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  
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 over the genome of the virus,  
with nine substitutions in ORFlab, ORF8 (4  
substitutions), the spike gene (3 substitutions), 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  
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, nspl to  
nspl0 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 sequences of other  
coronaviruses (16).  
  
The virus structure of SARS-CoV-2 is depicted in  
Fig. 2.  
  
Spike glycoprotein (S)  
(required for the entry of the  
infectious virion particle)  
  
Membrane protein (M)  
  
(most abundant viral protein) Major structural proteins  
  
Envelope glycoprotein (E)  
(smallest among the major  
structural proteins)  
  
Nucleocapsid protein (N)  
+ single-stranded positive  
sense RNA genome  
  
Lipid bilayer  
  
FIG 2 SARS-CoV-2 virus structure.  
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, with 221,216 cases and 30,911 deaths, but  
now, the United States is the country with the largest  
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  
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.  
  
Major events of current coronavirus COVID-19 disease outbreak  
  
B Jan, 2020 from Aapar WN Feb, 2020  
San. 2020 COOK announces 4 “151 pera WHO names the  
31 Dec, 2019 Wuhan health committee novel comonavirus 24 Jan, 2020 quarantined by the Japanese: \* sense COND.  
Wuhan notes 27 exclude SARS and MERS as reccosalenetonsiay Fest complete 9 ar Aacer the painting 6 Virus was named  
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22 Apr, 2020 02 Apr, 2020 flings  
  
The U.S. CDC and the USDA National Total confirmed cases Wo dadens CONDS1D roratogtontin pe ocipiomene  
Veterinary Services Laboratories peaches BR6A50 with a3 global pandemic Hoeloablg ryt from Tawan-  
  
(NVSL) confirms cases of SARS-Co¥-2 45,526 deaths workwide:  
  
scovowupater  
in two pet cats  
  
FIG 5 Timeline 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 hichest number of confirmed cases  
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 Ro  
value, since there is a possibility of bias due to  
insufficient data. The higher Rg value is indicative of  
the more significant potential of SARS-CoV-2  
transmission in a susceptible population. This is not  
the first time where 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), minks,  
soup from palm civet, ostriches, hamsters, snapping  
turtles, ducks, fish, Siamese crocodiles, and other  
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).  
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c  
  
nucleic acid test results as one of the additiona  
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  
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  
  
ye \* ye  
suffering trom 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  
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 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 novel SARS-CoV-2, with more than  
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 in 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 (Ro) 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  
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 Huanan  
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).  
responsible for MERS-COV and SARS-COV (5). Lhe  
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  
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 care 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 |  
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 (125days) (68). Immediately after the  
confirmation of viral etiology, the Chinese  
virologists 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). The  
possible origin of SARS-CoV-2 and the first mode of  
Splits Tree phylogeny analysis.  
  
In the unrooted phylogenetic tree of different  
betacoronaviruses based on the S protein, virus  
sequences from different subgenera 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 Sarbecovirus appeared  
jointly in SplitsTree and divided into three  
subclusters, 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 Nobecovorus 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 MegAlign 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 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  
(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).  
  
nsps and Accessory Proteins  
  
™ a | at e 4  
addGdpuve CVvOliullon, Close MONMOrimMye Of the viral  
mutations that occur during subsequent human-to-  
human transmission is warranted.  
  
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 terminus 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). The inactivation or  
CWOTOnNAaAVITUS S protein Is ad lal ee, ITU UIC LIOllal  
class I viral transmembrane protein. The size of this  
abundant S protein varies from 1,160 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 responses (45). The  
ectodomains in all CoVs S proteins have similar  
domain organizations, divided into two subunits, S1  
and §2 (43). The first one, S1, helps in host receptor  
binding, while the second one, $2, 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 $2  
  
cat and camels, respectively, act as amplifier hosts  
(40, 41).  
  
Coronavirus genomes and subgenomes encode  
six ORFs (31). The majority of the 5’ end is occupied  
by ORFla/b, which produces 16 nsps. The two  
polyproteins, ppla and pplab, are initially produced  
from ORFla/b by a —1 frameshift between ORFla  
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 with an  
alpha helix and beta-sheet with six strands encoded  
by ORF8 (31).  
  
Coronaviruses encode four major structural  
proteins, namely, spike (S), membrane (M), envelope  
(E), and nucleocapsid (N), which are described in  
detail below.  
  
