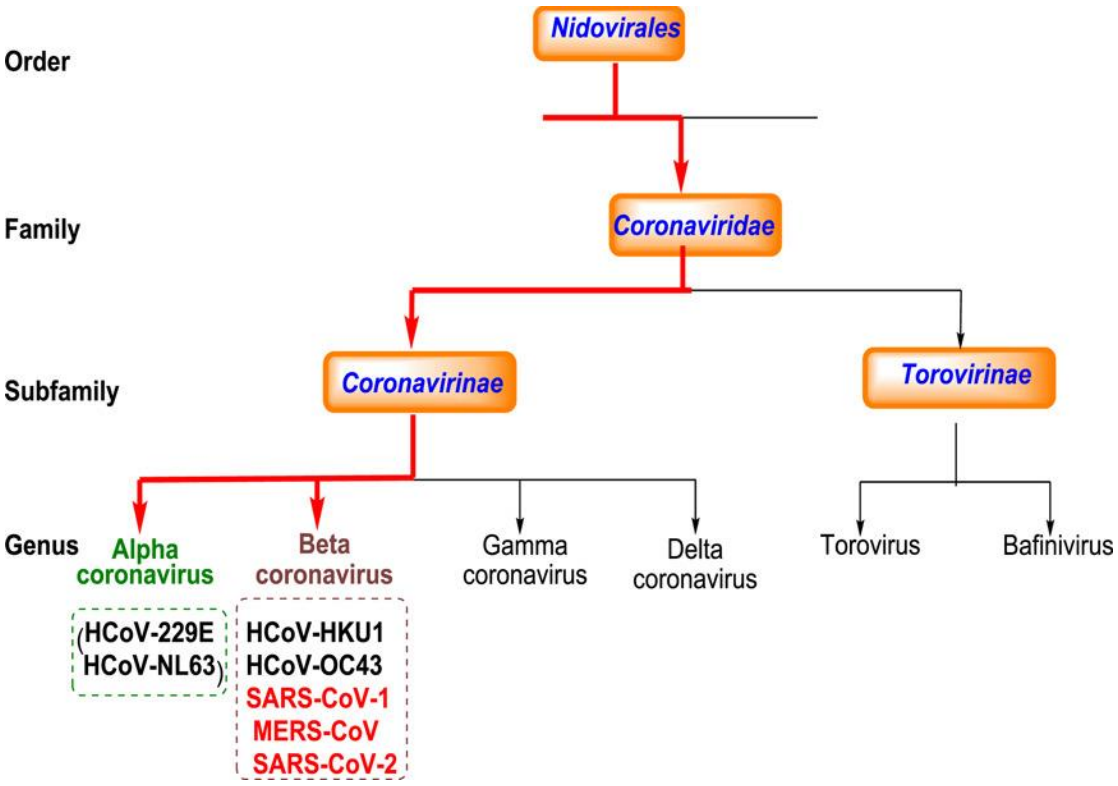


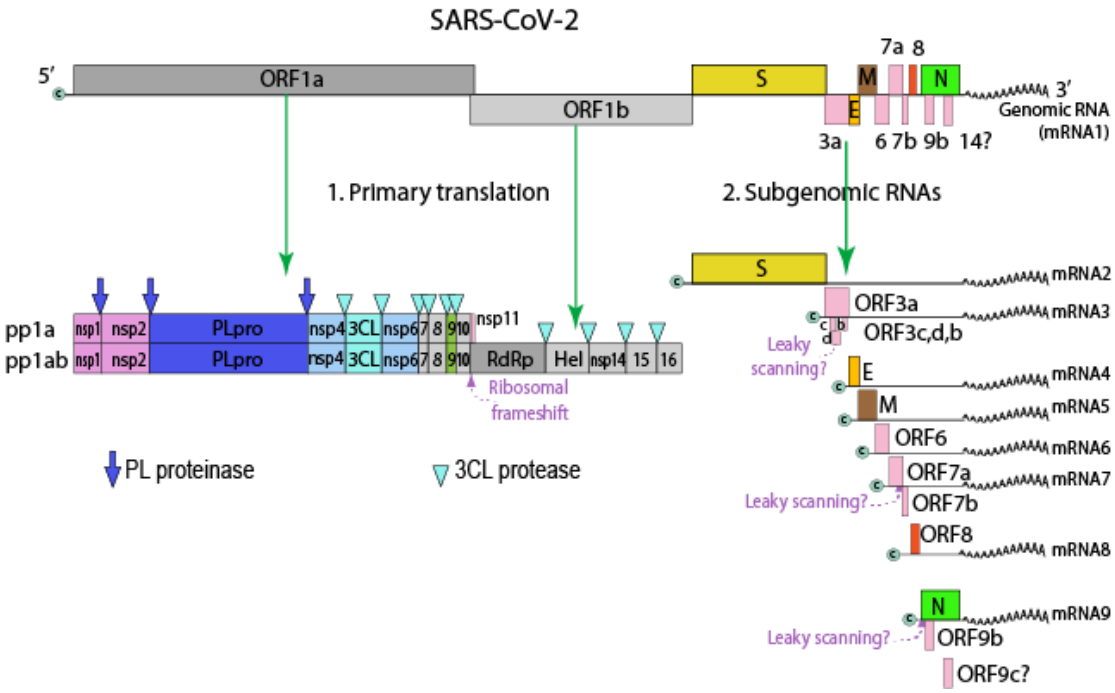
Figure (1.1) Classification of SARS-COV -2 (Pillaiyar *et al* .,2021**)**

For SARS-Cov-2, next-generation sequencing Genomic Structure and Function of Coronaviruses The organization of the coronavirus genome is 5′-leader-UTR- replicas-S (Spike)–E (Envelope)-M (Membrane)-N (Nucleocapsid)- 3′UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ end of the genome (Figure1 - 2). The four structural proteins are required by most CoVs to produce a structurally complete viral particle (Mortola and Roy., 2004;Masters., 2006).

suggesting that some CoVs may encode additional proteins with overlapping compensatory functions (Ruch and Machamer.,2012).

While each of the major protein plays a primary role in the structure of the virus particle, they are also involved in other aspects of the replication cycle. The S protein (~150 kDa) mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry (Song *et al*., 2004; Kirchdoerfer *et al* .,2016)

In some CoVs, the S protein also mediate cell-cell fusion between infected and adjacent, uninfected cells resulting in formation of multinucleated giant cells, a strategy that allows direct viral spread between cells while avoiding virus neutralizing antibodies (Qian *et al* .,2013).



Figure(1.2) Genomes of SARS-CoV-2 (Hemarajata .,2020)

The genome of SARS-CoV-2 is similar to that of typical CoVs and contains at least ten open reading frames (ORFs). The first ORFs(ORF1a/b), about two-thirds of viral RNA, are translated into two large polyproteins. In SARS-CoV and MERS-CoV, two polyproteins, pp1a and pp1ab, are processed into 16 non-structural proteins (nsp1-nsp16), which form the viral replicas transcriptase complex (Fehr and Perlman., 2015).

Those nsps rearrange membranes originating from the rough endoplasmic reticulum (RER) into double-membrane vesicles where viral replication and transcription occur (Masters, 2006; Knoops *et al*., 2008).

The other ORFs of SARS-CoV-2 on the one-third of the genome encode four main structural proteins: spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as several accessory proteins with unknown functions which do not participate in viral replication Several groups of scientists in China have all discovered

that SARS-CoV-2, just like SARS-CoV, requires the angiotensin converting enzyme 2 (ACE2).(Zhou *et al*., 2020).

1.2.3. SARS-CoV-2 Pathogenesis

CoVs induce inflammation in lung tissue. The histological examination of lung biopsy specimens received from COVID-19 infected patients revealed diffuse alveolar damage, desquamation of pneumocystis, hyaline membrane formation, and cellular fibromyxoidexudates connotative of acute respiratory distress syndrome (ARDS). The latest autopsies have confirmed that lungs are filled with clear liquid jelly, much like the lungs of wet drowning. (Xu *et al*., 2020).

Although the nature of the crystal clear jelly has not yet been recognized, there is a connection between it and ARDS (Hallgren *et al*., 1989).

which is the potential of death. In the healthy human lung, the ACE2 receptor is expressed on type I and II alveolar epithelial cells. Not only 83% of the type II alveolar cells have ACE2 receptor expression, but men also had a higher ACE2 receptor level in their alveolar cells than women. Moreover, the level of ACE2 receptor expression in Asians' alveolar cells is higher than that of white and African American populations. So, it is why Asian men are at high risk of the infection. The binding of SARS-CoV-2 to the ACE2 receptors causes an elevated expression of ACE2, which can lead to alveolar cell damages and, in turn, trigger a series of systemic reactions and even death. For preventing the alveolar cell damage and death, pulmonary mechanisms would be compromised via bronchoconstriction, airway congestion, secretions, and decreased mucociliary clearance (Giesbrecht., 1995).

1.2.4. Clinical Symptoms

The usual symptoms of COVID-19 include fever (83–98%), cough (59–82%), shortness of breath (19–55%), and muscle ache (11–44%), which are similar to those of SARS and MERS. Some patients may have sore throat, rhinorrhea, headache and confusion a few days before the onset of fever, indicating that fever is a critical symptom, but not the only initial manifestation of infection. (Huang *et al*., 2020).

The pattern of fever has not yet been fully understood. A small proportion of patients had hemoptysis (Wang *et al*., 2020).

And a number of cases were found relatively asymptomatic COVID-19 patients may have normal or lower white blood cell counts, lymphopenia, or thrombocytopenia, with the increased C-reactive protein level(Guan *et al*., 2020).

People who have fever and upper respiratory tract symptoms with leukopenia or lymphopenia should be suspected for this disease, especially for patients with travel history to the endemic area or close exposure record. However, the clinical course of COVID-19 pneumonia exhibits a broad spectrum of severity and progression patterns. In some patients, dyspnea develops within a median of 8 days after the onset of illness (range of 5–13 days), while in others, respiratory distress may be absent(Wang *et al*., 2020).

Around 3–29% patients may need the admission to the intensive care unit. Severely ill patients may have poor disease course of rapid progression to multiple organ dysfunction and even death (Huang *et al*., 2020).

those who have shortness of breath and hypoxemia can quickly progress into (ARDS), severe sepsis with shock, and even multiple organ dysfunction within one week.(Paraskevis *et al*., 2020).

ARDS was observed to develop in 17–29% of hospitalized patients approximately 8 days after symptoms onset, and the global mortality rate reached approximately 5.4% (Wang *et al*., 2020).

It is also worth noting that the gastrointestinal symptoms of COVID-19 may be caused by the direct viral damage to the intestine rather than the immune pathogenic response to the lung infection of the host. Since angiotensin-converting enzyme 2 (ACE2), the main cellular receptor of SARS-CoV-2 is expressed in the human gastrointestinal epithelial cells, it is believed that the viral shedding at the gastrointestinal tract and fecal–oral transmission is highly plausible (Hindson., 2020).

Indeed, it was reported that the rectal swabs showed positive results even after the nasopharyngeal tests were constitutively negative (Xu *et al*., 2020).

Besides, the live virus was also detected in stool samples of diseased patients. This evidence strongly indicate that stool can be contagious for a long time after the discharge of patients based on two negative nasopharyngeal swabs. Thus, adding rectal swabs to the discharge criteria should be considered for the prevention of both nosocomial and community spread of COVID-19.(Mao *et al*., 2020).

Though the loss of olfaction during SARS-CoV-2 infection could be explained by the swelling of the nasal mucosa, a larger population of patients should be included to determine whether hypogeusia and hyposmia could be a common neurological manifestation of COVID-19. Nevertheless, hyposmia and hypogeusia are now being recommended as

the early warning signs and an indication for early self-isolation.(Tu *et al*., 2020).

1.2.5. Transmission

1.2.5.1. Human to Human Transmission

The novel COVID-19 is found to be able to spread human to human by exposure to cough, sneeze, droplets, aerosols inhaled by mouth or nose, contact of subject with object infected with virus and transmission of virus to nose or mouth of subject (Shereen *et al.*,2020*;* Kumar *et al*., 2020).

In very few cases, it may have been the digestive route where ACE2 was considered expressive in ileum and colon (Adhikari *et al*., 2020).

Binding of the viral spike (S) protein to the host angiotensin-converting enzyme 2 (ACE2) receptor is a critical step for cell entry, and as a result, host ACE2 distribution determines viral tropism (Hoffmann *et al*., 2020; Walls *et al*., 2020).

1.2.5.2. Respiratory Transmission

When a virus spreads through respiratory transmission, it does so either with visions suspended on large droplets or fine aerosols expelled from the respiratory tract of the primary case patient. Droplets are classically considered to be particles larger than 5 μm that fall to the ground within about 6 feet and aerosols to be particles smaller than 5 μm that can remain suspended in the air for prolonged periods; however, this route may be an overs amplification, and distinguishing droplet and

aerosol transmission is difficult in clinical settings (Klompas *et al* .,2020; Fennelly ., 2020).

The dominant route of transmission of SARS-CoV-2 is respiratory (Lu *et al*., 2020).

1.2.5.3.Direct Contact and Fomites

In contrast, fomite transmission is the transfer of surface-borne virus-laden respiratory droplets to a susceptible person; this typically occurs when a person touches a surface bearing virus-laden droplets with his/her hand, and subsequently touches his/her exterior mucosa (e.g., eyes, nose, or mouth) with the same hand, resulting in self-inoculation. Fomite transmission of SARS-CoV-2 was first suspected following the reported survival ability of SARS-CoV-2 on common surfaces (Van Doremalen *et al*., 2020; Chin *et al*., 2021).

1.2.5.4. Domestic Pets and Farm Animals

Several studies have documented that SARS-CoV-2 can infect domestic animals, including cats, dogs, and ferrets(Shi *et al*., 2020; Sit *et al*., 2020).

The virus replicates well in cats (but not in dogs) and is transmissible between cats and ferrets (Garigliany *et al*., 2020).

There are no confirmed cases of transmission from domestic pets to humans. Minks are susceptible to SARS-CoV-2 infection and are farmed in some areas where cases of transmission from minks to human farm workers is suspected(Oreshkova *et al*., 2020; Munnink *et al*., 2020).

1.2.5.5.Vertical Transmission

Vertical transmission of SARS-CoV-2 from infected mothers to their neonates and other adverse neonatal outcomes related to in utero viral exposure. Reassuringly, the evidence to date has indicated a low risk of vertical transmission in exposed neonates (Mullins *et al*.,2021).

In addition, the small number of neonates who do test positive for SARS-CoV-2 are healthy and do not show significant morbidities related to SARS-CoV-2 infection(Norman *et al*., 2021).

SARS-CoV-2 also appears to be unlikely to infect the placenta or other biospecimens, including breastmilk, and studies reporting on neonates who were breastfed and allowed to room-in with mothers report similar rates of vertical transmission as those in which neonates were isolated from their mothers (Jafari *et al*., 2021).

There are several reports of positive SARS-CoV-2 IgM in neonates (Zeng *et al*., 2020).

1.2.5.6.Fecal–Oral (or Fecal Aerosol) Transmission

As the presence of SARS-CoV-2 genetic material and live virus in patients' faeces became apparent, apprehension increased as COVID-19 patients can also present gastrointestinal symptoms such as diarrhea (Livanos *et al*., 2021).

It is unclear whether the virus in feces is infectious and might be an additional source for transmission and whether the virus can directly infect the intestine by passing the respiratory system. Either way, the virus may infect, replicate, and shed from the enterocytes and possibly hepatocytes/cholangiocytes and be excreted as fecal materials into the environment, contaminating water and food supplies. The possibility of

culturing SARS-CoV-2 from stool opens discussions regarding the possibility of fecal-oral transmission and human health or ecological risks (Brogna *et al*., 2020).

Fecal–oral transmission was theorized early in the outbreak because of the known high concentration of ACE2 receptors in the small bowel (Gu *et al*., 2020).

1.2.5.7.Sexual Transmission:

No current evidence supports sexual transmission of SARS-CoV-2. Viral RNA has been found in semen, although infectious virus has not been isolated(Li *et al*., 2020).

Vaginal fluid has been negative except in a single case that reported RNA with a low viral level (Qiu *et al*., 2020;Scorzolini *et al*., 2020).

One study reported lack of transmission to a discordant partner among 5 couples who remained sexually active while 1 partner was in the period of infectiousness (Prazuck *et al*., 2020).

1.2.5.8.Bloodborne Transmission:

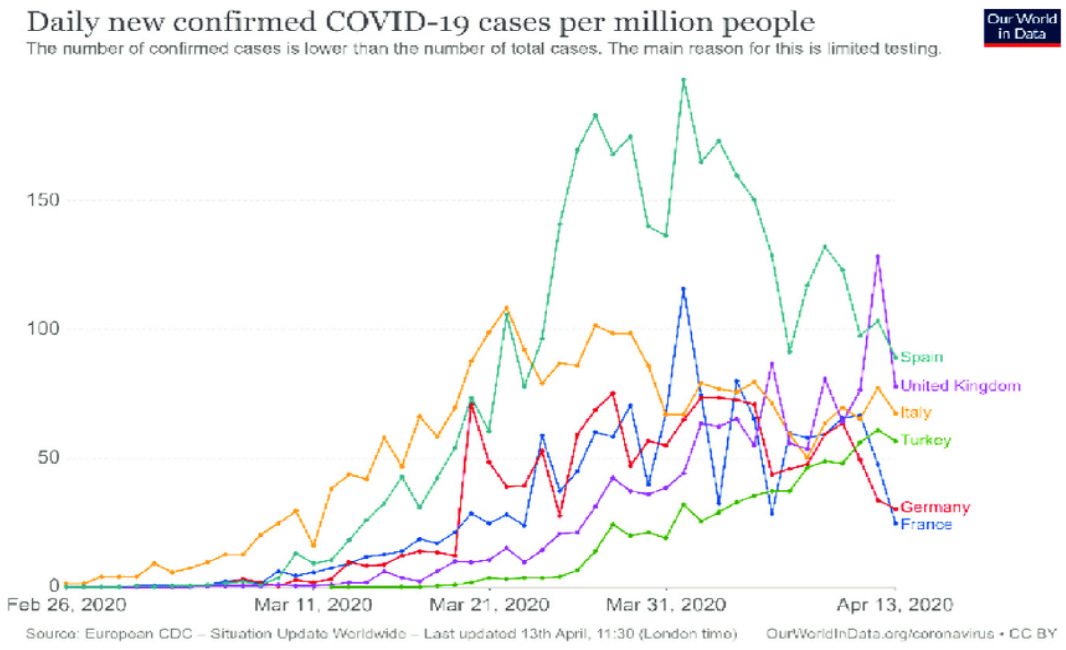
The proportion of persons with viral RNA detectable in blood is currently unknown. An early study found viral RNA in only 3 of 307 blood specimens (Wang *et al*., 2020).

1.2.6. Epidemiology

In epidemiology, an epidemic curve of infection is a statistical graphic that depicts the commencement of a coronavirus outbreak. There are three zones in an epidemic curve: growing, plateauing, and falling. Increasing phase: This stage is influenced by a variety of factors. Various

criteria such as country demographics, age distribution, health system preparedness for an outbreak, implementation of specific preventive measures, country pandemic response time, and societal attitude to new implementing policies (Bulut and Kato., 2020).

Distinct countries can have very different curve patterns, which can make things more complicated, to draw any assumptions regarding a country's epidemic pattern figure (1-3). However, it appears that COVID-19 takes 3 to 4 weeks to complete this process. The disease incidence remains steady during the plateau phase. COVID-19 takes 2 to 3 weeks, Phase of decrease: currently, only have China's data on this phase, which shows that disease activity could be detected at extremely low levels 2 or 3 weeks later (Li *et al*., 2020).



Figure(1.3) Epidemiological curve for some countries, there are daily new confirmed COVID-19 cases per million individuals (Source: Our World in data, accessed 13 / April 2020).

Case numbers and geographic dispersion COVID-19khas been verified in approximatelyy150 million people throughout the world. The

World Health Organization and the European Centre for Disease Prevention and Control have updated case counts in English on their websites. Cases have been recorded on all continents since the initial reports of cases from Wuhan, a city in China's Hubei Province, toward the end of 2019 (Sohrabi *et al*., 2020).

The published case counts understate the true burden of COVID-19 since only a small percentage of acute infections are discovered and reported. Seropositivity shows that the rate of past exposure to SARSCoV-2, as reflected by seropositivity, surpasses the incidence of reported cases by a factor of 10 or more after accounting for any false positives or negatives (Havers *et al*., 2020; Stringhini *et al*., 2020).

1.2.7. Replication Cycle

Target host receptors are found mainly in the human respiratory tract epithelium, including the oropharynx and upper airway. The conjunctiva and gastrointestinal tracts are also susceptible to infection and may serve as transmission portals (Hui *et al*., 2020).

Angiotensin-converting enzyme 2 (ACE2) is specifically expressed in certain organs and tissues, suggesting that it plays an important role in regulating cardiovascular, renal, and reproductive functions. The virus first affects the respiratory epithelial cells and alveolar cells, followed by the digestive system (Mao *et al*., 2020).

Severe acute respiratory syndrome coronavirus-2 attaches to the host cell cellular ACE2. The ACE2 receptor is expressed more in oral cavity than lung. This potentially could indicate that susceptibility and infectivity of SARS-CoV-2 is greater than oral mucosa surfaces (Hoffmann *et al*., 2020). Receptor binding domain (RBD) directly binds

to the peptide domain ACE2, which is also the cellular receptor for the SARS-CoV-2 (Zhang *et al*., 2020).

Following the binding of the RBD in the S1 subunit to the receptor ACE2, SARS-CoV-2 S protein is cleaved by the cell surface-associated trans membrane protease serine 2 (TMPRSS2), which activates S2 domain for membrane fusion between the viral and cell membrane (De Wilde *et al.*, 2018).

Following the release and uncoating of viral RNA to the cytoplasm, coronavirus replication starts with the translation of ORF1a and ORF1b into polyproteins pp1a and pp1ab via a frame shifting mechanism (Figure1- 3) (Weiss and Navas-Martin, 2005).

The resulting complex drives (-) RNA production through both replication and transcription. During replication, full-length (-) RNA copies of the genome are produced and used as a template for full-length (+) RNA genomes (Luk *et al.*, 2019).

The former serves as template for new full length genomic RNAs and the latter template the synthesis of the sub genomic mRNAs required to express the structural and accessory protein genes residing in the 3′-proximal quarter of the genome (Knoops *et al*., 2008).

