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Review

# A Comprehensive Study of SARS-CoV-2: From 2019-nCoV to COVID-19 Outbreak

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**The coronavirus disease 2019 (COVID-19) is a highly contagious pneumonia that has spread throughout the world. It is caused by a novel, single stranded RNA virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Genetic analysis revealed that, phylogenetically, the SARS-CoV-2 is related to severe acute respiratory syndrome-like viruses seen in bats. Because of this, bats are considered as a possible primary reservoir. The World Health Organization has declared the COVID-19 outbreak as a pandemic. As of May 27, 2020, more than 5,406,282 confirmed cases, and 343,562 confirmed deaths have been reported worldwide. Currently, there are no approved vaccines or antiviral drugs available against COVID-19. Newly developed vaccines are in the first stage of clinical trials, and it may take a few months to a few years for their commercialization. At present, remdesivir and chloroquine are the promising drugs for treating COVID-19 patients. In this review, we summarize the diversity, genetic variations, primary reservoirs, epidemiology, clinical manifestations, pathogenesis, diagnosis, treatment strategies, and future prospects with respect to controlling the spread of COVID-19.**

**Keywords:** **Coronavirus, 2019-nCoV, COVID-19 outbreak, SARS-CoV-2, diversity, epidemiology**

## Introduction

The name coronavirus comes from the Latin word "corona", meaning crown and referring to the crown-like spikes on the viral surface, similar to the solar corona. Coronaviruses belong to the family *Coronaviridae*, of the order Nidovirales. These are single stranded RNA viruses that contain 26–32 kbs of genomes, which are small in size ranging from 65–125 nm in diameter as shown in Figure 1. The family *Coronaviridae* has different genera: alpha, beta, gamma, and delta. These have a wide range of hosts including mammals and birds [1]. Until the outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in Guangdon, China in 2002, it was thought that these genera infect only animals [2]. Just a decade after this, in the Middle Eastern countries, an endemic was caused by another strand of pathogenic coronavirus that was called Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV and MERS-CoV as well as other members of *Coronavirida,* cause acute respiratory distress syndrome and Acute lung injury. These syndromes can cause pulmonary failure and result in death [3].

At the end of 2019, an outbreak of a novel pneumonia in Wuhan, a business city in the Hubei province of China, was caused by a coronavirus [4] belonging to the

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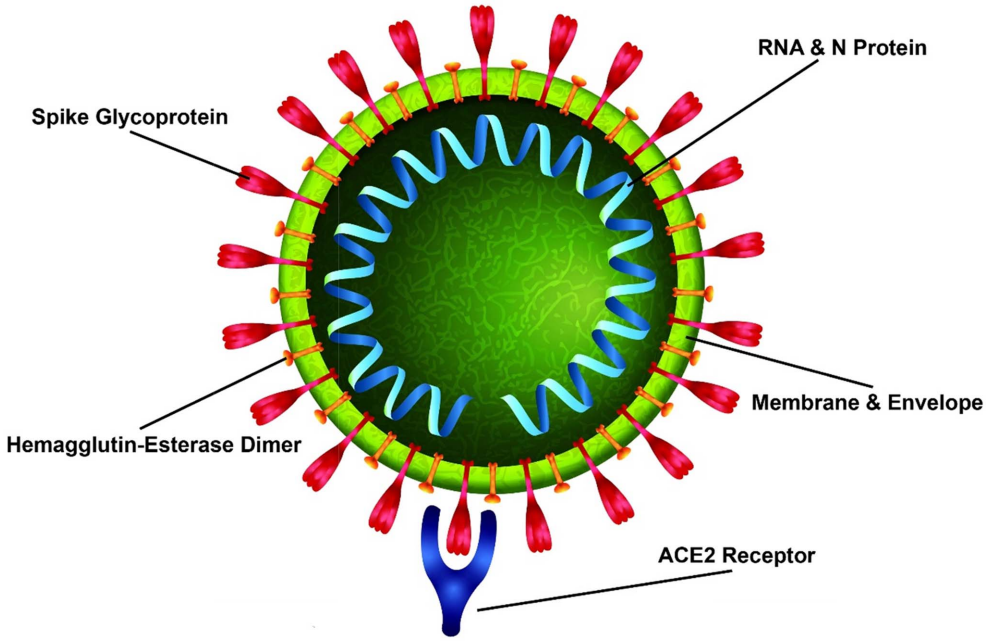


**Fig. 1. The general structure that shows the main components of SARS-CoV-2, which included RNA, N protein, Spike glycoprotein, hemagglutin-esterase dimer, membrane, and envelope.** The spike glycoprotein bound to the ACE2 receptor [8].

Betacoronavirus genus, which Chinese researchers provisionally called 2019-nCoV [5, 6]. The International Committee on Taxonomy of Viruses later named this virus as SARS-CoV-2. On the same day, the World Health organization (WHO) named the disease as the COVID-19 [7].

Within a month, this virus had spread through China at a high rate and killed over 1900, with about 75,000 people being infected with-in the first two months of the epidemic [9, 10]. COVID-19 is the third outbreak of severe respiratory disease, following SARS-CoV (November 2002, Guangdong, China) and MERS-CoV (June 2012, Jedda, Saudi Arabia) [11]. On January 31, 2020, the WHO announced COVID-19 as a Public Health Emergency of International Concern, meaning that it needed an international coordinated response as it might present risks to the entire world [12]. The WHO declared COVID-19 as a pandemic April 11, 2020 [13]. According to the data, from the Centre for Disease Control and Prevention, U.S.A. and the WHO, on May 27, 2020, there were 5,406,282 confirmed cases, with 343,562 confirmed deaths reported in 216 countries [14]. The literature has been reviewed with the aim of summarizing the epidemiological characteristics of COVID-19 in relation to the severity, diagnosis, drug development, and prevention based on recent research.

