

## INTRODUCTION

Over the past 2 decades, coronaviruses (CoVs) have been associated with significant disease outbreaks in East Asia and the Middle east. The severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) began to emerge in 2002 and 2012, respectively. Recently, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), emerged in late 2019, and it has posed a global health threat, causing an ongoing pandemic in many countries and territories(1).

Health workers worldwide are currently making efforts to control further disease outbreaks caused by the novel CoV (originally named 2019-nCoV), which was first identified in Wuhan City, Hubei Province, China, on 12 December 2019. On 11 February 2020, the World Health Organization (WHO) announced the official designation for the current CoV-associated disease to be COVID-19, caused by SARS-CoV-2. The primary cluster of patients was found to be connected with the Huanan South China Seafood market in Wuhan (2). CoVs belong to the family Coronaviridae (subfamily Coronavirinae), the members of which infect a broad trimeric S1 locates itself on top of the trimeric S2 stalk (45). Recently, structural analyses of the S proteins of COVID-19 have revealed 27 amino acid substitutions, within a 1,273-amino-acid stretch (16). Six substitutions are located in the RBD (amino acids 357 to 528), while four substitutions are in the RBM at the CTD of the S1 domain (16). Of note, no amino acid change is seen in the RBM, which binds directly to the angiotensin-converting enzyme-2 (ACE2) receptor in SARS-CoV (16,46). At present, the main emphasis is knowing how many differences would be required to change the host tropism.

Sequence comparison revealed 17 nonsynonymous changes between the early sequence of SARS-CoV-2 and the later isolates of SARS-CoV. The changes were found scattered in ORF1ab, ORF8 (4 situations), the spike gene (3 situations), and ORF7a (single substitution) (4). Notably, the same nonsynonymous changes were found in a familial cluster, indicating that the viral evolution happened during person-to-person transmission (4,47). Such adaptive evolution events are frequent and constitute a constantly ongoing process once the virus spreads among new hosts (47). Even though no functional changes occur in the virus associated with this adaptive evolution, close monitoring of the viral absence of this protein is related to the altered virulence of coronaviruses due to changes in morphology and tropism (54). The E protein consists of three domains, namely, a short hydrophilic amino terminal, a large hydrophobic transmembrane domain, and an efficient C-terminal domain (51). The SARS-CoV-2 E protein reveals a similar amino acid constitution without any substitution (16).

### N Protein

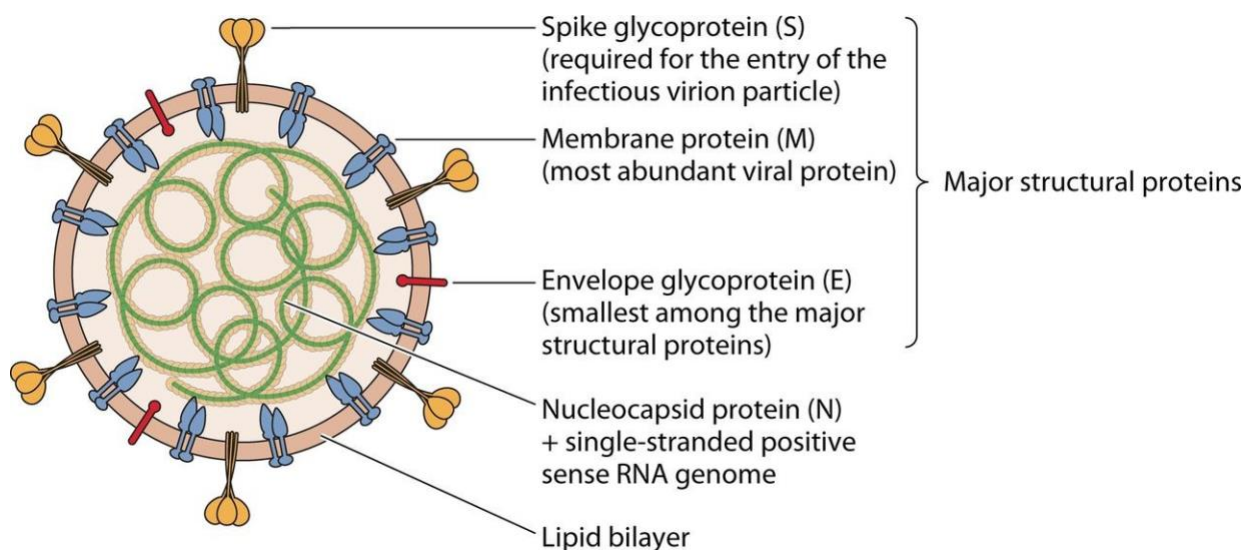
The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus (55, 56). It contains three highly conserved and distinct domains, namely, an NTD, an RNA-binding domain or a linker region (LKR), and a CTD (57). The NTD binds with the 3' end of the viral genome, perhaps via electrostatic interactions, and is highly diverged both in length and sequence (58). The charged LKR is serine and arginine rich and is also known as the SR (serine and arginine) domain (59).

The LKR is capable of direct interaction with in vitro RNA interaction and is responsible for cell signaling (60, 61). It also modulates the antiviral response of the host by working as an antagonist for interferon.

### Nsps and Accessory Proteins

Besides the important structural proteins, the SARS-CoV-2 genome contains 15 nsps, nsp1 to nsp10, and nsp12, to nsp16, and 8 accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b and ORF14) (16). All these proteins play a specific role in viral replication (27). Unlike the accessory proteins of SARS-CoV, SARS-CoV-2 does not contain 8a protein and has a longer 8b and shorter 3b protein (16). The nsp7, nsp13, envelope, matrix, and p6 and 8b accessory proteins have not been detected with any amino acid substitutions compared to the sequence of other coronaviruses (16).

The virus structure of SARS-CoV-2 is depicted in Fig.2.



Initially, the epicenter of the SARS-CoV-2 pandemic was China, which reported a significant number of deaths associated with COVID-19, with 84,458 laboratory-confirmed cases and 4,644 deaths as of 13 May 2020 ( Fig.4). As of 13 May 2020, SARS CoV-2 confirmed cases have been reported in more than 210 countries apart from China (Fig.3 and 4) (WHO Situation Report 114) (25, 64). COVID-19 has been reported on all continents except Antarctica. For many weeks, Italy was the focus of concerns regarding the large number of cases, 1,322,054, and 79,634 deaths. Now, the United Kingdom has even more cases (226,4671) and deaths (32,692) than Italy. A John Hopkins University web platform has provided daily updates on the basic epidemiology of the COVID-19 outbreak.

COVID-19 has also been confirmed on a cruise ship, named Diamond Princess, quarantined in Japanese waters (Port of Yokohama), as well as on other cruise ships around the world (239) (Fig.3). The significant events of the SARS-CoV-2/COVID-19 virus outbreak occurring since 8 December 2019 are presented as a timeline in Fig.5.

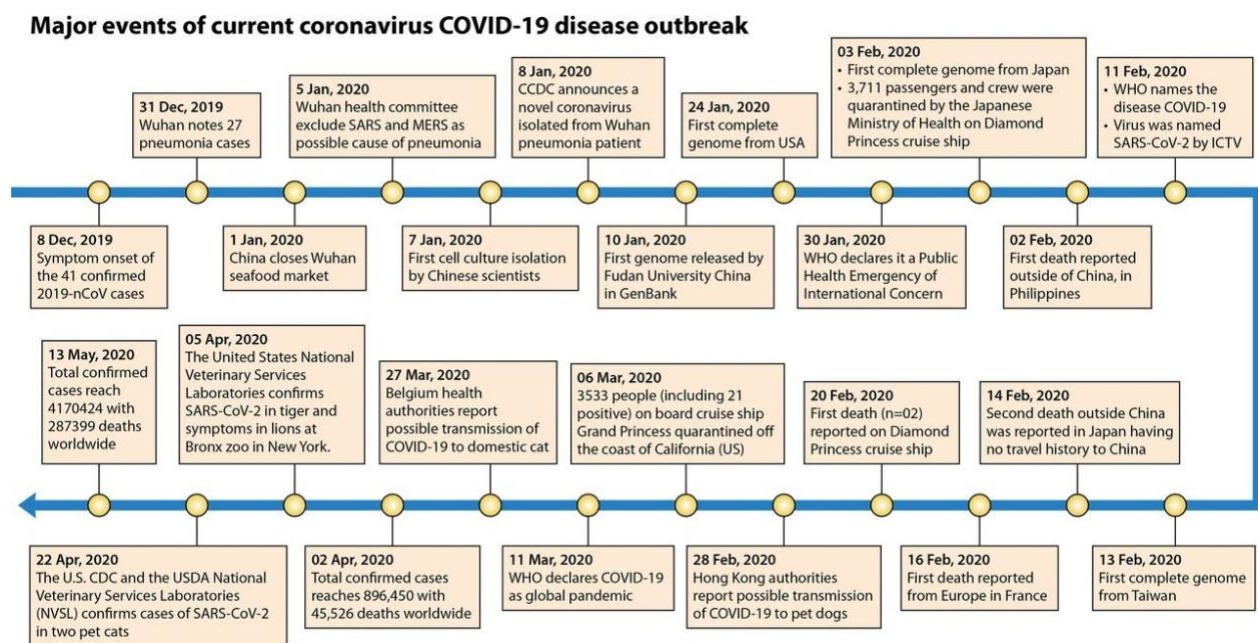


FIG 5 Time depicting the significant events that occurred during the SARS-CoV-2/COVID-19 virus outbreak. The timeline describes the significant events during the current SARS-CoV-2 outbreak, from 8 December 2019 to 13 May 2020.

At the beginning, China experienced the majority of the burden associated with COVID-19 in the form of disease morbidity and mortality (65), but over time the COVID-19 menace moved to Europe, particularly Italy and Spain, and now the United States has the highest number of confirmed cases.

In another study, the average reproductive number of COVID-19 was found to be 3.28, which is significantly higher than the initial WHO estimate of 1.4 to 2.5 (77). It is too early to obtain the exact  $R_0$  value, since there is a possibility of bias due to insufficient data. The higher  $R_0$  value is indicative of the more significant potential of SARS-CoV-2 transmission in a susceptible population. This is not the first time that the culinary practices of China have been blamed for the origin of novel coronavirus infection in humans. Previously, the animals present in the live-animal market were identified to be the intermediate hosts of the SARS outbreak in China (78). Several wildlife species were found to harbor potentially evolving coronavirus strains that can overcome the species barrier (79). One of the main principles of Chinese food culture is that live-slaughtered animals are considered more nutritious (5).

After 4 months of struggle that lasted from December 2019 to March 2020, the COVID-19 situation now seems under control in China. The wet animal markets have reopened, and people have started buying bats, dogs, cats, birds, scorpions, badgers, rabbits, pangolins (scaly anteaters), and minks. Soup from palm civets, ostriches, hamsters, snapping turtles, ducks, fish, Siamese crocodiles, and other creatures.

As an entry receptor while exhibiting an RBD similar to that of SARS-CoV (17, 87, 254, 255). Several countries have provided recommendations to their people traveling to China (88, 89). Compared to the previous coronavirus outbreaks caused by SARS-CoV and MERS-CoV, the efficiency of SARS-CoV-2 human-to-human transmission was thought to be less. This assumption was based on the finding that health workers were affected less than they were in previous outbreaks of fatal coronaviruses (2). Superspreading events are considered the main culprit for the extensive transmission of SARS and MERS (90,91). Almost half of the MERS-CoV cases reported in Saudi Arabia are of secondary origin that occurred through contact with infected asymptomatic or symptomatic individuals through human-to-human transmission (92). The occurrence of Superspreading events in the COVID-19 outbreak cannot be ruled out until its possibility is evaluated. Like SARS and MERS, COVID-19 can also infect the lower respiratory tract, with milder symptoms (27). The basic reproduction number of COVID-19 has been found to be in the range of 2.8 to 3.3 based on real time reports and 3.2 to 3.9 based on predicted infected cases (84). Route warrants the introduction of negative fecal viral nucleic acid test results as one of the additional discharge criteria in laboratory-confirmed cases of COVID-19 (326).

The COVID-19 pandemic does not have any novel factors, other than the genetically unique pathogen and a further possible reservoir. The cause and the likely future outcome are just repetitions of our previous interactions with fatal coronaviruses. The only difference is the time of occurrence and the genetic distinctness of the pathogen involved. Mutations on the RBD of CoVs facilitated their capability of infecting newer hosts, thereby expanding their reach to all corners of the world (85). This is a potential threat to the health of both animals and humans. Advanced studies using Bayesian phylogeographic reconstruction identified the most probable origin of SARS-CoV-2 as the bat SARS-like coronavirus, circulating in the *Rhinolophus* bat family (86).

