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 test 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-CoV-2.

Currently, our knowledge on the animal origin of SARS-CoV-2 remains incomplete to a large part. The reservoir hosts of the virus have not been clearly proven. It is unknown whether SARS-CoV-2 was transmitted to humans through an intermediate host and which animals may act as its intermediate host. Detection of RaTG13, RmYN02 and pangolin coronaviruses implies that diverse coronaviruses similar to SARS-CoV-2 are circulating in wildlife. In addition, as previous stud- ies showed recombination as the potential origin of some sarbecoviruses such as SARS-CoV, it cannot be excluded that viral RNA recombination among different related coronaviruses was involved in the evolution of SARS-CoV-2. Extensive surveillance of SARS-CoV-2- related viruses in China, Southeast Asia and other regions targeting bats, wild and captured pangolins and other wildlife species will help us to better understand the zoonotic origin of SARS-CoV-2.

Besides wildlife, researchers investigated the sus- ceptibility of domesticated and laboratory animals to SARS-CoV-2 infection. The study demonstrated exper- imentally that SARS-CoV-2 replicates efficiently in cats and in the upper respiratory tract of ferrets, whereas dogs, pigs, chickens and ducks were not susceptible to SARS-CoV-2 (REF.[43]). The susceptibility of minks was documented by a report from the Netherlands on an outbreak of SARS-CoV-2 infection in farmed minks. Although the symptoms in most infected minks were mild, some developed severe respiratory distress and died of interstitial pneumonia[44].

Another study, the average reproductive number of COVID-19 was found to be 3.28, which is significantly higher than the initial WHO estimate of 1.4 to 2.5 (77). It is too early to obtain the exact Ro value, since there is a possibility of bias due to insufficient data. The higher Ro 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.

Infections can be diagnosed clinically or through routine lab tests. Therefore, travel history becomes important. However, as the epidemic spreads, the travel history will become irrelevant.

Treatment [21,23]

Treatment is essentially supportive and symptomatic.

The first step is to ensure adequate isolation (discussed later) to prevent transmission to other contacts, patients, and healthcare workers. Mild illness should be managed at home with counseling about danger signs. The usual principles are maintaining hydration and nutrition and controlling fever and cough. Routine use of antibiotics and antivirals such as oseltamivir should be avoided in confirmed cases. In hypoxic patients, provision of oxygen through nasal prongs, face mask, high flow nasal cannula should be provided.

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.

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).

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).

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-virus.

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 ORF1b (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.

The interferon response is one of the major innate immunity defences against virus invasion. Interferons induce the expression of diverse interferon-stimulated genes, which can interfere with every step of virus replication. Previous studies identified type I interferons as a promising therapeutic candidate for SARS[149]. In vitro data showed SARS-CoV-2 is even more sensitive to type I interferons than SARS-CoV, suggesting the potential effectiveness of type I interferons in the early treatment of COVID-19 (REF.[150]). In China, vapor inhalation of interferon-a is included in the COVID-19 treatment guideline[151]. Clinical trials are ongoing across the world to evaluate the efficacy of different therapies involving interferons, either alone or in combination with other agents[152].

Immunoglobulin therapy. Convalescent plasma treatment is another potential adjunctive therapy for COVID-19. Preliminary findings have suggested improved clinical status after the treatment [153,154]. The FDA has provided guidance for the use of COVID-19 convalescent plasma under an emergency investigational new drug application. However, this treatment may have adverse effects by causing antibody-mediated enhancement of infection, transfusion-associated acute lung injury and allergic transfusion reactions.

Monoclonal antibody therapy is an effective immuno- therapy for the treatment of some viral infections in select patients. Recent studies reported specific monoclonal antibodies neutralizing SARS-CoV-2 infection.

Category A agents (cholera, plague). Patients should be placed in separate rooms or cohorted together. Negative pressure rooms are not generally needed. The rooms and surfaces and equipment should undergo regular decontamination preferably with sodium hypochlorite. Healthcare workers should be provided with fit tested N95 respirators and protective suits and goggles. Airborne transmission precautions should be taken during aerosol generating procedures such as intubation, suction and tracheostomies. All contacts including healthcare workers should be monitored for development of symptoms of COVID-19. Patients can be discharged from isolation once they are afebrile for atleast 3 d and have two consecutive negative molecular tests at 1 d sampling interval. This recommendation is different from pandemic flu where patients were.

However cases may be asymptomatic or even without fever. A confirmed case is a suspect case with a positive molecular test.

Specific diagnosis is by specific molecular tests on respiratory samples (throat swab/nasopharyngeal swab/sputum/ endotracheal aspirates and bronchoalveolar lavage). Virus may also be detected in the stool and in severe cases, the blood. It must be remembered that the multiplex PCR panels currently available do not include the COVID-19. Commercial tests are also not available at present. In a suspect case in India, the appropriate sample has to be sent to designated reference labs in India or the National Institute of Virology in Pune.