S Glycoprotein  
Coronavirus § protein is a large, multifunctional  
class I viral transmembrane protein. The size of this  
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 la/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  
BJO1 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). Of note,  
the other two zoonotic CoVs (MERS-related CoV  
and SARS-related CoV) also originated from bats  
(38, 39). Nevertheless, for SARS and MERS, civet  
nucleocapsid. 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-  
replicase-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 la/lab (ppla/lab) (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).  
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  
coronavirus category and found this virus is related  
to SARS-CoVs (26). SARS-CoV-2 is a member of  
the order WNidovirales, family Coronaviridae,  
subfamily Orthocoronavirinae, which is subdivided  
  
into four genera, viz., 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 30  
kb, enclosed by a 5'-cap and 3’-poly(A) tail (30). The  
genome of SARS-CoV-2 is 29,891 bp long, with a  
G+C content of 38% (31). These viruses are  
encircled with an envelope containing viral  
Some therapeutic options for treating COVID-19  
showed efficacy in in vitro studies; however, to date,  
these treatments have not undergone any randomized  
animal or 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 diseases 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  
  
Beiter tn Idwwid lation \_leadi  
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  
characteristic 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).  
new targeted drugs, and prevention of further  
epidemics (13). 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  
analyses have revealed that SARS-CoV-2 is closely  
related (88% similarity) to two SARS-like CoVs  
derived from bat SARS-like CoVs\_ (bat-SL-  
“OVZCAS | bat-SL-CoVZXC21) (Fj '  
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 CoVs 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. Although  
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 Fan et al.  
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 emeroency an 21 Jannaryv 000: enheeanently  
health emergency on 31 January 2020; 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 may 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), development of  
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 as 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 1s 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 incubation 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).  
  
Coronavirus is the most prominent example of a  
in the epidemic strain (104). 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  
  
‘sible si F infecti kino it challengi  
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).  
  
CLINICAL PATHOLOGY OF SARS-CoV-2  
  
(COVID-19)  
  
The 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  
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 occurred  
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  
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 also have been found circulating in the  
Chinese horseshoe bat (Rhinolophus \_ sinicus)  
populations. The in vitro and in vivo studies carried  
  
coronavirus results in an epidemic by jumping the  
so-called species barrier (287).  
  
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  
coronaviruses calls for the development of efficient  
molecular surveillance strategies for studying  
Betacoronavirus among animals (12), especially in  
the Rhinolophus bat family (86). Chinese bats have  
high commercial value, since they are used in  
ads y Lplulllaule UL SYLIPLULMALLY pauls Lidv Lille  
minimum signs and symptoms (82). 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, indicates the requirement of  
data for assessing SARS-CoV-2 transmission  
dynamics and updating the screening procedures in  
the clinics (82).  
sanitation practices needs to be given due emphasis  
(249-252). 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  
A suspected case of COVID-1Y 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.  
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 a mild form 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.  
sputum, nasal swabs, fibrobronchoscope — brush  
biopsy specimens, pharyngeal swabs, feces, and  
blood (246).  
  
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 stools of patients  
infected with SARS-CoV-2. Biowaste materials and  
sewage from hospitals must be adequately  
disinfected, treated, and disposed of properly. The  
significance of frequent and good hand hygiene and  
The results of the studies related to SARS-CoV-2  
viral loads reflect active replication of this 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 x 10° 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,  
of SARS-CoV-2 were measured using N-gene-  
specific quantitative RI-PCR in throat swab 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 ranged from 107 to 10’ 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 recorded 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). Another study,  
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SARAS-CoV-2 strains available in the National  
Center for Biotechnology Information and GISAID  
databases were subjected to multiple-sequence  
alignment and phylogenetic analyses 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%  
(99.79% to 100%) at the amino acid level. Overall  
variation was found to be low in ORF regions, with  
13 variation sites recognized in la, 1b, 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 (ORF 1a) 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). The viral loads  
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 an effective method 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 betacoronaviruses  
and specific detection of SARS-CoV-2, developed  
by the U.S. CDC (Table 1) (258, 259).  
ducks, and pigs are not at all susceptible to SARS-  
CoV-2 (329).  
  
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 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 human and animal health sectors is  
becoming a necessity to evaluate and identify the  
possible risk factors of transmission between animals  
and humans. Such cooperation will help to devise  
efficient strategies for the management of emerging  
zoonotic diseases (12).  
to-human transmission is lacking and \_ requires  
further studies (332). 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 cats 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). Experimental inoculation was  
also done in other animal species and found that the  
dogs have low susceptibility, while the chickens,  
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 the 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. However, evidence of cat-  
in CoV isolated from pangolin was almost identical  
(one amino acid difference) to that of SARS-CoV-2.  
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).  
These findings will not have any significance until a  
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 genes (90.4%). The RBD of S protein  
traditional Chinese medicine (TCM). Therefore, the  
handling of bats for trading purposes poses a  
considerable risk of transmitting zoonotic CoV  
epidemics (139).  
  