Phylogenetic analysis of 10 whole-genome sequences of SARS-CoV-2 showed that they are related to two CoVs of bat origin, namely, bat-SL-CoVZC45 and bat-SL-CoVZXC21, which were reported during 2018 in China (17). It was reported that SARS-CoV-2 had been confirmed to use ACE2 as an entry receptor while exhibiting an RBD similar fever, cough, and sputum (83). Hence, the clinicians must be on the look-out for the possible occurrence of atypical clinical manifestations

to avoid the possibility of missed diagnosis. The early transmission ability of SARS-CoV-2 was found to be similar to or slightly higher than that of SARS-CoV, reflecting that it could be controlled despite moderate to high transmissibility (84).

Increasing reports of SARS-CoV-2 in sewage and wastewater warrants the need for further investigation due to the possibility of fecal-oral transmission. SARS-CoV-2 present in environmental compartments such as soil and water will finally end up in the wastewater and sewage sludge of treatment plants (328). Therefore, we have to reevaluate the current wastewater and sewage sludge treatment procedures and introduce advanced techniques that are specific and effective against SARS-CoV-2. Since there is active shedding of SARS-CoV-2 in the stool, the prevalence of infections in a large population can be studied using wastewater-based epidemiology. Recently, reverse transcription-quantitative PCR (RT-qPCR) was used to enumerate the copies of SARS-CoV-2 RNA concentrated from wastewater collected from a wastewater treatment plant (327). The calculated viral RNA copy numbers determine the number of infected individuals. The whole world is suffering from novel SARS-CoV-2, with more than 4,170,424 cases and 287,399 deaths across the globe. There is an urgent need for a rational international campaign against the unhealthy food practices of China to encourage the sellers to increase hygienic food practices or close the crude live-dead animal wet markets. There is a need to modify food policies at national and international levels to avoid further life threats and economic consequences from any emerging or reemerging pandemic due to close animal-human interaction (285).

Even though individuals of all ages and sexes are susceptible to COVID-19, older people with an underlying chronic disease are more likely to become severely infected (80). Recently, individuals with asymptomatic infection were also found to act as a source of infection to susceptible individuals (81). Both the asymptomatic and symptomatic patients secrete similar viral loads, which indicates that the transmission capacity of asymptomatic or minimally symptomatic patients is very high. Thus, SARS-CoV-2 transmission can happen early in the course of infection (82). Atypical clinical manifestations have also been reported in COVID-19 in which the only reporting symptom was fatigue. Such patients may lack respiratory signs, such as fever, cough, and sputum (83). Hence, the clinicians  
Turtles, ducks, fish, Siamese, Crocodiles, and other animal meats without any fear of COVID-19. The Chinese government is encouraging people to feel they can return to normalcy. However, this could be a risk, as it has been mentioned in advisories that people should avoid contact with live-dead animals as much as possible, as SARS-CoV-2 has shown zoonotic spillover. Additionally, we cannot rule out the possibility of new mutations in the same virus being closely related to contact with both animals and humans at the market (284). In January, 2020, China imposed a temporary ban on the sale of live-dead animals in the wet markets. However, now hundreds of such wet markets have been reopened without optimizing standard food safety and sanitation practices (286).

With China being the most populated country in the world and due to its domestic and international food exportation policies, the whole world is now facing the menace of COVID-19, including China itself. Wet markets of live-dead animals do not maintain strict food hygienic practices. Fresh blood splashes are present everywhere, on the floor and tabletops, and such food customs could encourage many pathogens to adapt, mutate, and jump the species barrier. As a result, the whole world is suffering from the novel SARS-CoV-2, with more.

From experience with several outbreaks associated with known emerging viruses, higher pathogenicity of a virus is often associated with lower transmissibility. Compared to emerging viruses like Ebola virus, avian H7N9, SARS-CoV, and MERS-CoV, SARS-CoV-2 has relatively lower pathogenicity and moderate transmissibility (15). The risk of death among individuals infected with COVID-19 was calculated using the infection fatality risk (IFR). The IFR was found to be in the range of 0.3% to 0.6%, which is comparable to that of a previous Asian influenza pandemic (1957 to 1958) (73,277).

Notably, the reanalysis of the COVID-19 pandemic curve from the initial cluster of cases pointed to considerable human-to-human transmission. It is opined that the exposure history of SARS-CoV-2 at the Wuhan seafood market originated from human-to-human transmission rather than animal-to-human transmission (74); however, in light of the zoonotic spillover COVID-19, is too early to fully endorse this idea (1). Following the initial infection, human-to-human transmission has been observed with a preliminary reproduction number ( $R_0$ ) estimate of 1.4 to 2.5 (70,75) and recently it is estimated to be 2.24 to 3.58 (76). In another study, the average reproductive number of possible origin of SARS-CoV-2 and the first model of disease transmission are not yet identified (70). Analysis of the initial cluster of infections suggests that the infected individuals had a common exposure point, a seafood market in Wuhan, Hubei Province, China (Fig.6). The restaurants of this market are well-known for providing different types of wild animals for human consumption (71). The Hunan South China Seafood Market also sells live animals, such as poultry, bats, snakes, and marmots (72). This might be the point where zoonotic (animal-to-human) transmission occurred (71). Although SARS-CoV-2 is alleged to have originated from an animal host (zoonotic origin) with further human-to-human transmission (Fig. 6), the likelihood of foodborne transmission should be ruled out with further investigations, since it is a latent possibility (1). Additionally, other potential and expected routes would be associated with transmission, as in other respiratory viruses, by direct contact, such as shaking contaminated hands, or by direct contact with contaminated surfaces (Fig. 6). Still, whether blood transfusion and organ transplantation (276), as well as transplacental and perinatal routes, are possible routes for SARS-CoV-2 transmission needs to be determined (Fig.6). Substantity is entirely different from the viruses responsible for MERS-CoV- and SARS-CoV (3). The newly emerged SARS-CoV-2 is a group 2B coronavirus (2). The genome sequences of SARS CoV-2 obtained from patients share 79.5% sequence similarity to the sequence of SARS-CoV (63).

As of 13 May 2020, a total of 4,170,424, confirmed cases of COVID-19 (with 287,399 deaths) have been reported in more than 210 affected countries worldwide (WHO Situation Report 114). The COVID-19 outbreak has also been associated with severe economic impacts globally due to the sudden interruption of global trade and supply chains that forced multinational companies to make decisions that led to significant economic losses (66). The recent increase in the number of confirmed critically ill patients with COVID-19 has already surpassed the intensive care supplies, limiting intensive case services to only a small portion of critically ill patients (67). This might also have contributed to the increased case fatality rate observed in the COVID-19 outbreak.

## **Viewpoint on SARS-CoV-2 Transmission , Spread, and Emergence**

The novel coronavirus was identified within 1 month (28 days) of the outbreak. This is impressively fast compared to the time taken to identify SARS-CoV reported in Foshan, Guangdong Province, China (125 days) (68). Immediately after the confirmation of viral etiology, the Chinese virologist rapidly released the genomic sequence of SARS-CoV-2, which played a crucial role in controlling the spread of this newly emerged novel coronavirus to other parts of the world (69).

## **Splits Tree phylogeny analysis**

In the uprooted phylogenetic tree of different beta coronaviruses based on the S protein, virus sequences from different subgenera are grouped into separate clusters. SARS-CoV-2 sequences from Wuhan and other countries exhibited a close relationship and appeared in a single cluster (Fig.1). The CoVs from the subgenus Saebecovirus appeared jointly in SplitsTree and divided into three sub clusters, namely, SARS-CoV-2, bat-SARS-like-CoV (bat-SL-CoV), and SARS-CoV (Fig.1). In the case of other subgenera, like MERbecovirus, all of the sequences grouped in a single cluster, whereas in Embecovirus, different species, comprised of canine respiratory CoVs, bovine CoVs, equine CoVs, and human CoV strain (OC43), grouped in a common cluster. Isolates in the subgenera Nobecovirus and Hibecovirus were found to be placed separately away from other reported SARS-CoVs but shared a bat origin.

## **CURRENT WORLDWIDE SCENARIO OF SARS -CoV-2**

This novel virus, SARS-CoV-2, comes under the subgenus Sarbecovirus of the Orthocoronavirinae subfamily and is entirely different from the viruses.

We assessed the nucleotide percent similarity using the MehAlign software program, where the similarity between the novel SARS-CoV-2 isolates was in the range of 99.4% to 100%. Among the other Serbecovirus CoV sequences, the novel SARS-CoV-2 sequences revealed the highest similarity to bat-SL-CoV, with nucleotide percent identity ranges between 88.12 and 89.65%. Meanwhile, earlier reported SARS-CoVs showed 70.6 to 74.9% similarity to SARS-CoV-2 at the nucleotide level. Further, the nucleotide percent similarity was 55.4%, 45.5% to 47.9%, 46.2% to 46.6%, and 45.0% to 46.3% to the other four subgenera, namely, Hibecovirus, Nobecovirus, Merbecovirus, and Embecovirus, respectively. The percent similarity index of current outbreak isolates indicates a close relationship between SARS-CoV-2 isolates and bat-SL-CoV, indicating a common origin. However, particular pieces of evidence based on further complete genomic analysis of current isolates are necessary to draw any conclusions, although it was ascertained that the current novel SARS-CoV-2 isolates belong to the subgenus Sarbecovirus in the diverse range of betacoronaviruses. Their possible ancestor was hypothesized to be from bat CoV strains, wherein bats might have played a crucial role in harboring this class of viruses.

## **N Protein**

The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus (55, 56). It contains three highly conserved and distinct domains, namely, an NTD, an RNA-binding domain or a linker region (LKR), and a CTD(57). The NTD binds with the 3' end of the viral genome, perhaps via electrostatic

interactions, and is highly diverged both in length and sequence (58). The charged LKR is serine and arginine rich and is also known as the SR (serine and arginine) domain (59).

The LKR is capable of director interaction with in vitro RNA interaction and is responsible for cell signaling (60,61). It also modulates the antiviral response of the host by working as an antagonist for interferon (IFN) and RNA interference (62). Compared to that of SARS-CoV, the N protein of SARS-CoV-2 possess five amino acid mutations, where two are in the intrinsically dispersed region (IDR; positions 25 and 26), one each in the NTD (position 103), LKR (position 217), and CTD (position 334) (16).

### **M Protein**

The M protein is the most abundant viral protein present in the virion particle, giving a definite shape to the viral envelope (48). It binds to the nucleocapsid and acts as a central organizer of coronavirus assembly (49). Coronavirus M proteins are highly diverse in amino acid contents but maintain overall structural similarity within different genera (50). The M protein has three transmembrane domains, flanked by a short amino terminus outside the virion and a long carboxy terminal inside the virion (50). Overall, the viral scaffold is maintained by M-M interaction. Of note, the M protein of SARS-CoV-2 does not have an amino acid substitution compared to that of SARS-CoV (16).

### **E Protein**

The coronavirus E protein is the most enigmatic and smallest of the major structural proteins (51). It plays a multifunctional role in the pathogenesis, assembly, and release of the virus (52). It is a small integral membrane polypeptide that acts as a viroporin (ion channel) (53).

### **S protein**

Coronavirus S protein is a large, multifunctional class I viral transmembrane protein. The size of this abundant S protein varies from amino acids (IBV, infectious bronchitis virus, in poultry) to 1,400 amino acids (FCoV, feline coronavirus) (43). It lies in a trimer on the virion surface, giving the virion a corona or crown-like appearance. Functionally it is required for the entry of the infectious virion particles into the cell through interaction with various host cellular receptors (44).

Furthermore, it acts as a critical factor for tissue tropism and the determination of host range (45). Notably, S protein is one of the vital immunodominant proteins of CoVs capable of inducing host immune response (45). The ectodomains in all CoVs S proteins have similar domain organizations, divided into two subunits, S1 and S2 (43). The first one, S1, helps in host receptor binding, while the second one, S2, accounts for fusion. The former (S1) is further divided into two subdomains, namely, the N-terminal domain (NTD) and C-terminal domain (CTD). Both of these subdomains act as receptor-binding domains, interacting efficiently with various host receptors (45). The S1 CTD contains the receptor-binding motif (RBM). In each coronavirus spike protein, the trimeric S1 locates itself on top of the trimeric S2.

Coronavirus gammed and subgenomes encode six ORFs (31). The majority of the 5' end is occupied by ORF1 a/b, which produces 16 nsps. The two polyproteins, pp 1a and pp 1ab, are initially produced from ORF 1 a/b by a -1 frameshift between ORF 1a and ORF 1b (32). The virus-encoded proteases cleave polyproteins into individual nsps (main protease [Mpro],



chymotrypsin-like protease [3CLpro], and papain-like proteases [PLPs]) (42). SARS-CoV-2 also encodes these nsps, and their functions have been elucidated recently (31). Remarkably, a difference between SARS-CoV-2 and other CoVs is the identification of a novel short putative protein within the ORF3 band, a secreted protein within the ORF3 band, a secreted protein with an alpha helix and beta-sheet with six strands encoded by ORF8 (31).