## Diversity of Coronaviruses

Coronaviruses infect a wide range of animals including livestock, poultry, and mammals. The interspecies transmissions of coronaviruses between unusual hosts form a complicated ecosystem [15]. The occurrence of SARS and the latest identification of the new COVID-19 have highlighted the necessity for investigation of the ecology of the coronavirus, particularly with respect to occurrences in wild animals such as bats [15]. Before the outbreak of SARS, most of the information concerning coronaviruses had resulted from findings related to animal health. Therefore, the ecological and evolutionary elements of coronaviruses have not been explored.

After the SARS outbreak, SARS-CoV was recognized as a disease-causing agent responsible for infection in humans [16]. SCoV-like viruses were isolated from Himalayan palm civets found in a live-animal market in Guangdong, China [17]. It was corroborated through virological surveillance that the zoonotic initiations occurred from SARS contagion that came from these

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animal souks [18]. However, subsequent research indicated that these animal markets had been intermediate hosts rather than the original the reservoirs of SARS-CoV, as huge surveillance research did not discover the virus in either farmed or wild animals of the identical species [19].

The current research, looks at whether the *Rhinolophus* spp. (Horseshoe bat) was a reservoir of SARS-CoV [20]. However, the genetic homology was identified as 64% in the civet SARS-like coronavirus and bat SARS-like coronavirus, for which the statistics indicated the variation trail of the SARS-CoV [21]. Because of the large biodiversity of bats, which have huge geographical distribution and migration potential, and their large populace size with the detection of numerous growing viruses [21], it is reasonable to consider that bats may also be the direct progenitor of SARS-CoV. Moreover, a growing number of novel coronaviruses have currently been identified. The identified types of coronaviruses are human coronavirus 229E (HCoV-229E), human coronavirus OC43 (HCoV-OC43), human coronavirus NL63, human coronavirus, SARS-CoV, MERS-CoV, and SARS-CoV-2 [22]. These accumulated findings support the view that coronaviruses have a much wider distribution in the animal kingdom than they did before. Genetic evaluation indicate that bat coronaviruses are mainly clustered. Further characterization of bat coronaviruses shows widespread genetic variety throughout an enormous geographic distribution and that unique species of bats also have coronaviruses that are specific to them and the same species of bat from exclusive geographic areas can have similar kinds of coronaviruses [23].

## Comparative Analysis of Emergence and Spreading of Coronavirus

In regards to SARS-CoV-2-infected pneumonia, the transmission includes respiratory contact and droplets, as stated on NHC (National Health commission). Medical remarks revealed that a patient infected with COVID-19 pneumonia detected positive for nucleic acid of SARS-CoV-2 in the stool. A case boom also revealed that a doctor from the United States collected a stool sample for RT-PCR testing in a patient with COVID-19 infected pneumonia, and this patient had a history of visiting family in Wuhan [24]. They perceived high positive nucleic acid of SARS-CoV-2 in patients. Additionally, Chinese scientists collected patients' feces and detected positive SARS-CoV-2 in 6.5% (4/62) of patients [25]. However, in regard to the diagnosis and existence of viral nucleic acid in these patients, further studies were required and performed to discover the existence of viral particles and nucleic acid levels in patients to determine the fecal-oral transmission. Fomite transmission, the spread of a virus through entities such as door handles, door-bells, and respirators, also showed an important role in the outbreak of the virus [26].

Zhong *et al.*, stated that some cases infected with SARS-CoV-2 are possibly from fomite transmission between humans, in addition to respiratory droplets and direct contact [25]. A recent study found that samples collected from nine newborn babies whose mothers had COVID-19, were not positive for SARS-CoV-2 [27]. Yet the authors acknowledged that the negative results may have been affected by the limited sample size. The possibility of vertical transmission needs to be considered if the virus or nucleic acid was extracted from the blood.

Besides, the contact levels of fecal contamination, aerosol transmission, and close contact with the mother could boost neonatal COVID-19 pneumonia in a newborn baby [28]. However, no reports of COVID-19 through blood contact were found. On the basis of clinical data, officials working in the National Health Commission inform that there is a risk of aerosol transmission. Strictly speaking, aerosols refer to suspended particles in a gas. A survey in 2010 suggested that a good ventilation strategy and avoiding aerosol generation may decrease the chance of aerosol transmission of influenza. Currently, however, there are no indications for the transmission of COVID-19 by aerosols. Nevertheless, we believe that aerosol transmission is possible because of the high risk of cross-infection among doctors, nurses, and staff [28].

## Reservoirs and Host of Coronavirus

Coronavirus phylogeny and biology were shown during the SARS epidemic in 2002–2003, which was characterized by frequent host transfer events. Such host transfer may be from animals to humans (zoonosis), humans to animals (reverse zoonosis), between animals [28, 29]. In the last few decades, various coronavirus

cross-species transfers led, to changes in the tropism of the virus, which led to many novel anima and humans diseases. These involve HCoV-229E, bovine coronavirus, canine coronavirus, transmissible gastroenteritis virus, HCoV-OC43, porcine coronavirus, and feline coronaviru [29]. A coronavirus (SARS-CoV) was identified as the etiological agent of SARS [30]. Bats are reservoirs of various zoonotic viruses including the Nipah and Hendra viruses. These were found lately in East Asia and Australia. Bats get infected by various viruses, but their clinical symptoms are rarely diagnosed. These variations and the enhanced availability of bats, led to spike in the traditional medicine and food-markets involving bats. The major share in bats product market was not only in Southern China, but everywhere in Asia [30].