To assess the genetic variation of different SARS-CoV-2 strains, the 2019 Novel Coronavirus Resource of China National Center for Bioinformation aligned 77,801 genome sequences of SARS-CoV-2 detected globally and identified a total of 15,018 mutations, including 14,824 single-nucleotide polymorphisms (BIGD)[31]. In the S protein, four amino acid alterations, V483A, L4551, F456V and G476S, are located near the binding interface in the RBD, but their effects on binding to

the host receptor are unknown. The alteration D614G in the S1 subunit was found far more frequently than other S variant sites, and it is the marker of a major subclade of SARS-CoV-2 (clade G). Since March 2020, SARS-CoV-2 variants with G614 in the S protein have replaced the original D614 variants and become the dominant form circulating globally. Compared with the D614 variant, higher viral loads were found in patients infected with the G614 variant, but clinical data suggested no significant link between the D614G alteration and disease severity[32]. Pseudotyped viruses carrying the S protein with G614 generated higher infectious titres than viruses carrying the S protein with D614, suggesting the alteration may have increased the infectivity of SARS-CoV-2 (REF.[32]). However, the results of in vitro experiments based on pseudovirus models may not exactly reflect natural infection. This preliminary finding should be validated by more studies using wild-type SARS-CoV-2 variants to infect different target cells and animal models. Whether this amino acid change enhanced virus transmissibility is also to be determined. Another marker mutation for SARS-CoV-2 evolution is the single-nucleotide.

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).

A suspected case of COVID-19 infection is said to be confirmed if the respiratory tract aspirate or

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

Animal Models and Cell Cultures

For evaluating the potential of vaccines and therapeutics against CoVs, including SARS-CoV,MERS-CoVs, and the presently emerging SARS-CoV-2, suitable animal models that can mimic the clinical disease are needed (211, 212). Various animal models were assessed for SARS- and MERS-CoVs, such as mice, guinea pigs, golden Syrian hamsters, ferrets, rabbits, nonhuman primates like rhesus macaques and marmosets, and cats (185,213-218). The specificity of the virus to hACE2 (receptor of SARS-CoV) was found to be a significant barrier in developing animal models. Consequently, a SARS-CoV transgenic mouse model has been developed by inserting the hACE2 gene into the mouse genome (219). The inability of MERS-CoV to replicate in the

respiratory tracts of animals (mice, hamsters, and ferrets) is another limiting factor. However, with genetic engineering, a 288-330$^{+/+}$ MERS-CoV genetically modified mouse model was developed and now is in use for the assessment of novel drugs and vaccines against MERS-CoV (220). In the past, small animals (mice or hamsters) have been targeted for being closer to a humanized structure, such as mouse DPP4 altered with human DPP4 (hDPP4).

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).

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).

Only a matter of time before another zoonotic 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).

Practice Points from an Indian Perspective

At the time of writing this article, the risk of coronavirus in India is extremely low. But that may change in the next few weeks. Hence the following is recommended:

• Healthcare providers should take travel history of all patients with respiratory symptoms, and any international travel in the past 2 wks as well as contact with sick people who have travelled internationally.

• They should set up a system of triage of patients with respiratory illness in the outpatient department and give them a simple surgical mask to wear. They should use surgical masks themselves while examining such.

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).

Pieces of evidence are available that link NSAID 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).

Significance of frequent and good hand hygiene and 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.

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.

In vitro and in vivo[155-158]. Compared with convalescent plasma, which has limited availability and cannot be amplified, monoclonal antibodies can be developed in larger quantities to meet clinical requirements. Hence, they provide the possibility for the treatment and prevention of COVID-19. The neutralizing epitopes of these monoclonal antibodies also offer important information for vaccine design. However, the high cost and limited capacity of manufacturing, as well as the problem of bioavailability, may restrict the wide application of monoclonal antibody therapy.

Vaccines

Vaccination is the most effective method for a long-term strategy for prevention and control of COVID-19 in the future. Many different vaccine platforms against SARS-CoV-2 are in development, the strategies of which include recombinant vectors, DNA, mRNA in lipid nano-particles, inactivated viruses, live attenuated viruses and protein subunits[159-161]. As of 2 October 2020, ~174 vaccine candidates for COVID-19 had been reported and 51 were in human clinical trials (COVID-19 vaccine and therapeutics tracker). Many of these vaccine candidates are in phase II testing, and some have already advanced to phase III trials. A randomized double-blinded phase II trial of an adenovirus type vectored vaccine expressing the SARS-CoV-2 S protein, developed by CanSino Biologicals and the Academy of Military Medical Sciences of China, was conducted in 603 adult volunteers in Wuhan. The vaccine has proved to be safe and induced considerable humoral and cellular immune response in most recipients after a single immunization[162].

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).

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).