Coronaviruses encode four major structural proteins, name, spikes (S), membrane (M), envelope (E), and nucleocapsid (N), which are described in detail below.

### **S Glycoprotein**

Coronavirus S protein is a large, multifunctional class 1 viral transmembrane protein. Based on molecular characterization, SARS-CoV-2 is considered a new Betacoronavirus belonging to the subgenus Sarbecovirus (3). A few other critical zoonotic viruses (MERS-related CoV and SARS-related CoV) belong to the same genus. However, SARS-CoV-2 was identified as a distinct virus based on the percent identity with other Betacoronavirus; conserved open reading frame 1a/b (ORF 1a/b) is below 90% identity (3). An overall 80% nucleotide identity was observed between SARS-CoV-2 and the original SARS-CoV, along with 89% identity with ZC45 and ZXC21 SARS-related COVs of bats (2,31,36). In addition, 82% identity has been observed between SARS-CoV-2 and human SARS-CoV Tor2 and human SARS-CoV BJ01 2003 (31). A sequence identity of only 51.8% was observed between MERS-related CoV and the recently emerged SARS-CoV-2 (37). Phylogenetic analysis of the structural genes also revealed that SARS-CoV-2 is closer to bat SARS-related CoV. Therefore, SARS-CoV-2 might have originated from bats, while other amplifier hosts might have played a role in disease transmission to humans (31). Fio nite, the other two zoonotic CoVs (MERS-related CoV and SARS-related CoV) also originated from bats (38,39). Nevertheless, for SARS and MERS, civet encircled with an envelope containing viral nucleocapsids. The nucleocapsids in CoVs are arranged in helical symmetry, which reflects an atypical attribute in positive-sense RNA viruses (30). The electron micrographs of SARS-CoV-2 revealed a diverging spherical outline with some degree of pleomorphism, virion diameters varying from 60 to 140 nm, and distinct spikes of 9 to 12 nm, giving the virus the appearance of a solar corona (3). The CoV genome is arranged linearly as 5'-leader-UTR-replicate-structural genes (S-E-M-N)-3' UTR-poly (A) (32). Accessory genes, such as 3a/b, 4a/b, and the hemagglutinin-esterase gene (HE), are also seen intermingled with the structural genes (30). SARS-CoV-2 has also been found to be arranged similarly and encodes several accessory proteins, although it lacks the HE, which is characteristic of some betacoronaviruses (31). The positive-sense genome of CoVs serves as the mRNA and is translated to polyprotein 1a/1ab (pp1a/1ab) (33). A replication-transcription complex (RTC) is formed in double-membrane vesicles (DMVs) by nonstructural proteins (nsps), encoded by the polyprotein gene (34). Subsequently, the RTC synthesizes a nested set of subgenomic RNAs (sgRNAs) via discontinuous transcription (35).

Some therapeutic options for treating COVID-19 showed efficiency in vitro studies; however, to date, these treatments have not undergone any randomized animal and human clinical trials, which limit their practical applicability in the current pandemic (7,9,19-21).

The present comprehensive review describes the various features of SARS-CoV-2/COVID-19 causing the current disease outbreaks and advances in diagnosis and developing vaccines and therapeutics. It also provides a brief comparison with the earlier SARS and MERS CoVs, the

veterinary perspective of CoVs and this emerging novel pathogen, and an evaluation of the zoonotic potential of similar CoVs to provide feasible One Health strategies for the management of this fatal virus (22-367).

## **THE VIRUS (SARS-CoV-2)**

Coronaviruses are positive-sense RNA viruses having an extensive and promiscuous range of natural hosts and affect multiple systems (23,24). Coronaviruses can cause clinical disease in humans that may extend from the common cold to more severe respiratory diseases like SARS and MERS (17,279). The recently emerging SARS-CoV-2 has wrought havoc in China and caused a pandemic situation in the worldwide population, leading to disease outbreaks that have not been controlled to date, although extensive efforts are being put in place to counter this virus (25). This virus has been proposed to be designated/named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), which determined the virus belongs to the Severe acute respiratory syndrome-related to coronaviruses category and found this virus is related to SARS-CoVs (26). SARS-CoV-2 is a member of the order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, which is subdivided into four genera, Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (3, 27). The genera Alphacoronavirus and Betacoronavirus originate from bats, while Gammacoronavirus and Deltacoronavirus have evolved from bird and swine gene pools (24,28,29,275).

Coronaviruses possess an unsegmented single-stranded, positive-sense RNA genome of around 30kn, enclosed by a 5'-cap and 3'-poly (A) tail (30). The genome of SARS-CoV-2 is 29,891 bplong, with a G+C content of 38% (31). These viruses are encircled with an envelope containing viral. Furthermore, SARS-CoV-2 is genetically distinct from SARS-CoV (79% similarity) and MERS-CoV (nearly 50%) (17). COVID-19 is associated with afflictions of the lungs in all cases and generated characteristics chest computer tomography findings, such as the presence of multiple lesions in lung lobes that appear as dense, ground-glass opaque structures that occasionally coexist with consolidation shadows (18). The most common symptoms associated with COVID-19 are fever, cough, dyspnea, expectoration, headache, and myalgia or fatigue.

In contrast, less common signs at the time of hospital admission include diarrhea, hemoptysis, and shortness of breath(14). Recently, individuals with asymptomatic infections were also suspected of transmitting infections, which further adds to the complexity of disease transmission dynamics in COVID-19 infections (1). Such efficient responses require in-depth knowledge regarding the virus, which currently is a novel agent; consequently, further studies are required.

Comparing the genome of SARS-CoV-2 with that of the closely related SARS/SARS-like CoV revealed that the sequence coding for the spike protein, with a total length of 1,273 amino acids, showed 27 amino acid substitutions. Six of these substitutions are in the region of the receptor-binding domain (RBD), and another six substitutions are in the underpinning subdomain (SD) (16). Phylogenetic analysis have revealed that SARS-CoV-2 is closely related (88% similarity) to two SARS-like CoVs derived from bat SARS-like CoVs (bat-SL-CoVZC45 and bat-SL-CoVZXC21) (Fig.1).

Range of hosts, producing symptoms and diseases ranging from the common cold to severe and ultimately fatal illnesses, such as SARS, MERS, and, presently, COVID-19. SARS-CoV-2 is considered one of the seven members of the CoV family that infect humans (3), and it belongs to the same lineage of CoV that causes SARS; however, this novel virus is genetically distinct. Until 2020, six CoVs were known to infect humans, including human CoV 229E (HCoV-229E), HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV have resulted in outbreaks with high mortality, others remain associated with mild upper-respiratory-tract illnesses (4).

Newly evolved CoVs pose a high threat to global public health. The current emergence of COVID-19 is the third CoV outbreak in humans over the past 2 decades (5). It is no coincidence that all predicted potential SARS- or MERS-like CoV outbreaks in China following pathogen transmission from bats (6). COVID-19 emerged in China and spread rapidly

Throughout the country and, subsequently, to other countries. Due to the severity of this outbreak and the potential of spreading on an international scale, the WHO declared a global health emergency on 31 January; subsequently, on 11 March 2020, they declared it a pandemic situation. At present, we are not in a position to effectively treat COVID-19, since neither approved vaccines nor specific antiviral drugs for treating human CoV infections are available (7-9). Most nations are currently making efforts to prevent the further spreading of this potentially deadly virus by implementing preventive and control strategies.

In domestic animals, infections with CoVs are associated with a broad spectrum of pathological conditions. Apart from infectious bronchitis virus, canine respiratory CoV, and mouse hepatitis virus, CoVs are predominantly associated with gastrointestinal diseases (10). The emergence of novel CoVs have become possible because of multiple CoVs being maintained in their natural host, which could have favored the probability of genetic recombination (10). High genetic diversity and the ability to infect multiple host species are a result of high-frequency mutations in CoVs, which occur due to the instability of RNA-dependent RNA polymerases along with higher rates of homologous RNA recombination (10,11). Identifying the origin of SARS-CoV-2 and the pathogen's evolution will be helpful for disease surveillance (12).

### **Coronaviruses-in Humans----- SARS, MERS, and COVID-19**

Coronavirus infection in humans is commonly associated with mild to severe respiratory diseases, with high fever, severe inflammation, cough, and internal organ dysfunction that can even lead to death (92). Most of the identified coronaviruses cause the common cold in humans. However, this changed when SARS-CoV was identified, paving the way for severe forms of the disease in humans (22). Our previous experience with the outbreaks of other coronaviruses, like SARS and MERS, suggests that the mode of transmission in COVID-19 was mainly human-to-human transmission via direct contact, droplets, and fomites (25). Recent studies have demonstrated that the virus could remain viable for hours in aerosols and up to days on surfaces; thus, aerosol and fomite contamination could play potent roles in the transmission of SARS-CoV-2 (257).

The immune response against coronavirus is vital to control and get rid of the infection. However, maladjusted immune responses may contribute to the immunopathology of the disease, resulting in impairment of pulmonary gas exchange. Understanding the interaction between CoVs and host innate immune systems could enlighten our understanding of the lung inflammation associated with this infection (24).

SARS is a viral respiratory disease caused by a formerly unrecognized animal CoV that originated from the wet markets in southern China after adapting to the human host, thereby enabling transmission between humans (90). The SARS outbreak reported in 2002 to 2003 had 8,098 confirmed cases with 774 total deaths (9.6%) (93). The outbreak severely affected the Asia Pacific region, especially mainland China (94). Even though the case fatality rate (CFR) of SARS-CoV-2 (COVID-19) is lower than that of SARS-CoV, there exists a severe concern linked to this outbreak due to its epidemiological similarity to influenza viruses (95, 279). This can fail the public health system, resulting in a pandemic (96).

MERS is another respiratory disease that was first reported in Saudi Arabia during the year 2012. The disease was found to have a CFR of around 35% (97). The analysis of available data sets suggests that the inoculation period of SARS-CoV-2, SARS-CoV, and MERS-CoV is in almost the same range. The longest predicted incubation time of SARS-CoV-2 is 14 days. Hence, suspected individuals are isolated for 14 days to avoid the risk of further spread (98). Even though a high similarity has been reported between the genome sequence of the new coronavirus (SARS-CoV-2) and SARS-like CoVs, the comparative analysis recognized a furin-like cleavage site in the SARS-CoV-2 S protein that is missing from other SARS-like CoVs (99). The furin-like cleavage site is expected to play a role in the life cycle of the virus and disease pathogenicity and might even act as a therapeutic target for furin inhibitors. The highly contagious nature of SARS-CoV-2 compared to that of its predecessors might be the result of a stabilizing mutation that occurred in the endosome-associated-protein-like domain of nsp2 protein.

Similarly, the destabilizing mutation near the phosphatase domain of nsp3 proteins in SARS-CoV-2 could indicate a potential mechanism that differentiates it from other CoVs (100). Even though the CFR reported for COVID-19 is meager compared to those of the previous SARS and MERS outbreaks, it has caused more deaths than SARS and MERS combined (101). Possibly related to the viral pathogenesis is the recent finding of an 832-nucleotide (nt) deletion in ORF8, which appears to reduce the replicative fitness of the virus and leads to attenuated phenotypes of SARS-CoV-2 (256). Transmission can also occur directly from the reservoir host to humans without RBD adaptations. The bat coronavirus that is currently in circulation maintains specific “poised” spike proteins that facilitate human infection without the requirement of any mutations or adaptations (105). Altogether, different species of bats carry a massive number of coronaviruses around the world (106).

The high plasticity in receptor usage, along with the feasibility of adaptive mutation and recombination, may result in frequent interspecies transmission of coronavirus from bats to animals and humans (106). The pathogenesis of most bat coronaviruses is unknown, as most of these viruses are not isolated and studied (4). Hedgehog coronavirus HKU31, a Betacoronavirus, has been identified from amur hedgehogs in China. Studies show that hedgehogs are the reservoir of Betacoronavirus, and there is evidence of recombination (107).

The current scientific evidence available on MERS infection suggests that the significant reservoir host, as well as the animal source of MERS infection in humans, is the dromedary camels (97). The infected dromedary camels may not show any visible signs of infection, making it challenging to populations.

## CLINICAL PATHOLOGY OF SARS-CoV-2 (COVID-19)

This disease caused by SARS-CoV-2 is also named severe specific contagious pneumonia (SSCP), Wuhan pneumonia, and recently, COVID-19 (110). Compared to SARS-CoV, SARS-CoV-2 has less severe pathogenesis but has superior transmission capability, as evidenced by the rapidly increasing number of COVID-19 cases (111). The incubation period of SARS-CoV-2 in familial clusters was found to be 3 to 6 days (112). The mean incubation period of COVID-19 was found to be 6.4 days, ranging from 2.1 to 11.1 days (113). Among an early affected group of 425 patients, 59 years was the median age, of which more males were affected (114). Similar to SARS and MERS, the severity of this nCoV is high in age groups above 50 years (2, 115). Symptoms of COVID-19 include fever, cough, myalgia or fatigue, and, less commonly, headache, hemoptysis, and diarrhea (116,282). Compared to the SARS-CoV-2-infected patients in Wuhan during the initial stages of the outbreak, only mild symptoms were noticed in those patients that are infected by human-to-human transmission (14).

