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

route warrants the introduction of negative fecal viral nucleic acid test results as one of the additional discharge criteria in laboratory-confirmed cases of COVID-19 (326).

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

Phylogenetic analysis of 10 whole-genome sequences of SARS-CoV-2 showed that they are related to two CoVs of bat origin, namely, bat-SL-CoVZC45 and bat-SL-CoVZXC21, which were reported during 2018 in China (17). It was reported that SARS-CoV-2 had been confirmed to use ACE2 as an entry receptor while exhibiting an RBD similar

fever, cough, and sputum (83). Hence, the clinicians must be on the look-out for the possible occurrence of atypical clinical manifestations to avoid the possibility of missed diagnosis. The early transmission ability of SARS-CoV-2 was found to be similar to or slightly higher than that of SARS-CoV, reflecting that it could be controlled despite moderate to high transmissibility (84).

Increasing reports of SARS-CoV-2 in sewage and wastewater warrants the need for further investigation due to the possibility of fecal-oral transmission. SARS-CoV-2 present in environmental compartments such as soil and water will finally end up in the wastewater and sewage sludge of treatment plants (328). Therefore, we have to reevaluate the current wastewater and sewage sludge treatment procedures and introduce advanced techniques that are specific and effective against SARS-CoV-2. Since there is active shedding of SARS-CoV-2 in the stool, the prevalence of infections in a large population can be studied using wastewater-based epidemiology. Recently, reverse transcription-quantitative PCR (RT-qPCR) was used to enumerate the copies of SARS-CoV-2 RNA concentrated from wastewater collected from a wastewater treatment plant (327). The calculated viral RNA copy numbers determine the number of infected individuals. The

trimeric S1 locates itself on top of the trimeric S2 stalk (45). Recently, structural analyses of the S proteins of COVID-19 have revealed 27 amino acid substitutions within a 1,273-amino-acid stretch (16). Six substitutions are located in the RBD (amino acids 357 to 528), while four substitutions are in the RBM at the CTD of the S1 domain (16). Of note, no amino acid change is seen in the RBM, which binds directly to the angiotensin-converting enzyme-2 (ACE2) receptor in SARS-CoV (16, 46). At present, the main emphasis is knowing how many differences would be required to change the host tropism. Sequence comparison revealed 17 nonsynonymous changes between the early sequence of SARS-CoV-2 and the later isolates of SARS-CoV. The changes were found scattered over the genome of the virus, with nine substitutions in ORF1ab, 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

SplitsTree 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

adaptive evolution, close monitoring 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

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 (ORF1a/b) is below 90% identity (3). An overall 80% nucleotide identity was observed between SARS-CoV-2 and the original SARS-CoV, along with 89% identity with ZC45 and ZXC21 SARS-related CoVs of bats (2, 31, 36). In addition, 82% identity has been observed between SARS-CoV-2 and human SARS-CoV Tor2 and human SARS-CoV BJ01 2003 (31). A sequence identity of only 51.8% was observed between MERS-related CoV and the recently emerged SARS-CoV-2 (37). Phylogenetic analysis of the structural genes also revealed that SARS-CoV-2 is closer to bat SARS-related CoV. Therefore, SARS-CoV-2 might have originated from bats, while other amplifier hosts might have played a role in disease transmission to humans (31). 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

encircled with an envelope containing viral 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 1a/1ab (pp1a/1ab) (33). A replication-transcription complex (RTC) is formed in double-membrane vesicles (DMVs) by nonstructural proteins (nsps), encoded by the polyprotein gene (34). Subsequently, the RTC synthesizes a nested set of subgenomic RNAs (sgRNAs) via discontinuous transcription (35).

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 emergency on 31 January 2020; subsequently

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 is vital to control and get rid of the infection. However, maladjusted immune responses may contribute to the immunopathology of the disease, resulting in impairment of pulmonary gas exchange. Understanding the interaction between CoVs and host innate immune systems could enlighten our

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

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).

dogs have low susceptibility, while the chickens, 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).

system (30).

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

snakes, and various other wild animals (20, 30, 79, 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 HKU15) (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

with SARS and MERS (117).