The attempt to discover the origins of human coronaviruses identified SARS-CoV in horseshoe bats (*R. pearsoni, R. macrotis, R. sinicus,* and *R. ferrumequinum*) [30] and other coronavirus species were found in other bat species (*R. ferrumequinum, R. sinicus, Miniopterus magnate)* [31]. Studies on SARS-CoV, *P. pipistrellus, Pipistrellus abramus, Tylonycteris pachypus, Scotophilus kuhlii* and *Myotis ricketti* revealed that the phylogenetic and evolutionary relations among the coronaviruses and bats, as host, are unclear [32, 33]. These findings show that current pathogen hosts transfer as the inter-species transfer following by establishing the long term perseverance in the new hosts' specie. Various studies have described the relationship between geographic location and viral phylogeny by the identification of hosts [34].

## Genomic Variation in Coronavirus

The details inculcated below are based on the pathogenicity, traceability, and classification of a virus by studying its genetic information minutely. The entire genome sequence of SARS-CoV-2 is fifty percent similar to MERS-CoV, seventy-nine percent to SARS-CoV, eighty-eight percent to Bat-SL-CoVZXC21 and Bat-SL-CoVZC45, and about ninety-six percent to Bat-SARSr-CoV RaTG13 [35]. The genome of each SARS-CoV-2 strain comprises of about 29,900 nt. A minimum of 14 open reading frames (ORFs) of 5′ to 3′ were predicted of which the spike gene encoding a glycoprotein was more essential for defining transmission capability and host tropism, and when compared with Bat-SARSr-CoV, RaTG13 with an identity of 93.1% nt was found to be contradictory. Mostly, the RNA viruses were more rapid in substituting the nucleotide compared to their hosts, which mainly caused their evolution by natural selection. Among the viruses, gene mutations have often been reported in the SARS-CoV-2 [36].

**The mutation pattern between SARS-CoV-2 and its closely related coronaviruses.** The SARS-CoV-2 strain with genome sequence WIV04 was found to be closely related to the Bat-SL-CoVZC45 and Bat-SARSr-CoV RaTG13, of which the host specie *Rhinolophus affinis* matched with the genome sequence RaTG13. There were mutations observed in numerous nucleotide substitutions with only five mini delectations and insertions, while in the genome WIV04, which is closer to the border of S1 and S2 areas of spike protein, a large insert segment "CGGCGGGCACGT" was found. Synonymous mutations of insert sequence were observed. Compared with genome Bat-SL-CoVZC45, the insert segment was identified, however, non-synonymous mutations were also found around it [37, 38].

Subsequently, the synonymous mutations were found, compared, and analyzed, in between the RaTG13 and WIV04 of the spike gene. The observations showed increased synonymous mutations in the whole genome [39]. So, compared with that of RaTG13, this amplified T: C mutation indicates that the exon of SARS-CoV-2 could be deactivated. The SARS-CoV-2 with 108 strains of genome sequence was distinguished from December 2019 until February 2020, where 98 point mutations were identified at 93 nt sites on February 25, 2020. Thus, the current reported synonymous mutations (~40%) of COVID-19 strains were examined and similarities were found (39.1%) between the Bat-SL-CoVZC45 and WIV04, but it was significantly less than (90.7%) that between RaTG13 and WIV04 [36].

## Life Cycle of Coronavirus

The pneumonia caused by the novel coronavirus (SARS-CoV-2) is a highly contagious respiratory disease. Patients infected with COVID-19 show signs and symptoms such as high temperature (above 38℃), dry cough, fever, shortness of breathing, fatigue, and dyspnea. This pneumonia was first reported at the end of December,

2019, in Wuhan city of China. The infection is highly transmitted [40].

The life cycle of the SARS-CoV-2 in the host's cell; the virus' life cycle is initiated when the S protein attaches to the angiotensin-converting enzyme 2 (ACE2) cellular receptor. Due to the receptor attachment, conformation changing occurs by which the S protein helps the viral envelope to fuse via the endosomal pathway with the cell membrane. Subsequently, the SARS-CoV-2 releases its RNA into the cytoplasm of the host cell. The genomic RNA is translated into viral replicas polyproteins pp1a and 1ab, after which it is cleaved by the viral proteinases into mini products. The enzyme polymerase forms a series of subgenomic mRNAs by transcription that leads to the translation of viral proteins. On the endoplasmic reticulum and Golgi bodies, the genomic RNA and viral proteins assemble together, which forms versions and are then transported as well as released out of the cell with the help of vesicles [41].

## Epidemiology of Coronavirus

As of May 27, 2020, 5,406,282 confirmed cases of COVID-19 have been reported globally with 343,562 confirmed deaths. The confirmed cases in countries across Asia, Australia, Europe, and North America were warnings for the WHO to announce a COVID-19 global health emergency. These figures translate to a current global mortality rate of 3.04%; however, the number is liable to change the quantity of cases and influence the tolerant populaces to develop and change individually [42]. The outbreak of COVID-19 aligned with the festival of the Chinese Lunar New Year in late January 2020 and a related surmised 15 million visits to Wuhan City. At the beginning of COVID-19 outbreak, reports from emergency clinics in China showed that patients with severe disease and poor outcomes (as estimated by emergency units and mortality levels) were, in general, those with comorbid conditions for example, asthma, incessant obstructive aspiratory sickness, or advanced age [43]. In late January of 2020, the principal information about clinical highlights, the course of the disease, and the possible route of contamination with COVID-19 was dried from the past two destructive coronavirus outbreaks (MERS-CoV and SARS-CoV) [44].