The initial trends suggested that the mortality associated with COVID-19 was less than that of previous outbreaks of SARS (101). The updates obtained from countries like China, Japan, Thailand, and South Korea indicated that the COVID-19 patients had relatively mild manifestations compared to those with SARS and MERS (4). Regardless of the coronavirus type, immune cells, like mast cells, that are present in the submucosa of the respiratory tract and nasal cavity are considered the primary barrier against this virus (92). Advanced in-depth analysis of the genome has identified 380 amino acid substitutions between the amino acid sequences of SARS-CoV-2 and the SARS/SARS-like coronaviruses. These differences in the amino acid sequences might have contributed to the difference in the pathogenic divergence of SARS-CoV-2 (16). Further research is required to evaluate the possible differences in tropism, pathogenesis, and transmission of this novel agent associated with this change in the amino acid sequence. With the current outbreak of COVID-19, there is an expectancy of a significant increase in the number of published studies about this emerging coronavirus, as visible signs of infection, making it challenging to identify animals actively excreting MERS-CoV that has the potential to infect humans. However, they may shed MERS-CoV through milk, urine, feces, and nasal and eye discharge and can also be found in the raw organs (108). In a study conducted to evaluate the susceptibility of animal species to MERS-CoV infection, llamas' and pigs were found to be susceptible, indicating the possibility of MERS-CoV circulation in animal species other than the dromedary camels (109).

Following the outbreak of SARS in China, SARS-CoV-like viruses were isolated from Himalayan palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) found in a live-animal market in Guangdong, China. The animal isolates obtained from the live-animal market retained a 29-nucleotide sequence that was not present in most of the human isolates (78). These findings were critical in identifying the possibility of interspecies transmission in SARS-CoV. The higher diversity and prevalence of bat coronaviruses in this region compared to those in previous reports indicate a host/pathogen coevolution. SARS-like coronaviruses have also been found circulating in the Chinese horseshoe bat (*Rhinolophus sinicus*) populations. The in vitro and in vivo studies carried out on the isolated virus confirmed that there is a potential risk for the reemergence of SARS-CoV infection from the viruses that are currently circulating in the bat population (105).

The host spectrum of coronavirus increased when a novel coronavirus, namely, SW1, was recognized in the liver tissue of a captive beluga whale (*Delphinapterus leucas*) (138). In recent decades, several novel coronaviruses were identified from different animal species. Bats can harbor these viruses without manifesting any clinical disease but are persistently infected (30). They are the only mammals with the capacity for self-powered flight, which enables them to migrate long distances, unlike land mammals. Bats are distributed worldwide and also account for about a fifth of all mammalian species (6). This makes them the ideal reservoir host for many viral agents and also the source of novel coronaviruses that have yet to be identified. It has become a necessity to study the diversity of coronavirus in the bat population to prevent future outbreaks that could jeopardize livestock and public health. The repeated outbreaks caused by bat-origin coronavirus calls for the development of efficient molecular surveillance strategies for studying Betacoronavirus among animals (12), especially in the *Thinolophus* bat family (86). Chinese bats have high commercial value.

Another study conducted in South Korea, related to SARS-CoV-2 viral load, opined that SARS-CoV-2 kinetics were significantly different from those of earlier reported CoV infections, including SARS-CoV (253). SARS-CoV-2 transmission can occur early in the viral infection phase; thus, diagnosing cases and isolation attempts for this virus warrant different strategies than those needed to counter SARS-CoV. Studies are required to establish any correlation between SARS-CoV-2 viral load and cultivable virus. Recognizing patients with fewer or no symptoms, along with having modest detectable viral RNA in the oropharynx for 5 days, indicated the requirement of data for assessing SARS-CoV-2 transmission dynamics and updating the screening procedures in the clinics (82).

Future explorative research needs to be conducted with regard to the fecal-oral transmission of SARS-CoV-2, along with focusing on environmental investigations to find out if this virus could stay viable in situations and atmospheres facilitating such potent routes of transmission. The correlation of fecal concentrations of viral RNA with disease severity needs to be determined, along with assessing the gastrointestinal symptoms and the possibility of fecal SARS-CoV-2 RNA detection during the COVID-19 incubation period or convalescence phases of the disease (249-252). The lower respiratory tract sampling techniques, like bronchoalveolar lavage fluid aspirate, are considered the ideal clinical materials, rather than the throat swab, due to their higher positive rate on the nucleic acid test (148). The diagnosis of COVID-19 can be made by using upper-respiratory-tract specimens collected using nasopharyngeal and oropharyngeal swabs. However, these techniques are associated with unnecessary risks to health care workers due to close contact with patients (152). Similarly, a single patient with a high viral load was reported to contaminate an entire endoscopy room by shedding the virus, which may remain viable for at least 3 days and is considered a great risk for uninfected patients and health care workers (289). Recently, it was found that the anal swabs gave more positive results than oral swabs in the later stages of infection (153). Hence, clinicians have to be cautious while discharging any COVID-19 infected patient based on negative oral swab test results due to the possibility of fecal-oral transmission. Even though the viral loads in stool samples were found to be less than those of respiratory samples, strict precautionary measures have to be followed while handling stool samples of COVID-19 suspected or infected patients (151). Children infected with SARS-CoV-2 experience only mild forms of illness and recover immediately after treatment. It was recently found

that stool samples of SARS-CoV-2-infected children that gave negative throat swab results were positive within ten days of negative results. This could result in the fecal-oral transmission of SARS-CoV-2 infections, especially in children (290). Hence, to prevent the fecal-oral transmission of SARS-CoV-2, infected COVID-19 patients should only be considered negative when they test negative for SARS-CoV-2 in the stool sample.

A suspected case of COVID-19 infection is said to be confirmed if the respiratory tract aspirate or blood samples test positive for SARS-CoV-2 nucleic acid using RT-PCR or by the identification of SARS-CoV-2 genetic sequence in respiratory tract aspirate or blood samples (80). The patient will be confirmed as cured when two subsequent oral swab results are negative (153). Recently, the live virus was detected in the self-collected saliva of patients infected with COVID-19. These findings were confirmative of using saliva as a noninvasive specimen for the diagnosis of COVID-19 infection in suspected individuals (152). It has also been observed that the initial screening of COVID-19 patients infected with RT-PCR may give negative results even if they have chest CT findings that are suggestive of infection. Hence, for the accurate diagnosis of COVID-19, a combination of repeated swab tests using RT-PCR and CT scanning is required to prevent the possibility of false-negative results during disease screening (154). RT-PCR is the most widely used test for diagnosing COVID-19. However, it has some significant limitations from the clinical perspective, since it will not give any clarity regarding disease progression. Droplet digital PCR (ddPCR) can be used for the quantification of viral load in the samples obtained from lower respiratory tracts.

The presence of SARS-CoV-2 in fecal samples has posed grave public health concerns. In addition to the direct transmission mainly occurring via droplets of sneezing and coughing, other routes, such as fecal excretion and environmental and fomite contamination, are contributing to SARS-CoV-2 transmission and spread (249-252). Fecal excretion has also been documented for SARS-CoV and MERS-CoV, along with the potential to stay viable in situations aiding fecal-oral transmission. Thus, SARS-CoV-2 has every possibility to be transmitted through this mode. Fecal-oral transmission of SARS-CoV-2, particularly in regions having low standards of hygiene and poor sanitation, may have grave consequences with regard to the high spread of this virus. Ethanol and disinfectants containing chlorine or bleach are effective against coronaviruses (249-252). Appropriate precautions need to be followed strictly while handling the tools of patients infected with SARS-CoV-2. Biowaste materials and sewage from hospitals must be adequately disinfected, treated, and supposed to be properly.

The result of the studies related to SARS-CoV-2 viral loads reflects active replication of the virus in the upper respiratory tract and prolonged viral shedding after symptoms disappear, including via stool. Thus, the current case definition needs to be updated along with a reassessment of the strategies to be adopted for restraining the SARS-CoV-2 outbreak spread (248). In some cases, the viral load studies of SARS-CoV-2 have also been useful to recommend precautionary measures when handling specific samples, e.g., feces. In a recent survey from 17 confirmed cases of SARS-CoV-2 infection with available data (representing days 0 to 13 after onset), stool samples from nine cases (53%; days 0 to 11 after onset) were positive on RT-PCR analysis. Although the viral loads were lower than those of respiratory samples (range, 550 copies per ml to  $1.21 \times 10^5$  copies per ml), this has essential biosafety implications (151).

The samples from 18 SARS-CoV-2-positive patients in Singapore who had traveled from Wuhan to Singapore showed the presence of viral RNA in stool and whole blood but not in urine by real-time RT-PCR (288). Further, novel SARS-CoV-2 infections have been detected in a variety of clinical specimens, like bronchoalveolar lavage fluid. The viral loads of SARS-CoV-2 were measured using N-gene-specific quantitative RT-PCR in throat swabs and sputum samples collected from COVID-19 infected individuals. The results indicated that the viral load peaked at around 5 to 6 days following the onset of symptoms, and it ranges from  $10^4$  and  $10^7$

Copies /ml during this time (151). In another study, the viral load was found to be higher in the nasal swabs than the throat swabs obtained from COVID-19 symptomatic patients (82). Although initially it was thought that viral load would be associated with poor outcomes, some case reports have shown asymptomatic individuals with high viral loads (247). Recently, the viral load in nasal and throat swabs of 17 symptomatic patients was determined, and higher viral loads were recovered soon after the onset of symptoms, particularly in the nose compared to the throat. The pattern of viral nucleic acid shedding of SARS-CoV-2-infected patients was similar to that of influenza patients but seemed to be different from that of SARS-CoV patients. The viral load detected in asymptomatic patients resembled that of symptomatic patients as studied in China, which reflects the transmission perspective of asymptomatic or symptomatic patients having minimum signs and symptoms (82). Recently, 95 full-length genomic sequences of SARS-CoV-2 strains available in the National Center for Biotechnology Information and GISAID database were subjected to multiple-sequence alignment and phylogenetic analysis for studying variations in the viral genome (260). All the viral strains revealed high homology of 99.99% (99.91% to 100%) at the nucleotide level and 99.99% (99.97% to 100%) at the amino acid level. Overall variation was found to be low in ORF regions, with 13 variation sites recognized in 1a, S, 3a, M, 8, and N regions. Mutation rates of 30.53% (29/95) and 29.47% (28/95) were observed at nt 28144 (ORF8) and nt 8782 (ORF1a) positions, respectively. Owing to such selective mutations, a few specific regions of SARS-CoV-2 should not be considered for designing primers and probes. The SARS-CoV-2 reference sequence could pave the way to study molecular biology and pathobiology, along with developing diagnostics and appropriate prevention and control strategies for countering SARS-CoV-2 (260).

Nucleic acids of SARS-CoV-2 can be detected from samples (64) such as bronchoalveolar lavage fluid, sputum, nasal swabs, fiber bronchoscope brush biopsy specimen, pharyngeal swabs, feces, blood, and urine, with different levels of diagnostic performance (Table 2) (80, 245, 246).

## **DIAGNOSIS OF SARS-CoV-2 (COVID-19)**

RNA tests can confirm the diagnosis of SARS CoV-2 (COVID-19) cases with real-time RT-PCR or next generation sequencing (148,149,245,246). At present, nucleic acid detection techniques, like RT-PCR, are considered as effective methods for confirming the diagnosis in clinical cases of COVID-19 (148). Several companies across the world are currently focusing on developing and marketing SARS-CoV-2 specific nucleic acid detection kits. Multiple laboratories are also developing their own in-house RT-PCR. One of them is the SARS-CoV-2 nucleic acid detection kit produced by Shuoshi Biotechnology (double fluorescence PCR method) (150). Up to 30 March 2020, the U.S. Food and Drug Administration (FDA) had granted 22 in vitro diagnostics Emergency Use Authorizations (EUAs), including for the RT-PCR diagnostic panel for the universal detection of



SARS-like beta coronaviruses and specific detection of SARS-CoV-2, developed by the U.S.CDC (Table 1) (258, 259).

Similarly, the National Veterinary Services Laboratories of the USDA have reported COVID-19 in tigers and lions that exhibited respiratory signs like dry cough and wheezing. The zoo animals are suspected to have been infected by an asymptomatic zookeeper (335). The total number of COVID-19-positive cases in human beings is increasing at a high rate, thereby creating ideal conditions for viral spillover to other species, such as pigs. The evidence obtained from SARS-CoV-2 suggests that pigs can get infected with SARS-CoV-2 (336). However, experimental inoculation with SARS-CoV-2 failed to infect pigs (329).