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

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

Coronavirus is the most prominent example of a virus that has crossed the species barrier twice from wild animals to humans during SARS and MERS outbreaks (79, 102). The possibility of crossing the species barrier for the third time has also been suspected in the case of SARS-CoV-2 (COVID-19). Bats are recognized as a possible natural reservoir host of both SARS-CoV and MERS-CoV infection. In contrast, the possible intermediary host is the palm civet for SARS-CoV and the dromedary camel for MERS-CoV infection (102). Bats are considered the ancestral hosts for both SARS and MERS (103). Bats are also considered the reservoir host of human coronaviruses like HCoV-229E and HCoV-NL63 (104). In the case of COVID-19, there are two possibilities for primary transmission: it can be transmitted either through intermediate hosts, similar to that of SARS and MERS, or directly from bats (103). The emergence paradigm put 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

samples obtained from lower respiratory tracts. 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 ORF1b 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-

been controlled by adopting appropriate and strict 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

explored targeting molecular dynamic simulations, 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

might be lower. Further genetic analysis is required between SARS-CoV-2 and different strains of SARS-CoV and SARS-like (SL) CoVs to evaluate the possibility of repurposed vaccines against COVID-19. This strategy will be helpful in the scenario of an outbreak, since much time can be saved, because preliminary evaluation, including *in vitro* studies, already would be completed for such vaccine candidates.

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

helicase activity.

Among the evaluated compounds, 4-(cyclopent-1-en-3-ylamino)-5-[2-(4-iodophenyl)hydrazinyl]-4H-1,2,4-triazole-3-thiol and 4-(cyclopent-1-en-3-ylamino)-5-[2-(4-chlorophenyl)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-

anti-SARS-CoV-2 activity is far higher than the 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

COVID-19 patients showing severe signs are treated symptomatically along with oxygen therapy. In such cases where the patients progress toward respiratory failure and become refractory to oxygen therapy, mechanical ventilation is necessitated. The COVID-19-induced septic shock can be managed by providing adequate hemodynamic support (299). Several classes of drugs are currently being evaluated for their potential therapeutic action against SARS-CoV-2. Therapeutic agents that have anti-SARS-CoV-2 activity can be broadly classified into three categories: drugs that block virus entry into the host cell, drugs that block viral replication as well as its survival within the host cell, and drugs that attenuate the exaggerated host immune response (300). An inflammatory cytokine storm is commonly seen in critically ill COVID-19 patients. Hence, they may benefit from the use of timely anti-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 SARS, 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 (EK1), anti-inflammatory drugs, abidol, and Chinese traditional medicine (Lianhuaqingwen and ShuFengJieDu capsules) could aid in COVID-19 treatment. However, further clinical trials are being carried out concerning their safety and 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

Therapeutics and Drugs

There is no currently licensed specific antiviral treatment for MERS- and SARS-CoV infections, and the main focus in clinical settings remains on lessening clinical signs and providing supportive care (183–186). Effective drugs to manage COVID-19 patients include remdesivir, lopinavir/ritonavir alone or in a blend with interferon beta, convalescent plasma, and monoclonal antibodies (MAbs); however, 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

risk regions. It is derived from a live attenuated 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).

vaccine that can produce cross-reactive antibodies. 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 Ii-Key peptide COVID-19 vaccine are under preclinical 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 Ii-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.

proteins without the presence of S protein would not 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

other emerging viral diseases. Several therapeutic and preventive strategies, including vaccines, immunotherapeutics, and antiviral drugs, have been exploited against the previous CoV outbreaks (SARS-CoV and MERS-CoV) (8, 104, 164–167). These valuable options have already been evaluated for their potency, efficacy, and safety, along with several other types of current research that will fuel our search for ideal therapeutic agents against COVID-19 (7, 9, 19, 21, 36). The primary cause of the unavailability of approved and commercial vaccines, drugs, and therapeutics to counter the earlier SARS-CoV and MERS-CoV seems to owe to 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

therapeutics, and drug regimens to counter emerging 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

rates, disease outbreaks, community spread, clustered transmission events, hot spots, and superspreader potential of SARS-CoV-2/COVID warrant full exploitation of real-time disease mapping by employing geographical information systems (GIS), such as the GIS software Kosmo 3.1, web-based real-time tools and dashboards, apps, and advances in information technology (356–359). Researchers have also developed a few prediction tools/models, such as the prediction model risk of bias assessment tool (PROBAST) and critical appraisal and data extraction for systematic reviews of prediction modeling studies (CHARMS), which could aid in assessing the possibility of getting infection and estimating the prognosis in patients; however, such models may suffer from bias issues and, hence, cannot be considered completely trustworthy, which necessitates the development of new and reliable predictors (360).