The assembly of virions is quickly ensued with the accumulation of new genomic RNA and structural components. The N protein complexes with genome RNA, forming helical structures. Then, the trans membrane M protein, localized to the intracellular membranes of the ER-Golgi intermediate compartment (ERGIC), interacts with the other viral structural proteins (S, E, and M proteins) to allow the budding of virions (Huang *et al*., 2020).

Following assembly and budding, virions are transported in vesicles and eventually released by exocytosis (Ortiz-Prado et al., 2020).

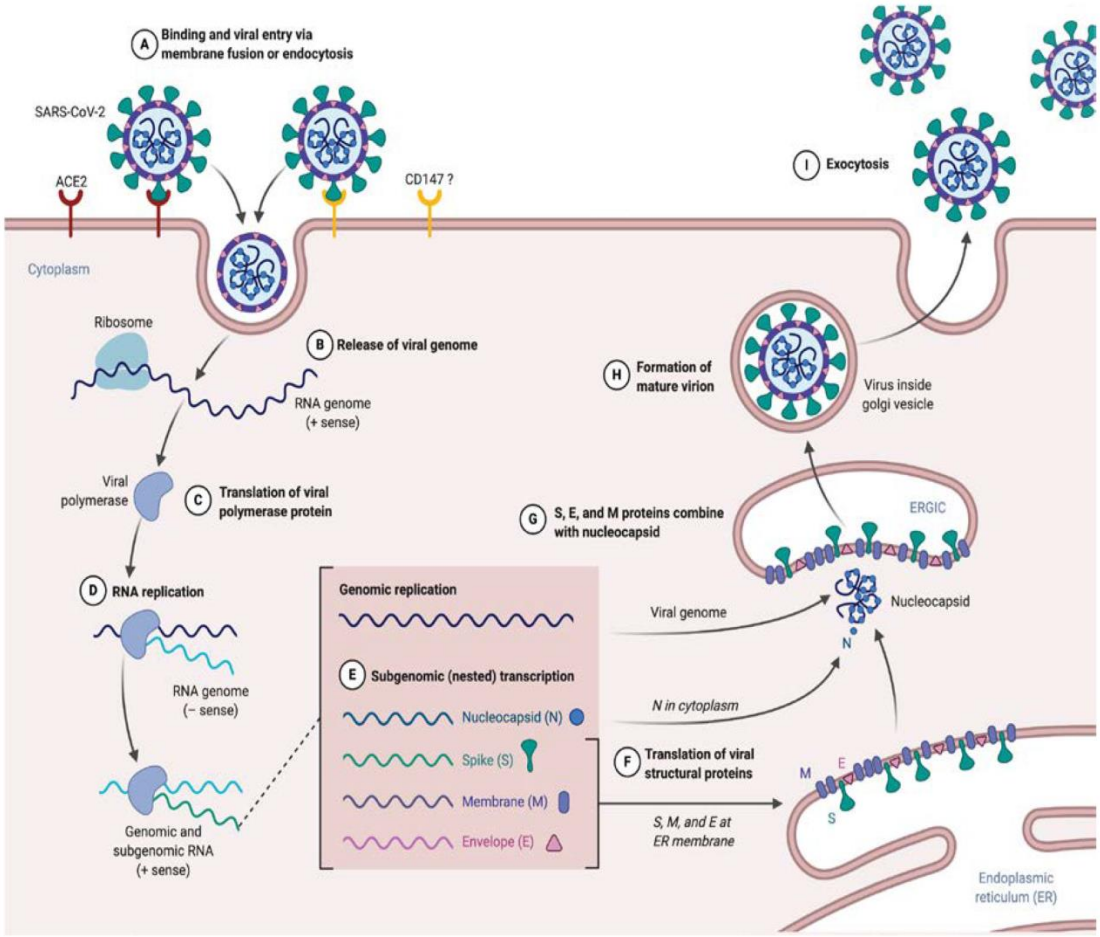


Figure (1.4) Replication Cycle of Severe Acute Respiratory Syndrome Coronavirus-2. The virus binds to ACE 2 as the host target cell receptor stage 1. Leads to membrane fusion and releases the viral genome into the host cytoplasm stage 2. Stages 3-7 show the remaining steps of viral replication, leading to viral assembly, maturation, and virus release (Cevik *et al*., 2020) .

1.2.8. Risk Factors

1.2.8.1. Age

Advancing age is increasingly recognized as one of the strongest predictors for severe SARS-CoV-2 (Zhou *et al.*, 2020).

Older adults (aged above 60 years) are at increasing risk of contracting severe SARS-CoV-2 with higher complication and case fatality rates (Verity *et al*., 2020). Patients with COVID-19 infection have shown that people with underlying diseases not only have a higher risk of developing the disease but also are more likely to die from the virus infection (Verity *et al*., 2020).

Although age has emerged as the most important risk factor for adverse health outcomes related to the development of the cytokine storm and mortality, some younger individuals also fall gravely ill and develop a similar cytokine storm pathology with COVID-19 (Qi *et al*., 2018).

1.2.8.2 Gender

Accumulating data also show the existence of a gender-associated predisposition to SARS-CoV-2, with men being more prone to develop severe disease than women. Possible explanations of male predominance among SARS-CoV-2 patients may be differences in exposure, smoking behavior, other lifestyle factors, differences in chromosomal ACE2 expression, ACE2 expression in testicular tissue, sex hormone-driven immune system regulation, or gender differences in renin-angiotensin aldosterone (RAAS) regulation (Cai, 2020).

1.2.8.3.Other Diseases

Interestingly, in two independent cohorts of patients with heart failure, plasma concentrations of ACE2 were higher in men than in women (Sama *et al*., 2020).

Patients with cardiovascular disease and over the age of 60, with a history of high blood pressure, obesity with a BMI above 25, and a history of smoking are at a higher risk of COVID-19 infection. Researchers at the Wuhan University of China found that of 416 patients admitted to the hospital, about 20% had severe muscle heart damage due to a severe COVID-19 infection, of whom more than half died (Chen *et al*., 2020).

Older adults are far more likely than younger adults to have the chronic conditions type 2 diabetes mellitus, hypertension, cardiovascular disease, renal failure, and chronic obstructive pulmonary disease. These chronic conditions greatly increase the likelihood that those infected with COVID-19 experience more severe illness adverse outcomes, including acute respiratory distress syndrome and respiratory failure, sepsis, acute cardiac injury, and hypercoagulability (Yang *et al*., 2020).

1.2.8.4.Obese

Obese patients with SARS-CoV-2 may have an increased risk of intensive care unit (ICU) admission and mortality. Although obese patients frequently present with mechanical hypoventilation (leading to hyper capnic respiratory failure), those with SARS-CoV-2 present with hypoxic respiratory failure. This led to discussions about a potential role of fat tissue in SARS-CoV-2 pathogenesis in relation to ACE2 expression. Granting that obesity predisposes to developing chronic

disease, obesity could also be an independent risk factor for SARS-CoV-2 (Ryan *et al*., 2020).

1.2.9. Immune Response

The natural history of the disease can be divided into four different phases, from incubation toward critical illness in which the direct cytotoxic effects of SARS-CoV-2, coagulopathy and exacerbated immune responses play critical roles in the progression to severe illness (Lagier *et al*., 2020).

Increasing data indicate that successful recovery from COVID-19 relies on antibody and T-cell responses. Importantly, there appears to be a strong correlation between circulating SARS-CoV-2 specific cluster of differentiation 4 (CD4) and cluster of differentiation 8 (CD8) T cells and IgG antibodies against the nuclear and/or the spike protein of SARS-CoV-2 (Grifoni *et al*., 2020).

1.2.9.1. Innate Immune Response

Innate immune cells play a major role in antiviral immunity, inflammatory signaling, and cytokine production, among the proinflammatory cytokines, IL-1β and IL-18 are key mediators of the inflammatory response, and increased amounts of IL-1β and IL-18 in plasma have been correlated with mortality or severity in patients with COVID-19 (Hadjadj *et al*., 2020).

Severe acute respiratory syndrome coronavirus 2 infection appears to target dendritic cells directly. It is key players in antigen presentation, cytokine production, priming specific T cell responses, and a loss of dendric cells (DCs) function could lead to delayed immune responses in COVID19 patients (Rao *et al*., 2020).

Macrophages in the lung and upper respiratory tract act as sentinel cells and are among the first immune cells to encounter incoming virions. In response, these macrophages can limit early viral replication through initiating IFN-I response, as well as through initiating an inflammatory response to recruit additional immune cells (Lavin *et al*., 2015).

Recent study found that significantly reduced activity of IFN-α in critical SARS-COV-2 patients compared with patients that had mild to moderate infection, possibly because of migration to sites of infection (Bordoni *et al*., 2020).

The number of natural killer (NK) reduce and monocyte significantly increase in patient with sever and mild SARS-COV-2. In addition, the percentage of NK cells was low in patients with fatal COVID-19, whereas it increased in those individuals who recovered from severe disease (Carsetti *et al*., 2020).

Recent preliminary data highlight the importance of complement system in patients with COVID-19 and showed the presence of placental complement deposits in 5 cases of pregnant patients with COVID-19, with histological evidence of avascular villi and thrombosis in fetal vessels, associated with complement deposits (Mulvey *et al*., 2020).

1.2.9.2 Adaptive Immune Response

Regarding the emergence of the new SARS-CoV-2, it has been observed that most patients generate high neutralizing antibody titers 10-14 days after the onset of symptoms; however, some patients had undetectable antibody titers in their blood (Wang *et al*., 2020).

Immunoglobulin production against nucleocapsid (N) protein that can be detected by serum as early as day 4 after the onset of disease and with most patients seroconverting by day 14 (Liu *et al*., 2006).

Current data indicate that anti-SARS-CoV-2 IgM antibodies appear within one week after infection and are present for a month before they gradually decrease. In contrast, anti-SARS-CoV-2 IgG antibodies appear within 10-21 days after infection and appear to remain more-or-less stable for up to 3 months (Zhang *et al*., 2020).

Antibody levels decrease by 11.7% in more than half of the patients after 2 months of recovery which suggests that they are not long-lasting and are not the only ones that play an important role in the resolution of the disease. Therefore, a vaccine should not focus solely on generating humoral immunity (Long *et al*., 2020).

1.2.10. Diagnosis Methods

Diagnosing viral infections currently relies on three major methodologies: immunological method for antibodies detection or viral antigen detection, real-time reverse transcription polymerase chain reaction (rRT-PCR), and computed tomography (CT) scanning (Benzigar *et al*., 2020).

Immunoassay is established diagnostic method. This method detects viral protein antigens or serum antibodies in patients who have been exposed to the SARS-CoV-2.The current gold standard for diagnosing COVID-19 is based on a molecular test of the reverse transcription polymerase chain reaction, aimed at detecting the RNA of the virus in respiratory samples such as nasopharyngeal swabs or bronchial aspirate (Corman *et al*., 2020).

Nucleic acid amplification tests (NAAT) such as real-time reverse transcription polymerase chain reaction are the methods of choice for SARS-CoV-2 diagnostic testing (Fang *et al*., 2020).

In addition to the above chemical diagnosis methods, CT is considered first line imaging in suspected cases to screen the respiratory pneumonia caused by coronavirus, CT is also used as clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA test (An *et al*., 2020).

1.2.11. Prevention and Control

1.2.11.1. Treatment

Until the diagnosis is confirmed, SARS-CoV-2 infected patients are treated in single rooms (Li *et al* .,2020).

As SARS-CoV-2 is an emerging virus, an effective antiviral treatment has not been identified. The main treatment of COVID-19 is symptomatic treatment. The antiviral drugs, including oseltamivir , ribavirin, ganciclovir, lopinavir, and ritonavir have been used in attempts to reduce viral load and to prevent the likelihood of respiratory complications in several studies (Chan *et al* .,2020- Wang *et al* ., 2020).

Remdesivir was reported in the treatment of a patient with COVID-19 in the United States and got an effective result (Holshue *et al* .,2020).

However, the efficacy of these antiviral drugs for COVID-19 need to be verified by randomized-controlled clinical trials. The antibiotics used generally covered common pathogens and some atypical pathogens. When secondary bacterial infection occurred, medication was

administered according to the results of bacterial culture and drug sensitivity(Chen *et al*., 2020).

Current evidence in patients with SARS and MERS suggests that receiving corticosteroids did not have a survival benefit, but rather delayed viral clearance (Arabi *et al*., 2018;Lansbury *et al*., 2019).

Therefore, routine corticosteroids should be avoided unless they are indicated for other reason. Arbidol is used empirically in China because of its direct antiviral effect on SARS-CoV-2 in cell culture (Khamitov *et al*., 2008).

Chinese herbal medicine formulae are used to prevent SARS-CoV-2 infection in 23 provinces in China(Luo *et al* .,2020).

Noninvasive or mechanical ventilation should be considered in patients with hypoxia despite oxygen supplement and worsening shortness of breath. Extracorporeal membrane oxygenation is used as a last resort. (Huang *et al*., 2020).

A study in United States, where Covid-19 patients hospitalized within 24 h of diagnosis was treated with hydroxychloroquine alone (HCQ) or with hydroxychloroquine and azithromycin (HCQ + AZM) or no HCQ as treatments. Among patients, there was no significant reduction in mortality rate or in the need of ventilation with hydroxychloroquine alone or with hydroxychloroquine and azithromycin (Magagnoli *et al*. 2020).

Dexamethasone may be useful for the short-term in severe SARSCoV-2 patients as it inhibit the protective function of T cells and block B cells from making antibodies (Theoharides and Conti., 2020).

1.2.11.2. Vaccination

The S gene is regarded as a key target for SARS-CoV-2 vaccines (Amanat and Krammer., 2020).

Inactivated vaccines and live-attenuated vaccines Due to the urgent need to combat COVID-19, diverse SARS-CoV-2 vaccine types are currently under development, including inactivated vaccines, nucleic acid vaccines, adenovirus-based vector vaccines, and recombinant subunits vaccines . Inactivated viruses are made non-infectious via physical or chemical approaches and are attractive because they present multiple viral proteins for immune recognition, have stable expression of conformation-dependent antigenic epitopes, and can be easily produced in large quantities (Al-Jighefee *et al* ., 2021).

1.2.11.2.1. Inactivated and Protein Subunit Vaccines

One approach for vaccine development is creation of inactivated vaccines derived from virus grown in culture and then chemically inactivated, which may deliver stably expressed, conformation ally native antigenic epitopes. Sinopharm and Sinovac are among the manufacturers farthest along in development of this type of vaccine. This approach can protect immunized animals in vivo but has the theoretic risk of generating a polarized (Th2 over Th1) immune response that can be overcome, depending on the adjuvant used (Zimmermann and Curtis., 2019).

1.2.11.2.2. Viral Vector Vaccines

Viral vector vaccines use replication-deficient viruses engineered to express the genetic sequence of the antigen of interest in host cells. Replication-incompetent adenoviruses have been developed for HIV, tuberculosis, malaria, and Ebola virus (Tatsis and Ertl., 2004).

This vaccination approach has had variable success, often limited by preexisting immunity to the adenovirus vector (Zak *et al*., 2012).

Using adenoviruses that have minimal preexisting immunity in the US and Europe, 2 vaccines have shown early promise: adenovirus serotype 26 vector vaccine (Ad26.CoV2.S; Johnson and Johnson) and chimpanzee adenovirus vector vaccine (ChAdOx; AstraZeneca).

Both appear efficacious in preventing COVID-19–related hospitalization and death, but have varying efficacy in preventing clinical disease, particularly disease caused by the novel SARS-CoV-2 variants (Creech *et al*., 2021).

1.2.11.2.3. mRNA Vaccines

New advancements harnessing mRNA for vaccine delivery have the potential to greatly improve vaccine development for many pathogens. In these vaccines, lipid nanoparticles are used to protect the perfusion-stabilized S protein encoding mRNA en route to the intracellular space. The host uses the mRNA to make the target protein (S protein in this case), which induces a coordinated immune response. Pfizer-BioNTech and Modern have developed mRNA-based vaccines that demonstrate more than 90% efficacy against COVID-`19 clinical disease in clinical trials (Patel *et al*., 2022)

This high vaccine efficacy is associated with very few adverse events, although local and systemic reactogenicity to vaccine are common. There are many advantages to this approach, including speed of vaccine manufacturing (weeks) and ability to generate a Th1 and Th2 response. Studies are underway or planned to assess the efficacy of currently authorized vaccines in children and against common SARS-

CoV-2 variants, and to assess whether repeat vaccinations containing mRNA coding for the variants can be effective (Creech *et al*., 2021).

1.2.12. Co-infection in COVID-19

Bacterial co-infections are frequently determined in viral respiratory tract infections, such as influenza, and are a significant cause of morbidity and mortality. Thus, timely diagnosis and antibacterial treatment are necessary (Clancy and Nguyen.,2020). The frequency, incidence and features of bacterial co-infection in patients infected with (SARS-CoV-2) are not clear; in these critical circumstances, this is a crucial knowledge gap (Langford *et al*., 2020).

Although antibiotics are ineffective treatment of COVID-19, physicians prescribed them for patients with suspected or documented COVID19 for a variety of reasons . In terms of the mortality rate of patients with bacterial supra-infection during influenza pandemics, several guidelines support the usage of empirical antibiotic therapy for COVID-19 patients, It is difficult to rule out bacterial co-infection on presentation, but also the possibility of bacterial secondary infection during the course of the disease. Nevertheless, this approach increases concerns about antibiotic overuse and subsequent detrimental consequences related to bacterial resistance. Given a rise in mortality in patients with bacterial super infection during influenza pandemics, several guidelines supporting the application of empirical antibiotics for patients with severe COVID-19 have been developed (Alhazzani *et al* .,2020).

Given the fact that Covid-19 patients can have a bacterial co-infection and bearing in mind the action of the pathogens, it is critical to

treat Covid-19 patients responsibly in terms of antibiotics in order to minimize the negative effects of overuse.(Huttner *et al*., 2020).

1.2.12.1 Nosocomial infection in COVID-19 Patient

The percentage of nosocomial infection among COVID-19 patients who have died was significantly higher than that of patients who were cured and discharged (P = .002)(Ruan *et al* ., 2020).

The mortality of COVID-19 patients with nosocomial infection was 15.4%, significantly higher than that of COVID-19 patients without nosocomial infection7.3%. Rational utilization of antibiotics and steroids to treat patients with COVID-19 is important in preventing nosocomial infection, and special attention should be given to diabetic patients and patients with invasive devices (ie: CVC or PICC). Future studies are warranted to evaluate the efficacy of implementing infection control strategies or protocols on COVID-19 patients to achieve better therapeutic outcomes The association between demographic and clinical factors and the treatment of nosocomial infection as determined by univariate and multivariable analyses was displayed in Supplementary (He *et al*., 2020).

1.2.12.2.Studies the most common bacteria isolated from COVID- 19 patients include

1.2.12.2.1.The most pathogenic bacteria

1.2.12.2.1.1.*Klebsiella pneumonia*

Klebsiella pneumoniae is a class of gram-negative bacterium that is ubiquitously found on the surface of mucosa in animals, or in the environment (such as water, soil, etc.). In humans, *K. pneumoniae* is

concentrated in the gastrointestinal tract, and a few in the nasopharynx, through which the bacteria can enter the blood circulation or other tissues, and then cause infection. In the era of pre-antibiotics, *K. pneumoniae* was a vital pathogen of community-acquired pneumonia (CAP), especially in diabetics and alcoholics. In the era of antibiotics that followed, it became a major cause of medical-related infections in hospitals (Podschun and Ullmann., 1998).

And a risk factor of severe community-acquired infections (Holt *et al* .,2015). In Singapore, mortality rates of *K. pneumoniae* bacteremia ranged from 20–26% (Chew *et al* .,2017).

In China, *K. pneumoniae* accounted for 11.9% of isolated pathogens from ventilator-associated pneumonia (VAP) and intensive care unit (ICU)-acquired pneumonia (Zhang *et al* .,2014).

In addition, carbapenem-resistant Enterobacteriaceae (CRE) caused by K. pneumoniae have been reported to account for 73.9% of 664 clinical samples in a multi-center clinical study that covered 25 “AAA” hospitals in 14 provinces of China(Zhang *et al* .,2018).

There is no doubt that such a high prevalence and mortality rate of *K. pneumoniae* infection caused a great burden on the country’s health system. *K. pneumoniae* has many accessory genomes of plasmids and chromosome gene loci. According to the accessory genome, *K. pneumoniae* strains are divided into three types: opportunistic, hypervirulent, and multidrug-resistant (MDR) (Martin and Bachman., 2018).

Most infections of *K. pneumoniae* were caused by the classic *K. pneumoniae* (cKp) strains which remained living in the hospitals, and then caused infection in weak patients. The cKp strain appears to be

different from the hvKp. Genetic factors of high virulence phenotype of hvKp are present on a large virulent plasmid, and there may be integrative conjugal elements. hvKp infection often occurs in multiple sites and subsequently spreads, which makes it more difficult to treat and control. The variant strain hvKp, firstly discovered in the Pacific Circle, can lead to community-acquired, aggressive, and metastatic infections in diabetes or normal immune function with liver abscesses, endophthalmitis, meningitis, and septic arthritis in young people in (Russo and Marr., 2019).

The inflammatory response is inhibited, and the clearance to bacteria is reduced by these modifications. At the same time, the ability to obtain iron is critical to the growth and replication of bacteria. There are four iron-absorbing molecules (iron carriers) in *K. pneumoniae*: *enterobactin*, *yersiniabactin*, *salmochelin*, and *aerobactin*, respectively. Existing in both typical and highly virulent strains, e*nteromycin* has the highest affinity for iron, and it is considered to be the main iron absorption system. Unlike enteromycin, gastrin and yersinide are more prevalent in hvKp than in cKp (Happel *et al* ., 2005).

1.2.12.2.1.2. *Acinetobacter baumannii*

The genus *Acinetobacter* is a large and diverse group of biochemically, physiologically, and naturally multi-skilled bacteria. *Acinetobacter spp*., a ubiquitous coccobacillus genus, is characterized as glucose non-fermentative, non-motile, catalase-positive, oxidase-negative, and non-fastidious Gram-negative bacteria (Lee *et al* .,2017)**.**

Even though these species differ in their pathogenicity, antimicrobial resistance, and epidemiology (Chen *et al* .,2014).

In the hospital environment, *Acinetobacter* is also easily isolated, especially the *A. baumannii* species. When recovered from clinical samples, most species may have some significance as human pathogens (Peleg et al .,2008).

A. baumannii is an opportunistic pathogen mainly associated with HAI. Opportunistic pathogens can also cause super infections, especially in combination with viral respiratory tract infections in hospitalized patients (Sharifipour *et al* .,2020).