## Clinical Manifestation

Recent literature has concluded that there have been patients infected with SARS-CoV-2 who did not show symptoms [45]. The infections of these patients were confirmed by the presence of viral nucleic acid in samples through real-time PCR. These patients had no symptoms of COVID-19, like dry cough, fever, or shortness of breath, but they were SARS-CoV-2 positive [46]. These people had been spreading the virus easily and posing a serious threat to society. Therefore, the identification of patients who were positive for SARS-CoV-2 had great importance. There was a need for thorough monitoring of the disease's course, and only the patients' contact histories helped in identification. However, from the current research, it is not clear whether these patients will be asymptomatic throughout their whole lives or if they were only asymptomatic in the initial phase of the disease [47].

According to Lai *et al.*, a pooled total of 970 patients had been analyzed with ARD based on two studies with clinical features conducted by Lui *et al.* and Guan *et al.* [47, 48]. In these studies, the ratio of males was higher than that of females, and the average age of the patients was 45. Among all the patients, diabetes mellitus and hypertension were the most common underlying diseases. The study concluded that 66% of patients had coughs while the percentage of those with fever was 44.7%. Furthermore, sore throat and sputum production were observed to be 14% and 33%, respectively. Gastrointestinal symptoms, like nausea, vomiting, and diarrhea were observed to be only 5%. Meanwhile, the patients that required oxygen therapy and mechanical ventilators were 32.4% and 0%, respectively. Only one patient with ARD died, hence the rate of mortality was 0.1% [49].

Another study was carried out by W.C. Ko *et al.*, who analyzed the clinical manifestations of 278 patients who were SARS-CoV-2 positive, also referred to as Wuhan pneumonia or novel coronavirus pneumonia. The ages of all patients were above 18 years. The male ratio was about double of the female. Among all the patients, hypertension and cardiovascular diseases were the common underlying diseases. It is summarized that the most common symptom was fever was 92.2%, followed

by cough, dyspnea, myalgia headache and diarrhea 69.8%, 34.5%, 27.7%, 7.2%, and 6.1%, respectively. The white blood cell count was normal in the majority of patients, however, 56.8% had leukopenia [50, 51].

## Severity/Pathogenesis of COVID-19

In December 2019, a respiratory disease emerged in China, caused by SARS-CoV-2 and termed as COVID-19. The severity of symptoms has multiplied day by day and resulted in more worldwide infections. The number of fatalities has also increased [52]. Patients who tested positive for the virus presented with clinical symptoms like fever (persistent or recurrent), dry cough (without sputum), fatigue, myalgia, and dyspnea [53]. Some patients' temperature increased to 93℃ with elevated or normal white blood cells counts. The virus targets the respiratory system hence the main pathogenesis is pneumonia. The blood profile of some serious intensive care unit (ICU) patients indicated high Pro-Inflammatory Cytokines. The symptoms are similar to the MERS-CoV and SARS-CoV infections [54]. Currently, the pathogenesis of COVID-19 is not clearly understood. However, the closed mechanism of MERS-CoV and SARS-CoV has given us clues about the pathogenesis of COVID-19 and helped us to identify it [55].

Initially, it was believed that the SARS-CoV entered the cells through direct contact with the cytoplasmic membrane [56]. Later, Belouzard et al., reported that the S protein of the coronavirus played an important role in its entry into the host cell [57]. The crown-like spikes bund to the host cell receptors helping facilitate entry of the virus. There are different types of cellular receptors, e.g., the MERS-CoV recognizes the DPP4 receptor on the surface of the host cell, while the SARS-CoV recognizes the CD209L [58]. The ACE2 receptor is recognized by both SARS-CoV and SARS-CoV-2. The virus enters into the host cell where it releases its viral genome in the cytoplasm. It then translates into structural proteins and polyproteins and the viral genomes begin to replicate [59, 60].

When the virus enters the host cell, the antigen presenting cells introduce the virus to the immune cells with the help of major histocompatibility complexes (class I and class II) molecules. The virus (antigen) is then recognized by the virus-specific cytotoxic T lymphocytes. Again, there is no information pertaining to the SARS-CoV-2 mechanisms; we have gathered data from previous research on MERS-CoV and SARS-CoV [61].

The antigen presentation led to the activation of the body cellular immunity which was mediated by the T lymphocytes and B lymphocytes, respectively [58]. The immune system produces antibodies against SARS-CoV: immunoglobulin G (IgG) and immunoglobulin M (IgM). The IgM almost disappear by day 70. However, the IgG remain present for a lengthy period of time in the body. This means that the IgG plays an important role. The IgG antibodies that were SARS-specific, N-specific and S-specific antibodies [62].