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  
diseases 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).  
  
The comprehensive sequence analysis of the  
(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 had 95% genomic similarity with  
horseshoe bat (Rhinolophus species) coronavirus  
HKU2, 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 coronavirus 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). It is  
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 (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 can affect the  
gastrointestinal tract, abdominal cavity (peritonitis),  
respiratory tract, and central nervous system (128).  
Canines are also affected by 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 diseases. of  
respiratory, urinary, and reproductive systems, with  
substantial economic losses in chickens (131, 132).  
In small laboratory animals, mouse hepatitis virus,  
rat sialodacryoadenitis coronavirus, and guinea pig  
and rabbit coronaviruses are the major CoVs  
associated with disease manifestations like enteritis,  
hepatitis, and respiratory infections (10, 133).  
  
Swine acute diarrhea syndrome coronavirus  
93, 124, 125, 287). 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 HKUI15) (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  
(244). Middle-aged and elderly patients with primary  
chronic diseases, especially high blood pressure and  
diabetes, were found to be more susceptible to  
respiratory failure and, therefore, had poorer  
prognoses. 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  
ZOONOTIC 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,  
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 postbirth; 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  
  
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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). However,  
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 forward 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). Transmission can also  
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 diagnostic 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 ORFlb and N regions of the SARS-CoV-2  
genome (156). That assay was found to achieve the  
rapid detection of SARS-CoV-2. Nucleic acid-based  
assays offer high accuracy in the diagnosis of SARS-  
prevention and control measures, and patients for  
clinical trials will not be available. The newly  
developed drugs cannot be marketed due to the lack  
of end users.  
  
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 (BHPIV3). Of note, the result  
was conclusive that the expression of M, E, or N  
  
proteins without the presence of S protein would not  
vaccine for COVID-19 in partnership with the  
Vaccine Research Center (VRC) of the National  
Institute of Allergy and Infectious Diseases (NIAID),  
part of the National Institutes 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 vaccine’s safety and ability to trigger  
immune responses (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, INO-4800  
DNA coronavirus vaccine, and adenovirus type 5  
vector vaccine candidate (Ad5-nCoV), are a few  
examples under phase I clinical trials, while self-  
amplifying RNA vaccine, oral recombinant COVID-  
19 vaccine, BNT162, plant-based COVID-19  
  
vaccine, and li-Key peptide COVID-19 vaccine are  
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). CEPI has also funded Moderna to develon a  
evaluating their interaction with corresponding major  
histocompatibility complex class I molecules. 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  
degrees 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). The intranasal  
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. Jn 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 and B\_ cells from \_ the surface  
glycoprotein of SARS-CoV-2. Recently, a few  
epitopes have been recognized from the SARS-CoV-  
2 surface glycoprotein. The selected epitopes  
explored targeting molecular dynamic simulations,  
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 efficacy 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 SI 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. Further genetic analysis is required  
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-1B 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  
  
— sas ,  
Among the evaluated compounds, 4-(cyclopent-  
1-en-3-ylamino)-5-[2-(4-  
iodopheny1)hydrazinyl ]-4H-1,2,4-triazole-3-thiol and  
4-(cyclopent- 1-en-3-ylamino)-5-[2-(4-  
chloropheny])hydrazinyl]-4H-1,2,4-triazole-3-thiol  
were found to be the most potent. These compounds  
were used for 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-  
maximum plasma \_ concentration achieved by  
administering the approved dose (340). 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  
might have a synergistic effect, since ivermectin  
reduces viral replication, while 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, for their ability to inhibit  
possibility of misinterpretation could arise. 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, thereby 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 than the  
efficacy in the treatment of COVID-19 infection in  
humans. 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 600 mg 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 and, hence, the  
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-y treatment in MERS-CoV  
infection (8). Remdesivir also inhibits a diverse  
range of coronaviruses, including circulating human  
CoV, zoonotic bat CoV, and prepandemic zoonotic  
CoV (195). Remdesivir is also considered the only  
therapeutic drug that significantly reduces  
pulmonary pathology (8). All these findings indicate  
that remdesivir has to be further evaluated for its  
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  
antipolymerase 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). A  
broad-spectrum antiviral drug that was developed in  
the United States, tilorone dihydrochloride (tilorone),  
relaxation drugs to prevent ventilator-related lung  
injury associated with human-machine  
incoordination (122). 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). The in  
vitro antiviral potential of FAD-approved drugs, viz.,  
uses with the occurrence of respiratory and  
cardiovascular adverse effects. 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 is not indicated in COVID-19, since  
there are some concerns associated with the 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).  
  