Belonging to the ESKAPE group (which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.)*, *A. baumannii* stands out with its ability to effectively escape antibiotic treatments, affecting mainly immunocompromised and critically ill patients in ICUs (Monem *et al* .,2020).

While *A. baumannii* is an important nosocomial pathogen that can cause various diseases, community-acquired infections by this microorganism (including pneumonia and bacteremia) are less common, but it is associated with relatively high mortality (Chusri *et al* .,2019).

Mainly, carbapenem resistance in A. baumannii is an essential concern because this type of antimicrobial is the last line of defense used to treat infections caused by multidrug-resistant Gram-negative bacteria. Infections caused by carbapenem-resistant *Acinetobacter baumannii* (CRAb) cause more extended hospitalization, adverse outcomes, and increased costs than

infections caused by carbapenem-susceptible strains (Boinett *et al* .,2019).

CRAb readily contaminates the hospital environment and health care providers' hands, can survive for prolonged periods on dry surfaces, and can be spread by asymptomatic colonization; these factors make CRAb outbreaks in acute care hospitals challenging to control (Nutman *et al* .,2016).

Co-infection with *A. baumannii* secondary to SARS-CoV-2 infections has been reported multiple times in literature during the COVID-19 pandemic including Wuhan (China), France, Spain, Iran, Egypt, New York (USA), Italy, and Brazil . The incidence of secondary infections (mostly lower respiratory tract infections) due to *A. baumannii* was said to be as high as 1% of hospitalized COVID-19 patients in an Italian hospital (Ripa *et al* .,2021).

A systematic review and meta-analysis of bacterial co-infection and secondary infection in patients with COVID-19 reported 3.5% and 14.3% for co-infection and secondary infection, respectively. However, in general, bacterial infection was 6.9%, varying slightly in the patient population, ranging from 5.9% in hospitalized patients to 8.1% in critically ill patients (Langford *et al* .,2020).

1.2.12.2.1.3. *Streptococcus pneumonia*

Streptococcus pneumoniae (*S. pneumoniae*) is an opportunistic extra-cellular Gram-positive bacterium that usually colonizes the mucosa of the human upper respiratory organs. S. pneumoniae can cause many diseases, including diseases that have mild symptoms but are common,

such as otitis media, sinusitis, and bacterial pneumonia, as well as severe invasive pneumococcal diseases (IPD) such as bacteremia and meningitis. *S. pneumoniae* is the most common pathogen in community-acquired pneumonia (CAP) and it is also the major pathogen in nosocomial pneumonia (Weiser *et al* .,2018).

Because the incidence and mortality of CAP among elderly people are both high, *S. pneumoniae* pneumonia has al-ways been a focus of attention. The main symptoms are mostly fever and cough as well as dyspnea and shortness of breath. Although COVID-19 and *S. pneumoniae* pneumonia are characterized by pulmonary inflammation caused by different pathogens, they have similar clinical symptoms and incidence rates. The incidence of COVID- 19 seems to be higher in older men and patients with comorbidities. Particularly when the reverse transcription–polymerase chain reaction (RT-PCR) detection result is negative, there are some difficulties in distinguishing between COVID-19 and *S. pneumoniae* pneumonia. Furthermore, although COVID-19 is somewhat under control in China, the number of COVID-19 patients worldwide still shows an increasing trend. Therefore, there are many reports on chest computed tomography (CT) findings of COVID-19.(Lin *et al* .,2020).

The drugs for *S. pneumoniae* pneumonia are mainly antibiotics, such as β-lactams (penicillin and cephalosporin), quinolones, and macrocyclic lipids (Pallares *et al*., 2020).

The Diagnosis and Treatment of COVID-19 (the provisional 6th edition) mentioned using α-interferon combined with antiviral drugs, such as lopinavir, for treatment and avoiding blind or inappropriate use of antimicrobial agents (Lu ., 2020).

Therefore, in the COVID-19 outbreak, familiarity with the CT signs of COVID-19 and its differential diagnosis from *S. pneumoniae* pneumonia not only can provide powerful imaging evidence for diagnosis but also can screen the patients who have symptoms but do not receive timely nucleic acid detection. Suspected patients should be isolated for treatment as soon as possible to avoid disease progression into severe illness, which is conducive to controlling the development of the disease and alleviating the shortage of medical resources (Machnicki *et al*., 2021)

There are some limitations in our study. Because of time and sample- size constraints, dynamic imaging data of COVID-19 and *S. pneumoniae* pneumonia after treatment were not analyzed in this study, which could be included in future studies. In summary, the findings of GGO, the crazy paving sign, and abnormally thickened interlobular septa on chest CT were higher in COVID-19 than *S. pneumoniae* pneumonia in this study, whereas the findings of consolidation lesions, bronchial wall thickening, pleural effusion, and centrilobular nodule on chest CT were lower in COVID-19 than *S. pneumoniae* pneumonia (Dirkx *et al*., 2021).

In addition, disease foci in *S. pneumoniae* pneumonia mainly showed a lung lobular and segmental distribution. The most important differential points were whether the disease foci had the CT features of lung lobular and segmental distribution, the crazy paving sign, abnormally thickened interlobular septa, and consolidation lesion (Zhou *et al*., 2021).

1.2.12.2.2.Other pathogenic and nosocomial bacterial isolates in current study

Streptococcus parasanguinis , Staphylococcus lentus, Serratia marcescens , Lactococcus garvieaeare , Raoultella planticola, staphylococcus hominis, Sphingomonas paucimobilis.

1.2.12.2.3. Normal flora isolates in current study

Staphyloccous hemlyticus , Kocuria kristinae , Staphylococcus epidermidis , Granulicatella elegans , Streptococcus mitis , Rothia dentocariiosa , Granulicatella adiacens.

Chapter Two

Materials and Methods

Materials and Methods

2.1.Patients Specimens

2.1.1.Study Design

A total of (100) samples were taken from patient who suffering from SARS-COV2, during the period from September 2021 to November 2021, who admitted to Marjan Medical City and Imam Al-Sadiq Teaching Hospital in Babylon Governorate . samples were taken from COVID-19 patients divided in to two groups, 50 Nasopharyngeal swabs 28 males (56%) and 22 females (44%) to detect SAR-COV-2 infection by RT-PCR and the same patients take another Nasopharyngeal and oral pharyngeal swabs for bacterial infections with (50) samples as healthy (control) These study including questionnaire as (**Appendix1**).

2.1.2. Collection of Samples

Nasopharyngeal swabs were collected from all subject included all patients and was conducted directly on them. Placed in tube contain 2ml of viral transport medium (VTM) then stored at -20 ºC until the time RNA extraction and reverse transcriptase polymerase chain reaction processing. Nasopharyngeal and oral pharyngeal swabs for bacterial infections diagnostic bacteria by vitik2 system to Gram-positive and Gram-negative these bacteria co-infection to SARS-cov2 patients and increase severity of COVID-19**.**

2.1.3.Ethical approval

All subjects involved in this work are informed and the agreement will obtained verbally from each one before the collection of samples. This study was approved by the committee on research ethics at college of medicine, University of Babylon, Iraq.

2.2. Materials

2.2.1. Equipment and Instruments

Table (2.1) The Equipment and Instruments that used in this study with their companies and countries of origin.

	Instruments and Equipment	Manufacturing	Origin
1.	AURA TM PCR Cabinet	EuroClone	Italy
2.	Autoclave	Stermite	Japan
3.	Amies transport media	Zahrat Al-Raw	China
4.	Bio TDB-100, Dry block thermostatbuilt	BioSan	Lativa
5.	Benson burner	Membrane	Germany
6.	Combi-spin	Biosan	Lativa
7.	Disposable syringe 10 ml,5ml and 3ml	Sterile EO.	China
8.	Digital camera	Samsung	China
9.	Disposable pipette tips	VWR internatic	USA
10.	Eppendorf tubes	Bioneer	Korea
11.	Exispin vortex centrifuge	Bioneer	Korea
12.	Gel electrophoresis	Shandod Scient	UK
13.	Facial mask	Unimed	K.S.A.
14.	Face Shield	ROHS	China
15.	Forceps	Spin react	Spain
16.	High Speed Cold Centrifuge	Eppendorf	Germany

17.	Incubator	Memmert	Germany
18.	Inoculating loop	HiMedia	India
19.	Laminar Hood	Labogene,	Denmark
20.	Latex gloves	Unimed,	K.S.A.
21.	Light Microscope	Olympus	Japan
22.	Micropipettes 5-50, 0.5-10,100-1000µl	CYAN	Belgium
23.	Miniopticon Real-Time PCR	BioRad	USA
24.	Mini-Power Supply 300V, 2200V	Labnet	China
25.	McFarland tube standard 0.5	Mastgroup	England
26.	Micro tube 2ml and 1.5ml (for reverse transcription)	Bioneer	Korea
27.	N95 mask	Amir Teb	IRAN
28.	Nano drop	Nabi	Korea
29.	Petri dishes	Dolphin	Syria
30.	Refrigerator	Concord	Lebanon
31.	RT-PCR	Sacace	Italy
32.	Sensitive Balance	Sartorius	
33.	Sterile test tube	Superestar	India
34.	Thermocycler PCR	Bio-Rad	USA
35.	UV Transilluminator	ATTA	Korea
36.	Vitek 2 system	Biomerieux	France
37.	Vortex	CYAN	Belgium

38.	Water Bath	CYAN	Belgium
39.	Water distilater	Rovsun	China

2.2.2.Kits

Table (2.2) The kits used in this study with their companies and countries of origin

1.	**Maxime PCR PreMix**	**iNtRON**	**Korea**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris-HCl pH 9.0		
	KCl		
	$MgCl_2$		
	Stabilizer and Tracking dye		
2.	**Prime Script TM RT reagent Kit**	**Genes labs**	**Korea**
	Prime Script TM RT Enzyme RNase Free dH2O EASY Dilution Buffer (for Real Time PCR)		
	HS-Taq DNA Polymerase, PCR buffer, MgCl2 and dNTP's		
3.	**GP- ID card -Vitek 2 syste compact kit**	**Biomeriux**	**France**
4.	**GN -ID card -Vitek 2 system compact Kit**	**Biomeriux**	**France**

2.2.3. Primers

The PCR primers for detection SARS-COV-2 based on ORF1ab protein were design in this study using NCBI-Genbank Sequence database and primer 3 plus. primers was provided from Macrogen Company, Korea as shown in tables (2.3)

Table (2.3) The new designed PCR primers for ORF1ab genes of SARS-COV2

Primer	Sequence (5'-3')		Product Size
ORF1ab gene	F	CGGATGGCTTATTGTTGGCG	639bp
	R	ACAACTCCGGATGAACCGTC	

2.2.4. Chemical and Biological Materials

2.2.4.1. Chemical Materials

The chemical and reagents and their manufacturer which have been used in the study are listed in **table (2.4)**

Table (2.4) Chemical and reagents have been used in the study

	Materials	Manufacturing	Origin
1.	5 × PrimeScriptTM mix	Intron	Korea
2.	6X Loading dye	Intron	Korea
3.	70% alcohol(Ethanol)	JOUD TOL Al – Kafeel	Iraq
4.	Absolute Ethanol	Scharlau	Spain
5.	Agarose	BioBasic	Canada
6.	Ethidium Bromide	BioBasic	Canada

7.	Free Nuclease Water	Bioneer	Korea
8.	Gram stain set (Crystal violet, Methylene blue, iodine, absolute alcohol, safranine)	HiMedia	India
9.	H2O2 (3%)	Panreac	Spain
10.	Ladder 1000bp and Ladder 2000bp	Intron	Korea
11.	TBE buffer 10X	BioBasic	Canada

2.2.4.2. Culture Media

A group of culture media was prepared according to the instructions of the companies and sterilized by autoclaving at 121^{o}C for 15 minutes.

Table (2.5) Culture Media Used During This Study

Culture media	Company	Country
Blood Agar	**CondaLab**	**Spain**
MacConkey Agar	**Liofilchem**	**Italy**

2.3. Methods

The main methods included in this study are shown in the figure (2-1).

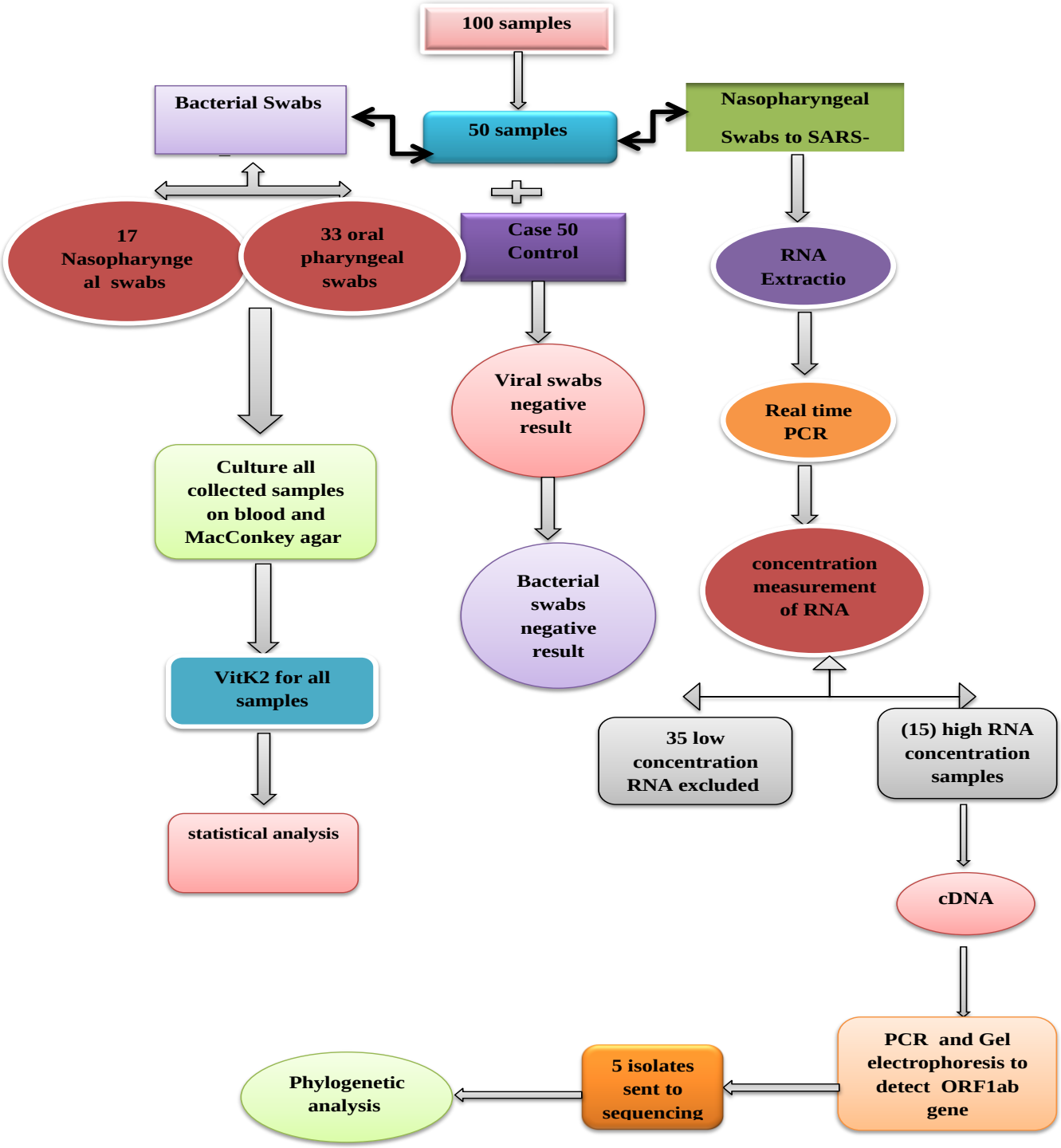


Figure (2.1) Step of study

2.3.1 . Preparation of media for isolation and identification of bacterial isolates

A group of culture media was prepared according to the instructions of the companies and sterilized by autoclaving at 121^{o}C for 15 minutes**.**

2.3.1.1.Blood agar medium (PH: 7.1)

Blood agar medium was prepared by dissolving 40 gm of blood agar base in 1000 ml D.W., this media was autoclaved at 121^{o}C for 15 minute, and then cooled to 45^{o}C 5% of fresh human blood was supplemented and mixed with base medium . This medium was used as enriched and differentiate Gram positive bacteria and Gram negative bacteria (Niederstebruch *et al*., 2017). **Appendix (2).**

2.3.1.2. MacConkey agar medium

MacConkey agar medium was prepared by dissolving 49.53 grams in 1000 ml of purified / distilled water . The medium is boiled for a few seconds until the ingredients are completely dissolved. Sterilize by autoclaving at (121 ° C) pressure for 15 minutes. Cool to 47 ° C, mixed well before pouring into sterile Petri dishes. This medium was used as a selective medium Gram negative bacteria (Allen., 2005). **Appendix (3).**

2.3.2.Bacterial Transport Medium

Amie's media were used for transport the clinical bacterial swabs to the laboratory. **Appendix (4).**

2.3.3. Viral Transport Medium

Media used for viral transport. It is containing N-2hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), gelatin, bovine serum albumin (BSA), sucrose and compatible antibiotics.(Vircell,Spain). **Appendix (5).**

2.3.4. Nasopharyngeal and Oral pharyngeal Swab Procedure

To correctly perform the nasopharyngeal swab, the patient must be seated comfortably with the back of their head against the headrest. Furthermore, it can helpful to lift the tip of the patient's nose. From the side view of the patient, the swab is inserted in the nose horizontally, along an imaginary line between the nostril and the ear. Upon reaching the posterior wall of the nasopharynx, rotate the tip of the swab continuously for a few seconds, before gently removing the swab. Be careful not to insert the swab in an upwards direction or limit the sampling to only the anterior portion of the nasal cavity as this would reduce the probability of taking a significant amount of viral RNA.Oral pharyngeal sampling is easier to perform. The swab is directed toward the rear wall of the oropharynx and it is rotated a few times before removal. After taking the sample, it is necessary to insert both swabs in the same tube, breaking the rod with one swift and controlled movement. Finally, carefully reset the cap. The specimen were kept frozen at – 20 C° for real time reverse transcriptase –PCR test (rRT-PCR) .(Petruzzi et al ., 2020).

For bacterial studies, nasopharyngeal and oral pharyngeal swabs were collected and transmitted by bacterial transport media then inoculated on to blood agar and MacConkey agar . Thereafter, bacterial isolates for further tested for biochemical tests by Vitek 2 System (Biomerieux, France).**(Figure 2.2)**

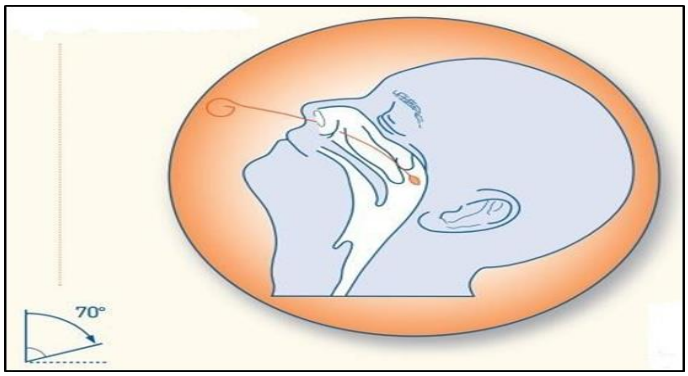


Figure (2.2) Nasopharyngeal swab technique (LeBlanc *et al* .,2020).

2.3.5. Genomic Viral RNA Extraction

RNA were extracted manually as below:

1- Lysis buffer (extraction kit) 300 µl were added to 100 µl from sample.
2- Four hundred µl from prep were added and mixed by centrifuge 3mint.
3- Five hundred µl of wash1 was used, it was centrifuged at 1100 rpm for 1 minute.
4- Five hundred µl of wash 2 was added, then centrifuged at 1100 rpm for 1 minute.
5- Two hundred ul of wash 2 was added, then centrifuged 1100 rpm for 1 minute.
6- Centrifuge lid was closed at 65 c^{o} for 5 minutes.
7- Ninety µl preheat dilution was added, it was centrifuged for 1 minute and mixed well. RNA ready to be used for next part.

2.3.5.1. Real -Time Polymerase Chain Reaction

Real Line SARS-CoV-2(A format) part1(BI1019-96)

Real-Time PCR technique was performed for detection of SARS-COV-2 based amplification for a major gene: Fam gene and nucleocapsid protein N gene and

envelope E gene with internal control using Kit. This technique was carried out following steps:

Table (2.6) The Real Line Cycler should be programmed as follows

Step no.	Temperature	Time	No. of cycle
Step1	35C^0	20 min	1 cycle
Step2	95C^0	5 min	1 cycle
Step3	94C^0 64C^0	10 sec 15 sec	50 cycles
Step 4	80C^0	1sec	1 cycle
Step 5	10 C^0	Hold	
Measurement of Fluorescence			
Rotor Gene Cyclers			
Step1	32C^o	20 min	1 cycle
Step2	95C^o	5 min	1 cycle
Step3	94C^o 64C^o	10 sec 15 sec	50 cycles

2.3.5.2. Real-Time PCR Data Analysis

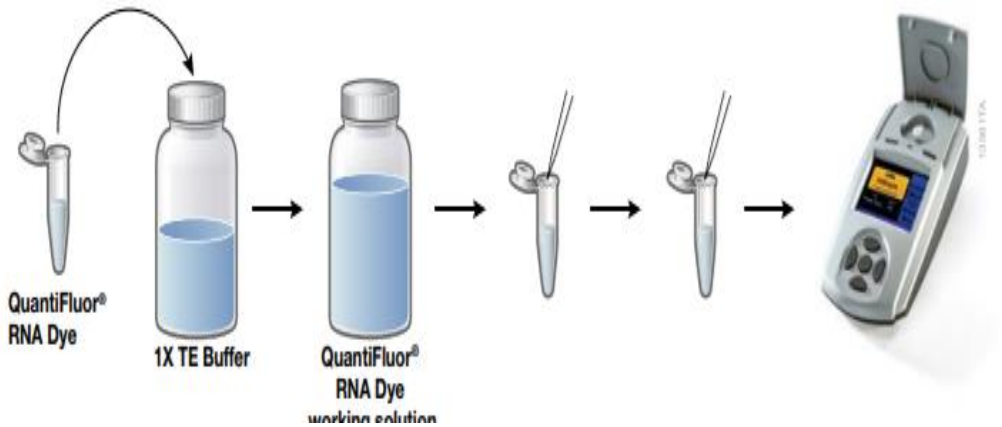
The analysis of Real-Time data was done by looking at the threshold cycle number (CT value) that showed positive amplification in the Real-Time PCR cycle number as shown in **table(2.7**)

Table (2.7) PCR results interpretation

Detection Channel				
FAM/Green	HEX/Yellow	ROX/orang		Cy5/Red
SARS-COV	IC	SARS-CoV-2 E- gene		SARS-CoV2 N-gene
Analyzed Samples				
+	Not considered	+	+	RNA of SARS-CoV2 is considered*
+	Not considered	-	-	RNA of SARS-like coronaviruses is detected, RNA of SARS –CoV2 is not detected
-	+	-	-	RNA of SARS-like coronaviruses is detected, RNA of SARS –CoV2 is not detected
Positive control sample				
+	Not considered	+	+	Positive Result
-	+	-	-	Negative Result

2.3.5.3. Measuring of RNA concentration

A fluorescent RNA-binding dye in the QuantiFluor® RNA System allows for sensitive quantification of tiny quantities of RNA in solution. Many biological applications require the detection and quantification of small amounts of RNA, including determining the yield of in vitro transcribed RNA and measuring RNA concentration :



Figure(2.3) Illustration of the QuantiFluor® RNA System's single-tube format quantification.(Kocabas *et al* .,2015)

2.3.5.4. Procedure

1- 20X TE Buffer was diluted 20 times with nuclease-free water to make 1X TE buffer (not provided). Mix 1 mL of 20X TE Buffer with 19 mL of Nuclease-Free Water (Cat.# P1195).