Prevent further spread of disease at mass gatherings, functions remain canceled in the affected cities, and persons are asked to work from home (232). Hence, it is a relief that the current outbreak of COVID-19 infection can be brought under control with the adoption of strategic preventive and control measures along with the early isolation of subsequent cases in the coming days. Studies also report that since air traffic between China and African countries increased many times over in the decade after the SARS outbreak, African countries need to be vigilant to prevent the spread of novel coronavirus in Africa (225). Due to fear of virus spread, Wuhan City was completely shut down (233). The immediate control of the ongoing COVID-19 outbreaks appears a mammoth task, especially for developing countries, due to their inability to allocate quarantine stations that could screen infected individuals' movements (234). Such underdeveloped countries should divert their resources and energy to enforcing the primary level of preventive measures, like controlling the entry of individuals from China or countries where the disease has flared up, isolating the infected individuals, and quarantining individuals with suspected infection. Most of the sub-Saharan African countries have a fragile health system.

Repurposed broad-spectrum antiviral drugs having proven uses against other viral pathogens can be employed for SARS-CoV-2-infected patients. These possess benefits of easy accessibility and recognized pharmacokinetic and pharmacodynamics activities, stability, doses, and side effects (9). Repurposed drugs have been studied for treating CoV infections, like lopinavir/ritonavir, and interferon-1ß 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 reauire sedatives, analgesics.

Between 4 and -70°C. Urine samples must also be collected using a sterile container and stored in the laboratory at a temperature that ranges between 4 and -70°C.[32]

7 PREGNANCY

Currently, there is a paucity of knowledge and data related to the consequences of COVID-19 during pregnancy.[40-42] However, pregnant women seem to have a high risk of developing severe infection and complications during the recent 2019-nCoV outbreak [41-43] This speculation was based on previous available scientific reports on coronaviruses during pregnancy (SARS-CoV and MERS-CoV) as well as the limited number of COVID-19 cases.[41-43]

Analysing the clinical features and outcomes of 10 newborns (including two sets of twins) in China, whose mothers are confirmed cases of COVID-19, revealed that perinatal infection with 2019-nCoV may lead to adverse outcomes for the neonates, for example, premature labour, respiratory distress, thrombocytopenia with abnormal liver function and even death.[44] It is still unclear whether

or not the COVID-19 infection can be transmitted during pregnancy to the foetus through the transplacental route.[42]

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.

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).

Prevention [21, 30]

Since at this time there are no approved treatments for this infection, prevention is crucial. Several properties of this virus make prevention difficult namely, non-specific features of the disease, the infectivity even before onset of symptoms in the incubation period, transmission from asymptomatic people, long incubation period, tropism for mucosal surfaces such as the conjunctiva, prolonged duration of the illness and transmission even after clinical recovery.

Isolation of confirmed or suspected cases with mild illness at home is recommended. The ventilation at home should be good with sunlight to allow for destruction of virus. Patients should be asked to wear a simple surgical mask and practice cough hygiene.

Exponentially in other countries including South Korea, Italy and Iran. Of those infected, 20% are in critical condition, 25% have recovered, and 3310 (3013 in China and 297 in other countries) have died [2]. India, which had reported only 3 cases till 2/3/2020, has also seen a sudden spurt in cases. By 5/3/2020, 29 cases had been reported; mostly in Delhi, Jaipur and Agra in Italian tourists and their contacts. One case was reported in an Indian who traveled back from Vienna and exposed a large number of school children in a birthday party at a city hotel. Many of the contacts of these cases have been quarantined.

These numbers are possibly an underestimate of the infected and dead due to limitations of surveillance and testing.

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.

Using sequences of five conserved replicative domains in pplab (3C-like protease (3CLpro), nidovirus RNA-dependent RNA polymerase (RdRp)-associated nucleotidyltransferase (NIRAN), RdRp, zinc-binding domain (ZBD) and HEL1), the *Coronaviridae* Study Group of the International Committee on Taxonomy of Viruses estimated the pairwise patristic distances between SARS-CoV-2 and known coronaviruses, and assigned SARS-CoV-2 to the existing species SARSr-CoV[17]. Although phyloge- netically related, SARS-CoV-2 is distinct from all other coronaviruses from bats and pangolins in this species.

The SARS-CoV-2 S protein has a full size of 1,273 amino acids, longer than that of SARS-CoV (1,255 amino acids) and known bat SARSr-CoVs (1,245-1,269 amino acids). It is distinct from the S proteins of most members in the subgenus Sarbecovirus, sharing amino acid sequence similarities of 76.7- 77.0% with SARS-CoVs from civets and humans.

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).

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,).

The exploration of fully human antibodies (human single-chain antibodies; HuscFvs) or humanized nanobodies (single-domain antibodies; sdAb, VH/VHH) could aid in blocking virus replication, as these agents can traverse the virus-infected cell membranes (transbodies) and can interfere with the biological characteristics of the replicating virus proteins. Such examples include transbodies to the influenza virus, hepatitis C virus, Ebola virus, and dengue virus (206). Producing similar transbodies against intracellular proteins of coronaviruses, such as papain-like proteases (PLpro), cysteine-like protease (3CLpro), or other nsps, which are essential for replication and transcription of the virus, might formulate a practical move forward for a safer and potent passive immunization approach for virus-exposed persons and rendering therapy to infected patients.