VACCINES, THERAPEUTICS, AND DRUGS

Recently emerged viruses, such as Zika, Ebola, and Nipah viruses, and their grave threats to humans have begun a race in exploring the designing and developing of advanced vaccines, prophylactics, therapeutics, and drug regimens to counter emerging

developed for rapid and colorimetric detection of this virus (354). RT-LAMP serves as a simple, rapid, and sensitive diagnostic method that does not require sophisticated equipment or skilled personnel (349). An interactive web-based dashboard for tracking SARS-CoV-2 in a real-time mode has been designed (238). A smartphone-integrated home-based 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,

major problem associated with this diagnostic kit is that it works only when the test subject has an active infection, limiting its use to the earlier stages of infection. Several laboratories around the world are currently developing antibody-based diagnostic tests against SARS-CoV-2 (157).

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

assays offer high accuracy in the diagnosis of SARS-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 1 month, indicating a prolonged stage of virus replication in SARS-CoV-2-infected patients. The IgG levels were found to increase only in the later stages of the disease. These findings indicate that the specific antibody profiles of SARS-CoV-2 and SARS-CoV were similar (325). These findings can be utilized for the development of specific diagnostic tests against COVID-19 and can be used for rapid screening. Even though diagnostic test kits are already available that can detect the genetic sequences of SARS-CoV-2 (95), their availability is a concern, as the number of COVID-19 cases is skyrocketing (155, 157). A major problem associated with this diagnostic kit is

considerable protection in mice against a MERS-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 U.S. biotechnology company Regeneron is attempting to recognize potent and specific MAbs to combat COVID-19. An ideal therapeutic option suggested for SARS-CoV-2 (COVID-19) is the combination therapy comprised of MAbs and the drug remdesivir (COVID-19) (201). The SARS-CoV-specific human MAb CR3022 is found to bind with SARS-CoV-2 RBD, indicating its potential as a therapeutic agent

countries. Large-scale screening programs might 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-19 outbreak is 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 R_0 number and reduce the transmission risk (228). Considering the zoonotic

particularly in bats. Both *in vitro* and *in vivo* studies (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 (α-, β-, γ- and δ-). 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.[13, 14] Two of these genera belong to human coronaviruses (HCoVs): α-coronaviruses, which comprise human coronavirus 229E (hcov229E) and human coronavirus NL63 (hcovNL63), and β-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).[15]

The severe acute respiratory syndrome CoV-2 (SARS-CoV-2) is now named novel COVID-19 (coronavirus disease 2019).[16] 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

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.[19, 20] 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.[21]