2- workable solution: Calibration with a Low Standard; To make the QuantiFluor® RNA Dye working solution, dilute the QuantiFluor® RNA Dye 1:2,000 in 1X TE buffer, combine 2µl of QuantiFluor® RNA Dye with 3,998 µl of 1X TE buffer and well mix . The working solution kept away from light by covering with foil or placing in the dark.

3- a blank sample by filling an empty 0.5ml PCR tube with 200 µl of QuantiFluor® RNA Dye working solution. This is the blank sample that will be utilized in Step 8. The tube kept away from the light.

4- the RNA Standard Sample as follows: Calibration with a Low Standard: Prepare a 10ng standard by first diluting the RNA Standard (100ng/µl) 1:100 in 1X TE buffer (10µl of RNA Standard + 990µl of 1X TE buffer). Next, add 10µl of diluted standard to 200µl of QuantiFluor® RNA Dye working solution in a 0.5ml PCR tube.

5- Start preparing RNA: Add 1–20µl of unknown sample to 200µl of QuantiFluor® RNA Dye working solution in a 0.5ml PCR tubes. For example, add 1µl sample to 200µl QuantiFluor® RNA Dye working solution. Vortex well, and protect tube from light.

6- Incubate the prepared samples at room temperature for 5 minutes, protected from light.

7- On the QuantusTM Fluorometer, select the RNA procedure with the low" depending on the standard calibration supplied.

8- Calibrate the QuantusTM Fluorometer by reading the blank (made in Step 3) and standard (produced in Step 4) RNA in the Calibration screen, then selecting "Save."

9- The volume of the sample and the concentration units desired 2 µl were entered.

10- The QuantusTM Fluorometer is used to measure the fluorescence of RNA concentration. The value given represents the original sample's concentration

Figure: (2.4) Quantus™ Fluorometer.(Kocabas *et al* .,2015)

2.4. Conversion of RNA to cDNA

The Prime Script TM RT reagent Kits in current study we used intended for reverse transcription that is optimized for real-time RT-PCR. It employs Prime Script TM RTase, which has a wide range of applications and allows for quick and efficient cDNA template synthesis for real time PCR. The step-by-step experimental approach is straight orward and well-suited to high-throughput analysis. This kit can be used in conjunction with SYBR® Premix, a real time PCR reagent. used for the Kit Components :

1. Prime Script TMRT Enzyme MixI
2. RNase Free H_2O
3. EASY Dilution Buffer (for Real Time PCR

2.4.1.Principle

1-Allows for the rapid and efficient synthesis of cDNA templates for Real Time PCR. This kit is ideal for two-step real-time RT-PCR

2-Included in the kit are Random 6 mers and Oligo dT Primer for use as reverse transcription primers. The reaction can be carried out using a combination of these two primers, or the primer based on the objective of the experiment. Gene Certain Primers can also be used to detect specific genes.

3-For Real-Time RT-PCR quantitation, a standard curve must be established. Because low amounts are required for a valid standard curve, whole RNA or cDNA must be diluted after reverse transcription. Dilution with water or TE, on the other hand, can limit the range of the curve due to unstable dilution at low concentrations. When using EASY Dilution Solution (for Real Time PCR) for dilution, the results are accurate at lower concentrations and the production of a wide-range standard curve is facilitated.

2.4.2. Procedure

1- On ice, prepare the following reaction mixture was mixed as shown below To compensate for pipetting losses, prepare a little larger volume of master mix than is required. Add the RNA sample after dispensing aliquots of this mixture into the micro tubes < For 1 reaction >.

Reagents	Volumes
5 × Prime Script TM mix	**2µl**
Total RNA	**l µl**
RNase Freed H_2O	**Total to 10µl**

2- The reaction mixture was uploaded in real-time pcr for 1 step only condition 37℃, 15 minutes*3 (reverse transcription) , 85℃, 5 sec (inactivation of reverse transcriptase with heat treatment), 4°C in.

2.4.3.PCR master mix preparation

PCR master mix was prepared by using (**Maxime PCR PreMix Kit**) and this master mix done according to company instructions as Table**(2.8)**

Table (2.8) PCR master mix preparation for ORF1abgene

PCR Master mix	Volume
DNA template	5µL
ORF1ab gene Forward primer (10pmol)	1µL
ORF1ab gene Reverse primer (10pmol)	1µL
PCR water	13µL
Total volume	**20µL**

After that, these PCR master mix component that mentioned in table above placed in standard **PCR PreMix Kit** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (T100 Thermal cycler. BioRad USA).

2.4.4. PCR thermo cycler conditions

PCR thermocycler conditions by using convential PCR thermo cycler system as following Table **(2.9).**

Table (2.9) PCR thermo cycler conditions

PCR Step	Temp.	Time	Repeat
Initial Denaturation	94ºC	5min	1
Denaturation	94 ºC	30sec.	30 cycle
Annealing	58 ºC	30sec	
Extension	72 ºC	1min	
Final extension	72 ºC	5min	1
	4 ºC	Forever	-

2.4.5. PCR product analysis

The PCR products of was analyzed by agarose gel electrophoresis following steps:

1- 1.5 % Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5µl of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1 hour.

5- PCR products were visualized by using UV Transilluminator.

2.5. Isolation and identification of bacterial co-infection in SARS – COV2 patient by Vitek2 compact system

The Vitek 2 compact system was used to confirm the biochemical and deferential tests for bacteria . The system had been performed according to the manufacturer's instructions (Biomerieux –France). **Appendix (6**).

2.5.1.Principle

The VITEK 2 compact is an automated microbiology system utilizing growth-based technology. The system is based on accomm- odate the same colorimetric reagent cards that are incubated and interpreted automatically(Pincus ., 2007) .

2.5.2. Assay procedure

Three ml of normal saline are placed in a khan tube and inoculated with a loop full of isolated colony for suspension. The colony must be aged 24 hr. .The khan tube inserted into a densities plus instrument to measure the optical density which is 0.5-0.6 of microorganism suspension. The standardization inoculums placed into the cassette and a sample identification number entered into the computer software via barcode. Thus, the Vitek 2 card connected to the sample ID number. Then the cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader / incubator module **Appendix (7)**

2.6. Statistical Analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Pearson's chi square (X2) and Fisher-exact tests were used to find the association between categorical variables. A p-value of ≤ 0.05 was considered as significant (Daniel and Cross, 2018)

Chapter Three

Results and Discussion

Result and Discussion

3.1. Distribution of study population according to gender

The total number of the patients who were positive SARA-COV-2 were 50 samples ,N= 28 (56 %) of them were males and N= 22(44%) were females, in comparing with 50 apparently healthy control individuals N=25 (50%) males and N= 25 (50%) females. The current study show not significant difference at p (0.54) and the incidence of males is higher than females as shown in table **(3.1).**

Table (3.1) Distribution of SARS-COV-2 according to gender

Gender * study groups						
			Groups		Total	P Value
			Patient	Control		
Gender	Male	N	28	25	53	0.54
		%	56 %	50 %	53 %	
	Female	N	22	25	47	
		%	44 %	50.0%	47 %	
Total		N	50	50	100	
		%	100 %	100 %	100 %	

Also, when the relationship between gender and occupation was shown, it showed that the highest percentage was of women housewives N=19 (38.0%) females , retired N=13 (26.0%) males and show significant difference at p value (0.001), as shown in the **table(3.2)**

Table (3.2) Correlation occupation and gender

gender * occupation Cross tabulation							
			occupation				
			retired	employee	free job	house wife	P value
gender	male	N	13	5	10	0	0.001
		%	26.0%	10.0%	20.0%	0.0%	
	Female	N	1	2	0	19	
		%	2.0%	4.0%	0.0%	38.0%	
Total		N	14	7	10	19	
		%	28.0%	14.0%	20.0%	38.0%	

This result is comparable with result of previous studies done show not resemble with result study neighboring countries male 300 (48.9%) and female 313(51.1%) show significant difference at p(0.018) (Alamri *et al*., 2021).

The total number of the patients who were positive SARA-COV-2 was 78. 50(64.10%) of them were males and 28(35.90%) were females, in comparing with 10 apparently healthy control individuals, males were 7(70%) and 3(30%) were females. The result of statistical analysis demonstrated that statistically significant differences (Alia and Areej., 2021).

Furthermore, this result is consistent with several research conducted in Wuhan, China, which indicated that, males have a considerably higher risk of infection than females such as (Wu *et al*., 2020).

The result were not similar to recent findings for gender and its relationship to patient condition, which found significant results for both groups, with male out performing females with a p value of (0.001). Males died at a rate (10.6%) higher than females, which was a significant according to data from Germany, Italy, Spain, and Switzerland, the male case fatality rate was higher than the female case fatality rate (Gebhard and Klein., 2020).

While other studies have explained the sex difference in COVID-19 mortality by higher expression of ACE2 receptor which was found in Asian males . (Zheng *et al* ., 2020).

This might be due to the X chromosome and sex hormones present in women that play a protective role (via innate and adaptive immunity) in susceptibility to viral infections (Jaillon *et al* ., 2019).

Consistent with the current findings, it has been reported that males had higher mortality than females(Wenham *et al* ., 2020).

The angiotensin-converting enzyme 2 (ACE2) allows SARS-COV-2 to enter the blood stream (Hoffmann, 2020).

These gender differences could be related to changes in ACE2 expression caused by hormonal influences. Because females have a larger degree of heterodimer construction than males, male SARS-COV2 spike receptors have a higher affinity for ACE binding (Joyce *et al*., 2020).

Angiotensin-converting enzyme 2 genes are present on the X-chromosome, allowing females to be heterozygous and genetically diverse compared to men who are homozygous enhance in SARS-COV-2 infection (Gemmati, and Veronica ., 2020).

Preliminary studies have suggested that while the prevalence of infection is the same in men and women, male patients are more likely to be hospitalized, have a more severe course of disease and higher mortality (Leung *et al* ., 2004).

Sex differences are well known in innate and adaptive immunity with resultant sex-specific responses to vaccines and infections (Scully *et al* ., 2020).

In response to viral infection, females have stronger humeral and cell-mediated immunity than males (Mössner *et al*., 2010 ; Syrett *et al*., 2019).

In addition, the sex hormone interaction to specific receptors on immune cells plays a critical role in initiating the process. They have the potential to grow and estrogen boosts antibody production (Gemmati and Veronica, 2020).

This could be as a result of estrogen has a beneficial effect and promotes IgG and IgM production. The opposing hand's expression testosterone hormone has a suppressive impact. SARS-COV-2 enters the body via the trans membrane protease serine-2, which is required for SARS-COV-2 to spread throughout the body, according to another method. Androgen receptor is more highly expressed in males than females, with higher ACE2 levels in various organs, and androgen receptor has a role TMPRSS2 transcription. This could explain why males are more severely affected and die from SARS-COV-2 infections than females (Hoffmann., 2020).

Frequency of occupational of patients can be used to highlight gender inequalities in the biological repercussions of health and illness. Until recently, gender variations in occupational health received little

attention, and the majority of social science literature concentrated on inequalities in exposure to health risks.(Kwon *et al*., 2021)

3.2. Distribution of study population according to age

The results revealed that the highest COVId-19 infection rate was in persons >61in the rate of 44.0% and the lowest number of infected people were at the age of <30 in the rate of 8.0% . The result show significant difference at p value (0.080) **as in table (3.3).**

Table (3.3)Distribution of SARS-COV-2 according to age

Age group and study					P value
			patient	Control	
Age group	<30	N	4	13	0.080
		%	8.0%	26.0%	
	31-40	N	4	6	
		%	8.0%	12.0%	
	41-50	N	5	6	
		%	10.0%	12.0%	
	51-60	N	15	13	
		%	30.0%	26.0%	
	>61	N	22	12	
		%	44.0%	24.0%	
Total		N	50	50	

It was found in other not similar studies, who reported that, most cases were between the ages of 29 and 50, with those aged (30-39) years old being the most infected age range in Iraq (Goldstein and Lipsitch., 2020)

reported that, younger adults, particularly those under the age of 35, frequently have a high incidence of SARS-CoV-2 infection in the community, and the mortality rate in very elderly is significantly higher

than elderly using serological tests in the United States . Another study conducted by Tang *et al*., (2020)

discovered that, the majority of infections occurred in young people aged 482(18-29), 1097(30-39), 841(40-49), 1011(50-59), 886(60-69), and > 70 (563) years during an assessment of 4880 confirmed cases SARS-COV2 infection using RT-PCR in China. The similar study reveal, the elderly were more vulnerable to the diseases with severe stages (Crespo *et al.*, 2020) .

That, the case fatality rate of patients aged 70 to 79 years was (8.0%) compared to 14.8% for patients aged 80 years and older. A results of (Niu *et al*., 2020) .

has been found that, older patients (> 65 years) with comorbidities and acute respiratory distress syndrome (ARDS) have an increased risk of death(Gold., 2020).

reported that, the elevated infection incidence of SARS-COV2 among the elderly may be attributable to a number of comorbidities . Among those infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), elderly patients have had the most severe outcomes, including the highest death rates, whereas infected younger persons, particularly children aged 1–18 years, if symptomatic at all, are far more often mildly ill (Cevik *et al* ., 2020).

While this age-dependent pattern of illness severity has become well established, the roles of different age groups in transmission has not been as clear. Recently, evidence has accumulated that susceptibility to infection generally increases with age (Jing *et al* ., 2020).

This, however, does not suggest that the oldest individuals necessarily have the highest SARS-CoV-2 incidence in fact, serological studies suggest that younger adults, particularly those younger than 35 years, often experience the highest cumulative rates of infection (Takita *et al* ., 2020).

reported that older patients (> 65 years) with comorbidities and ARDS are at increased risk of death(Bialek *et al*., 2020).

report that SARS-COV-2 occurs more frequently in old age individuals and those in high susceptibility to ICU admission and mortality in United States. The high infection rate of SARS-COV-2 among old age may be due to several comorbidities among them was observed (Pahan and Pahan., 2020; Zhou *et al*., 2020).

3.3.Type of bacteria co -infection to SARSCOV-2

The results showed that the highest infection was with bacteria *Klebsiella pneumonia* followed by bacteria *Streptococcus pneumonia* and *Acinetobacter baumannii* as in **figure 3-1.**

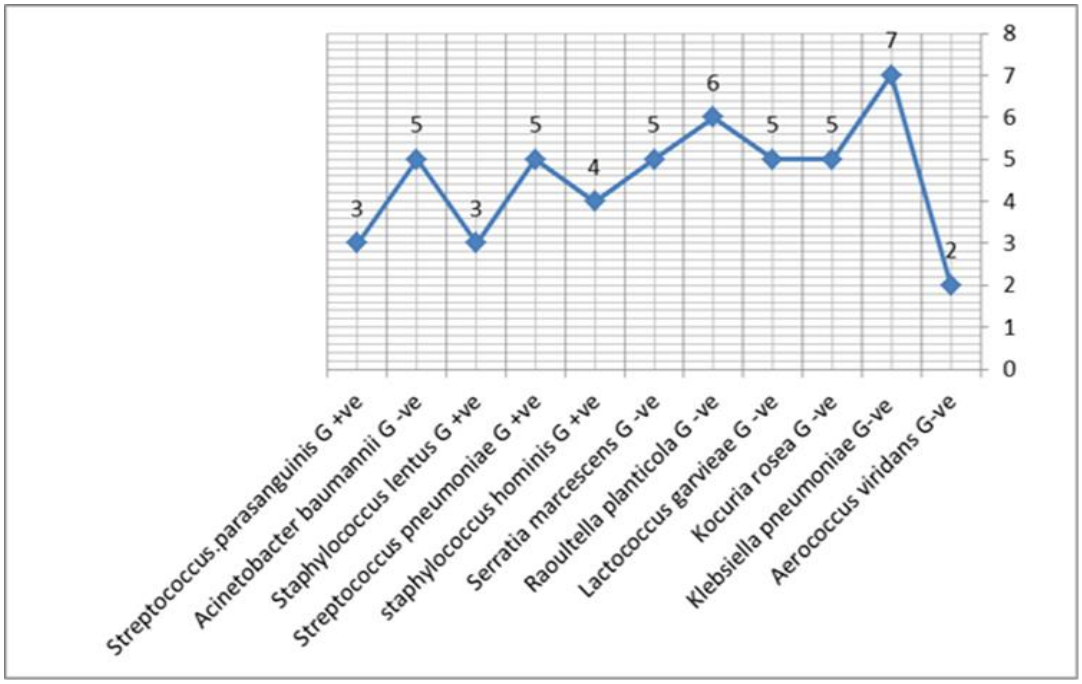


Figure (3.1) Type of bacteria co- infection in COVID-19 patients

The results were compared with the results of other research and it was found that, With regard to the unit of admission, COVID-19 patients with bacterial infections were more frequently admitted to the intensive care unit (ICU) (56%) compared to patients without bacterial infections who were mostly admitted to the ward (38%) ($p < 0.001$). The use of invasive devices such as endotracheal tube and central venous catheters were also more frequent among cases compared to controls ($p < 0.001$) .Patients with bacterial infections were managed with invasive ventilation in 56% of cases compared to 14% controls ($p < 0.001$) and with noninvasive ventilation in 64% of cases compared to 34% controls ($p = 0.003$). A comparatively higher proportion of patients who had bacterial infections had received treatment with systemic steroids (92%) ($p = 0.001$).(Du *et al* ., 2020)

All patients with bacterial infections had received antibiotics. The choice of empiric antibiotics was based on local anti biogram and institutional guidelines for community-acquired pneumonia and definitive

antibiotic treatment was decided based on the identification and sensitivity pattern of the isolated organism (Nasir *et al* ., 2021)

Admission to the ward unit was found infection and treatment with steroids was also a significant risk factor to be protective (odds ratio [OR]: 0.15; 95% CI: 0.02–0.75). bacteremia and one had candidemia. The most frequently isolated organism from blood was multi-drug resistant (MDR) *Acinetobacter* in (3/10) patients, followed by ceftriaxone resistant. Escherichia coli in two patients, vancomycin-resistant Enterococcus in two patients and ceftriaxone-resistant *Klebsiella pneumonia* in one patient. In patients who developed hospital-acquired pneumonia, the most commonly isolated pathogen was MDR *Acinetobacter* species in (13/28), followed by MDR Pseudomonas aeruginosa in 24% (5/28), *Stenotrophomonas maltophilia* in(5/28), methicillin-resistant *Staphylococcus aureus* in 3/28, *K. pneumoniae* in2/28 patients, and E. coli in 2/28 patients. Among patients with community-acquired pneumonia, the most common organism was *Staphylococcus aureus* (4/8) in which two were methicillin-resistant and *P. aeruginosa* in 3/8 ceftriaxone resistant *K. pneumoniae* in (1/8). (Nasir *et al* .,2021).

The COVID-19 pandemic caused a large number of immunocompromised individuals to be hospitalized and some reports indicated that some COVID-19 patients were diagnosed with secondary infections (Rasmussen *et al*., 2020).

The specific source and nature of these infections have not yet been fully investigated; however, there is evidence indicating that multidrug-resistant bacteria are among those microbes responsible for the development of these secondary infections. In one study, five cases

(5.1%) with bacterial confections including *Acinetobacter baumannii* and *Klebsiella pneumoniae* were found among 99 patients (Chen *et al*., 2020).

While in another study, four cases (9.8%) with secondary bacterial infections were reported among 41 patients (Huang *et al* .,2020). 221 patients with SARSCoV-2 pneumonia were admitted to Zhongnan Hospital, Wuhan, China. Among them, 25.8% (57/221) patients were afflicted with co-infections, and among these patients with co-infections, 29.8% (17/57) were co-infected with bacteria. In a study conducted (Blasco *et al* .,2020). They detected one patient who was positive for *M. pneumoniae* co-infection among patients with COVID-19 pneumonia. (Duployez *et al*., 2020).

Reported a fatal case of necrotizing pneumonia induced by Panton-Valentine leukocidin secreting *S. aureus* in a patient who was affected by COVID-19. Some patients infected with SARS-CoV-2 showed the increased levels of biomarkers and inflammatory cytokines related to co-infection by bacteria, caused by deregulation in the immune system (Zhang *et al*., 2020).

3.4. Correlation between age groups and type of bacteria co-infection to SARS-COV-2 patients

The results revealed that most of the ages that were infected with pathogenic bacteria co- infection with COVID-19 were at the age of >61 where the number of pathogenic bacteria N= 18 (46.2%) and the age of 51-60 where the number of pathogenic bacteria was N=13 (33.2%).The result show significant difference at p value (0.016) as in table **(3.4)**

Table (3.4) Correlation between age groups and type of bacteria

			Type of bacteria			Total	P Value
			pathogen	non pathogen	no growth		
Age groups	<30	N	4	0	13	17	0.016
		%	10.3%	0 %	26.0%	17 %	
	31-40	N	3	1	6	10	
		%	7.7%	9.1%	12 %	10 %	
	41-50	N	1	4	6	11	
		%	2.6%	36.4%	12 %	11 %	
	51-60	N	13	2	13	28	
		%	33.2 %	18.2 %	26 %	28 %	
	>61	N	18	4	12	34	
		%	46.2%	36.3%	24.0%	34.0%	
Total		N	39	11	50	100	
		%	100 %	100 %	100 %	100 %	

This result is comparable with other result ,reveal resemble study, Similar co-infection rate and pathogens in females was found when

compared with males which mean both males and females are susceptible to other respiratory pathogens. The species of co-infected pathogens were higher between patients of 15–64 years old than that of below 15 years and above 65 years of age. The highest Co-infection rate and the most pathogen species were detected in cases of 1–4 days after onset. Along with the course of disease, both the rates and pathogen species of co-infection among COVID-19 patients were decreased significantly, which may due to the treatment (Zhu *et al*., 2020).