In a case study on five grimly sick patients having symptoms of severe pneumonia due to COVID-19, convalescent plasma administration was found to be helpful in patients recovering successfully. The convalescent plasma containing a SARS-CoV-2-specific ELISA (serum) antibody titer higher than 1:1,000 and neutralizing antibody titer more significant than 40 was collected from the recovered patients and used for plasma transfusion.

Had >95% homology with the bat coronavirus and > 70% similarity with the SARS- COV. Environmental samples from the Huanan sea food market also tested positive, signifying that the virus originated from there [7]. The number of cases started increasing exponentially, some of which did not have exposure to the live animal market, suggestive of the fact that human-to-human transmission was occurring [8]. The first fatal case was reported on 11th Jan 2020. The massive migration of Chinese during the Chinese New Year fuelled the epidemic. Cases in other provinces of China, other countries (Thailand, Japan and South Korea in quick succession) were reported in people who were returning from Wuhan. Transmission to healthcare workers caring for patients was described on 20th Jan, 2020. By 23rd January, the 11 million population of Wuhan was placed under lock down.

Comorbidities, it may progress to pneumonia, acute respiratory distress syndrome (ARDS) and multi organ dysfunction. Many people are asymptomatic. The case fatality rate is estimated to range from 2 to 3%. Diagnosis is by demonstration of the virus in respiratory secretions by special molecular tests. Common laboratory findings include normal/ low white cell counts with elevated C- reactive protein (CRP). The computerized tomographic chest scan is usually abnormal even in those with no symptoms or mild disease. Treatment is essentially supportive; role of antiviral agents is yet to be established. Prevention entails home isolation of suspected cases and those with mild illnesses and strict infection control measures at hospitals that include contact and droplet precautions. The virus spreads faster than its two ancestors.

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).

All of these therapeutic approaches have revealed both in *vitro* and in *vivo* anti-CoV potential. Although *in vitro* research carried out with these therapeutics showed efficacy, most need appropriate support from randomized animal or human trials. Therefore, they might be of limited

applicability and require trials against SARS-CoV-2 to gain practical usefulness. The binding of SARS-CoV-2 with ACE2 leads to the exacerbation of pneumonia as a consequence of the imbalance in the renin- angiotensin system (RAS). The virus-induced pulmonary inflammatory responses may be reduced by the administration of ACE inhibitors (ACEI) and angiotensin type-1 receptor (AT1R) (207).

Several investigations have suggested the use of small-molecule inhibitors for the potential control of SARS-CoV infections. Drugs of the FDA-approved compound library were screened to identify four small-molecule inhibitors of MERS-CoV (chlorpromazine, chloroquine, loperamide, and lopinavir) that inhibited viral replication. These compounds also hinder SARS-CoV and human CoVs (208). Therapeutic strategies involving the use of specific antibodies or compounds that neutralize cytokines and their receptors will help to restrain the host inflammatory responses.

Shedding the virus, which may remain viable for at least 3 days and is considered a great risk for uninfected patients and health care workers (289). Recently, it was found that the anal swabs gave more positive results than oral swabs in the later stages of infection (153). Hence, clinicians have to be cautious while discharging any COVID-19-infected patient based on negative oral swab test results due to the possibility of fecal-oral transmission. Even though the viral loads in stool samples were found to be less than those of respiratory samples, strict precautionary measures have to be followed while handling stool samples of COVID-19 suspected or infected patients (151). Children infected with SARS-CoV-2 experience only 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.

Swine acute diarrhea syndrome coronavirus
(SADS-CoV) was first identified in suckling piglets having severe enteritis and belongs to the genus *Alphacoronavirus* (106). The outbreak was associated with considerable scale mortality of piglets (24,693 deaths) across four farms in China (134). The virus isolated from the piglets was almost identical to and 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).

Individuals with asymptomatic and atypical clinical manifestations were also identified recently, further adding to the complexity of disease transmission dynamics. Atypical clinical manifestations may only express symptoms such as fatigue instead of respiratory signs such as fever, cough, and sputum. In such cases, the clinician must be vigilant for the possible occurrence of asymptomatic and atypical clinical manifestations to avoid the possibility of missed diagnoses.

The present outbreak caused by SARS-CoV-2 was, indeed, expected. Similar to previous outbreaks, the current pandemic also will be contained shortly. However, the real question is, how are we planning to counter the next zoonotic CoV epidemic that is likely to occur within the next 5 to 10 years or perhaps sooner? Our knowledge of most of the bat CoVs is scarce, as these viruses have not been isolated and studied, and extensive studies on such viruses are typically only conducted when they are associated with specific disease outbreaks. The next step following the control of the COVID-19 outbreak in China should be focused on screening, identification, isolation, and

characterization of CoVs present in wildlife species of China, particularly in bats. Both in *vitro* and in *vivo* studies (using suitable animal models) should be conducted.

The intranasal administration of the recombinant adenovirus-based vaccine in BALB/c mice was found to induce long- lasting neutralizing immunity against MERS spike pseudotyped virus, characterized by the induction of systemic IgG, secretory IgA, and lung-resident memory T-cell responses (177). Immunoinformatics methods have been employed for the genome-wide screening of potential vaccine targets among the different immunogens of MERS-CoV (178). The N protein and the potential B-cell epitopes of MERS- CoV E protein have been suggested as immunoprotective targets inducing both T-cell and neutralizing antibody responses (178, 179).