## Cytokine Storm in COVID-19

Studies revealed that that pro-inflammatory chemokines and cytokines including interleukin 1β (IL-1β), tumor necrosis factor (TNF) α, granulocyte-colony stimulating factor (G-CSF), IL-6, monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory proteins (MIP) 1-α, and interferon-γ-induced protein-10 (IP10) were significantly elevated in COVID-19 patients [63, 64]. The increased production of these cytokines results in a cytokine storm, leaving patients vulnerable to an increased risk of multiorgan failure, vascular hyperpermeability, and eventually death when not counteracted on time [65]. Cytokine storm is associated with high serum levels of TNFα, IFNγ, G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, MCP1, IP10, MIP1A, and MIP1B. Comparative analysis showed that ICU patients have elevated concentration TNFα, IP10, MCP1, MIP1A, G-CSF, IL-2, IL-7, and IL-10 [66]. TNFα and IL-1β promote TH17 responses, leakage, and vascular permeability. TH17 cells produce GM-CSF, IL-17, IL-21, and IL-22 by themselves. IL-17 has wide pro-inflammatory effects on the stimulation of cytokines G-CSF (involved in neutrophils recruitment and granulopoiesis), TNFα, IL-1β, IL-6, (leads to systemic inflammatory symptoms, including fever); MIP2A, IP10, MIP3A, chemokines KC, IL-8, (attracting and employing additional immune intruders); and matrix metalloproteinases (they take part in tissue damage and remodeling). GM-CSF and IL-17 are linked with inflammatory and autoimmune diseases. IL-21 is a prerequisite for TH17

cell maintenance, IL-22 upregulates fibrinogen, mucins, serum amyloid A, anti-apoptotic proteins, and LPS binding protein [67]; hence, IL-22 may lead to the establishment of life-threatening edema, observed in SARS-CoV-2. It has been concluded that TH17 type response leads to the cytokine storm in viral infection including SARS-CoV-2, which causes tissue damage and possibly stimulates pulmonary edema [68].

## Receptors of SARS-CoV-2

The coronaviruses bind to a number of host receptors. Spike protein of coronaviruses consists of S1 and S2 subunits. S1 subunit acts as a receptor-binding domain and further comprises of two more domains, the N-terminal domain and the C-terminal domain while the S2 subunit is required for the fusion of virus and host cell membrane [69]. The virus enters the cell by first attaching itself to the ACE2 receptor, and then entering the cell by either of the two pathways, endocytosis or by cleaving the receptor attached spike protein [70]. Coronavirus spike S protein was reported to be a major determinant of virus entry into the host cell. Entry relies on the binding of S protein to the cellular receptor. SARS-CoV-2 hijacked the ACE2 as a target for entry [71]. According to the Cryo-EM structure-based studies of spike protein, it is found that the SARS-CoV-2 Spike protein has a 10 to 20-fold greater affinity to the ACE2 receptor than the SARS-CoV receptor which potentially leads to the rapid virus spreading [72]. The discovery of ACE2 as the SARS-CoV-2 receptor also shows that some human organs have a high degree of ACE2 expression, such as intestinal tract, lungs, alveolar cells, gallbladder, testis and renal tubule [73].

## Diagnosis of COVID-19

The diagnosis of COVID-19 is based on the clinical manifestation, epidemiological history, and some other examinations like CT scan, nucleic acid detection, blood culture, enzyme-linked immune-sorbent assay (ELISA), and immune identification technology of IgG/IgM [74, 75]. However, the clinical signs and symptoms of a SARS-CoV-2 positive individual are highly atypical. The symptoms and signs include high fever, a dry cough, shortness of breath, viral pneumonia, and dyspnea. In addition, sufferers might also have a sore throat, vomiting, and diarrhea. This is why an auxiliary examination is required for the SARS-CoV-2 virus diagnosis [76].

Currently, there are two technologies commonly used for the detection of SARS-CoV-2. These are high-throughput sequencing and real-time quantitative polymerase chain reaction (RT-qPCR) [75, 77]. However, high-throughput sequencing technology is not commonly used in the diagnosis of SARS-CoV-2 because of its high cost and because it is totally dependent on equipment. Instead, RT-qPCR is the most common, straightforward, and effective technology for the clinical diagnosis of SARS-CoV-2 from blood or respiratory secretions [77].

The China Center for Disease Control and Prevention (China CDC) has suggested a specific probe and primer for the clinical detection of SARS-CoV-2. They are the ORF1ab and N gene regions which can be detected by real-time PCR. During the real-time PCR examination, if these two targets are positive, the patient is confirmed to be COVID-19 positive [78]. Although the RT-qPCR is highly specific, it did sometimes give false negative results, an issue that should not be ignored. That is why an auxiliary method should also be used. A patient with signs and symptoms of SARS-CoV-2 may still have a negative result from RT-qPCR. For this, both a chest CT scan and high-resolution computed tomography (HRCT), for early diagnosis and disease severity, and repeated RT-qPCR would be helpful [79, 80].

F. Zhang of MIT has developed a rapid detection test paper for SARS-CoV-2 and this test will give results in just one hour. However, this technology has not been clinically verified [81]. Different Nano based diagnosing techniques such as point of care testing, optical biosensor technology, and nanopore target sequencing have also been developed [82].

## Serological Feature of COVID-19

The most specific diagnostic method which uses uniformly is nucleic acid detection by real-time RT-PCR. Rapid lateral flow assays for both IgM and IgG antibodies undoubtedly will play an important role in the COVID-19 outbreak, asymptomatic infections, the basic reproduction number, and the overall mortality to be determined. However, IgM responses are notoriously nonspecific, and weeks required to develop specific IgG

responses. Serology detection is not the same to play a role in active case management, except to diagnose/confirm late COVID-19 cases or to determine the immunity of health care workers as the outbreak progresses. Detection of COVID-19 can be difficult due to the nonspecific mild respiratory symptoms in many affected individuals. Because some people might recover without being diagnosed [83]. Although, it may be lead to a false-negative rate of the procedure is unelectable and the serological techniques are urgently warranted. The quick detection approach targeting viral IgM or IgG antibody, the colloidal gold-based immune chromatographic (ICG) strip assay, in comparison with RT-PCR testing [84].