The distribution of all co-infections (bacterial and viral) in the SARS-CoV-2-positive population suggests a positive correlation with age Even though the overall median age of the SARS-CoV-2-positive patients was 45 years, the co-infections were significantly higher in the older age group (60+ years) when compared to any other age group. Detailed analyses of the COVID-19 pandemic assert that the most severe outcomes of the disease are observed in older patients (Wu *et al* ., 2020).

Patients aged ⩾60 years showed heavier clinical manifestations, greater severity and longer disease courses compared with those aged (Liu *et al*., 2020).

3.5. Distribution of study population according to infection ratio of CT scan in COVID -19

The results showed that the highest number of infected people was at the rate of infection <20 % of CT scan in percent N=32 (64.0%) and the lowest number of infected people was at the rate of 76-80% of CT scan in percent N= 1(2.0 %), the result show significant difference at p value (0.000). **as in table (3.5).**

Table (3.5) Distribution of study population according to infection ratio in CT scan

infection ratio * patients					
			groups		P value
			patients	control	
infection ratio	<20	Count	32	50	0.000
		%	64.0%	100.0%	
	21-50	Count	8	0	
		%	16.0%	0.0%	
	51-75	Count	6	0	
		%	12.0%	0.0%	
	76-80	Count	1	0	
		%	2.0%	0.0%	
	>81	Count	3	0	
		%	6.0%	0.0%	
Total			50	50	

The result compare with other result show the same significant , Chest CT findings and SARSCoV-2 RT-PCR Ct values at hospital admission, and occurrence of secondary infection during hospitalization are factors able to predict mortality in COVID-19 patients. The major CT features of COVID-19 pneumonia patients were bilateral, peripheral or mixed distributed GGO, consolidation, and GGO with consolidation .Patients in the progressive group had more lobes and segments involved, with a higher proportion of crazy paving sign and higher CT severity score compared with those in the stable group (all $P < 0.05$). And other not similar However, no significant difference was found in hospital length of stay ($P = 0.398$) and duration of viral shedding after illness onset ($P = 0.087$) between the two groups (Feng *et al* ., 2020).

follow-up chest CT scan can accurately reflect the disease evolution and monitor the treatment response (Pan *et al*., 2020).

Just as clinicians are evaluating more suspected patients, radiologists are similarly interpreting more chest CTs in those suspected of infection. Chest CT is a vital component in the diagnostic algorithm for patients with suspected COVID-19 infection. Indeed, given the limited number of rRT-PCR kits in some centers and the possibility of false negative rRT-PCR results, the National Health Commission of the People's Republic of China has encouraged diagnosis based on clinical and chest CT findings alone (Yijiu ., 2020).

Chest CT therefore has limited sensitivity and negative predictive value early after symptom onset, and is thereby unlikely a reliable standalone tool to rule out COVID19 infection Other findings of this work largely concur with early radiology investigative efforts . Our investigative efforts have demonstrated that frequency of CT findings is related to infection time course. Our data largely concur with work by Pan et al that demonstrated preponderance of ground-glass abnormality in early disease, followed by development of crazy paving, and finally increasing consolidation later in the disease course (Pan *et al* .,2020).

3.5.1. Correlation between infection ratio of CT scan and type of bacteria

The results revealed that the highest rate of infection with pathogenic bacteria was N= 25 (64.1%) for people infected with COVID-19 by <20 based on reading Ct scan, The lowest rate of infection with pathogenic bacteria is N= 1(2.6 %) for people who were infected 76- 80 % with COVID-19 .The result show significant difference at p value (0.002). There is no relationship to the increase in infection rate with COVID-19 depending on the CT Scan and the type of pathogenic bacteria as in **Table (3.6)**

Table (3.6) Correlation between infection ratio of CT scan and type of bacteria

			Type of bacteria			Total	P value
			pathogen	non pathogen	no growth control		
infection ratio	<20	N	25	7	50	82	0.002
		%	64.1%	63.6%	100.0%	82.0 %	
	21-50	N	7	1	0	8	
		%	17.9%	9.1%	0.0%	8.0%	
	51-75	N	4	2	0	6	
		%	10.3%	18.2%	0.0%	6.0%	
	76-80	N	1	0	0	1	
		%	2.6%	0.0%	0.0%	1.0%	
	>81	N	2	1	0	3	
		%	5.1%	9.1%	0.0%	3.0%	
Total		N	39	11	50	100	
			100.0%	100.0%	100.0%	100.0 %	

The results were compared with other results, and it was found that they are similar result , Some of the subjects tested positive for more than one co-pathogen, with co-infection rates being greater in SARS-CoV-2 patients. Co-infection with *S. aureus* + *M. pneumoniae* was more prevalent in SARS-CoV-2 . Analysis also revealed that SARS-CoV-2 patients are more likely to have multiple co-infections, whereas the percentage of patients with a single co-infected pathogen is higher in the SARS-CoV-2 .Finally, secondary infection occurrence can be associated with worse outcomes in COVID-19 patients (Zhou *et al*., 2020) .

even though it is unclear whether secondary infections definitively worsen COVID-19 patient outcomes. However, historical data from pandemics suggest that bacterial secondary infections can worsen viral diseases (Klein *et al*., 2016).

3.6. Distribution of study population according to chronic disease.

The results showed that the highest percentage of people infected with the COVID-19 were have chronic diseases N=34 (68.0%) ,The result revealed significant difference at p value (0.001) ,odd Ratio for chronic disease (24.438) as in **table (3.7)and(3.8).**

Table(3.7) Distribution of study population according to chronic disease

chronic disease * pat +cont. Cross tabulation					
			Pat +cont.		P value
			patient	control	
chronic disease	Found	N	34	4	0.001
		%	68.0%	8.0%	
	not found	N	16	46	
		%	32.0%	92.0%	
Total		Count	50	50	
		% of Total	50.0%	50.0%	

Table(3.8) Estimated Risk for chronic disease

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for chronic disease (found / not found)	24.438	7.495	79.682

The results obtained were compared with other results, and it was found that they are similar, patients who have pre-existing cardiovascular disease, specifically hypertension and atherosclerosis, are at a substantially higher risk of developing severe and fatal cardiovascular disease (Flaherty *et al*., 2020).

Infection with COVID-19 according to Blair study, roughly (85%) of people infected with COVID-19 experience minor disease (Blair *et al*., 2021).

previously suggested that preexisting heart disease could be a potential risk factor for SARS-CoV-2-infected patients being admitted to the ICU. To test this, a Met analysis was performed to investigate whether cardiovascular disease (CVD) and / or hypertension was significantly associated with increased disease severity in patients infected with SARS-CoV-2. In one of the studies the percentage of SARS-COV-2 patients with comorbidity diseases such as hypertension, diabetic mellitus and asthma were (14.10%, 16.66%, 3.84%) respectively. This result is in an agreement with a study done by Huang *et al*., (2020).

who reported that 41 patients with SARSCOV-2 that less than half number of patients with comorbidities as following including diabetes mellitus 20%, hypertension 15%, and cardiovascular disease 15% in Wuhan City and the current study reported 34 patients were chronic diseases including hypertension and diabetic patients table **(3.7)** (Guan *et al*., 2020b)

reported that among 1099 patients, 179 had severed disease with comorbidity hypertension 23.7%, diabetes mellitus 16.2%, coronary heart diseases 5.8%, and cerebrovascular disease 2.3%. According to result of several studies on presence of comorbidities disease recorded that 20-

51% of SARS-COV-2 at time of admission had at least one disease; these include 10-15% hypertension, 10-20% diabetic mellitus and 7-40% cardiovascular disease (Chen *et al*., 2020; Liu *et al*., 2020).

Also agreed with study of Zhang *et al*., (2020d) demonstrates that 30% of the 140 SARS-COV-2 patients were admitted to the hospital had hypertension and 12% had diabetes. High-risk patients requiring hospitalization for SARS-COV-2 infections are those with common comorbidities including hypertension as their percentage 58%, cardiovascular disease 59%, and diabetes 71% (Yang *et al* ., 2020).

However, the study stated that diabetic patients with type 1 or type 2 diabetes are at a higher risk of developing a severe form of COVID-19 and also have a higher death rate than that of the non-diabetic people. Diabetes patients have chronic, low-level inflammatory processes, which leads to global cellular dysfunction, which underpins the disease's vast range of symptoms, including an elevated risk of pneumonitis, while the higher severity of COVID-19 in diabetic patients is not fully understood, deregulated immunological and inflammatory responses are shared by both disorders (Roberts and Megson., 2021).

This may be due to immune status, atrophies and declines in functions of the thymus gland during aging, which is a primary lymphoid organ responsible for the production of immunocompetent T cells (Rezzani *et al*., 2014).

which is critically important to the immune system, which serves as the body’s defense mechanism, providing surveillance and protection against diverse pathogens (Thapa and Farber., 2019).

The severity of COVID-19 depends more on the individual immune status, as lymphocyte level, including absolute T cell and B cell

numbers.SARS-CoV-2 enters the epithelial cells of the respiratory tract mucosal surface via ACE2. Infected epithelial cells release types I and III interferon and IL-1, which in turn activate three pathways that counter virus infection: autophagy, innate immunity, and adaptive immunity (Braciale and Hahn ., 2013).

3.6.1 Correlation between chronic disease and type of bacteria in COVID-19

The results revealed that people with chronic diseases were infected with the highest percentage of pathogenic bacteria, which is N=28 (71.8%). The result show Highly significant difference at p value (0.0001)as in **table 3-9**

Table (3.9) Correlation between chronic disease and type of bacteria in COVID-19

chronic disease and Type of bacteria Correlation						P value
			Type of bacteria			0.0001 *
			pathogen	non pathogen	no growth control	
chronic disease	Found	N	28	6	4	
		%	71.8%	54.5%	8.0%	
	not found	N	11	5	46	
		%	28.2%	45.5%	92.0%	
Total		N	39	11	50	
		%	100.0%	100.0%	100.0%	

The first study reported focusing on the results of airway microbiological sampling of COVID-19 patients admitted to the French intensive care unit due to acute respiratory failure. The main results are as follows: 28% of the patients admitted to ICU for acute respiratory failure

related to severe SARS-CoV-2 pneumonia might have a respiratory bacterial co-infection upon ICU admission; the leading involved bacteria were methicillin-sensitive *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus infuenzae* and *Enterobacteriaceae* with no infection related to atypical bacteria and no viral co-infection especially no influenza infection. The prevalence of bacterial or viral co-infections in patients admitted to the ICU for acute respiratory failure related to SARS-CoV-2 pneumonia is poorly studied (Huttner *et al*., 2020).

In diabetes patients and all the complications associated with this disease, such as weakened immune response or hyperglycemia, present a higher risk for infected patients . Not only for the COVID-19 progression, but also as a higher risk to develop secondary infections and therefore worsening of the health state. For those reasons, an effective and tight management of diabetes specifically of glucose plasma levels, is extremely important in, infected patients with diabetes (Cristelo *et al* .,2020).

3.7 . Correlation between get vaccine and type of bacteria

The results showed that people who were not vaccinated against the Corona virus were infected with a large number of pathogenic bacteria, with a percentage of N=34 (87.2%) .The result show significant difference at p value (0.0001*).**table (3.10**) .

Table (3.10) Correlation between get vaccine and type of bacteria

Get vaccine and type of bacteria correlation							
			Type Of Bacteria			Total	P Value
			Pathogen	Non Pathogen	No Growth Control		
Get Vaccine	Yes	N	5	0	33	38	0.0001*
		%	12.8%	0.0%	66.0%	38.0 %	
	No	N	34	11	17	62	
		%	87.2%	100.0%	34.0%	62.0 %	
Total		N	39	11	50	100	
		%	100.0%	100.0%	100.0%	100.0 %	

•* High significant at 0.05

The highest percentage of recipients of the vaccine was Sino pharm at an age greater than 60 and Pfizer at an age younger than 30, as shown in the **figure 3-1 .**

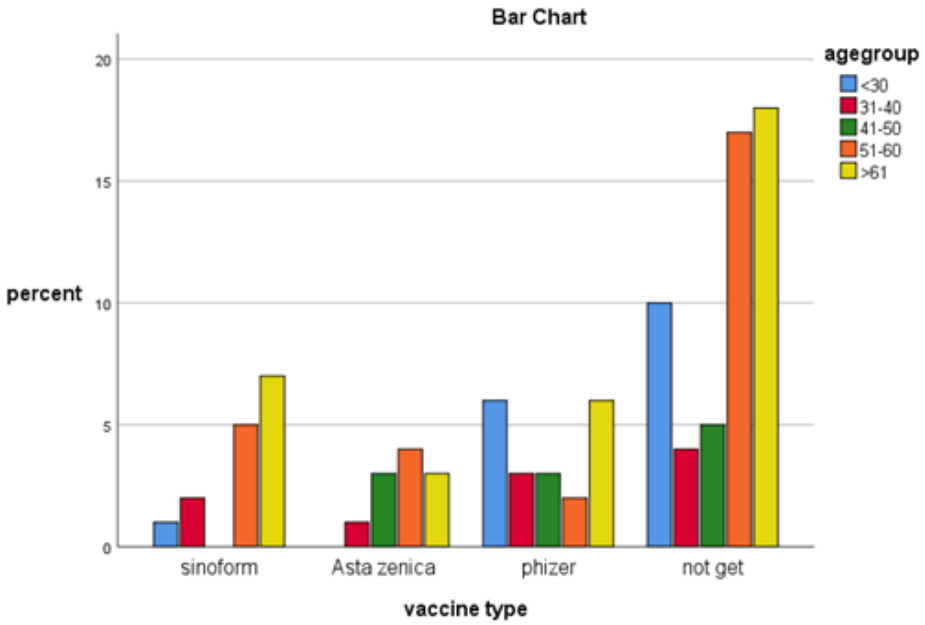


Figure (3.2) percentage of vaccinated COVID-19 patients according to age group

COVID-19 vaccine mediated immune activation is intended to mimic hat of natural SARS-CoV-2 infection in order to develop the same effector and memory subsets but without infecting the host or triggering severe inflammatory side effects (Teijaro and Farber., 2021).

provide an overview as to how the various COVID-19 vaccines elicit an immune response and consequent immunity to SARS-CoV-2. Vaccines generally contain an immunogenic that encodes antigenic viral peptides and an adjuvant that triggers a highly orchestrated immune response (Liang *et al* ., 2020).

During clinical trials, COVID-19 mRNA vaccines were found to induce the maturation of CD4+ and CD8+ T-lymphocytes (Park *et al* ., 2021).

And more than 70% of vaccinated individuals have memory T lymphocyte responses (Naaber *et al*., 2021).

Similarly, individuals who received COVID-19 mRNA vaccines developed B-lymphocytes and high levels of IgM and IgG antibodies which were detected eight weeks after the second dose . Moreover, RBD memory B lymphocyte levels were equivalent to those found in individuals who had acquired antibodies from natural SARS-CoV-2 infection.(Wang *et al*., 2021).

Antibody-mediated protection is well described, and antibody detection is used to determine effectiveness of many human vaccines. However, past experience with other viruses suggests that antibodies might enhance inflammatory responses. This is termed antibody-dependent enhancement (ADE) of the disease, and is due to the presence of poorly neutralizing cross-reactive antibodies that bind to the virus and enhance viral entry into cells (Yang *et al*., 2020).

3.8.Correlation between antibiotic take and type of bacteria.

The result found that the people who took antibiotics(ceftazidime) had more pathogenic bacteria N=4 (10.2%), ceftazidime +Amikacin N=3 (7.7%), ceftazidime +Amikacin +meropenem N=6 (15.2%), cefteriaxon and meropenem N=4 (10.2%), meropenem N=5 (12.8%), meropenem+vancomycin +ceftazdim N=3 (7.7%) . Used ceftriaxone only , meropenem +Amikacin , meropenem +vancomycin +tazocin , vancomycin only , vancomycin +meropenem, vancomycin +meropenem only not found pathogenic bacteria . The correlation between type of bacteria and take antibiotic showed significant difference at p value (0.0001)as in **table (3.11) .**

Table(3.11) Correlation between take antibiotic and type of bacteria

Take Antibiotic And Type Of Bacteria Correlation						
			Type Of Bacteria			P value
			pathogen	non pathogen	no growth control	
Take antibiotic	Ceftriaxone	N	0	1	0	0.0001
		%	0.0%	9.1%	0.0%	
	Azithromycin	N	2	0	1	
		%	5.1%	0.0%	2.0%	
	Ceftazidime	N	4	0	1	
		%	10.2%	0.0%	2.0%	
	Ceftazidime +Amikacin	N	3	0	1	
		%	7.7%	0.0%	2.0%	
	Ceftazidime +Amikacin +Meropenem+	N	6	0	1	
		%	15.2%	0.0%	2.0%	
	Ceftazdime +Vancomycin	N	1	0	0	
		%	2.6%	0.0%	0.0%	
	Cefteriaxon and	N	4	1	0	
		%	10.2%	9.1%	0.0%	

	Meropenem					
	Ceftriaxon +Levofloxacin	**N**	**3**	**1**	**0**	
		%	**7.7%**	**9.1%**	**0.0%**	
	Ceftriaxon +Tazocin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Ceftriaxon+ Amikacin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Gentamycin+ Ceftriaxon	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Gramycin +Tazocin +Vancomycin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Meropenem	**N**	**5**	**1**	**0**	
		%	**12.8%**	**9.1%**	**0.0%**	
	Meropenem +Amikacin	**N**	**0**	**1**	**0**	
		%	**0.0%**	**9.1%**	**0.0%**	
	Meropenem +Vancomycin +Amikacin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Meropenem +Vancomycin +Tazocin	**N**	**0**	**1**	**0**	
		%	**0.0%**	**9.1%**	**0.0%**	
	Meropenem+ Vancomycin +Ceftazdim	**N**	**3**	**0**	**0**	
		%	**7.7%**	**0.0%**	**0.0%**	
	Meropenem+ Vancomycin +Levofloxacin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Tazocin +Vancomycin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Tazocin +Vancomycin + Ceftriaxon +Gramycin	**N**	**1**	**0**	**0**	
		%	**2.6%**	**0.0%**	**0.0%**	
	Vancomycin	**N**	**0**	**2**	**0**	
		%	**0.0%**	**18.1%**	**0.0%**	
	Vancomycin +Levofloxacin +Moxifloxacin ceftriaxon+	**N**	**0**	**1**	**0**	
		%	**0.0%**	**9.1%**	**0.0%**	

	Amikacin					
	Vancomycin +Meropenem	**N**	**0**	**1**	**0**	
		%	**0.0%**	**9.1%**	**0.0%**	
	Vancomycin+ Ceftriaxon	**N**	**0**	**1**	**0**	
		%	**0.0%**	**9.1%**	**0.0%**	
Total		**N**	**39**	**11**	**50**	
		%	**100%**	**100%**	**8%**	

- *** High significant at 0.05**

The current study significant resemble the previous study and medication orders and administration were obtained electronically from the medical records. The recent systematic review and meta-analysis by Lansbury et al. only 7% of hospitalised patients with COVID-19 were reported as having evidence of bacterial co-infection, yet >90% received empirical antibiotic (Lansbury *et al* ., 2020).

Is hardly surprising given the challenges associated with distinguishing bacterial from viral pneumonia and that bacterial coinfection is likely to worsen an already poor prognosis in these patients (Youngs et al ., 2020).

found that bacterial coinfection was more common for those in intensive care (ICU) (14%, 95% CI 5–26, vs. 4%, 95% CI 1–9) but only one study provided data on the timing of infection in relation to admission. Distinguishing between bacterial co-infection acquired prior to or following ICU admission is essential when developing antibiotic prescribing policies. Some of the concern over bacterial co-infection in COVID-19 stems from experience with influenza where bacterial co-infection is well recognized and often the factor precipitating admission to ICU (Lansbury *et al* ., 2020).

The following antibiotics were considered “CAP antibiotics”: ceftriaxone or cefdinir + azithromycin or doxycycline, ampicillin/sulbactam + azithromycin, cefepime + azithromycin or

doxycycline, piperacillin/tazobactam + azithromycin or doxycycline, vancomycin + piperacillin/tazobactam, vancomycin + cefepime, or ceftriaxone alone. Patient records were reviewed to adjudicate the indication of the following antibiotic combinations: vancomycin + piperacillin/tazobactam, vancomycin + cefepime, or vancomycin + ceftriaxone. For calculating duration of therapy, 1 antibiotic day was any dose of any number of antibiotics given to a patient on 1 calendar day. The primary outcome was the proportion of respiratory co-infections among SARS-CoV-2-infected individuals. Secondary outcomes included antibiotic use for respiratory tract bacterial co-infections and non-respiratory co-infections. Patients who died within 5 days of hospitalization were excluded from duration of antibiotic therapy calculations as 5 days is the typical duration of therapy for cap treatment (Westerhof *et al* ., 2021).

In previous study The most frequently detected pathogen was *Staphylococcus aureus* which was found in 26% of SARS-CoV-2 negative patients (*n* = 9) and as a co-infection in 25% of SARS-CoV-2 positive patients (*n* = 7). In only 2 (11%) of the 18 patients with a clinically known COVID-19 diagnosis, *Staphylococcus aureus* was detected in post mortem lung tissues, and histology showed acute bronchopneumonia without DAD. Both patients did not receive empirical antibiotics nor did they get blood culture testing for growth of bacteria during hospitalization. In the remaining 16 COVID-19 patients, no pathogens other than SARS-CoV-2 were detected in lung tissues, and all of these 16 patients had received prophylactic empirical antibiotics (Musuuza *et al* ., 2021).

This indicates a protective effect of empirical antibiotic therapy in COVID-19 patients . In the 10 patients who were unexpectedly positive for SARS-CoV-2 *post mortem*, 5 (50%) autopsy lungs showed co-

infections. Only 2 of these 5 patients with bacterial co-infections had received empirical antibiotics, further supporting the beneficial effect of antibiotics during SARS-CoV-2 infection (Schwab et al ., 2022).