The collaborative effort of the researchers of Rocky Mountain Laboratories and Oxford University is designing a chimpanzee adenovirus-vectored vaccine to counter COVID-19 (180). The Coalition for Epidemic Preparedness Innovations (CEPI) has initiated three programs to design SARS-CoV-2 vaccines (181). CEPI has a collaborative project with Inovio for designing a MERS-CoV DNA vaccine that could potentiate effective immunity. CEPI and the University of Queensland are designing a molecular clamp vaccine platform for MERS-CoV and other pathogens, which could assist in the easier identification of antigens by the immune system (181).

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.

Identified angiotensin receptor 2 (ACE2) as the receptor through which the virus enters the respiratory mucosa [11].

The basic case reproduction rate (BCR) is estimated to range from 2 to 6.47 in various modelling studies [11]. In comparison, the BCR of SARS was 2 and 1.3 for pandemic flu H1N1 2009 [2].

Clinical Features [8, 15-18]

The clinical features of COVID-19 are varied, ranging from asymptomatic state to acute respiratory distress syndrome and multi organ dysfunction. The common clinical features include fever (not in all), cough, sore throat, headache, fatigue, headache, myalgia and breathlessness. Conjunctivitis has also been described. Thus, they are indistinguishable from other respiratory infections In a subset

Glass opacities and sub segmental consolidation. It is also abnormal in asymptomatic patients/ patients with no clinical evidence of lower respiratory tract involvement. In fact, abnormal CT scans have been used to diagnose COVID-19 in suspect cases with negative molecular diagnosis; many of these patients had positive molecular tests on repeat testing [22].

Differential Diagnosis [21]

The differential diagnosis includes all types of respiratory viral infections [influenza, parainfluenza, respiratory syncytial virus (RSV), adenovirus, human metapneumovirus, non COVID- 19 coronavirus], atypical organisms (mycoplasma, chlamydia) and bacterial infections. It is not possible to differentiate COVID-19 from these infections clinically or through routine.

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).

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]

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).

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

16 CONCLUSION

The corona virus (COVID-19) spreads at an alarming rate all over the world. The outbreak of the virus has confronted the world's economic, medical and public health infrastructure. Elderly and immunocompromised patients also are susceptible to the virus's mortal impacts. Currently, there is no documented cure for the virus and no vaccine has been created, although some treatment protocols have been promising. Therefore, the virus can be controlled with the appropriate prevention strategies. Also, attempts have to be made to formulate systematic strategies to prevent such future zoonotic outbreaks.

SARS- or MERS-CoV outbreak (120). However, there has been concern regarding the impact of SARS-CoV-2/COVID-19 on pregnancy. Researchers have mentioned the probability of in utero transmission of novel SARS-CoV-2 from COVID- 19-infected mothers to their neonates in China based upon the rise in IgM and IgG antibody levels and cytokine values in the blood obtained from newborn infants immediately 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.

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.

In addition, transmission of the virus through the ocular surface and prolonged presence of SARS-CoV-2 viral RNA in faecal samples were also documented[101, 102]. Coronaviruses can persist on inanimate surfaces for days, which could also be the case for SARS-CoV-2 and could pose a prolonged risk of infection[103]. These findings explain the rapid geographic spread of COVID-19, and public health interventions to reduce transmission will provide benefit to mitigate the epidemic, as has proved successful in China and several other countries, such as South Korea[89,104,105].

Diagnosis

Early diagnosis is crucial for controlling the spread of COVID-19. Molecular detection of SARS-CoV-2 nucleic acid is the gold standard. Many viral nucleic acid detection kits targeting ORF1b (including RdRp), N, E or S genes are commercially available[11,106-109]. The detection time ranges from several minutes to hours depending on the technology [106, 107,109-111]. The molecular detection can be affected by many factors. Although SARS-CoV-2 has been detected from a variety of respiratory sources, including throat swabs, posterior oropharyngeal saliva, nasopharyngeal swabs, sputum and bronchial fluid, the viral load is higher in lower respiratory tract samples[11,96,112-115]. In addition, viral nucleic acid was also found in samples from the intestinal tract or blood even when respiratory samples were negative [116]. Lastly, viral load may already drop from its peak level on disease onset[96,97]. Accordingly, false negatives can be common when oral swabs and used, and so multiple detection methods should be adopted to confirm a COVID-19 diagnosis[117,118]. Other detection methods were there- fore used to overcome this problem. Chest CT was used to quickly identify a patient when the capacity of molecular detection was overloaded in Wuhan.

Recently, 95 full-length genomic sequences of 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 1a, 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 (ORF1a) positions, respectively. Owing to such selective mutations, a few specific regions of SARS-CoV-2 should not be considered for designing primers and probes. The SARS-CoV-2 reference sequence could pave the way to study molecular biology and pathobiology, along with developing diagnostics and appropriate prevention and control strategies for countering SARS-CoV-2 (260). Nucleic acids of SARS-CoV-2 can be detected from samples (64) such as bronchoalveolar lavage fluid, sputum, nasal swabs, fiber bronchoscope brush biopsy specimen, pharyngeal swabs, feces, blood, and urine, with different levels of diagnostic performance (Table 2) (80, 245, 246).