Further studies are required to identify the possible animal reservoirs of SARS-CoV-2 and the seasonal variation in the circulation of these viruses in the animal population. Research collaboration between a necessity to evaluate and identify the possible risk factors for transmission between animals and humans. Such cooperation will help to devise efficient strategies for the management of emerging zoonotic disease (12).

Rather than waiting for firmer evidence on animal-to-human transmission, necessary preventive measures are advised, as well as following social distancing practices among companion animals of different households (331). One of the leading veterinary diagnostic companies, IDEXX, has conducted large-scale testing for COVID-19 in specimens collected from dogs and cats. However, none of the tests turned out to be positive (334).

In a study conducted to investigate the potential of different animal species to act as the intermediate host of SARS-CoV-2, it was found that both ferrets and cats can be infected via experimental inoculation of the virus. In addition, infected cast efficiently transmitted the disease to naive cats (329). SARS-CoV-2 infection and subsequent transmission in ferrets were found to recapitulate the clinical aspects of COVID-19 in humans. The infected ferrets also shed virus via multiple routes, such as saliva, nasal washes, feces, and urine, postinfection, making them an ideal animal model for studying disease transmission (337). These findings will not have any significance until a significant outbreak occurs due to a virus-like SARS-CoV-2.

There is a steady increase in the reports of COVID-19 in companion and wild animals around the world. Further studies are required to evaluate the potential of animals (especially companion animals) to serve as an efficient reservoir host that can further alter the dynamics of human-to-human transmission (330). To date, two pet dogs (Hong Kong) and four pet cats (one each from Belgium and Hong Kong, two from United States ) have tested positive for SARS-CoV-2 (335). The World Organization for Animal Health (OIE) has confirmed the diagnosis of COVID-19 in both dogs and cats due to human-to-animal transmission (331). The similarity observed in the gene sequence of SARS-CoV-2 from an infected pet owner and his dog further confirms the occurrence of human-to-animal transmission (333). Even though asymptomatic, feline species should be considered a potential transmission route from animals to humans (326). However, currently, there are no reports of SARS-CoV-2 transmission from felines to human beings. Based on the current evidence, we can conclude that cats are susceptible to SARS-CoV-2 and can get infected by human beings. A comparison of the genomes suggests recombination between pangolin-CoV-like viruses with the bat-CoV-RaTG13-like virus. All this suggests the potential of pangolins to act as the intermediate host of SARS-CoV-2 (145).

Human-wildlife interactions, which are increasing in the context of climate change (142), are further considered high risk and responsible for the emergence of SARS-CoV. COVID-19 is also suspected of having a similar mode of origin. Hence, to prevent the occurrence of another zoonotic spillover (1), exhaustive coordinated efforts are needed to identify the high-risk pathogens harbored by wild animal populations, conducting surveillance among the people who are susceptible to zoonotic spillover events (12), and to improve the biosecurity measures associated with the wildlife trade (146). The serological surveillance studies conducted in people living in proximity to bat caves had earlier identified the serological confirmation of SARS-related CoVs in humans. People living at the wildlife-human interface, mainly in rural China, are regularly exposed to SARS-related CoVs (147).

The comprehensive sequence analysis of the SARS-CoV-2 RNA genome identified that the CoV from Wuhan is a recombinant virus of the bat coronavirus and another coronavirus of unknown origin. The recombination was found to have happened within the viral spike glycoprotein, which recognizes the cell surface receptor. Further analysis of the genome based on codon usage identified the snake as the most probable animal reservoir of SARS-CoV-2 (143). Contrary to these findings, another genome analysis proposed that the genome of SARS-CoV-2 is 96% identical to bat coronavirus, reflecting its origin from bats (63). The involvement of bat-derived materials in causing the current outbreak cannot be ruled out. High risk is involved in the production of bat-derived materials for TCM practices involving the handling of wild bats. The use of bats for TCM practices will remain a severe risk for the occurrence of zoonotic coronavirus epidemics in the future (139).

Furthermore, the pangolins are an endangered species of animals that harbor a wide variety of viruses, including coronaviruses (144). The coronavirus isolated from Malayan pangolins (*Manis javanica*) showed a very high amino acid identity with COVID-19 at E (100%), M (98.2%), N (96.7%), and S (90.4%).

Due to the possible role played by farm and wild animals in SARS-CoV-2 infection, the WHO, in their novel coronavirus (COVID-19) situation report, recommended the avoidance of unprotected contact with both farm and wild animals (25). The live-animal markets, like the one in Guangdong, China, provides a setting for animal coronaviruses to amplify and to be transmitted to new hosts, like humans (78). Such markets can be considered a critical place for the origin of novel zoonotic disease and have enormous public health significance in the event of an outbreak. Bats are the reservoirs for several viruses; hence, the role of bats in the present outbreak cannot be ruled out (140). In a qualitative study conducted for evaluating the zoonotic risk factors among rural communities of southern China, the frequent human-animal interactions along with the low levels of environmental biosecurity were identified as significant risks for the emergence of zoonotic disease in local communities (141,142).

Swine acute diarrhea syndrome coronavirus (SADS-CoV) was first identified in suckling piglets having severe enteritis and belongs to the genus Alphacoronavirus (106). The outbreak was associated with considerable scale mortality of piglets (24,693 deaths) across four farms in China (134). The virus isolated from the piglets was almost identical to and has 95% genomic similarity with horseshoe bat (*Rhinolophus* species) coronavirus KHU2, suggesting a bat origin of the pig virus (106, 134,135). It is also imperative to note that the SADS-CoV outbreak started in Guangdong province, near the location of the SARS pandemic origin (134). Before this outbreak, pigs were not

known to be infected with bat-origin coronaviruses. This indicates that the bat-origin coronaviruses jumped to pig by breaking the species barrier. The next step of this jump might not end well, since pigs are considered the mixing vessel for influenza A viruses due to their ability to be infected by both human and avian influenza A viruses (136).

Similarly, they may act as the mixing vessel for coronaviruses, since they are in frequent contact with both humans and multiple wildlife species. Additionally, pigs are also found to be susceptible to infection with human SARS-CoV and MERS-CoV, making this scenario a nightmare (109, 137).

Bovine coronaviruses (BoCoVs) are known to infect several domestic and wild ruminants (126). BoCoV inflicts neonatal calf diarrhea in adult cattle, leading to bloody diarrhea in adult cattle, leading to bloody diarrhea (winter dysentery) and respiratory disease complex (shipping fever) in cattle of all age groups (126). BoCoV-like viruses have been noted in humans, suggesting its zoonotic potential as well (127). Feline enteric and feline infectious peritonitis (FIP) viruses are the two major feline CoVs (128), where feline CoVs that fall under different genera, namely, canine enteric coronavirus in Alphacoronavirus and canine respiratory coronavirus in Betacoronavirus, affecting the enteric and respiratory tract, respectively (129,130). IBV, under Gammacoronavirus, causes disease of respiratory, urinary, and reproductive systems, with substantial economic losses in chickens (131,132). In small laboratory animals, mouse hepatitis virus, rat sialodacryoadenitis coronaviruses, and guinea pig and rabbit coronaviruses are the major CoVs associated with disease manifestations like enteritis, hepatitis, and respiratory infections (10,133). Coronavirus infection is linked to different kinds of clinical manifestations, varying from enteritis in cows and pigs, upper respiratory disease in chickens, and fatal respiratory infections in humans (30).

Among the CoV genera, Alphacoronavirus and Betacoronavirus infect mammals, while Gammacoronavirus and Deltacoronavirus mainly infect birds, fishes, and, sometimes, mammals (27,29,106). Several novel coronaviruses that come under the genus Deltacoronavirus have been discovered in the past from birds, like Wigeon coronavirus HKU20, Bulbul coronavirus HKU11, Munia coronavirus HKU13, white-eye coronavirus HKU16, night-heron coronavirus HKU19, and common moorhen coronavirus HKU21, as well as from pigs (porcine coronavirus HKU 15) (6,29). Transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and porcine hemagglutinating encephalomyelitis virus (PHEV) are some of the coronaviruses of swine. Among them, TGEV and PEDV are responsible for causing severe gastroenteritis in young piglets with noteworthy morbidity and mortality. Infection with PHEV also causes enteric infection but can cause encephalitis due to its ability to infect the nervous aminotransferase, bilirubin, and, especially , D-dimer (244). Middle-aged and elderly patients with primary chronic disease, especially high blood pressure and diabetes, were found to be more susceptible to respiratory failure and, therefore, had poorer prognosis. Providing respiratory support at early stages improved the disease prognosis and facilitated recovery (18). The ARDS in COVID-19 is due to the occurrence of cytokine storms that results in exaggerated immune response, immune regulatory network imbalance, and, finally, multiple-organ failure (122). In addition to the exaggerated inflammatory response seen in patients with COVID-19 pneumonia, the bile duct epithelial cell-derived hepatocytes upregulate ACE2 expression in liver tissue by compensatory proliferation that might result in hepatic tissue injury (123).

## **CORONAVIRUSES IN ANIMALS AND ZONOTIC LINKS— A BRIEF VIEWPOINT**

Coronavirus can cause disease in several species of domestic and wild animals, as well as humans (23). The different animal species that are infected with CoV include horses, camels, cattle, swine, dogs, cats, rodents, birds, ferrets, minks, bats, rabbits, snakes, and various other wild animals (20,30,79). However there has been concern regarding the impact of SARS-CoV-2/COVID-19 on pregnancy. Researchers have mentioned the probability of in utero transmission of novel SARS-CoV-2 from COVID-19-infected mothers to their neonates in China based upon the rise in IgM and IgG antibody levels and cytokine values in the blood obtained from newborn infants immediately post birth; however, RT-PCR failed to confirm the presence of SARS-CoV-2 genetic material in the infants (283). Recent studies show that at least in some cases, preterm delivery and its consequences are associated with the virus. Nonetheless, some cases have raised doubts for the likelihood of vertical transmission (240 - 243).

COVID-19 infection was associated with pneumonia, and some developed acute respiratory distress syndrome (ARDS). The blood biochemistry indexes, such as albumin, lactate dehydrogenase, C-reactive protein, lymphocytes (percent), and neutrophils (percent) give an idea about the disease severity in COVID-19 infection (121). During COVID-19, patients may present leukocytosis, leukopenia with lymphopenia (244), hypoalbuminemia, and an increase of lactate dehydrogenase, aspartate transaminase, alanine aminotransferase, bilirubin, and especially, D-dimer (244).

SARS-COV-2 invades the lung parenchyma, resulting in severe interstitial inflammation of the lungs. This is evident on computed tomography (CT) images as ground-glass opacity in the lungs. This lesion initially involves a single lobe but later expands to multiple lung lobes (118). The histological assessment of lung biopsy samples obtained from COVID-19 infected patients revealed diffuse alveolar damage, cellular fibromyxoid exudates, hyaline membrane formation, and desquamation of pneumocytes, indicative of acute respiratory distress syndrome (119). It was also found that the SARS-CoV-2 infected patients often have lymphocytopenia with or without leukocyte abnormalities. The degree of lymphocytopenia gives an idea about disease prognosis, as it is found to be positively correlated with disease severity (118). Pregnant women are considered to have a higher risk of getting infected by COVID-19. The coronaviruses can cause adverse outcomes for the fetus, such as intrauterine growth restriction, spontaneous abortion, preterm delivery, and perinatal death.

Nevertheless, the possibility of intrauterine maternal-fetal transmission (vertical transmission) of CoVs is low and was not seen during either the SARS- or MERS-CoV outbreak (120).

Coronavirus is the most prominent example of a virus that has crossed the species barrier twice from wild animals to humans during SARS and MERS outbreaks (79, 102). The possibility of crossing the species barrier for the third time has also been suspected in the case of SARS-COV-2 (COVID-19). Bats are recognized as a possible natural reservoir host of both SARS-COV and MERS-CoV infection. In contrast, the possible intermediary host is the palm civet for SARS-CoV and

the dromedary camel for MERS-CoV infection (102). Bats are considered the ancestral hosts for both SARS and MERS (103). Bats are also considered the reservoir host of human coronaviruses like HCoV-229E and HCoV-NL63 (104). In the case of COVID-19, there are two possibilities for primary transmission: it can be transmitted either through intermediate hosts, similar to that of SARS and MERS, or directly from bats (103). The emergence paradigm put forwards in the SARS outbreak suggests that SARS-CoV originated from bats (reservoir host) and later jumped to civets (intermediate host) and incorporated changes within the receptor-binding domain (RBD) to improve binding to civet ACE2. This civet-adapted virus, during their subsequent exposure to humans at live markets, promoted further adaptations that resulted in the epidemic strain (104).