Previous epidemic and pandemic outbreaks of viral respiratory infections have reported bacterial infections complicating initial viral illness. Bacterial co-infection was reported in up to 30% of critically ill patients and in 12% of hospitalized patients not requiring ICU admission (MacIntyre, *et al*., 2018).

When reported, the most commonly identified bacterial co-pathogens were *Staphylococcus aureus* and *Streptococcus pneumoniae* It can be especially difficult to withhold antibiotics from patients with COVID-19 cytokine release syndrome, which can mimic bacterial sepsis. In such patients, the ongoing need for antibiotics should be continuously reviewed in light of response to immune modulatory therapy and limited to as short a duration as possible. The impact of immune modulatory therapies such as corticosteroids (now standard of care for ventilated patients in light of recovery trial findings (Horby, *et al* 2020)..

3.9.Correlation between the type of bacteria and occupation

The result of present study show significant difference at p value(0.001). The results showed that the highest percentage of pathogenic bacteria was among the housewives, with a percentage of N= 16 (16.0%) as in **table (3.12)**

Table (3.12) Correlation between the type of bacteria and occupation

			occupation				P value
			retired	employee	free job	house wife	
Type of bacteria	pathogen	N	11	5	7	16	0.001
		%	11.0%	5.0%	7.0%	16.0%	
	non pathogen	N	3	2	3	3	
		%	3.0%	2.0%	3.0%	3.0%	
	no growth	N	2	27	12	9	
		%	2.0%	27.0%	12.0%	9.0%	
Total		N	16	34	22	28	
		%	16.0%	34.0%	22.0%	28.0%	

The results in table **(3.12)** related to the relationship between occupation and underlying disease of patients infected with COVID-19 similar to study done at the Imam Khomeini Hospital in Ardabil. According to the results, there is a significant relationship between occupation and the underlying disease of patients infected with coronavirus ($P < 0.05$).

The purpose of this study was to investigate the prevalence of the disease in people with different occupations to gain a clearer understanding of the situation of employed people (Roy *et al* .,2020). Due to the fact that the symptoms of the disease are different in different sexes and ages, it is important to study individual factors among different people in society like their occupations (Lechien *et al*., 2020).

In some occupations, it may be difficult to follow health measures such as social; for example, taxi driver are in this group of occupations and are at risk to have contact with sick passengers. Therefore, it is very

important to study the disease in such occupations (Pongpirul *et al*., 2020).

3.10 . Multiple correlation

3.10.1.Correlation infection ratio and SARS-COV2 infection (Nasopharyngeal swab)

The result show significant difference at p value (0.000) **as in table 3.13) .**

The results in previous study were compared and found to be similar, The SARS-CoV-2 detection rate was significantly higher for NPS [46.7% (56/120)] than OPS [10.0% (12/120)] ($P < 0.001$). The sensitivity of NPS was also significantly higher than that of OPS ($P < 0.001$).

At the time of sampling, the time of detectable SARS-CoV-2 had a longer median duration (25.0 vs. 20.5 days, respectively) and a longer maximum duration (41 vs. 39 days, respectively) in NPS than OPS. The mean cycle threshold (Ct) value of NPS (37.8, 95% CI 37.0-38.6) was significantly lower than that of OPS (39.4, 95% CI 38.9-39.8) by 1.6 (95% CI 1.0-2.2, $P < 0.001$), indicating that the SARS-CoV-2 load was significantly higher in NPS specimens than OPS. Patient discomfort was low in both sampling methods. During NPS sampling, patients were significantly less likely to have nausea and vomit.(Wang *et al* ., 2020).

Repeated samples may improve yield. For example, among patients with a high pretest probability for COVID-19 and a negative NP swab, repeating the NP swab and also collecting a saliva sample may be considered, as saliva sampling is noninvasive and 11% of patients in this study with at least 1 positive specimen were only positive in their saliva.

There are several limitations to this analysis. As these patients were originally diagnosed using NP, mid turbinate, or nasal swabs, it is possible that there is a bias towards subsequent NP swabs versus other specimens being positive. We used a single detection system (See gene), and other platforms may have yielded different results (Fan *et al* .,2021).

We simply asked patients to spit a teaspoon of saliva into a specimen container; many patients were unable to provide a full teaspoon of saliva, and this may in part explain the gap in sensitivity between NP swabs and saliva. It is also possible that other methods, such as throat washing with normal saline, would have improved yield. One small study found throat washing to be significantly more sensitive than NP swabs for SARS-CoV-2 detection, possibly enabling the acquisition of more epithelial cells (Guo *et al* ., 2020).

Throat washing is easy to self-collect and should be further investigated as a noninvasive alternative to NP swabs and other invasive swabs such as oropharyngeal swabs. In conclusion, NP swabs were more sensitive than saliva for SARS-CoV-2 detection, particularly among patients beyond the first week of illness. Notably, however, NP swabs were only 6% more sensitive than saliva among the 18 sample pairs collected in the first week of illness in this study (Jamal *et al*., 2021).

3.10.2. Correlation specific treatment with antibiotic and type of bacteria.

The result show significant at p value (0.000**). as in table 3-13 and 3-14 show**

The current study resemble show significant , of all reported studies, only 30.8% discussed the presence of bacterial co-infection, of

which 15 different bacterial species were reported. From evaluated studies, co-infection with *Staphylococcus aureus (S. aureus)* was the highest (75%), followed by *Escherichia coli (E. coli*) (58.3%), *Klebsiella pneumonia* (41.6%), then *Pseudomonas aeruginosa* (33.3%). Furthermore, 25% of the studies recorded other pathogens such as *Acinetobacter baumannii*, *Streptococcus pneumonia*, and *Haemophilus influenza* .On the other hand, no clear data on bacterial co-infection were reported in most reviewed studies (69.2%) (Anibijuwon *et al* ., 2019).

Antibiotic exposure and mortality rates were one of critical analysis outcomes throughout the review. Evaluated data from the reviewed studies demonstrated strong positive Pearson correlation coefficients ($r = 0.925$), between admission to ICU and exposure to antibiotic treatment, which are statistically significant ($p < 0.001$). In addition, a moderate Pearson positive correlation coefficient ($r = 0.393$) was recorded between prescribing antimicrobial and the outcome of patient death, which is statistically significant, $p = 0.029$.(Abu-Rub *et al* ., 2021).

The unprecedented recorded numbers of patients infected with SARS-CoV-2 continues to escalate worldwide. As observed from the early stages of the pandemic, the extensive practice of prescribing antimicrobials for the treatment of COVID-19 infected patients might lead to the increased adverse events and long-term consequences such as antimicrobial resistance (Al-Hadidi *et al* ., 2021).

Several studies confirm that most hospitalized patients with CO VID-19 are managed with broad-spectrum antimicrobials with unproven efficacy (Nassef *et al* ., 2020). Highlighted that the

percentage of antibiotic prescriptions for patients with both severe/critical disease nearly equaled the percentage for patients with mild/moderate disease, even though more critically ill patients are at greater risk of developing secondary bacterial infections (Cong *et al* ., 2021).

These unnecessary antimicrobials and excessive prescribing for mild and moderate COVID-19 will probably increase risks for adverse events and selective development of multidrug-resistant bacterial pathogens at local healthcare and regional levels. To our knowledge, the presented study is the first systematic review to evaluate the prevalence of antibiotic prescribing for patients admitted to ICU settings with confirmed SARS-CoV-2 infection. Our review involved 16 different countries, including major countries, where the plight of the pandemic was evident: China, USA, Italy, and France. The demographic data of this review demonstrate that 2715 ICU patients with ages ranging from 1 day to 92 years and a mean age of 62.7 were exposed to antibiotic therapy (Majumder *et al* ., 2020).

Analogous to our findings, many previous studies concluded that old age patients are more susceptible to severe and critical illness leading to ICU admission. In addition to the clear age-related morbidity and mortality, older patients are more prone to an increase of long-term complications (Haas *et al* .,2020).

Table (3.13) Multiple correlation

Correlations					
		Type of bacteria	Infection ratio	SARS-COV2 infection (Nasopharyngeal swab)	Specific treatment
Type of bacteria	Pearson Correlation		-.364-**	-.950-**	.823**
	Sig. (2-tailed)		.000	.000	.000
	N		100	100	100
infection ratio	Pearson Correlation	-.364-**		.400**	-.367-**
	Sig. (2-tailed)	.000		.000	.000
	N	100		100	100
SARS-COV2 infection (Nasopharyngeal swab)	Pearson Correlation	-.950-**	.400**		-.841-**
	Sig. (2-tailed)	.000	.000		.000
	N	100	100		100
Specific treatment	Pearson Correlation	.823**	-.367-**	-.841-**	
	Sig. (2-tailed)	.000	.000	.000	
	N	100	100	100	

**. Correlation is significant at the 0.01 level (2-tailed).

Figure (3.14)specific treatment

Specific treatment	Frequency
ceftazdime+amikacine+meropenem	19
meropenem	12
ceftriaxon+amikacine	13
Azethromycine	3
ceftriaxone+meropenem	3
not get	50
Total	100

3.11.Molecular Assay to detected SARS-COV-2

3.11.1 .Genetic analysis of Sever Acute Respiratory Syndrome2 (ORF- 1ab gene)

To the best of our knowledge, this is the first phylogenetic analysis of SARS-COV2 study in Babylon province (Iraq) on ORF1ab gene. A phylogenetic study of SARS-COV2 done by RNA extracted from identified patient's distinct groups. Out of 50 positive SARS-COV2 (15) samples with high RNA concentration .The study were obtained and sequenced for (ORF1abgenes) findings SARS-COV2 for the ORF1ab gene as a critical positive component indicating in the results SARS-CoV2 RNA is now identified utilizing real-time RT-PCR as shown in Figure (3-14) detection of three target channel FAM, neucleocapsid protein (N), and envelope E gene. In this study, focus on the orf 1abgene as it the the largest proportion of positive effects. Positive SARS-COV2 sample amplified by PCR and sequenced by Sanger Deoxy Method

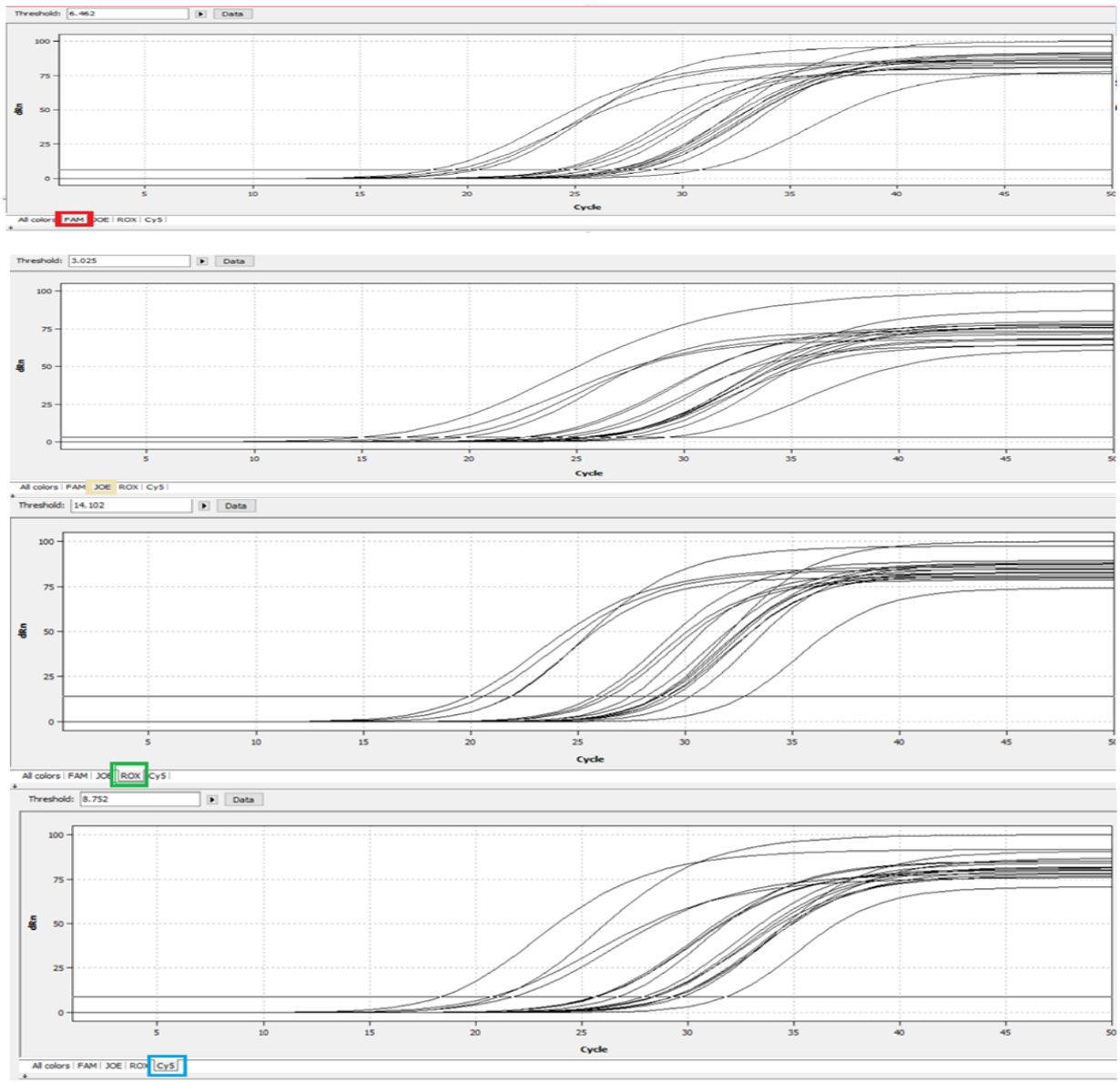


Figure (3.3) SARS-CoV-2 RT-PCR result lineage B-betacoronavirus FAM for (E gene), SARS-CoV-2 Specific Cy5 (S gene), internal control JOE and ROX (reference due)

After amplify of the ORF1ab gene for local isolates from nasopharyngeal of SARS-COV2 The results displayed by electrophoresis on (1.5%) agarose gel electrophoresis stained with Ethidum. The as seen in the Figure **(3.4)** to amplify ORF1ab gene area of SARS-COV-2 were analyzed. M: 100bp ladder marker.

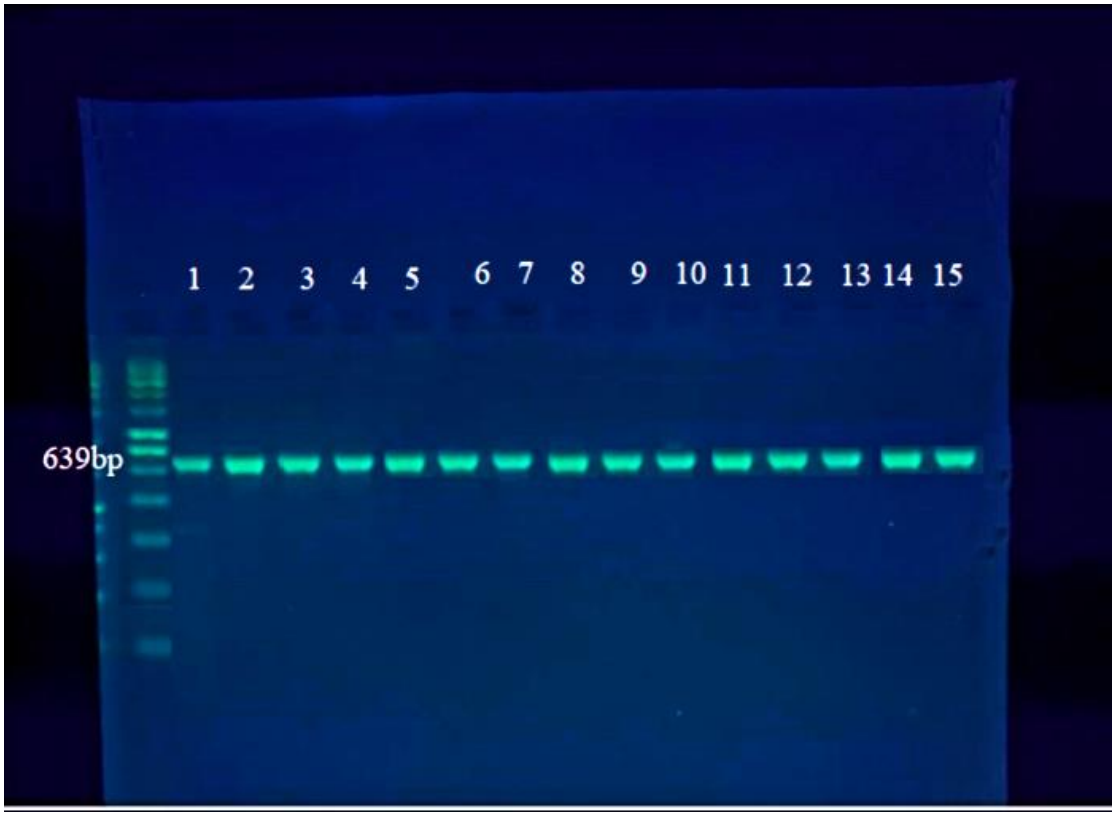


Figure (3.4) An Agarose gel electrophoresis demonstrating PCR product size (639bp) analysis for the SARS-COV2 ORF1ab gene. The results of ORF1ab gene amplification in COVID-19 samples were fractionated on 1.5 percent agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker.

The nucleotide sequence was generated by amplification of ORF1 ab genes in five isolates of ORF1ab, which were then compared using Blast software, translated to a specific amino acid, and produced a substantial difference. Using the NCBI site's GenBank library (https://blast.ncbi.nlm.nih.gov/Blast.cgi), alignment was performed against reference isolates (OL539215.1, OL525506 and OL538734

). All of the local isolates from patients had nucleotide sequence identities with the reference strain that ranged between 99 and 100 percent appeard in the **table (3.15**) .

Table(3.15) (NCBI - National Center for Biotechnology Information)
SARS-COV2 ORF1ab identification according NCBI –Blast Identity

SARS-COV2(ORF1ab) isolates (accession NO)	**Identification ORF1ab**	
	SARS-COV2 genotype	**Identity to NCBI – Blast SARS-COV2-ORF1ab genotype**
OL665115	ORF1ab	100%
OL665116	ORF1ab	100%
OL665117	ORF1ab	100%
OL639726.1	ORF1ab	100%
OL639727.1	ORF1ab	100%

Isolates were registered after correspondence with the National Center for Biotechnology Information (NCBI - National Center for Biotechnology Information) and were assigned an accession number **shown in the table (3.15)**

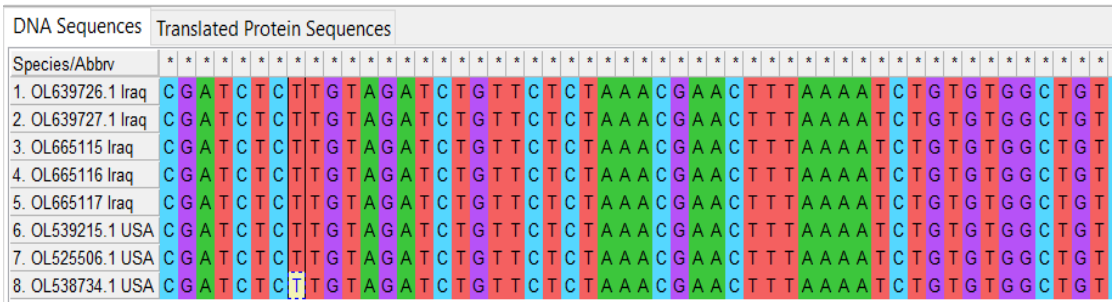


Figure (3.5)(NCBI - National Center for Biotechnology Information): Multiple sequence alignment analysis of ORF 1ab gene sequence between NCBI-Genbank SARS-COV2 ORF 1ab and local SARS-COV2 isolates by using MEGAX program3.1. Population Study Appendix(12)

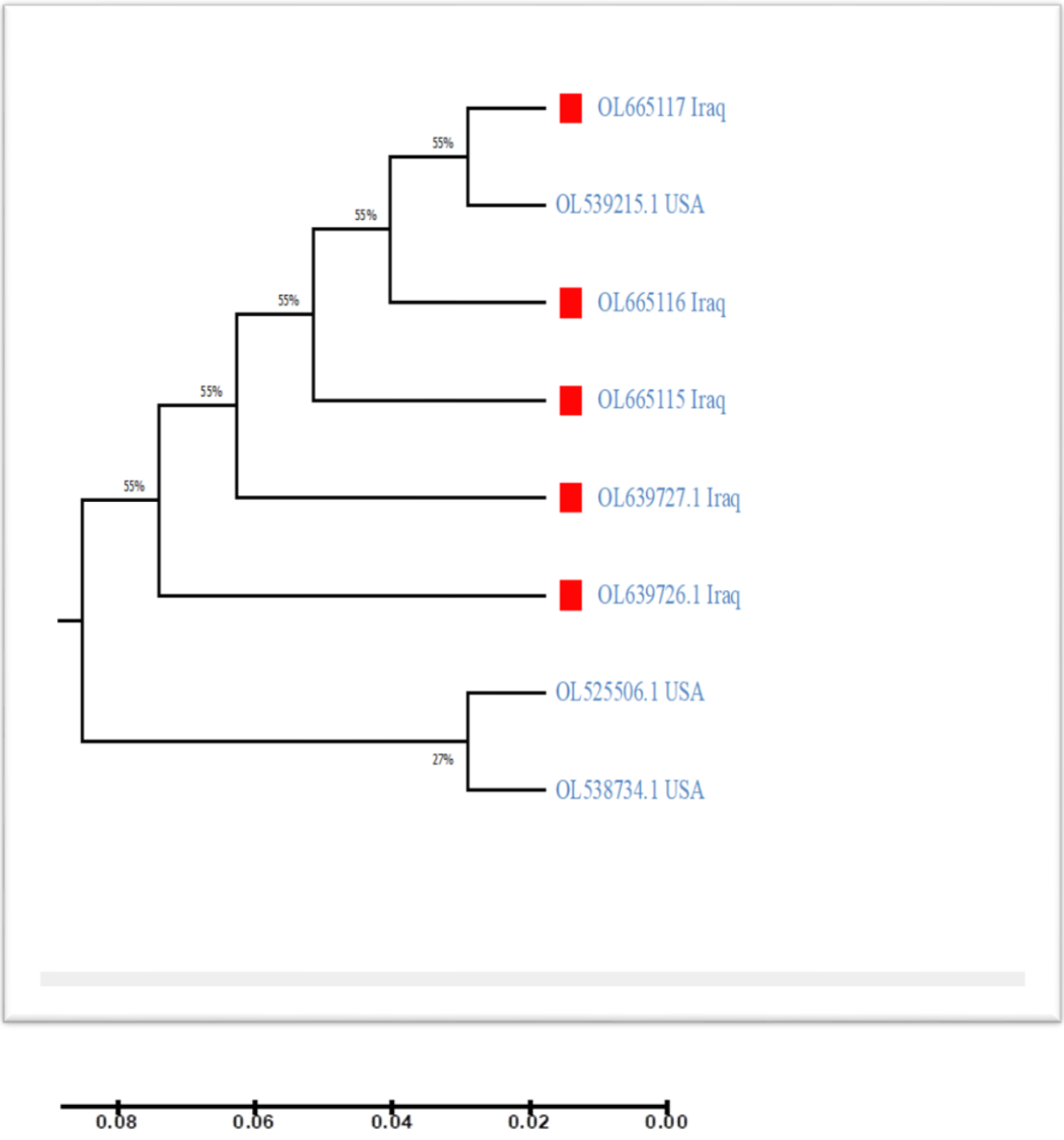


Figure (3.6) (NCBI - National Center for Biotechnology Information): Phylogenetic tree analysis for international isolates based ORF1ab gene partial sequence by using Test UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean) . Where the results show that the local SARS-COV2 (ORF1ab (OL665117and OL665116) close related to USA isolates (ORF1ab), the localOL639726.1) close related to USA OL525506, OL538734.1