Visible signs of infection, making it challenging to identify animals actively excreting MERS-CoV that has the potential to infect humans. However, they may shed MERS-CoV through milk, urine, feces, and nasal and eye discharge and can also be found in the raw organs (108). In a study conducted to evaluate the susceptibility of animal species to MERS-CoV infection, llamas and pigs were found to be susceptible, indicating the possibility of MERS- CoV circulation in animal species other than 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.

CEPI has also funded Moderna to develop a vaccine for COVID-19 in partnership with the Vaccine Research Center (VRC) of the National Institute of 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.

Such an S1-S2 cleavage site is not observed in all related viruses belonging to the subgenus Sarbecovirus, except for a similar three amino acid insertion (PAA) in RmYN02, a bat-derived coronavirus newly reported from Rhinolophus malayanus in China[28] (FIG. 3A). Although the insertion in RmYN02 does not functionally represent a polybasic cleavage site, it provides support for the notion that this characteristic, initially considered unique to SARS-CoV-2, has been acquired naturally[28]. A structural study suggested that the furin-cleavage site can reduce the stability of SARS-CoV-2 S protein and facilitate the conformational adaption that is required for the binding of the RBD to its receptor[29]. Whether the higher trans- missibility of SARS-CoV-2 compared with SARS-CoV is a gain of function associated with acquisition of the furin-like cleavage site is yet to be demonstrated[26].

An additional distinction is the accessory gene *orf*8 of SARS-CoV-2, which encodes a novel protein showing only 40% amino acid identity to ORF8 of SARS-CoV. Unlike in SARS-CoV, this new ORF8 protein does not contain a motif that triggers intracellular stress pathways[25]. Notably, a SARS-CoV-2 variant with a 382-nucleotide deletion covering the whole of ORF8 has been discovered in a number of patients in Singapore, which resembles the 29- or 415-nucleotide deletions in the ORF8 region observed in human SARS-CoV variants from the late phase of the 2002-2003 outbreak[30]. Such ORF8 deletion may be indicative of human adaptation after cross-species transmission from an animal host.

The virus can remain viable on surfaces for days in favourable atmospheric conditions but are destroyed in less than a minute by common disinfectants like sodium hypochlorite, hydrogen peroxide etc. [13]. Infection is acquired either by inhalation of these droplets or touching surfaces contaminated by them and then touching the nose, mouth and eyes. The virus is also present in the stool and contamination of the water supply and subsequent transmission via aerosolization/feco oral route is also hypothesized [6]. As per current information, transplacental transmission from pregnant women to their fetus has not been described [14]. However, neonatal disease due to post natal transmission is described [14]. The incubation period varies from 2 to 14 d [median 5 d).

Exponentially in other countries including South Korea, Italy and Iran. Of those infected, 20% are in critical condition, 25% have recovered, and 3310 (3013 in China and 297 in other countries) have died [2]. India, which had reported only 3 cases till 2/3/2020, has also seen a sudden spurt in cases. By 5/3/2020, 29 cases had been reported; mostly in Delhi, Jaipur and Agra in Italian tourists and their contacts. One case was reported in an Indian who traveled back from Vienna and exposed a large number of school children in a birthday party at a city hotel. Many of the contacts of these cases have been quarantined.

These numbers are possibly an underestimate of the infected and dead due to limitations of surveillance and testing.

Abstract

There is a new public health crises threatening the world with the emergence and spread of 2019 novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus originated in bats and was transmitted to humans through yet unknown intermediary animals in Wuhan, Hubei province, China in December 2019. There have been around 96,000 reported cases of coronavirus disease 2019 (COVID-2019) and 3300 reported deaths to date (05/03/2020). The disease is transmitted by inhalation or contact with infected droplets and the

incubation period ranges from 2 to 14 d. The symptoms are usually fever, cough, sore throat, breathlessness, fatigue, malaise among others.

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).

6.5 Specimen collection and storage

A Nasopharyngeal and oropharyngeal swab should be collected using Dacron or polyester flocked swabs. It should be transported to the laboratory at a temperature of 4°C and stored in the laboratory between 4 and -70°C on the basis of the number of days and, in order to increase the viral load, both nasopharyngeal and oropharyngeal swabs should be placed in the same tube. Bronchoalveolar lavage and nasopharyngeal aspirate should be collected in a sterile container and transported similarly to the laboratory by maintain a temperature of 4°C.

Sputum samples, especially from the lower respiratory tract, should be collected with the help of a sterile container and stored, whereas tissue from a biopsy or autopsy should be collected using a sterile container along with saline. However, both should be stored in the laboratory at a temperature that ranges between 4 and -70°C. Whole blood for detecting the antigen, particularly in the first week of illness, should be collected in a collecting tube and stored in the laboratory between 4 and -70°C. Urine samples must also be collected using a sterile container and stored.