The antibodies are produced and secreted by B lymphocytes of the adaptive immune system when the pathogens invaded. IgM is usually the first responded antibody that eliminating pathogens before sufficient IgG is produced, while IgG serves as the most robust antibody-based immunity. The retrospective study of immunoglobulins against SARS-CoV, IgM, and IgG were started to be detected after 7 days of onset and persistent for 2–3 years. Similar to SARS-CoV, COVID-19 patients also showed similar characteristics. Both IgM and IgG can be detected after 5 days of onset by anti-SARS-CoV-2 ELISA assay. Both (IgM and IgG) were firstly detected on day 4 among the confirmed cohort and the rate positivity of IgM and IgG were 11.1% and 3.6% in early stage patients, respectively. Noteworthy, combining IgM and IgG detection results could reach the maximal testing efficacy, especially in the intermediate stage. A similar trend could be captured in clinically diagnosed patients. In addition, the consistency rate of whole blood and plasma samples in IgM and IgG assays were 100% and 97.1%, respectively [85].

## Treatment Strategies

The transmission of SARS-CoV-2 from one person to another is very high, which leads to the isolation of an infected person for proper care and treatment. Just as for MERS-CoV and SARS-CoV, currently, no vaccines, drugs, or other antiviral agents are available for SARS-CoV-2. The work on vaccine development and drug development is in progress [86]. Currently, different strategies have been used to treat patients. These strategies include the use of broad-spectrum antibiotics, antiviral drugs, interferons-α nebulization, and oxygen therapy to decrease the viral load [41].

**Antiviral treatment.** Different types of antiviral drugs have been used to treat SARS-CoV-2-infected patients. The recently reported drug remdesivir showed some strong antiviral properties against RNA viruses that target RNA-dependent RNA polymerases which lead to the blockage of RNA synthesis and hence stop virus replication. It was a promising drug against RNA viruses such as SARS-CoV, SARS-CoV-2, and MERS-CoV in mice and nonhuman primates [41]. Holshue *et al.* were the first to report that the use of remdesivir against COVID-19-infected patients gives good results [81, 87]. Remdesivir, a nucleotide analogue prodrug that inhibits viral RNA polymerases, has shown in vitro activity against SARS-CoV-2. The Washington State Department of Health also demonstrated that remdesivir in combination with chloroquine effectively inhibits SARS-CoV-2 in vitro. Chloroquine has immune-modulating activity and effectively inhibits RNA viruses in vitro. It was reported that in vitro, chloroquine possessed different inhibitory effects against a wide range of RNA viruses, including polio-virus, HIV, hepatitis A virus, rabies, influenza A and B virus, Hepatitis C virus, chikungunia virus, dengue virus and zika virus, as well as DNA viruses, including herpes simplex virus and hepatitis B virus [88]. Other viral drugs were also being used. These include nitazoxanide, penciclovir, ribavirin, ritonavir, nafamostat, baricitinib, and favipiravir [89, 41]. Combinations such as antiviral drugs with antibiotics and chines medicines are also evaluated in mice and humans against SARS-CoV-2-induced infections. In the current literature, the remdesvir show high effectiveness and considered as a promising drug against COVID-19. In Shanghai, doctors isolated plasma from the recovered infected person and inject it to the patient for rapid recovery. Recently it was also identified that monoclonal antibody (CR3022) binds with the spike receptor binding domain of SARS-CoV-2 [87, 90].

Clinical trials on remdesivir. The remdesivir is a potential COVID-19 therapy drug. It is a phosphoramidite prodrug of an adenosine C-nucleoside and a broad-spectrum antiviral agent synthesized and developed in

2017 by Gilead Science to treat Ebola virus infection [91]. The studies show that the remdesivir can effectively reduce the viral load of MERS-CoV infected mice in the lung tissue, enhanced the lung function, and mitigated pathological damage to the lung tissue [92]. RDV is strongly antiviral in primary human lung epithelial cell culture toward the transmission of contemporary human CoVs, SARS-CoV (EC50 = 0.07 μM), MERS-CoV (EC50 = 0.07 μM) and associated zoonotic batCoVs1. The recent studies stated that therapeutic RDV improve disease outcomes and reduce the viral load in mice infected with SARS-CoV. Wang et al found that Remdesivir blocks infection with SARS-CoV-2 at low micromolar concentrations and has a high selectivity index (Half maximum Effective Concentration (EC50), 0.77 μM; Half Cytotoxic concentration (CC50)>100 μM; SI>129.87) [91]. Between 6 Feb 2020 and 12 March 2020, 237 patients were registered and randomly assigned to the treatment group (158 to Remdesivir and 79 to placebo), one placebo patient who withdrew after randomization was not included in the ITT population. Remdesivir use was not correlated with a clinical improvement gap in time (hazard ratio 1.23 [95% CI 0.87–1.75]). Although not statistically important, but there was a numerically faster for clinical improvement in patients receiving remdesivir than those receiving placebo in patients with symptoms length of 10 days or less [93]. Remdesivir decrease the virus title of mice infected with MERS-CoV more significantly and minimized damage to the lung tissue. Remdesivir treatment has improved disease outcome and reduced viral load in infected mice with SARS-CoV, and is inhibitory for in vitro SARS-CoV-2 [94]. For COVID-19 patients, a study reported that 36 out of 53 patients (68%) with sever COVID-19 who taken Remdesivir had seen clinical improvement. A case of COVID-19 infection was also recorded successfully treated by Remdesivir in the US, and the patient shows substantial improvement in clinical symptoms within 24 hours of treatment. However, a randomized, double-blind, placebo-controlled multicenter study of 237 patients found that intravenous Remdesivir did not significantly improve the time for clinical improvement. However, significant in patients treated within 10 days of the onset of symptoms, but the median time for clinical improvement was associated with a five days' reduction [95].