Hence, based on the viral load, we can quickly evaluate the progression of infection (291). In addition to all of the above findings, sequencing and phylogenetics are critical in the correct identification and confirmation of the causative viral agent and useful to establish relationships with previous isolates and sequences; as well as to know, especially during an epidemic, the nucleotide and amino acid mutations and the molecular divergence. The rapid development and implementation of diagnosis tests against emerging novel diseases like COVID-19 pose significant challenges due to the lack of resources and logistical limitations associated with an outbreak (155).

SARS-CoV-2 infection can also be confirmed by isolation and culturing. The human airway epithelial cell culture was found to be useful in isolating SARS-CoV-2 (3). The efficient control of an outbreak depends on the rapid diagnosis of the disease. Recently, in response to the COVID-19 outbreak, 1-step quantitative real-time reverse transcription-PCR assays were developed that detect the ORF1b and N regions of the SARS-CoV-2 genome (156). That assay was found to achieve the rapid detection of SARS-CoV-2.

## **Vaccines**

The S protein plays a significant role in the induction of protective immunity against SARS-CoV by mediating T-cell responses and neutralizing antibody production (168). In the past few decades, we have seen several attempts to develop a vaccine against human coronaviruses by using S protein as the target (168, 169). However, the developed vaccines have minimal application, even among closely related strains of the virus, due to a lack of cross-protection. That is mainly because of the extensive diversity existing among the different antigenic variants of the virus (104). The contributions of the structural proteins, like spike (S), matrix (M), small envelope (E), and nucleocapsid (N) proteins, of SARS-CoV to induce protective immunity has been evaluated by expressing them in a recombinant parainfluenza virus type 3 vector (BHPV3). CEPI has also funded Moderna to develop a vaccine for COVID-19 in partnership with the Vaccine Research Center (VRC) of the National Institute of Health (NIH) (182). By employing mRNA vaccine platform technology, a vaccine candidate expressing SARS-CoV-2 spike protein is likely to go through clinical testing in the coming months (180). On 16 March 2020, Jennifer Haller became the first person outside China to receive an experimental vaccine, developed by Moderna, against this pandemic virus. Moderna, along with China's CanSino Biologics, became the first research group to launch small clinical trials of vaccines against COVID-19. Their study is evaluating the vaccines's safety and ability to trigger immune response (296).

Scientists from all over the world are trying hard to develop working vaccines with robust protective immunity against COVID-19. Vaccine candidates, like mRNA-1273 SARS-CoV-2 vaccine, IN-4800 DNA coronavirus vaccine, and adenovirus type 5 vector vaccine candidate (Ad5-nCoV), are a few examples under phase 1 clinical trials, while self-amplifying RNA vaccine, oral recombinant COVID-19 vaccine, and li-key peptide COVID-19 vaccine are challenge with MERS-CoV (169). The intranasal administration of the recombinant adenovirus-based vaccine in BALB/c mice was found to induce long-lasting neutralizing immunity against MERS spike pseudotyped virus, characterized by the induction of systemic IgG, secretory IgA, and lung-resident memory T-cell responses (177). Immunoinformatics methods have been employed for the genome-wide screening of potential vaccine targets among the different immunogens of MERS-CoV (178). The N protein and the potential B-cell epitopes of MERS-CoV E protein have been suggested as immunoprotective targets inducing both T-cell and neutralizing antibody responses (178,179).

The collaborative effort of the researchers of Rocky Mountain Laboratories and Oxford University is designing a chimpanzee adenovirus-vectored vaccine to counter COVID-19 (180). The Coalition for Epidemic Preparedness Innovations (CEPI) has initiated three programs to design SARS-CoV-2 vaccines (181). CEPI has a collaborative project with Inovio for designing a MERS-CoV DNA vaccine that could potentiate effective immunity. CEPI and the University of Queensland are designing a molecular clamp vaccine platform for MERS-CoV and other pathogens, which could assist in the easier identification of antigens by the immune system (181). They potentially induce immune responses (176). The recombinant vaccine can be designed by using rabies virus (RV) as a viral vector. RV can be made to express MERS-CoV S1 protein on its surface so that an immune response is induced against MERS-CoV. The RV vector-based vaccines against MERS-CoV can induce faster antibody response as well as higher degree of cellular immunity than the Gram-positive enhancer matrix (GEM) particle vector-based vaccine. However, the latter can induce a very high antibody response at lower doses (167). Hence, the degree of humoral and cellular immune responses produced by such vaccines depends upon the vector used.

Dual vaccines have been getting more popular recently. Among them, the rabies virus-based vectored vaccine platform is used to develop vaccines against emerging infectious diseases. The dual vaccine developed from inactivated rabies virus particles that express the MERS-CoV S1 domain of S protein was found to induce immune responses for both MERS-CoV and rabies virus. The vaccinated mice were found to be completely protected from challenge with MERS-CoV (169). Further genetic analysis is required between SARS-CoV-2 and different strains of SARS-CoV and SARS-like (SL) CoVs to evaluate the possibility of repurposed vaccines against COVID-19. This strategy will be helpful in the scenario of an outbreak, since much time can be saved, because preliminary evaluation, including in vitro studies, already would be completed for such vaccine candidates.

Multiepitope subunit vaccines can be considered a promising preventive strategy against the ongoing COVID-19 pandemic. In silico and advanced immunoinformatic tools can be used to develop multiepitope subunit vaccines. The vaccines that are engineered by this technique can be further evaluated using docking studies and, if found effective, then can be further evaluated in animal models (365). Identifying epitopes that have the potential to become a vaccine candidate is critical to developing an effective vaccine against COVID-19. The immunoinformatics approach has

been used for recognizing essential epitopes of cytotoxic T lymphocytes of SARS-CoV-2. Recently, a few epitopes have been recognized from the SARS-CoV-2 surface glycoprotein.

Hence, knowledge and understanding of S protein-based vaccine development in SARS-CoV will help to identify potential S protein vaccine candidates in SARS-CoV-2. Therefore, vaccine strategies based on the whole S protein, S protein subunits, or specific potential epitopes of S protein appear to be the most promising vaccine candidates against coronaviruses. The RBD of the S1 subunit of S protein has a superior capacity to induce neutralizing antibodies. This property of the RBD can be utilized for designing potential SARS-CoV vaccines either by using RBD-containing recombinant proteins or recombinant vectors that encode RBD (175). Hence, the superior genetic similarity existing between SARS-CoV-2 and SARS-CoV can be utilized to repurpose vaccines that have proven in vitro efficiency against SARS-CoV to be utilized for SARS-CoV-2. The possibility of cross-protection in COVID-19 was evaluated by comparing the S protein sequences of SARS-CoV-2 with that of SARS-CoV. The comparative analysis confirmed that the variable residues were found concentrated on the S1 subunit of S protein, an important vaccine target of the virus (150). Hence, the possibility of SARS-CoV specific neutralizing antibodies providing cross-protection to COVID-19 might be lower.

Repurposed broad-spectrum antiviral drugs having proven uses against other viral pathogens can be employed for SARS-CoV-2 infected patients. These possess benefits of easy accessibility and recognized pharmacokinetic and pharmacodynamic activities, stability, doses, and side effects (9). Repurposed drugs have been studied for treating CoV infections, like lopinavir/ritonavir, and interferon- $\beta$  revealed in vitro anti-MERS-CoV action. The in vivo experiment carried out in the nonhuman primate model of common marmosets treated with lopinavir/ritonavir and interferon beta showed superior protective results in treated animals than in the untreated ones (190). A combination of these drugs is being evaluated to treat MERS in humans (MIRACLE trial) (191). These two protease inhibitors (lopinavir and ritonavir), in combination with ribavirin, gave encouraging clinical outcomes in SARS patients, suggesting their therapeutic values (165). However, in the current scenario, due to the lack of specific therapeutic agents against SARS-CoV-2, hospitalized patients confirmed for the disease are given supportive care, like oxygen and fluid therapy, along with antibiotic therapy for managing secondary bacterial infections (192). Patients with novel coronavirus or COVID-19 pneumonia who are mechanically ventilated often require sedatives, analgesics, and even muscle relaxant activity.

Among the evaluated compounds, 4-(cyclopent-1-en-3-yl amino)-5-[2-(4-iodophenyl)hydrazinyl]-4H-1,2,4-triazole-3-thiol and 4-(cyclopent-1-en-3-yl amino)-5-[2-(4-chlorophenyl)hydrazinyl]-4H-1,2,4-triazole-3-thiol were found to be the most potent. These compounds were used in silico studies, and molecular docking was accomplished into the active binding site of MERS-CoV helicase nsp13 (21). Further studies are required for evaluating the therapeutic potential of these newly identified compounds in the management of COVID-19 infection.

### **Passive Immunization / Antibody Therapy / MAb**

Monoclonal antibodies (MAbs) may be helpful in the intervention of disease in CoV-exposed individuals. Patients recovering from SARS showed robust neutralizing antibodies against this CoV infection (164). A set of MAbs aimed at the MERS-CoV S protein-specific domains, comprising six specific epitope groups interacting with receptor-binding, membrane fusion, and sialic acid-binding

sites, make up crucial entry tasks of S protein (198,199). Passive immunization employing weaker and strongly neutralizing antibodies provided considerable protection in mice against a MERS-CoVs.

However, ivermectin, being a host-directed agent, exhibits antiviral activity by targeting a critical cellular process of the mammalian cell. Therefore, the administration of ivermectin, even at lower doses, will reduce the viral load at a minor level. This slight decrease will provide a great advantage to the immune system for mounting a large-scale antiviral response against SARS-CoV-2 (341). Further, a combination of ivermectin and hydroxychloroquine inhibits the entry of the virus in the host cell (339). Further, in vivo studies and randomized clinical control trials are required to understand the mechanism as well as the clinical utility of this promising drug.

Nafamostat is a potent inhibitor of MERS-CoV that acts by preventing membrane fusion. Nevertheless, it does not have any sort of inhibitory action against SARS-CoV-2 infection (194). Recently, several newly synthesized halogenated triazole compounds were evaluated, using fluorescence resonance energy transfer (FRET)-based helicase assays. However, in another case study, the authors raised concerns over the efficacy of hydroxychloroquine-azithromycin in the treatment of COVID-19 patients, since no observable effect was seen when they were used. In some cases, the treatment was discontinued due to the prolongation of the QT interval (307). Hence, further randomized clinical trials are required before concluding this matter.

Recently, another FDA-approved drug, ivermectin, was reported to inhibit the in vitro replication of SARS-CoV-2. The findings from this study indicate that a single treatment of this drug was able to induce an 5,000-fold reduction in the viral RNA at 48 h in cell culture.(308). One of the main disadvantages that limit the clinical utility of ivermectin is its potential to cause cytotoxicity. However, altering the vehicles used in the formulations, the pharmacokinetic properties can be modified, there having significant control over the systemic concentration of ivermectin (338). Based on the pharmacokinetic simulation, it was also found that ivermectin may have limited therapeutic utility in managing COVID-19, since the inhibitory concentration that has to be achieved for effective anti SARS-CoV-2 activity is far higher. The broad-spectrum activity exhibited by remdesivir will help control the spread of disease in the event of a new coronavirus outbreak.

Chloroquine is an antimalarial drug known to possess antiviral activity due to its ability to block virus-cell fusion by raising the endosomal pH necessary for fusion. It also interferes with virus-receptor binding by interfering with the terminal glycosylation of SARS-CoV cellular receptors, such as ACE2 (196). In a recent multicenter clinical trial that was conducted in China, chloroquine phosphate was found to exhibit both efficacy and safety in the therapeutic management of SARS-CoV-2 associated pneumonia (197). This drug is already included in the treatment guidelines issued by the National Health Commission of the People's Republic of China. The preliminary clinical trials using hydroxychloroquine, another aminoquinoline drug, gave promising results. The COVID-19 patients received 600mg of hydroxychloroquine daily along with azithromycin as a single-arm protocol. This protocol was found to be associated with a noteworthy reduction in viral load. Finally, it resulted in a complete cure (271); however, the study comprised a small population. A broad-spectrum antiviral drug that developed in the United States, tilorone dihydrochloride (tilorone), was previously found to possess potent antiviral activity against MERS, Marburg, Ebola, and Chikungunya viruses (306). Even though it had broad-spectrum activity, it was neglected for an extended period. Tilorone is another antiviral drug that might have activity against SARS-CoV-2.