Repurposed broad-spectrum antiviral drugs  
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-inflammation  
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. However, reasonable  
pieces of evidence are available that link NSAID  
and SAKS, along with adopting and strengthening a  
few precautionary measures owing to the unknown  
nature of this novel virus (36, 189). 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 (EK 1),  
anti-inflammatory drugs, abidol, and Chinese  
traditional medicine (Lianhuagingwen and  
ShuFengJieDu capsules) could aid in COVID-19  
treatment. However, further clinical trials are being  
carried out concerning their safety and efficacy (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.  
  
COVID-19 patients showing severe signs are  
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, efficacy 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).  
  
The efforts to control SARS-CoV-2 infection  
utilize defined strategies as followed against MERS  
and SARS, along with adopting and strengthening a  
strain of Mycobacterium bovis. 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 nonvaccinated 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 Guérin (BCG) vaccination is a  
promising strategy for controlling the COVID-19  
pandemic and requires further investigations. BCG is  
a widely used vaccine against tuberculosis in high-  
vaccine, and li-Key peptide COVID-19 vaccine are  
under = eepaticted trials (297). 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 a 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.  
confer any noticeable protection, with the absence of  
detectable serum SARS-CoV-neutralizing antibodies  
(170). 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 S1  
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). Hence, knowledge and understanding of  
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  
the 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 vaccines 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, the virus would have  
been controlled by adopting appropriate and strict  
viruses (161—163, 280). 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  
LdiCs, UISCdsec UULUICAKDS, COLTTIUTILY oplreda,  
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  
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 point-  
of-care testing (POCT) tool, a paper-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).  
  
Tracking and mapping of the rising incidence  
rates, disease outbreaks, community — spread,  
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).  
  
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. These abnormalities  
assay) S OFTel High ac CUlAaC) IT} Lie Glapnosis OT SANRD-  
CoV-2, but the current rate of spread limits its use  
due to the lack of diagnostic assay kits. 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-throughput 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 | 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 a concern, as the number  
of COVID-19 cases is skyrocketing (155, 157). A  
major problem associated with this diagnostic kit is  
CoV lethal challenge. 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 WIV1  
(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 USS.  
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). The SARS-CoV-specific human  
MAb CR3022 is found to bind with SARS-CoV-2  
RBD, indicating its potential as a therapeutic agent  
help us to control the spread of this 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).  
Immediate 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). Making a  
permanent and bold decision on the trade of wild  
animal species is necessary to prevent the possibility  
involved in the COVID-1Y outbreak 1S OF great  
importance, because the strain on their mental well-  
being will affect their attention, concentration, and  
decision-making capacity. Hence, for control of the  
COVID-19 outbreak, 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 of 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 Rpg number and reduce the  
transmission risk (228). Considering the zoonotic  
(using suitable animal models) should be conducted  
to evaluate the risk of future epidemics. 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 deltacoronaviruses (a-, B-, y- and 5-).  
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.'\* '\* Two of  
these genera belong to human coronaviruses  
(HCoVs): a-coronaviruses, which comprise  
human coronavirus 229E (hcov229E) and  
human coronavirus NL63 (hcovNL63), and B-  
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 receptor-binding gene region  
  
, . , a foal ee a  
primary anti-genic epitopes mainly those  
recognised by neutralising antibodies. 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.\*'  
  
FIGURE 1  
observed through both in vivo and in vitro  
experiments. 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.\*\* 2°  
  
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.'° 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  
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.?°  
  
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  
carrier of SARS-CoV2.\*” 2° With the data and  
evidence provided by the CDC, the usual  
incubation period is probably 3 to 7 days,  
sometimes being prolonged up to even 2  
weeks, and the typical symptom occurrence  
6.1 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.°?  
  
Itis 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  
weeks, 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, but COVID-19 was devoid of such  
symptoms. When examined, unilateral or  
bilateral involvement compatible with viral  
pneumonia is observed in the patients, and  
bilateral multiple lobular and sub-segmental  
consolidation areas were observed in patients  
hospitalised 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 3°  
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, amino  
peptidase-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 process 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 burgeoning from internal cell  
membranes.?\* 7°  
  
5 PATHOGENESIS  
  
Coronaviruses are tremendously precise and  
mature in most of the airway epithelial cells as  
observed through both in vivo and in vitro