Clinical trials on chloroquine. Chloroquine inhibits SARS-CoV-2 in-vitro, and a Chinese commentary mentioning 15 trails stated that "Studies from over 100 patients to date have shown that chloroquine phosphate is superior in control medication inhibiting pneumonia exacerbation" without providing any further information [96]. A Chinese study involving more than 100 COVID-19 patients found chloroquine superior to the control group to minimize the length of the symptoms, worsen pneumonia including radiological improvement and encourage virus-negative seroconversion without significant side effects. This was the first human trial ever performed against COVID-19 with chloroquine; this early outcome has led China to use chloroquine in COVID-19 prevention and treatment [97]. In the absence of serious side effects, early findings from more than 100 patients participating in studies performed in China demonstrated the superiority of chloroquine relative to control in terms of reduction of pneumonia exacerbation, length of symptoms and delay in viral clearance [98]. In in-vitro studies, chloroquine was found to inhibit contamination with COVID-19 at low micromolar concentrations, with a half-maximum effective concentration (EC50) of 1.13 μM and a half cytotoxic concentration (CC50) greater than 100 μM. A number of subsequent clinical trials (ChiCTR2000029939, ChiCTR2000029935, ChiCTR2000029899, ChiCTR2000029898, ChiCTR2000029868, ChiCTR2000029837, ChiCTR2000029826, ChiCTR2000029803, ChiCTR2000029762, ChiCTR2000029761, ChiCTR2000029760, ChiCTR2000029740, ChiCTR2000029609, ChiCTR2000029559, and ChiCTR2000029542), the efficiency and safety of chloroquine in the treatment of COVID-19 associated pneumonia was quickly tested in more than 10 hospitals of Wuhan, Jingzhou, Guangzhou, Beijing, Shangai, Chongqing and Ningbo [99].

**Chinese Medicine-Based Strategies.** Chinese medicine plays an important role in the treatment of COVID-19 [100]. A number of Chinese medicine prescriptions have been published by medical institutions and the local government. The Wuhan Institute of Virology, Chinese Academy of Sciences along with the Shanghai Institute of Materia Medica concluded that the SARS-CoV-2 could be inhibited with the help of Shuanghuanglian oral liq-

uid. The Shuanghuanglian contains some useful compounds such as chlorogenic acid, forsythin, and baicalin and these compounds showed an inhibitory effect on different bacteria and viruses [101, 102].

**Vaccines based strategies.** SARS-CoV-2 vaccines were required to limit the transmission and disease severity. Currently, there were no vaccines available for SARS-CoV-2 virus, which a big challenge to developed vaccines [41]. There was a different type of strategies against MERS-CoV and SARS-CoV tested on nonhuman primates including inactivated vaccines, Subunit vaccines, live attenuated vaccines, protein vaccines, recombinant DNA vaccines, and viral vectors. These strategies were in progress but it needs much time (from months to years) to devolve vaccines against the SARS-CoV-2 virus [81, 86]. The WHO and Chinese scientists work to launch eighty plus to clinical trials for the treatment of COVID-19. US Institute of Allergy and Infectious Diseases developed an RNA based vaccines for SARS-CoV-2 which are under clinical trials. The China CDC works to develop inactivated vaccines. In addition, Clover Biopharmaceuticals work on the recombinant 2019-nCoV S protein subunit-trimer based vaccine. Soon the vaccines against COVID-19 will be available in the market [103].

## Antibody based Therapeutics and mRNA based Vaccines under Clinical Trials

Scientists across the world are putting significant efforts in developing therapeutic agents against coronavirus infection. A variety of therapeutic agents have been screened against the SARS-CoV-2 including interferons, peptides, monoclonal antibodies, antivirals, cell-based therapeutics, and vaccines of different kinds. The development of these therapeutic and preventive agents may take several months. In order to contain the epidemic quickly and efficiently, we must exploit every potential therapeutic agent. Scientists have isolated a neutralizing antibody targeting the surface S protein of SARS-CoV-2 is likely the first therapy anticipated by biomedical researchers, giving passive immunity to COVID-19 [104]. Antibody-based therapeutic agents are under clinical trials in huge amounts and most of them have passed the preliminary assessment of safety and efficacy. Data mining reports that up to 72 different antibodies based agents in various countries are under clinical development [105].

One of the early developed and potent vaccine candidates is an antibody based vaccine under development by the University of Oxford. This vaccine, AZD1222, is presently in Phase II/III clinical trial in the United Kingdom, with remaining late-stage trials to be carried in various countries. Company AstraZeneca™ that will mass produce the AZD1222, will provide up to 400 million doses of the said vaccine to Europe by the end of this year. The company also aims to provide one billion doses to low and middle-income countries through a joint venture established with the Serum Institute of India [106]. WHO has recognized AZD1222 of Oxford University-AstraZeneca and mRNA-1273 of Moderna Inc™ as the most advanced vaccine candidates amongst others [107].