Remdesivir, a novel nucleotide analog prodrug, was developed for treating Ebola virus disease (EVD), and it was also found to inhibit the replication of SARS-CoV and MERS-CoV in primary human airway epithelial cell culture systems (195). Recently, in vitro study has proven that remdesivir has better antiviral activity than lopinavir and ritonavir. Further, in vivo studies conducted in mice also identified that treatment with remdesivir improved pulmonary function and reduced viral loads and lung pathology both in prophylactic and therapeutic regimens compared to lopinavir/ritonavir-IFN- $\gamma$  treatment in MERS-CoV infection (8). Remdesivir also inhibits a diverse range of coronaviruses, including circulating human CoV, zoonotic bat CoV, and pre pandemic zoonotic CoV (195). Remdesivir is also considered the only therapeutic drug that significantly reduces pulmonary pathology (8). In vitro antiviral potential of FDA-approved drugs, viz; ribavirin, penciclovir, nitazoxanide, nafamostat, and chloroquine, tested in comparison to remdesivir and favipiravir (broad-spectrum antiviral drugs) revealed remdesivir and chloroquine to be highly effective against SARS-CoV-2 infection in vitro (194). Ribavirin, penciclovir, and favipiravir might not possess noteworthy in vivo antiviral actions for SARS-CoV-2, since higher concentrations of these nucleoside analogs are needed in vitro to lessen the viral infection. Both remdesivir and chloroquine are being used in humans to treat other diseases, and such safer drugs can be explored for assessing their effectiveness in COVID-19 patients.

Several therapeutic agents, such as lopinavir/ritonavir, chloroquine, and hydroxychloroquine, have been proposed for the clinical management of COVID-19 (299). A molecular docking study, conducted in the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 using different commercially available anti polymerase drugs, identified that drugs such as ribavirin, remdesivir, galidesivir, tenofovir, and sofosbuvir bind RdRp tightly, indicating their vast potential to be used against COVID-19 (305). The result obtained from a clinical study of four patients infected with COVID-19 claimed that combination therapy using lopinavir/ritonavir, arbidol, and Shufeng Jiedu capsules (traditional Chinese medicine) was found to be effective in managing COVID-19 pneumonia (193). It is difficult to evaluate the therapeutic potential of a drug or a combination of drugs for managing a disease based on such a limited sample size. Before choosing the ideal therapeutic agent for the management of COVID-19, randomized clinical control studies should be performed with a sufficient study population.

### **Antiviral Drugs**

Several classes of routinely used antiviral drugs, like oseltamivir (neuraminidase inhibitor), acyclovir, ganciclovir, and ribavirin, do not have any effect on COVID-19 and, hence, are not recommended (187). Oseltamivir, a neuraminidase inhibitor, has been explored in Chinese hospitals for treating suspected COVID-19 cases, although proven efficacy against SARS-CoV-2 is still lacking for this drug (7). Hence, as a cautionary approach, it is better to recommend the use of NSAIDs as the first-line option for managing COVID-19 symptoms (302). The use of corticosteroids in COVID-19 patients is still a matter of controversy and requires further systematic clinical studies. The guidelines that were put forward to manage critically ill adults suggest the use of systemic corticosteroids in mechanically ventilated adults with ARDS (303). The generalized use of corticosteroids in viral pneumonia. Stem cell therapy using mesenchymal stem cells (MSCs) is another hopeful strategy that can be used in clinical cases of COVID-19 owing to its potential immunomodulatory capacity. It may have a beneficial role in attenuating the cytokine storm that is

observed in severe cases of SARS-CoV-2 infection, thereby reducing mortality. Among the different types of MSCs, expanded umbilical cord MSCs can be considered a potential therapeutic agent that requires further validation for managing critically ill COVID-19 patients (304).

COVID-19 patients showing severe signs are treated symptomatically along with oxygen therapy. In such cases where the patients progress toward respiratory failure and become refractory to oxygen therapy, mechanical ventilation is necessitated. The COVID-19 induced septic shock can be managed by providing adequate hemodynamic support (299). Several classes of drugs are currently being evaluated for their potential therapeutic action against SARS-CoV-2. Therapeutic agents that have anti-SARS-CoV-2 activity can be broadly classified into three categories; drugs that block virus entry into the host cell, drugs that block viral replication as well as its survival within the host cell, and drugs that attenuate the exaggerated host immune response (300). An inflammatory cytokine storm is commonly seen in critically ill COVID-19 patients. Hence, they may benefit from the use of timely anti-inflammatory treatment. Anti-inflammatory therapy using drugs like glucocorticoids, cytokine inhibitors, JAK inhibitors, and chloroquine/hydroxychloroquine should be done only after analyzing the risk/benefit ratio in COVID-19 patients (301). There have not been any studies concerning the application of nonsteroidal anti-inflammatory drugs (NSAID) to COVID-19-infected patients.

Presently, the main course of treatment for severely affected SARS-CoV-2 patients admitted to hospitals includes mechanical ventilation, intensive care unit (ICU) admittance, and symptomatic and supportive therapies. Additionally, RNA synthesis inhibitors (lamivudine and tenofovir disoproxil fumarate), remdesivir, neuraminidase inhibitors, peptide (EKI), anti-inflammatory drugs, abidol, and Chinese traditional medicine (Lianhuaqingwen and ShuFengJieDu capsules) could aid in COVID-19 treatment. However, further clinical trials are being carried out concerning their safety and efficiency (7). It might require months to a year(s) to design and develop effective drugs, therapeutics, and vaccines against COVID-19, with adequate evaluation and approval from regulatory bodies and moving to the bulk production of many millions of doses at commercial levels to meet the timely demand of mass populations across the globe (9). Continuous efforts are also warranted to identify and assess viable drugs and immunotherapeutic regimens that revealed proven potency in combating other viral agents similar to SARS-CoV-2.

## **Therapeutics and Drugs**

There is no currently licensed specific antiviral treatment for MERS-and SARS-CoV infections, and the main focus in clinical settings remains on lessening clinical signs and providing supportive care (183-186). Effective drugs to manage COVID-19 patients include remdesivir, lopinavir/ritonavir alone or in a blend with interferon beta, convalescent plasma, and monoclonal antibodies (MAbs); however, efficiency and safety issues of these drugs require additional clinical trials (187, 281). A controlled trial of ritonavir-boosted lopinavir and interferon alpha 2b treatment was performed on COVID-19 hospitalized patients (ChiCTR2000029308) (188). In addition, the use of hydroxychloroquine and tocilizumab for their potential role in modulating inflammatory responses in the lungs and antiviral effect has been proposed and discussed in many research articles. Still, no fool-proof clinical trials have been published (194,196,197,261-272). Recently, a clinical trial conducted on adult patients suffering from severe COVID-19 revealed no benefit of lopinavir-ritonavir treatment over standard care (273).

At present, three new clinical trials have been registered to evaluate the protective role of BCG vaccination against SARS-CoV-2 (363). Recently, a cohort study was conducted to evaluate the impact of childhood BCG vaccination in COVID-19 PCR positivity rates. However, childhood BCG vaccination was found to be associated with a rate of COVID-19-positive test results similar to that of the non-vaccinated group (364). Further studies are required to analyze whether BCG vaccination in childhood can induce protective effects against COVID-19 in adulthood. Population genetic studies conducted on 103 genomes identified that the SARS-CoV-2 virus has evolved into two major types, L and S. Among the two types, L type is expected to be the most prevalent ( ~ 70%), followed by the S type ( ~ 30 % ) (366). This finding has a significant impact on our race to develop an ideal vaccine, since the vaccine candidate has to target both strains to be considered effective. At present, the genetic differences between the L and S types are very small and may not affect the immune response. However, we can expect further genetic variations in the coming days that could lead to the emergence of new strains (367).

However, the success of such a vaccine relies greatly on its ability to provide protection not only against present versions of the virus but also the ones that are likely to emerge in the future. This can be achieved by identifying antibodies that can recognize relatively conserved epitopes that are maintained as such even after the occurrence of considerable variations (362). Even though several vaccine clinical trials are being conducted around the world, pregnant women have been completely excluded from these studies. Pregnant women are highly vulnerable to emerging diseases such as COVID-19 due to alterations in the immune system and other physiological systems that are associated with pregnancy. Therefore, in the event of successful vaccine development, pregnant women will not get access to the vaccines (361). Hence, it is recommended that pregnant women be included in the ongoing vaccine trials, since successful vaccination in pregnancy will protect the mother, fetus, and newborn. The heterologous immune effects induced by Bacillus Calmette Guerin (BCG) vaccination is a promising strategy for controlling the COVID-19 pandemic and requires further investigations. Similarly, the WHO, on its official website, has mentioned a detailed list of COVID-19 vaccine agents that are under consideration. Different phases of trials are ongoing for live attenuated virus vaccines, formaldehyde alum inactivated vaccine, adenovirus type 5 vector vaccine, LNP-encapsulated mRNA vaccine, DNA plasmid vaccine, and S protein, S-trimer, and li-Key peptide as s subunit protein vaccine, among others (298). The process of vaccine development usually takes approximately ten years, in the case of inactivated or live attenuated vaccines, since it involves the generation of long-term efficacy data. However, this was brought down to 5 years during the Ebola emergency for viral vector vaccines. In the urgency associated with the COVID-19 outbreaks, we expect a vaccine by the end of this year (343). The development of an effective vaccine against COVID-19 with high speed and precision is the combined result of advancements in computational biology, gene synthesis, protein engineering, and the invention of advanced manufacturing platforms (342).

The recurring nature of the coronavirus outbreaks calls for the development of a pan-coronavirus vaccine that can produce cross-reactive antibodies. Antigenic determinant sites present over S and N structural proteins of SARS-CoV-2 can be explored as suitable vaccine candidates (294). In the Asian population, S, E, M, and N, proteins of SARS-CoV-2 are being targeted for developing subunit vaccines against COVID-19 (295).

The identification of the immunodominant region among the subunits and domains of S protein is critical for developing an effective vaccine against the coronavirus. The C-terminal domain of the SI subunit is considered the immunodominant region of the porcine deltacoronavirus S protein (171). Similarly, further investigations are needed to determine the immunodominant regions of SARS-CoV-2 for facilitating vaccine development.

However, our previous attempts to develop a universal vaccine that is effective for both SARS-CoV and MERS-CoV based on T-cell epitope similarity pointed out the possibility of cross-reactivity among coronaviruses (172). That can be made possible by selected potential vaccine targets that are common to both viruses. SARS-CoV-2 has been reported to be closely related to SARS-CoV (173, 174). Several therapeutic and preventive strategies, including vaccines, immunotherapeutics, and antiviral drugs, have been exploited against the previous CoV outbreaks (SARS-CoV and MERS-CoV) (8, 104, 164-167). These valuable options have already been evaluated for their potency, efficacy, and safety, along with several other types of current research that will fuel our search for ideal therapeutic agents against COVID-19 (7, 9, 19, 21, 36). The primary cause of the unavailability of approved and commercial vaccines, drugs, and therapeutics to counter the earlier SARS-CoV and MERS-CoV seems to owe to lesser attention of the biomedicine and pharmaceutical companies, as these two COVs did not cause much havoc, global threat, and panic like those posed by the SARS-CoV-2 pandemic (19). Moreover, for such outbreak situations, the requirement for vaccine and therapeutics/drugs exists only for a limited period, until the outbreak is controlled. The proportion of the human population infected with SARS-CoV and MERS-CoV was also much lower across the globe, failing to attract drug and vaccine manufacturers and producers. Therefore, by the time an effective drug or vaccine is designed against such disease outbreaks. Several attempts are being made to design and develop vaccines for CoV infection, mostly by targeting the spike glycoprotein. nevertheless, owing to extensive diversity in antigenic variants, cross protection rendered by the vaccines is significantly limited, even within the strains of a phylogenetic subcluster (104). Due to the lack of effective antiviral therapy and vaccines in the present scenario, we need to depend solely on implementing effective infection control measures to lessen the risk of possible nosocomial transmission (68). Recently, the receptor for SARS-CoV-2 was established as the human angiotensin-converting enzyme 2 (hACE2), and the virus was found to enter the host cell mainly through endocytosis. It was also found that the major components that have a critical role in viral entry include PIKfyve, TPC2, and cathepsin L. These findings are critical, since the components described above might act as candidates for vaccines or therapeutic drugs against SARS-CoV-2 (293).

The majority of the treatment options and strategies that are being evaluated for SARS-CoV-2 (COVID-19) have been taken from our previous experiences in treating SARS-CoV, MERS-CoV, and other emerging viral diseases. Several therapeutic rates, disease outbreaks, community spread, clustered transmission events, hot spots, and superspreader potential of SARS-CoV-2/COVID warrant full exploitation of real-time disease mapping by employing geographical information systems (GIS), such as the GIS software Kosmo 3.1, web-based real-time tools and dashboards, apps, and advances in information technology (356-359). Researchers have also developed a few prediction tools/models, such as the prediction model risk of bias assessment tool (PROBAST) and critical appraisal and data extraction for systematic reviews of prediction

modeling studies (CHARMS), which could aid in assessing the possibility of getting infection and estimating the prognosis in patients; however, such models may suffer from bias issues and, hence, cannot be considered completely trustworthy, which necessitates the development of new and reliable predictors (360).