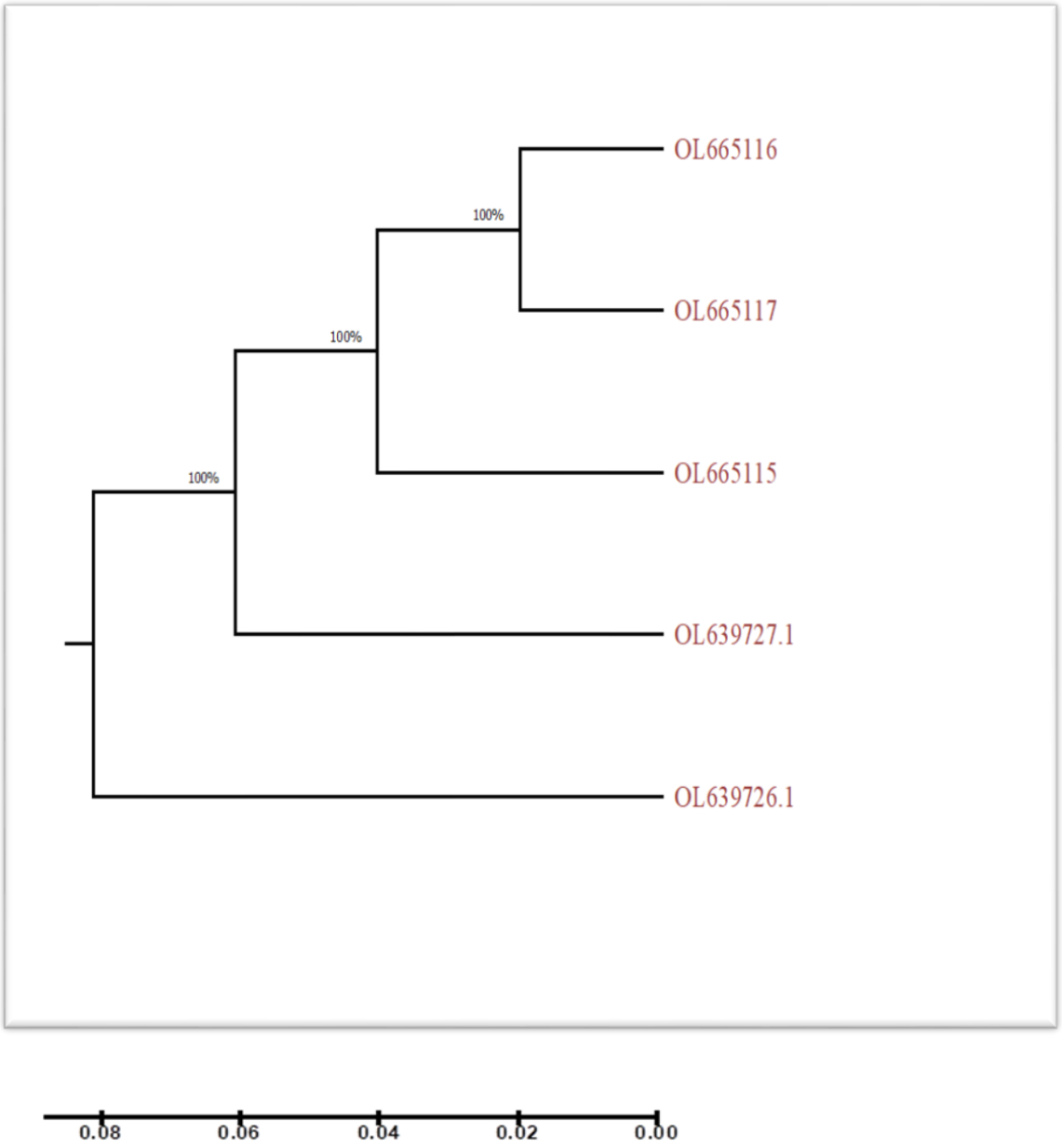


Figure (3.7) Phylogenetic tree analysis for local isolates based ORF1ab gene partial sequence by using Test UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean) . Where the results show that all local were 100% similar to each other's(TamuraStecherPetersonFilipski and Kumar, 2013).

The UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean) is put to the test. approach utilizing NCBI and MEGA 6 Software for alignment for all positive SARS COV-2 isolates (5 isolates in ORF1ab gene) using 3 reference isolates during phylogenetic tree The

construction for all positive SARS COV-2 isolates. The sequencing of genes is quite similar to a global isolate from a different nation

As seen in, compatibility ranged from 99 to 100 percent as in t**able 3-15** , phylogenetic tree **figure (3-6) and (3- 7).**

3.11. 2.Phylogenetic Analysis

A phylogenetic analysis of SARS-COV-2 RNA isolated from Nasopharyngeal swabs revealed 50 different groups, with the ORF1ab gene found in isolates 5 were recorded with accession number as in **table 3-15 .**

These local isolates were aligned with 3 reference isolates using the Mega version 6 program **(Figure 3-5).**

All of these isolates appear to be similar to global reference isolates from various countries around the world, with five local samples of ORF 1ab gene clustered with global isolates from the United States, China, and Saudi Arabia, this may help to understand why all of these global issues exist. Analyses of isolates from the same years and era, as well as the Iraq pandemic arrived from a different country Furthermore, in relation to the transmission route of SARS-COV-2 is exceedingly fast and, according to various sequencing analysis, the virus has a modest mutation rate.(Ullah *et al* ., 2021).

The findings of this study are analogous to the findings of another study conducted in the United States to illustrate the genetic geographic distribution of SARS-COV-2 in Los Angeles, which discovered that 15% of isolates were very close to a reference isolate from the same city, China and 85 percent comparable to the European reference isolate conducted a study and discovered that phylogenic analysis of the SARS-

COV-2 genomic sequence was compared to a reference isolate from a different country demonstrate that the Indian isolate is very closely connected to a neighboring isolate Bangladesh, Nepal, and China (Hong Kong) are only a few examples. (Zhang *et al.*, 2020). (NCBI - National Center for Biotechnology Information, Tushir *et al.*, 2021)

These discrepancies could be due to the usage of various genes and the size of the sample. The genes utilized to detect SARS-COV-2, which has a global distribution and affects every country on the planet, have a high level of stability and resistance various routes of transmission Several studies have been conducted to evaluate the genomic data. To determine the distinctive trait of SARS-COV-2, researchers looked at the characteristics of the entire worldwide population in various countries that assist health systems in comprehending epidemiology by (Aydillo et al., 2020 ;Forster et al., 2020).

In correlation, a study on the first three weeks of the pandemic in Canada found that 35 percent of SARS-COV-2 nucleotide sequences in the Canadian population are similar to isolates from Asia and 42 percent are similar to isolates from Europe, and the authors suggest that the reasons for these seminaries are due to most patients having a history of travel to these countries (Zhang and Yoo .,2016).

Another study used one-step RT-PCR as an alternative method for diagnosing SARS-COV-2 in Mexico. They used four sets of primers targeting the ORF1ab gene, which is the most variable region between humanSARS-COV-2 and bat coronavirus, to show that COV-2 is more closely related to bat coronavirus than SARS-COV-1 and MERS-COV during phylogenetic analysis (Meza-Robles *et al.*, 2020) .

Conclusions
And
Recommendations

Conclusions

1. All of the individuals tested positive for bacterial co infection to SARS-COV-2 , included the most common isolates were : *Klebsiella pneumonia* (7), *Streptococcus pneumonia* (5) , *Acinetobacter baumannii* (5) and Other bacteria are opportunistic and others are normal flora.
2. Distribution of SARS-COV-2 according to gender show not significant difference at p value (0.54), The infection rate for male was higher than for female .
3. Correlation between occupation and gender show significant at p value (0.001).
4. The result show significant difference at p value (0.000). in correlation between type of bacteria with infection ratio, SARS-COV2 infection (Nasopharyngeal swab) and specific treatment (take antibiotic)
5. Correlation between chronic disease and type of bacteria co infection show significant at p value (0.0001).
6. Among patients with COVID-19, the overall proportion of bacterial co-infection was low, but usage of antibiotics was high. There is insufficient evidence to support widespread use of empirical antibiotics in patients hospitalized for COVID-19, particularly those without critical illness-advocate against routine empiric antibiotic use in patients hospitalized with COVID-19 infection.

7. These unnecessary antimicrobials and excessive prescribing for mild and moderate COVID-19 will probably increase risks for adverse events and selective development of multidrug-resistant bacterial pathogens at local healthcare and regional levels.
8. Phylogenic analysis reveals that 5 local isolates of ORF 1ab gene related similar to global isolates from USA.

Recommendations

1. Future large cohort to assess the risk factor of mortality and hospitalization in SARS-COV-2 infection in Iraq**.**

2. There is a needed for measuring the level of cytokines and risk of development to sever disease in non-ICU patients.

3. Study the local immune reactivity by estimation of mucosal IgA level in acute and chronic infection.

4. A more intensive study on bacteria *Klebsiella pneumonia* , *Acinetobacter baumannii* and *Streptococcus pneumonia* its resistance to antibiotics and the wrong use of antibiotics to treat this bacteria co infection to SARS-COV-2.

5. Studying of the role of (T, B cells) on immunity in convalescent patients with COVID-19

6. Not to use antibiotics indiscriminately against bacteria associated with infection with the Corona virus, and to conduct a sensitivity test to antibiotics

7. Study of other accessory protein associated with pathogenicity such as ORF8 , ORF3a, ORF9b and ORF9c

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Appendices

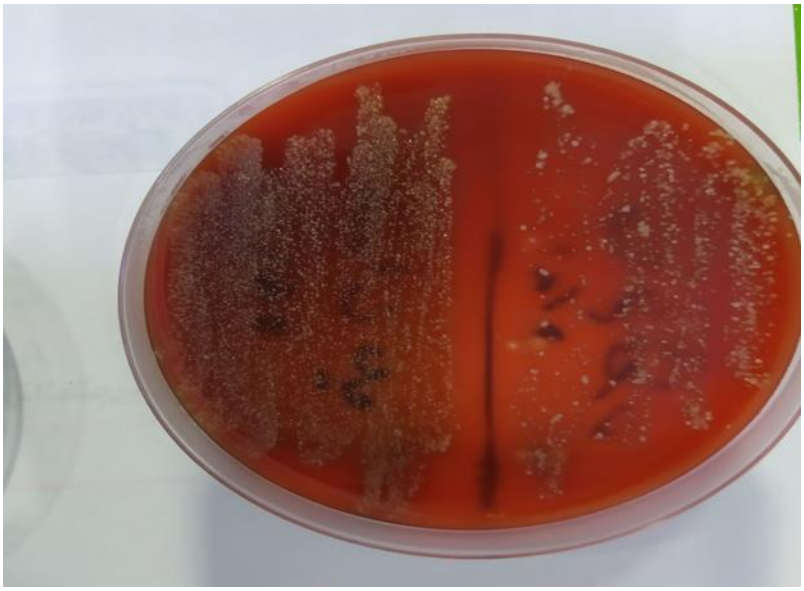
Appendix

Appendix1:Case history was taken from each patient using formula Sheet

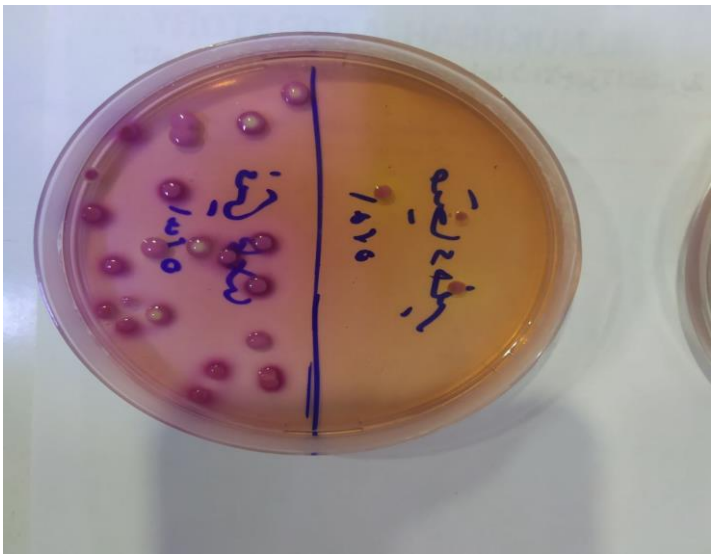
chronic disease	found
	not found
get vaccine	**yes**
	no
vaccine type	sinoform
	AstraZeneca
	Pfizer
	not get
Smoking	yes
	no
healthy tip	yes
	no
Occupation	retired
	employee
	free job
	house wife
Infection ratio	<20
	21-50
	51-75
	76-80
	>81
	<20
Samples collected	nasal
	oral
	oral and nasal
SARS-COV2 infection (Nasopharangeal swab)	Negative
	positive
Gender	male
	Female
Age group	<30
	31-40
	41-50

	51-60	28
	>61	34

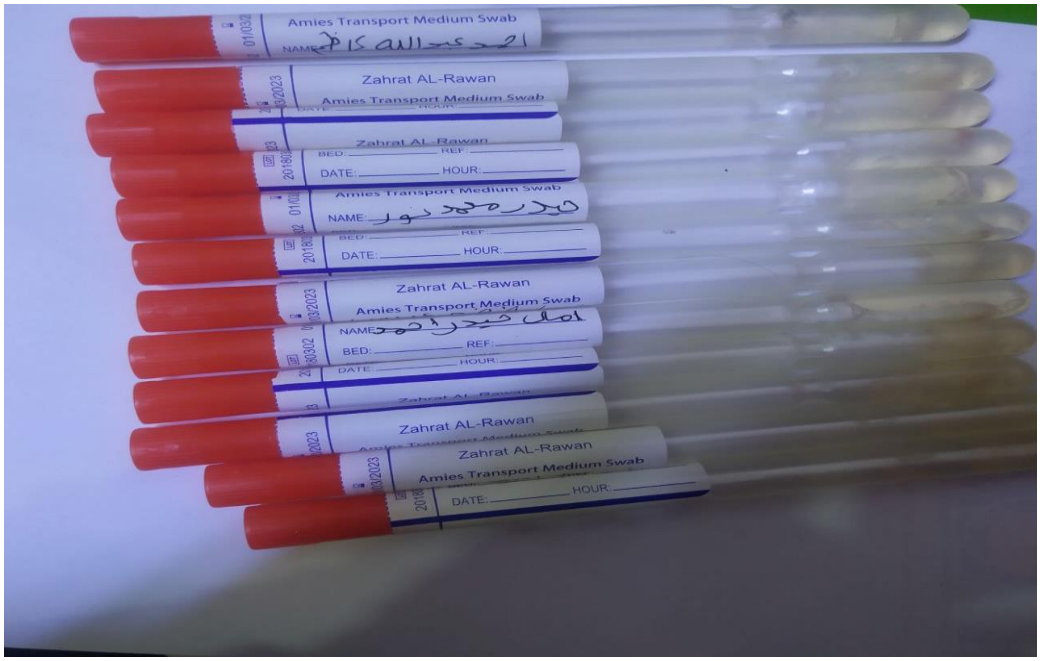
Appendix 2 :Blood agar medium



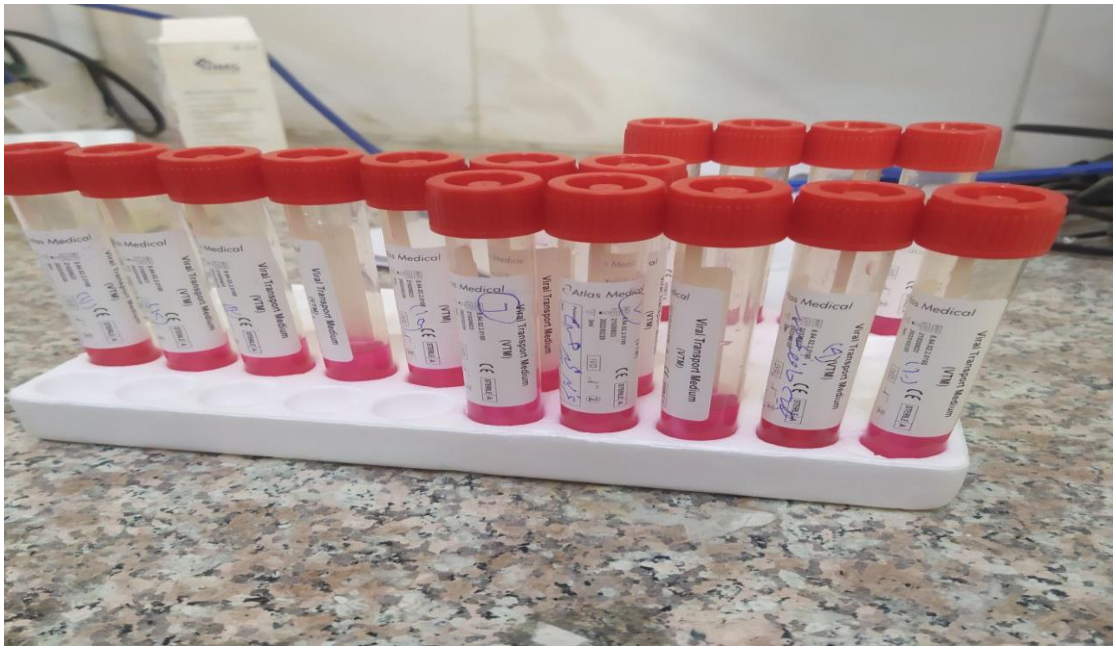
Appendix 3: Maconkey agar medium



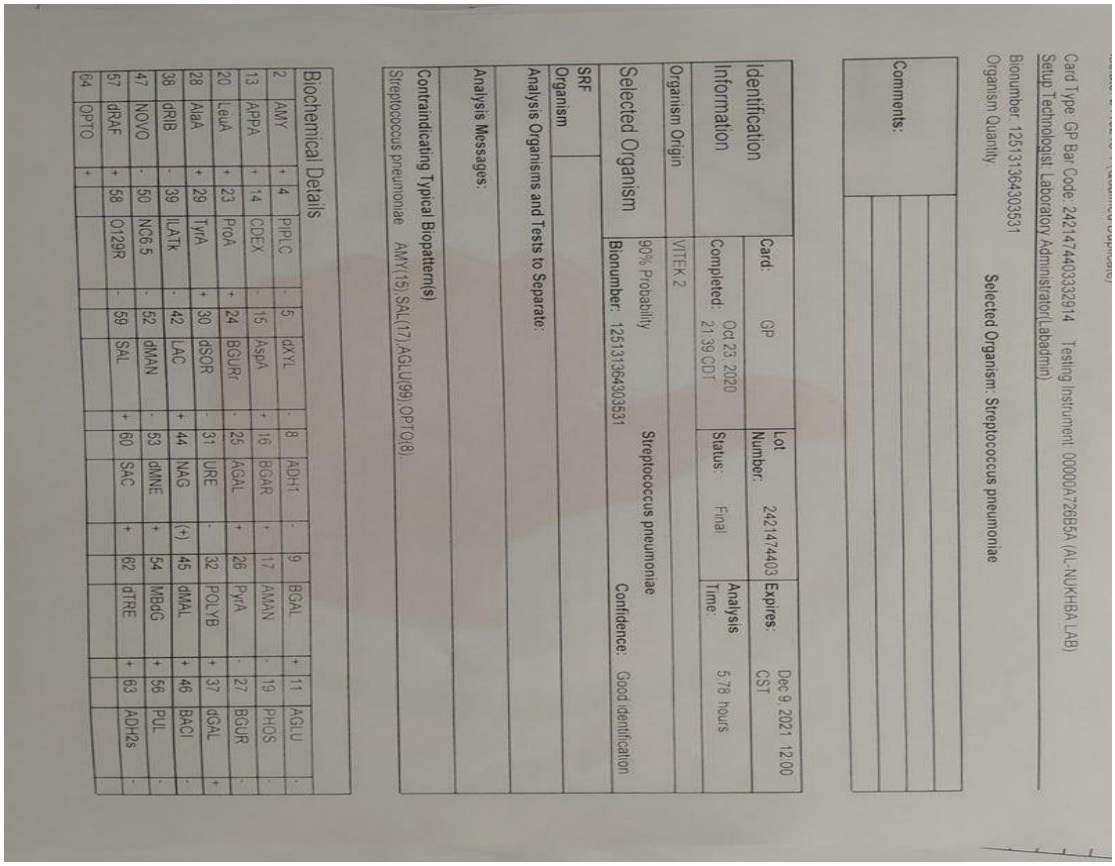
Appendix 4 : Transport medium for bacteria



Appendix 5 : Viral transport medium



Appendix 6 : Isolation and Identification bacterial co-infection in SARS –COV2 patient by Vitek2 Compact system



Card Type: GP Bar Code: 242147440333291 4 Testing Instrument: 00000A72685A (AL-NJKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 1251316430353 1

Organism Quantity:

Selected Organism: Streptococcus pneumoniae

Comments:

Identification Information	Card: GP	Lot Number: 242147440	Expires: Dec 9 2021 12:00 CST
	Completed: Oct 23 2020 21:39 CDT	Status: Final	Analysis Time: 5.78 hours
Organism Origin	VITEK 2		
Selected Organism	90% Probability	**Streptococcus pneumoniae**	
	Bionumber: 1251316430353 1		**Confidence:** Good identification

SRF

Organism

Analysis Organisms and Tests to Separate:

Analysis Messages:

Contraindicating Typical Biopatterns)

Streptococcus pneumoniae AMY(15) SAL(17) AGLU(99) OPTO(8)

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	+	11	AGLU	-
13	APPA	+	14	CDEX	-	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	+	24	BGUR	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	(+)	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Isolate 710216-1 (Qualified)

Card Type: GP Bar Code: 242147440333275S Testing Instrument: 00000A72695A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 1600000443603310
Organism Quantity: Selected Organism: Aerococcus viridans

Comments:

Identification Information					
	Card: GP	Lot Number: 242147440	Expires: CST	Dec 9 2021 12:00	
	Completed: Oct 6 2020 18:20 CDT	Status: Final	Analysis Time:	5.82 hours	
Organism Origin	VITEK 2				
Selected Organism	93% Probability	Aerococcus viridans			
	Bionumber: 1600000443603310		Confidence:	Very good identification	
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Aerococcus viridans	dMNE(90) AMY(20) PyrA(76)				

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	(-)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-
20	LeuA	-	23	ProA	-	24	BGUR	-	25	AGAL	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	-
57	dRAF	+	58	O129R	+	59	SAL	(-)	60	SAC	+
64	OPTO	+									

9	BGAL	+	11	AGLU	+						
17	AMAN	-	19	PHOS	(-)						
26	PyrA	-	27	BGUR	-						
32	POLYB	-	37	dGAL	+						
45	dMAL	+	46	BACI	-						
54	MBdG	-	56	PUL	(-)						
62	dTRE	+	63	ADH2s	-						

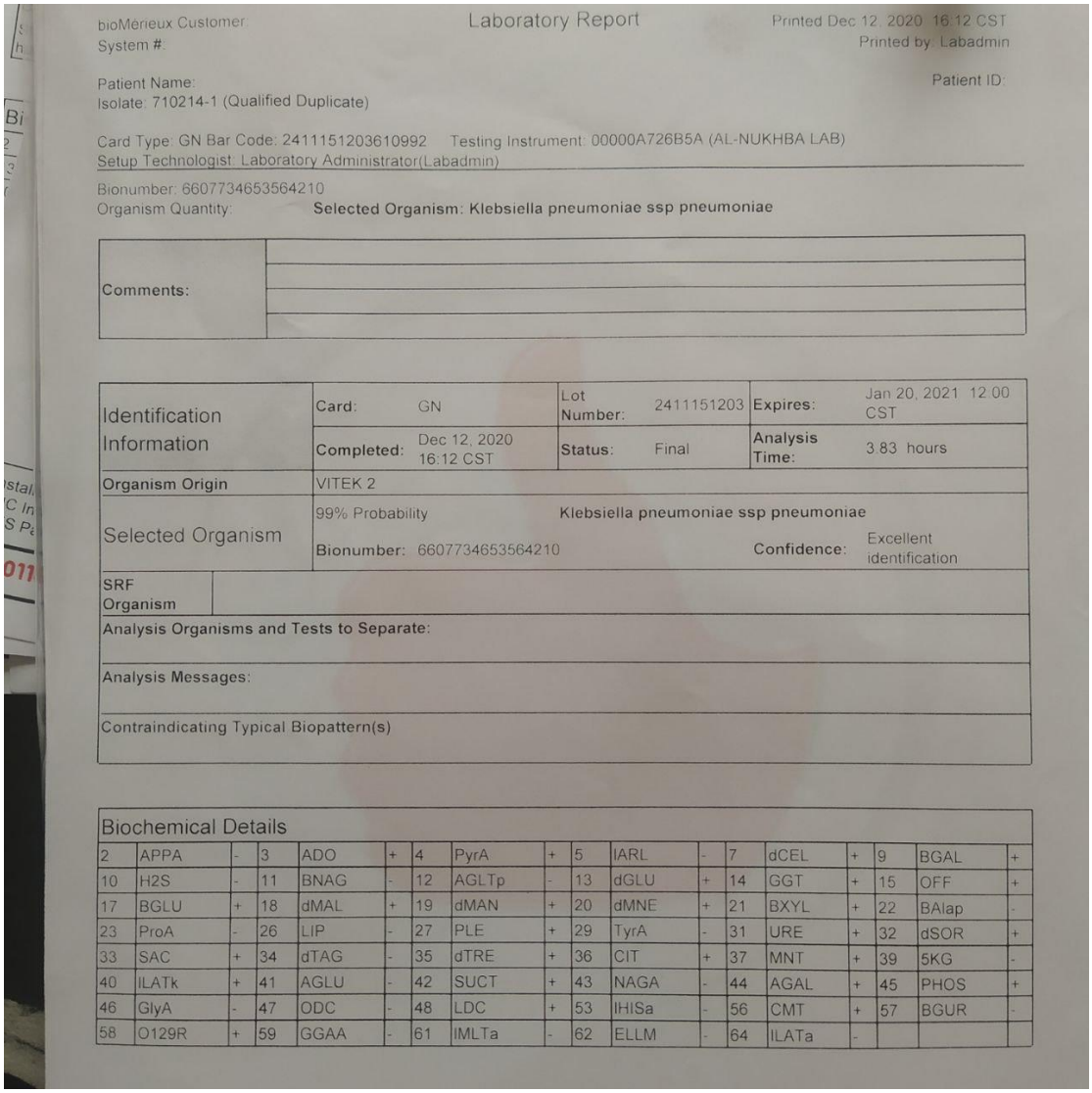
Card Type: GN Bar Code: 241153040332625?9 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0241010303500210
Organism Quantity:

Selected Organism: Acinetobacter baumannii complex

Comments:

Identification Information					
	Card: GN	Lot Number: 241153040	Expires: CST	Feb 3 2022 12:00	
	Completed: Oct 23 2020 20:47 CDT	Status: Final	Analysis Time:	4.88 hours	
Organism Origin	VITEK 2				
Selected Organism	99% Probability	Acinetobacter baumannii complex			
	Bionumber: 0241010303500210		Confidence:	Excellent identification	
SRF Organism					
Analysis Organisms and Tests to Separate:					
Acinetobacter baumannii complex					
Acinetobacter nosocomialis	44C(1);41C(99).				
Acinetobacter pittii	44C(99);41C(99).				
Acinetobacter baumannii	41C(1).				
Acinetobacter calcoaceticus					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					



bioMérieux Customer:
System #:

Laboratory Report

Printed Dec 12, 2020 16:12 CST
Printed by: Labadmin

Patient Name:
Isolate: 710214-1 (Qualified Duplicate)

Patient ID:

Card Type: GN Bar Code: 2411151203610992 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 6607734653564210
Organism Quantity: **Selected Organism: Klebsiella pneumoniae ssp pneumoniae**

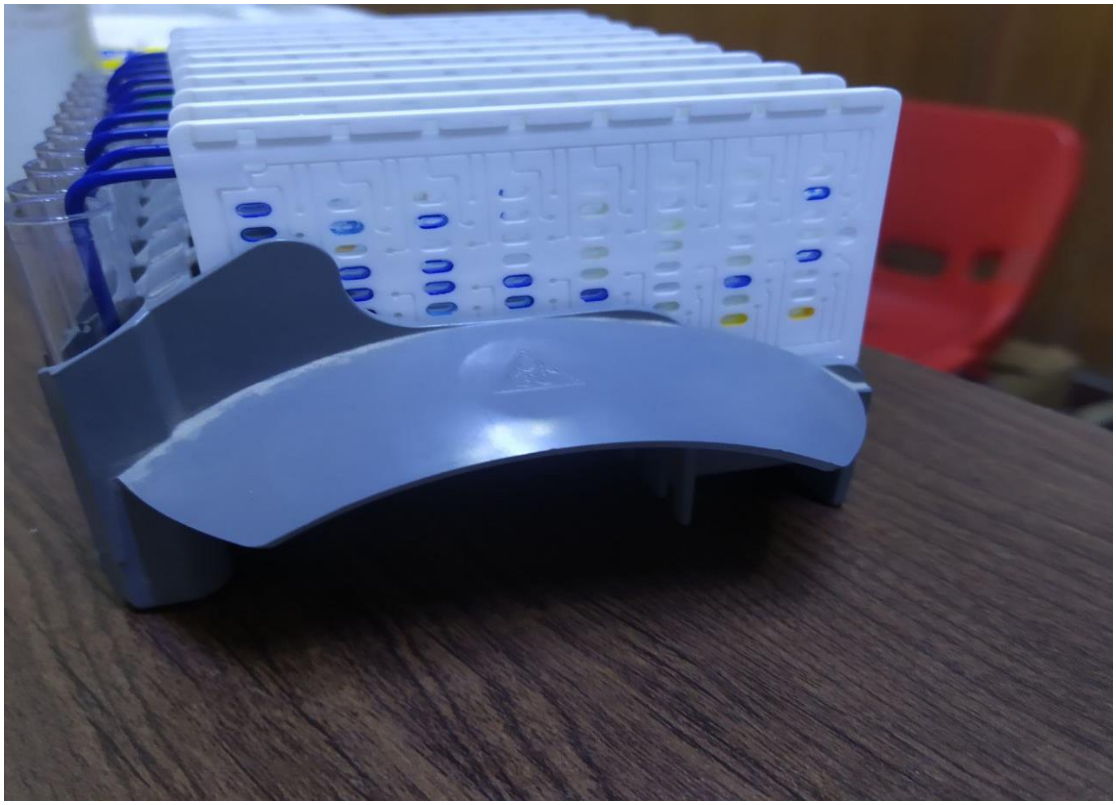
Comments:	

Identification Information	**Card:**	GN	**Lot Number:** 2411151203	**Expires:**	Jan 20, 2021 12:00 CST
	Completed:	Dec 12, 2020 16:12 CST	**Status:** Final	**Analysis Time:**	3.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability		**Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber: 6607734653564210			**Confidence:**	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Appendix 7:procedure VITEK 2 compact



Appendex 8: multiple sequence alignment (Omega Clustal)

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CLUSTAL O(1.2.4) multiple sequence alignment (Omega Clustal)


OL525506.1
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OL538734.1      -------------------------------------
CTTTCGATCTCTTGTAGATCT  21
OL639726.1      ----------------------------------------
TCGATCTCTTGTAGATCT     18
OL639727.1      ----------------------------------------
TCGATCTCTTGTAGATCT     18
OL665115        ----------------------------------------
TCGATCTCTTGTAGATCT     18
OL665116        ----------------------------------------
TCGATCTCTTGTAGATCT     18
OL665117        ----------------------------------------
TCGATCTCTTGTAGATCT     18
OL539215.1      ----------------------------------------
TCGATCTCTTGTAGATCT     18

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OL525506.1
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OL538734.1
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 81
OL639726.1
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 78
OL639727.1
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 78
OL665115
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OL665116
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 78
OL665117
GTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 78
OL539215.1
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OL525506.1
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OL538734.1
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OL639726.1
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138
OL639727.1
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138
OL665115
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138
OL665116
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138
OL665117
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138
OL539215.1
CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATC 138

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TTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTT 201
OL639726.1
TTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTT 198
OL639727.1
TTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTT 198
OL665115
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OL665116
TTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTT 198
OL665117
TTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTT 198
OL539215.1
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OL525506.1
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OL538734.1
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OL639726.1
TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAC 258
OL639727.1
TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAC 258
OL665115
TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAC 258
OL665116
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OL665117
TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAC 258
OL539215.1
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OL525506.1
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OL538734.1
ACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGG 321
OL639726.1
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OL639727.1
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OL665115
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OL665116
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OL665117
ACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGG 318
OL539215.1
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OL525506.1
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OL639726.1
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OL639727.1
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OL665115
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OL665117
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OL639726.1
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OL639727.1
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OL665115
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OL539215.1
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OL639726.1
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OL665117
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OL639726.1
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OL665117
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OL639726.1
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OL665115
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OL665117
CGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGG 618
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OL639727.1
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OL665116
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OL665117
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OL639727.1
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OL665115
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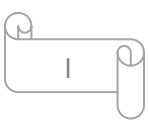

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OL525506.1      ----------------------------------------------------
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OL639727.1
ACTCATGCGTGAGCTTAACGGAGGGGCATACACTCGCTATGTCGATAACAACTTCTGTGG 798
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OL665116
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OL639727.1
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OL525506.1      ----------------------------------------------------
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OL639726.1
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OL639727.1
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OL665115
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OL525506.1      ----------------------------------------------------
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TGAACATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCA 978
OL665116
TGAACATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCA 978
OL665117
TGAACATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCA 978
OL539215.1
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OL525506.1      ----------------------------------------------------
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OL639726.1
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OL665117
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OL639727.1
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OL525506.1      ----------------------------------------------------
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OL639726.1
GCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAATG 1158
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OL639727.1
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OL665116
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OL639727.1      CAACCAAATGTGCCTTTCAACTCTCATGAAGT--------------------
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OL665115        CAACCAAATGTGCCTTTCAACTCTCATGAAGT--------------------
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OL665116        CAACCAAATGTGCCTTTCAACTCTCATGAAGT--------------------
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OL665117        CAACCAAATGTGCCTTTCAACTCTCATGAAGT--------------------
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OL539215.1
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OL639727.1      ----------------------------------------------------
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OL665115        ----------------------------------------------------
------- 1190
OL665116        ----------------------------------------------------
------- 1190
OL665117        ----------------------------------------------------
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OL665115        ----------------------------------------------------
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OL665116        ----------------------------------------------------
------- 1190
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OL639727.1      ----------------------------------------------------
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OL665116        ----------------------------------------------------
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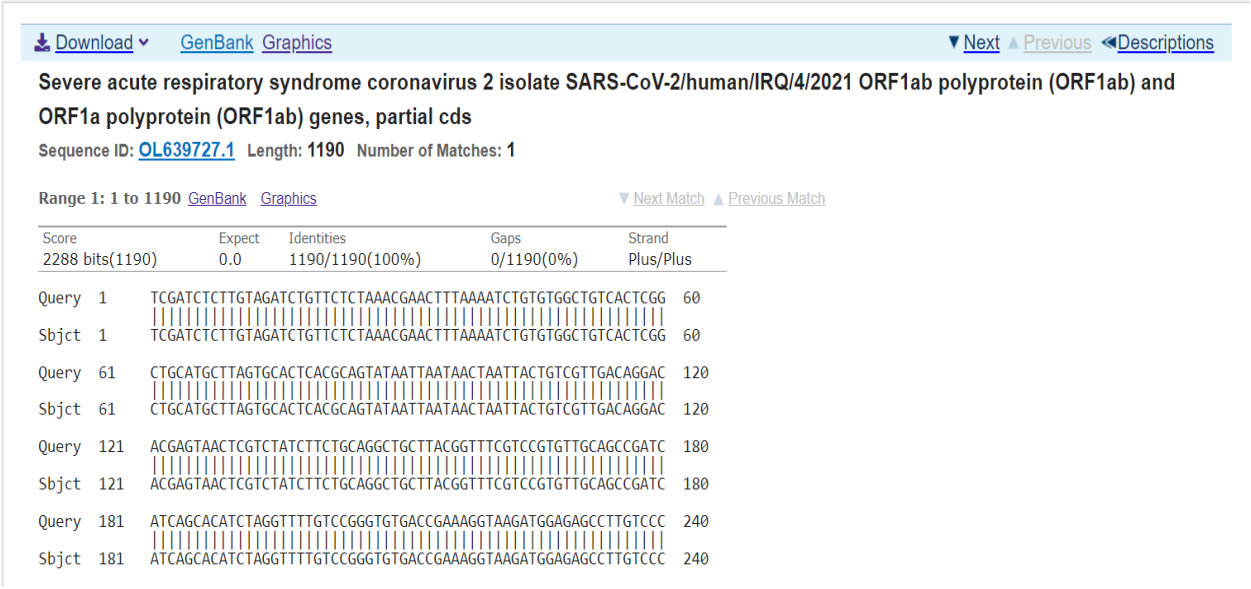

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Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/4/2021 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds

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Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/4/2021 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds

GenBank: OL639727.1

FASTA Graphics

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AUTHORS Jawad,N.A. and Alkhafaji,Z.A.
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LinkOut to external resources

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Nucleotide

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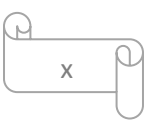

الخلاصة

المتلازمة التنفسية الحادة الشديدة - فيروس كورونا -2 المسؤول عن الجائحة المستمرة في جميع أنحاء العالم ولا يزال يُسجل عدد كبير جدًا من الإمراضية والوفيات. يسبب فايروس كورونا SARS- COV-2 مرضًا تنفسيًا يهدد الحياة . تهدف الدراسة الحالية إلى تحديد معدل وتسلسل عدوى فايروس كورونا - SARS-COV-2 بين المرضى الذين يعانون من أعراض تنفسية في محافظة بابل ، بينت الدراسة الإصابة بفايروس كورونا والعدوى البكتيرية المصاحبة للإصابة .

تضمنت الدراسة 50 مريضاً مصاباً بـ COVID-19 الذين تم ادخالهم الى وحدة العناية المركزة الوبائية في قسم الطوارئ في مستشفى الإمام الصادق التعليمي ومدينة المرجان الطبية في محافظة بابل ، 28 منهم من الذكور و 22 من الإناث ، وتتراوح أعمارهم بين 20- 80. عامًا ومقارنتها بـ 50 فردًا يتمتعون بصحة جيدة خلال الفترة من سبتمبر إلى نوفمبر 2021 ، وتم تأكيد ذلك من خلال تفاعل البوليمير المتسلسل للنسخ العكسي . تم جمع مسحات البلعوم الأنفي من هذه الدراسة التي شملت جميع المرضى وأجريت عليهم مباشرة. يوضع في أنبوب يحتوي على 2 مل من وسط النقل الفيروسي (VTM) ثم يخزن في درجة حرارة -20 درجة مئوية حتى وقت استخراج الحمض النووي الراي بوسي ومعالجة تفاعل البوليمير المتسلسل للنسخة العكسية. مسحات البلعوم الأنفي والبلعوم الفموي للعدوى البكتيرية للبكتيريا التشخيصية عن طريق جهاز vitik2 إلى موجبة لصبغة غرام وسالبة لصبغة غرام هذه البكتيريا مصاحبة مع COVID-19.

يحتاج جائحة COVID-19 ، الذي يحدث في جميع أنحاء العالم ، إلى اهتمام جاد. يمكن أن يتسبب COVID-19 الذي بدأه فيروس من نوع RNA في حدوث مشاكل أكثر خطورة لاحقًا بسبب قدرته على التحور. كود المادة الوراثية لفايروس SARS-COV-2 لبروتينات ORF1a ORF1ab / وأربعة بروتينات هيكلية تمت دراستها على نطاق واسع كأهداف دوائية رئيسية. تحتوي المادة الوراثية أيضًا على عدد متغير من إطارات القراءة المفتوحة (ORFs) لترميز البروتينات الإضافية التي ليست ضرورية لتكرار الفيروس ، ولكن يبدو أن لها دورًا في التسبب في المرض. أجريت هذه الدراسة على 50 مسحة من البلعوم الأنفي باستخدام RT-PCR و PCR لتضخيم الجين ORF1ab بعد ذلك ، خضعت خمس عينات لتحليل النشوء والتطور. تم تأكيد نتائج الدراسة الحالية من خلال تحليل ORF1ab الذي يُظهر أن ORF1ab يختلف

ويتحور ولديه عدد من الطفرات التي يمكن أن تؤثر عليه في التشابه بين البلدان بهدف الكشف عن عزلنا المحلي كان له 100 هوية مع الولايات المتحدة استنتجت أن التأثير المتطرف لانتقال الفيروس وهو حيازة وراثية متحركة.

من خمسين بمتوسط عمر (20-80) سنة ، تم تقسيم مرضى COVID-19 إلى مجموعتين : 28 (56%) من الذكور و 22 (44%) من الإناث. جميع الأفراد ثبتت إصابتهم بالعدوى البكتيرية بشكل إجمالي ، بما في ذلك ، (7) *Klebsiella pneumonia* عينات ، *Streptococcus pneumonia* (5)، *Acinetobacter baumannii* (5) ،مع وجود البكتيريا الأخرى انتهازية والبعض الآخر نبات طبيعي.

أظهر توزيع مجتمع الدراسة حسب الجنس عدم وجود فرق معنوي عند القيمة (0.54) p ، وبحسب العمر كشفت الدراسة الحالية عن وجود فرق معنوي عند قيمة (0.080)p ، تظهر نسبة الإصابة بفيروس SARS-COV-2 فرقًا معنويًا عند القيمة(0.000) p ، يظهر المرض المزمن فرقاً معنوياً عند قيمة (0.001) p ، النسبة الفردية للأمراض المزمنة (24.438).

يظهر الارتباط بين الفئة العمرية ونوع العدوى المشتركة للبكتيريا بلمريض المصاب بفايروس كورونا- SARS- COV-2 فرقًا معنويًا عند قيمة(0.016) p ، تظهر نسبة الإصابة ونوع البكتيريا فرقًا معنويًا عند قيمة(0.002) p ، الارتباط بين المرض المزمن ونوع البكتيريا يظهران فرقاً معنوياً عالياً عند قيمة (0.0001) p. يظهر الارتباط بين الملقح ونوع البكتيريا فرق معنوي عند قيمة(0.0001) p *. يظهر الارتباط بين نوع البكتيريا والمهنة فرقاً معنوياً عند قيمة (0.001) p. نسبة الإصابة بالارتباط وعدوى فايروس كورنا SARS- COV-2 المسحة الأنفية البلعومية ، تظهر فرق معنوي عند قيمة (0.000) p. المعاملة النوعية للارتباط مع المضاد الحيوي ونوع البكتيريا ، تظهر معنوية عند القيمة (0.000) p.

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كلية الطب/ فرع الأحياء المجهرية

الكشف عن اصابات (SARS-COV-2) والبكتريا المشتركة في مرضى الجهاز التنفسي في محافظة بابل

رسالة

مقدمة إلى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم / الأحياء المجهرية الطبية

من قبل

نور عباس جواد عبد الزهره

بكالوريوس علوم حياة / أحياء مجهرية / كلية العلوم / جامعة بابل

2016

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2022 م

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