However, evidence of cat- 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.

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.

Prongs, face mask, high flow nasal cannula (HFNC) or non-invasive ventilation is indicated. Mechanical ventilation and even extra corporeal membrane oxygen support may be needed. Renal replacement therapy may be needed in some. Antibiotics and antifungals are required if co-infections are suspected or proven. The role of corticosteroids is unproven; while current international consensus and WHO advocate against their use, Chinese guidelines do recommend short term therapy with low-to- moderate dose corticosteroids in COVID-19 ARDS [24, 25]. Detailed guidelines for critical care management for COVID-19 have been published by the WHO [26]. There is, as of now, no approved treatment for COVID-19. Antiviral drugs such as ribavirin, lopinavir ritonavir have been used based on the experience with SARS and MERS.

Article gives a bird's eye view about this new virus. Since knowledge about this virus is rapidly evolving, readers are urged to update themselves regularly.

History

Coronaviruses are enveloped positive sense RNA viruses ranging from 60 nm to 140 nm in diameter with spike like projections on its surface giving it a crown like appearance under the electron microscope; hence the name coronavirus [3]. Four corona viruses namely HKU1, NL63, 229E and OC43 have been in circulation in humans, and generally cause mild respiratory disease.

There have been two events in the past two decades wherein crossover of animal betacorona viruses to humans has resulted in severe disease.

Prevailing chronic medical conditions such as lung disease, heart failure, cancer, cerebrovascular disease, renal disease, diabetes, liver disease and immunocompromising conditions and pregnancy are risk factors for developing severe illness. Management includes implementation of prevention and control measures and supportive therapy to manage the complications, together with advanced organ support.[57]

Corticosteroids must be avoided unless specified for chronic obstructive pulmonary disease exacerbation or septic shock, as it is likely to prolong viral replication as detected in MERS-CoV patients.[58]

12 EARLY SUPPORTIVE THERAPY AND MONITORING

Management of patients with suspected or documented COVID-19 consists of ensuring appropriate infection control and supportive care. WHO and the CDC posted clinical guidance for COVID-19.[59]

Immediate therapy of add-on oxygen must be started for patients with severe acute respiratory infection (SARI).

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]

For example, a cohort study in London revealed 44% of the frontline health-care workers from a hospital were infected with SARS-CoV-2 (REF.[94]).

The high transmissibility of SARS-CoV-2 may be attributed to the unique virological features of SARS-CoV-2. Transmission of SARS-CoV occurred mainly after illness onset and peaked following disease severity[95]. However, the SARS-CoV-2 viral load in upper respiratory tract samples was already highest during the first week of symptoms, and thus the risk of pharyngeal virus shedding was very high at the beginning of infection[96,97]. It was postulated that undocumented infections might account for 79% of documented cases owing to the high transmissibility of the virus during mild disease or the asymptomatic period[89]. A patient with COVID-19 spreads viruses in liquid droplets during speech. However, smaller and much more numerous particles known as aerosol particles can also be visualized, which could linger in the air for a long time and then penetrate deep into the lungs when inhaled by someone else[98-100]. Airborne trans- mission was also observed in the ferret experiments mentioned above.

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 Ro number and reduce the transmission risk (228).

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): a-coronaviruses, which comprise human coronavirus 229E (hcov229E) and human coronavirus NL63 (hcovNL63), and ß- 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 betacoronavirus that belongs to the same subtypes as SARS virus, but still exists in a variant group.

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.

Among the first 27 documented hospitalized patients, most cases were epidemiologically linked to Huanan Seafood Wholesale Market a wet market located in downtown Wuhan, which sells not only seafood but also live animals, including poultry and wildlife[4,8]. According to a retrospective study, the onset of the first known case dates back to 8 December 2019 (REF.[9]). On 31 December, Wuhan Municipal Health Commission notified the public of a pneumonia out break of unidentified cause and informed the World Health Organization (WHO)[9] (FIG. 1).

By metagenomic RNA sequencing and virus isolation from bronchoalveolar lavage fluid samples from patients with severe pneumonia, independent teams of Chinese scientists identified that the causative agent of this emerging disease is a betacoronavirus that had never been seen before[6,10,11]. On 9 January 2020, the result of this etiological identification was publicly announced (FIG. 1). The first genome sequence of the novel coronavirus was published on the Virological website on 10 January, and more nearly complete genome sequences determined by different research institutes were then released via the GISAID database on 12 January". Later, more patients with no history of exposure to Huanan Seafood Wholesale Market were identified. Several familial clusters of infection were reported. and nosocomial infection also occurred in health-care facilities. All these cases provided clear evidence for human-to-human transmission of the new virus 4,12-14 As the outbreak coincided with the approach of the lunar New Year, travel between cities before the festival facilitated virus transmission in China. This novel coronavirus pneumonia soon spread to other cities in Hubei province and to other parts of China.

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.

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.

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.

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.