As of July 2, 2020, there are 179 vaccine candidates of various kinds under pre-clinical and clinical development. Researchers around the globe are using different platforms for vaccine development such as DNA-based, inactivated virus, live attenuated virus, non-replicating viral vectors, replicating viral vectors, protein subunits, virus-like particles and RNA-based vaccine [105]. The mRNA based vaccine candidate (mRNA-1273) of Moderna Inc™ has advanced to the late stages of clinical development [108]. Moderna's mRNA-1273, is one of the fastest and potent vaccine candidate as it entered into clinical trials just 66 days after SARS-CoV-2 was first sequenced. This vaccine carries a genetic sequence into a host cell, and co-opt host system to code antigens of interest. Moderna's vaccine uses a synthetic lipid nanoparticle for the transport of mRNA templates [109].

## Unresolved Issues of COVID-19

Worldwide, researchers trying to understand COVID-19, but there is vague information about the pandemic [110]. Coronavirus can be fully inactivated by using surface disinfectants with 62–71 percent ethanol, 0.5 percent hydrogen peroxide, or 0.1 sodium hypochlorite within a minute. However, according to some reports, the impact of disinfectants lacks recent investigations [111]. Some issues surrounding the recombination of coronavirus are unresolved particularly the recombination mechanism, e.g., what is the sequence requirement for recombination? What are the proteins required for

http://dx.doi.org/10.4014/mbl.2004.04006

recombination? [87, 112]. Whether the initial leader to body fusion occurs during positive-strand or negative-strand RNA synthesis, and whether the negative-strand single guide RNA species are active template or dead end products are still unanswered issues [81]. Although the reservoirs of the animals are likely to be a bat species that is not described nor the intermediate host. The routes for the infection are not completely characterized and the morbidity and mortality rates undetermined. The role of asymptomatic or mildly symptomatic infection in disease transmission is unknown, especially in children, who are potential super-spreaders, as we saw in the SARS-CoV epidemic. The current knowledge available about the temporal regulation of viral transcription and translation is minimal and information regarding the course of infection is a major unresolved problem of COVID-19. DMVs' (double-membrane vesicles) and CVs' (convoluted membranes) functioDMVsns and the exact location of the site of active viral RNA synthesis remain unresolved [41]. The clinical presentation of COVID-19 varies from asymptomatic to severe pneumonia; however, in most studies, the incidence of SARS-CoV-2 pneumonia in the male patient is higher than female patients, that mortality in males is higher which is also unclear. Subsequent to the publication of studies in patients with only ARD or moderate pneumonia [80]. We observed a decrease in the ratio of male to female patient, children, or neonate being able to contract COVID-19 and a decrease in mortality compared to previous reports. However, it remains unclear whether the children is less prone to SARS-CoV-2 or whether their appearance was mostly asymptomatic or difficult to detect. In addition, asymptomatic were confined in areas such as China with a high rate of infected population [41, 81, 90].

## Future Directions

Under the current scenario, numerous developments have occurred in different countries, including treatment and vaccines. Based on the results of minor experiments, the accessory protein was found to have a potential modulating effect on the viral replication [41]. More studies on the interaction between the accessory protein and the viral proteins, as well as the cellular factor, will lead to a better understanding of the complex interplay between the SARS-CoV. Human hosts can contribute to the development of antiviral therapy and the design of successful disease control strategies. Through clinical trials, the synthetic recombinant interferon α has proven to be effective in the treatment of SARS patients. Intravenous immunoglobulin may be the best available immunomodulator for long-term use for all ages of infected patients, and might help inhibit the development of proinflammatory cytokines and increase the production of anti-inflammatory mediators [81, 100, 102]. If a vaccine can be developed, it will be added to the Extended Program on Immunization (EPI). However, if an inadequate number of vaccines and experimental medications are developed, convalescent plasma therapy for seriously infected patients could be an effective method for alleviating the course of the disease. In retrospect, convalescent plasma therapy in patients with severe SARS is more effective than intense doses of hormonal shock treatment, reducing the mortality rate and shortening the length of hospitalization [41, 110, 112].

## Conclusion

The SARS-CoV-2 causes a disease called COVID-19, originated at a seafood market in Wuhan, China, where different animals were sold, including snakes, palm civets, dogs, and bats, etc., has spread rapidly around the world. Sequences-based analysis has revealed that the key reservoir is bats, however, this is not yet confirmed. Different diagnosis tests are available but require effective methods for the current testing strategies which could be expensive. Currently, no promising drugs or vaccines have been developed against COVID-19. Researchers are trying to develop different types of therapeutic strategies to combat this virus. Different types of antiviral drugs that were used for the treatment of SARS-Cov and MERS-CoV have been used alone or in combination with other antivirals, broad-spectrum antibiotics, or Chinese medicines. Remdesivir is considered the most promising drug as it blocks the replication of the virus in infected patients. A number of companies are working to develop vaccines against COVID-19. These include Inovio Pharmaceuticals, Moderna Therapeutics, Novavax, Vir Biotechnology, Johnson & Johnson, Clover Biopharmaceuticals, VIDO-InterVac, Stermirna Therapeutics, Johnson & Johnson, and

VIDO-InterVac. But vaccines take months to years to come to commercialization.

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## Conflict of Interest

The authors have no financial conflicts of interest to declare.

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