## **VACCINES, THERAPEUTICS, AND DRUGS**

Recently emerged viruses, such as Zika, Ebola, and Nipah viruses, and their grave threats to humans have begun a race in exploring the designing and developing of advanced vaccines, prophylactics, therapeutics, and drug regimens to counter emerging developed for rapid and colorimetric detection of this virus (354). RT-LAMP serves as a simple, rapid, and sensitive diagnostic method that does not require sophisticated equipment or skilled personnel (349). An interactive web-based dashboard for tracking SARS-CoV-2 in a real-time mode has been designed (238). A smartphone-integrated home-based POCT combined with LAMP, is a useful point-of-care diagnostic (353). An Abbott ID Now COVID-19 molecular POCT-based test, using isothermal nucleic acid amplification technology, has been designed as a point-of-care test for very rapid detection of SARS-CoV-2 in just 5 min (344). A CRISPR-based SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) diagnostic for rapid detection of SARS-CoV-2 without the requirement of specialized instrumentation has been reported to be very useful in the clinical diagnosis of COVID-19 (360). A CRISPR-Cas12-based lateral flow assay also has been developed for rapid detection of SARS-CoV-2 (346). Artificial intelligence, by means of a three-dimensional deep-learning model, has been developed for sensitive and specific diagnosis of COVID-19 via CT images (332).

Chest CT is an ideal diagnostic tool for identifying viral pneumonia. The sensitivity of chest CT is far superior to that of X-ray screening. The chest CT findings associated with COVID-19-infected patients include characteristic patchy infiltration that later progresses to ground-glass opacities (158). Early manifestations of COVID-19 pneumonia might not be evident in X-ray chest radiography. In such situations, a chest CT examination can be performed, as it is considered highly specific for COVID-19 pneumonia (118). Those patients having COVID\_19 pneumonia will exhibit the typical ground-glass opacity in their chest CT images (154). The patients infected with COVID-19 had elevated plasma angiotensin 2 levels. The level of angiotensin 2 was found to be linearly associated with viral load and lung injury, indicating its potential as a diagnostic biomarker (121). The chest CT imaging abnormalities associated with COVID-19 pneumonia have also been observed even in asymptomatic patients. This will further result in the extensive transmission of COVID-19, since only a portion of suspected cases can be diagnosed. In such situations, conventional serological assays, like enzyme-linked immunosorbent assay (ELISA), that are specific to COVID-19 IgM and IgG antibodies can be used as a high-throughout alternative (149). At present, there is no diagnostic kit available for detecting the SARS-CoV-2 antibody (150). The specific antibody profiles of COVID-19 patients were analyzed, and it was found that the IgM level lasted more than 1 month, indicating a prolonged stage of virus replication in SARS-CoV-2-infected patients. The IgG levels were found to increase only in the later stages of the disease. These findings indicate that the specific antibody profiles of SARS-CoV-2 and SARS-CoV were similar (325). These findings can be utilized for the development of specific diagnostic tests against COVID-19 and can be used for rapid screening. Even though diagnostic test kits are already available that can detect the

genetic sequences of SARS-CoV-2 (95), their availability is common, as the number of COVID-19 cases is skyrocketing (155,157). A major problem associated with this diagnostic kit is that it works only when the test subject has an active infection, limiting its use to the earlier stages of infection. Several laboratories around the world are currently developing antibody-based diagnostic tests against SARS-CoV-2 (157). Such antibodies may play a crucial role in enhancing protective humoral responses against the emerging COVs by aiming appropriate epitopes and functions of the S protein. The cross-neutralization ability of SARS-CoV RBD-specific neutralizing MABs considerably relies on the resemblance between their RBDs; therefore, SARS-CoV RBD-specific antibodies could cross-neutralized SL CoVs, i.e., bat-SL-CoV strain WIVI (RBD with eight amino acid differences from SARS-CoV) but not bat-SL-CoV strain SHC014 (24 amino acid differences) (200).

Appropriate RBD-specific MABs can be recognized by a relative analysis of RBD of SARS-CoV-2 to that of SARS-CoV, and cross-neutralizing SARS-CoV RBD-specific MABs could be explored for their effectiveness against COVID-19 and further need to be assessed clinically. The U.S. biotechnology company Regeneron is attempting to recognize potent and specific MABs to combat COVID-19. An ideal therapeutic option suggested for SARS-CoV-2 (COVID-19) is the combination therapy comprised of MABs and the drug remdesivir (COVID-19) (201). Large-scale screening programs might help us to control the spread of Thai virus. However, this is both challenging as well as time-consuming due to the present extent of infection (226). The current scenario demands effective implementation of vigorous prevention and control strategies owing to the prospect of COVID-19 for nosocomial infections (68). Follow-ups of infected patients by telephone on day 7 and day 14 are advised to avoid any further unintentional spread or nosocomial transmission (312). The availability of public data sets provided by independent analytical teams will act as robust evidence that would guide us in designing interventions against the COVID-19 outbreak. Newspaper reports and social media can be used to analyze and reconstruct the progression of an outbreak. They can help us to obtain detailed patient-level data in the early stages of an outbreak (227). Immediately travel restrictions imposed by several countries might have contributed significantly to preventing the spread of SARS-CoV-2 globally (89, 228). Following the outbreak, a temporary ban was imposed on the wildlife trade, keeping in mind the possible role played by wild animal species in the origin of SARS-CoV-2/ COVID-19 (147). Hence, for control of the COVID-19 outbreaks, rapid steps should be taken to protect the mental health of medical workers (229).

Since the living mammals sold in the wet market are suspected to be the intermediate host of SARS-CoV-2, there is a need for strengthening the regulatory mechanism for wild animal trade (13). The total number of COVID-19 confirmed cases is on a continuous rise and the cure rate is relatively low, making disease control very difficult to achieve. The Chinese government is making continuous efforts to contain the disease by taking emergency control and prevention measures. They have already built a hospital for patients affected by this virus and are currently building several more for accommodating the continuously increasing infected population (230). The effective control for SARS-CoV-2/COVID-19 requires high-level interventions like intensive contact tracing, as well as the quarantine of people with suspected infection and the isolation of infected individuals. The implementation of rigorous control and preventive measures together might control the  $R_0$  number and reduce the transmission risk (228). Presently, licensed antiviral drugs

or vaccines against SARS-CoV, MERS-CoV, and SARS-CoV-2 are lacking. However, advances in designing antiviral drugs and vaccines against several other emerging diseases will help develop suitable therapeutic agents against COVID-19 in a short time. Until then, we must rely exclusively on various control and prevention measures to prevent this new disease from becoming a pandemic.

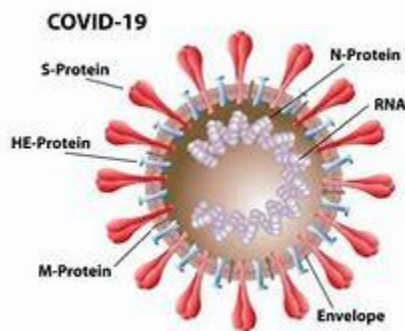
#### 4 VIROLOGY

Coronaviruses, a family of viruses within the nidoviruses superfamily, are further classified according to their genera, alpha, beta, gamma, and delta coronaviruses ( $\alpha, \beta, \gamma, \delta$ ).

Among those, alpha and beta species are capable of contaminating only mammals, whereas the other two genera can infect birds and could also infect mammals.<sup>13, 14</sup> Two of these genera belong to human coronaviruses (HCoVs):  $\alpha$ -coronaviruses, which comprise human coronavirus 229E (hCoV 229E) and human coronavirus NL63 (hCoVNL63), and  $\beta$ -coronaviruses, which are human coronavirus HKU1, human coronavirus OC43, MERS-CoV (known as Middle East respiratory syndrome coronavirus) and SARS-CoV (referred to as severe acute respiratory syndrome coronavirus).

The severe acute respiratory syndrome CoV-2 (SARS-CoV-2) is now named novel COVID-19 (coronavirus disease 2019). Genome sequencing and phylogenetic research revealed that the COVID-19 causing coronavirus is a beta-coronavirus that belongs to the same subtypes as SARS virus, but still exists in a variant group.

The spike S-protein being in a spike form is subjected to a structural rearrangement process so that fusing the outer membrane of the virus with the host-cell membrane becomes easier. Recent SARS-CoV work has also shown that the membrane exopeptidase ACE enzyme (angiotensin-converting enzyme) functions as a COVID-19 receptor to enter the human cell.



There is an enhanced nasal secretion observed along with local oedema because of the damage of the host cell, which further stimulates the synthesis of inflammatory mediators. In addition, these reactions can induce sneezing, difficulty breathing by causing airway inhibition and elevate mucosal temperature. These viruses, when released, chiefly affect the lower respiratory tract, with the signs and symptoms existing clinically. Also, the virus further affects the intestinal lymphocytes, renal cells, liver cells and T-lymphocytes. Furthermore, the virus induces T-cell apoptosis, causing the reaction of the T-cell to be erratic, resulting in the immune system's complete collapse<sup>(24,25)</sup>.

## **5.1 Mode Of Transmission**

In fact it was accepted that the original transmission originated from a seafood market, which had a tradition of selling live animals, where the majority of the patients had either worked or visited, although up to now the understanding of the COVID-19 transmission risk remains incomplete(16). In addition, while the newer patients had no exposure to the market and still got the virus from the humans present there, there is an increase in the outbreak of this virus through human-to-human transmission, with the fact that it has become widespread around the globe. This confirms the fact similar to the previous epidemics, including SARS and MERS, that this coronavirus exhibited potential; human-to-human transmission, as it was recently declared a pandemic by WHO.(26)

Respiratory droplets are the major carrier for coronavirus transmission. Such droplets can either stay in the nose or mouth or enter the lungs via the inhaled air. Currently, it is known that COVID-19's transmission from one person to another also occurs through touching either an infected surface or even an object. With the current scant awareness of the transmission systems however, airborne safety measures with a high-risk procedure have been proposed in many countries. Transmission levels, or the rates from one person to another, reported differ by both location and interaction with involvement in infection control. It is stated that even asymptomatic individuals or those individuals in their incubation period can act as carriers of SARS-CoV2. With the data and evidence provided by the eCDC, the usual incubation period is probably 3 to 7 days, sometimes being prolonged up to even 2 weeks, and the typical symptom occurrence.

### **Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases**

The assessment of the patients with COVID-19 should be based on the clinical features and also epidemiological factors. The screening protocols must be prepared and followed per the native context. Collecting and testing of specimen samples from the suspected individual is considered to be one of the main principles for controlling and managing the outbreak of the disease in a country. The suspected cases must be screened thoroughly in order to detect the virus with the help of nucleic acid amplification tests such as reverse transcription polymerase chain reaction (RT-PCR). If a country or a particular region does not have the facility to test the specimens, the specimens of the suspected individual should be sent to the nearest reference laboratories per the list provided by WHO.

It is also recommended that the suspected patients be tested for the other respiratory pathogens by performing the routine laboratory investigation per the local guidelines, mainly to differentiate from other viruses that include influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus, rhinovirus, human herpes, and the typical symptom occurrence from incubation period to infection takes an average of 12.5 days.



## **6 CLINICAL DIAGNOSIS**

The symptoms of COVID-19 remain very similar to those of the other respiratory epidemics in the past, which include SARS and MERS, but here the range of symptoms includes mild rhinitis to septic shock. Some intestinal disturbances were reported with the other epidemics. When examined, unilateral or bilateral involvement compatible with viral pneumonia is observed in the patients, and bilateral multiple lobular and subsegmental consolidation areas were observed in patients hospitalized in the intensive care unit.

Comorbid patients showed a more severe clinical course than predicted from previous epidemics. Diagnosis of COVID-19 includes the complete history of travel; and touch, with laboratory testing. It is more preferable to choose serological screening, which can help to analyse even the asymptomatic infections; several serological tests are in progress for SARS-CoV-2.

### **4.2 Viral replication**

Usually replication of coronavirus occurs within the cytoplasm and is closely associated with endoplasmic reticulum and other cellular membrane organelles. Human coronaviruses are thought to invade cells, primarily through different receptors. For 229E and OC43, aminopeptidase-N (AP-N) and a sialic acid containing receptor, respectively, were known to function in this role. After the virus enters the host cell and uncoating progress occurs, the genome is transcribed, and then, translated. A characteristic feature of replication is that all mRNAs form an enclosed group of typical 3 ends; only the special portions of the 5 ends are translated. In total, about 7 mRNAs are produced. The shortest mRNA codes and the others can express the synthesis of another genome segment for nucleoprotein. At the cell membrane, these proteins are collected and genomic RNA is initiated as a mature particle type by budding from internal cell membranes.

## **5 PATHOGENESIS**

Coronaviruses are tremendously precise and mature in most of the airway epithelial cells as observed through both in vivo and in vitro.