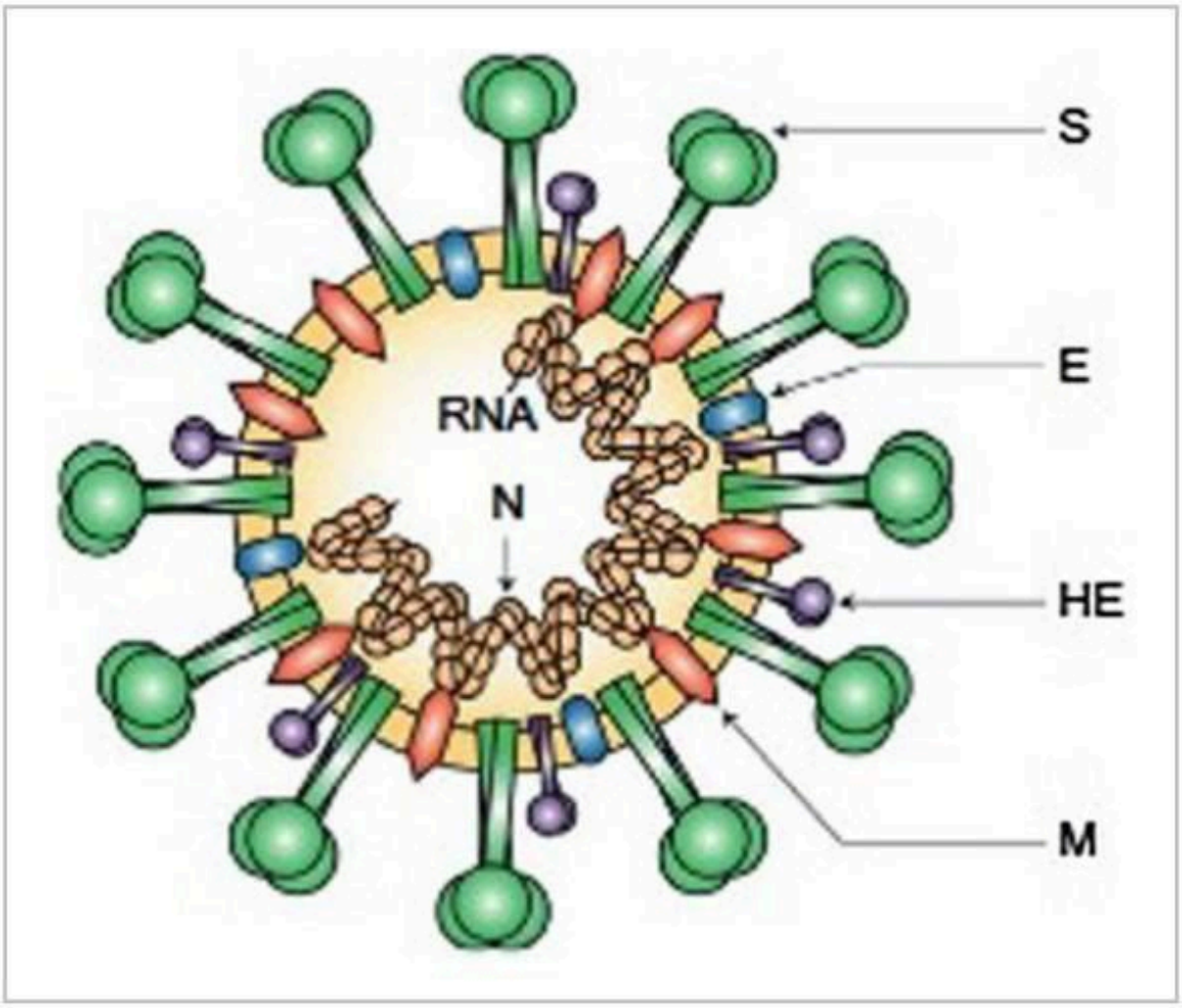

FIGURE 1

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.[24, 25]

5.1 Mode of transmission

In fact it was accepted that the original transmission originated from a seafood market, which had a tradition of selling live animals, where the majority of the patients had either worked or visited, although up to now the understanding of the COVID-19 transmission risk remains incomplete.[16] In addition, while the newer patients had no exposure to the market and still got the virus from the humans present there, there is an increase in the outbreak of

there, there is an increase in the outbreak of this virus through human-to-human transmission, with the fact that it has become widespread around the globe. This confirms the fact similar to the previous epidemics, including SARS and MERS, that this coronavirus exhibited potential human-to-human transmission, as it was recently declared a pandemic by WHO.[26]

Respiratory droplets are the major carrier for coronavirus transmission. Such droplets can either stay in the nose or mouth or enter the lungs via the inhaled air. Currently, it is known that COVID-19's transmission from one person to another also occurs through touching either an infected surface or even an object. With the current scant awareness of the transmission systems however, airborne safety measures with a high-risk procedure have been proposed in many countries. Transmission levels, or the rates from one person to another, reported differ by both location and interaction with involvement in infection control. It is stated that even asymptomatic individuals or those individuals in their incubation period can act as carrier of SARS-CoV2.[27, 28] 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.[31] 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.[32]

It is also recommended that the suspected patients be tested for the other respiratory pathogens by performing the routine laboratory investigation per the local guidelines, mainly to differentiate from other viruses that include influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus, rhinovirus, human

weeks, and the typical symptom occurrence from incubation period to infection takes an average of 12.5 days.[29]

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.[14, 30]

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.[22, 23]

5 PATHOGENESIS

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