The pathogenesis of SARS-CoV-2 infection in humans manifests itself as mild symptoms to severe respiratory failure. On binding to epithelial cells in the respiratory tract, SARS-CoV-2 starts replicating and migrating down to the airways and enters alveolar epithelial cells in the lungs. The rapid replication of SARS-CoV-2 in the lungs may trigger a strong immune response. Cytokine storm syndrome causes acute respiratory distress syndrome and respiratory failure, which is considered the main cause of death in patients with COVID-19 (REFS[60,61]). Patients of older age (>60 years) and with serious pre-existing diseases have a greater risk of developing acute respiratory distress syndrome and death[62-64] (FIG. 4). Multiple organ failure has also been reported in some COVID-19 cases[9,13,65].

Histopathological changes in patients with COVID-19 occur mainly in the lungs. Histopathology analyses showed bilateral diffused alveolar damage, hyaline membrane formation, desquamation of pneumocytes and fibrin deposits in lungs of patients with severe COVID-19. Exudative inflammation was also shown in some cases. Immunohistochemistry assays detected SARS-CoV-2 antigen in the upper airway, bronchiolar epithelium and submucosal gland epithelium, as well as in type I and type II pneumocytes, alveolar macrophages and hyaline membranes in the lungs [13,60,66,67].

Animal models used for studying SARS-CoV-2 infection pathogenesis include non-human primates (rhesus macaques, cynomolgus monkeys, marmosets and African green monkeys), mice (wild-type mice (with mouse-adapted virus) and human ACE2-transgenic or human ACE2-knock-in mice), ferrets and golden hamsters[43,48,68-74]. In non-human primate animal models, most species display clinical features similar to those of patients with COVID-19, including virus shedding, virus replication and host responses to SARS-CoV-2 infection[69,72,73].

Another serological study detected SARS-CoV-2 neutralizing antibodies in cat serum samples collected in Wuhan after the COVID-19 outbreak, providing evidence for SARS-CoV-2 infection in cat populations in Wuhan, although the potential of SARS-CoV-2 transmission from cats to humans is currently uncertain[46].

Receptor use and pathogenesis

SARS-CoV-2 uses the same receptor as SARS-CoV, angiotensin-converting enzyme 2 (ACE2) [11,47]. Besides human ACE2 (hACE2), SARS-CoV-2 also recognizes ACE2 from pig, ferret, rhesus monkey, civet, cat, pangolin, rabbit and dog[11,43,48,49]. The broad receptor usage of SARS-CoV-2 implies that it may have a wide host range, and the varied efficiency of ACE2 usage in different animals may indicate their different susceptibilities to SARS-CoV-2 infection. The S1 subunit of a corona- virus is further divided into two functional domains, an N-terminal domain and a C-terminal domain. Structural and biochemical analyses identified a 211 amino acid region (amino acids 319-529) at the S1 C-terminal domain of SARS-CoV-2 as the RBD, which has a key role in virus entry and is the target of neutralizing antibodies[50,51] (FIG. 3A). The RBM mediates con- tact with the ACE2 receptor (amino acids 437-507 of SARS-CoV-2 S protein), and this region in SARS-CoV-2 differs from that in SARS-CoV in the five residues critical residues.

Immunomodulatory agents. SARS-CoV-2 triggers a strong immune response which may cause cytokine storm syndrome[60,61]. Thus, immunomodulatory agents that inhibit the excessive inflammatory response may be a potential adjunctive therapy for COVID-19. Dexamethasone is a corticosteroid often used in a wide range of conditions to relieve inflammation through its anti-inflammatory and immunosuppressant effects. Recently, the RECOVERY trial found dexamethasone reduced mortality by about one third in hospitalized patients with COVID-19 who received invasive mechanical ventilation and by one fifth in patients receiving oxygen. By contrast, no benefit was found in patients without respiratory support[146].

Tocilizumab and sarilumab, two types of interleukin-6 (IL-6) receptor-specific antibodies previously used to treat various types of arthritis, including rheumatoid arthritis, and cytokine release syndrome, showed effectiveness in the treatment of severe COVID-19 by attenuating the cytokine storm in a small uncontrolled trial[147]. Bevacizumab is an anti-vascular endothelial growth factor (VEGF) medication that could potentially reduce pulmonary oedema in patients with severe COVID-19. Eculizumab is a specific monoclonal antibody that inhibits the proinflammatory complement protein C5. Preliminary results showed that it induced a drop of inflammatory markers and C-reactive protein levels, suggesting its potential to be an option for the treatment of severe COVID-19 (REF.[148]).

Origin and Spread of COVID-19 [1, 2, 6]

In December 2019, adults in Wuhan, capital city of Hubei province and a major transportation hub of China started presenting to local hospitals with severe pneumonia of unknown cause. Many of the initial cases had a common exposure to the Huanan wholesale seafood market that also traded live animals. The surveillance system (put into place after the SARS outbreak) was activated and respiratory samples of patients were sent to reference labs for etiologic investigations. On December 31st 2019, China notified the outbreak to the World Health Organization and on 1st January the Huanan sea food market was closed. On 7th January the virus was identified as a coronavirus that had >95% homology with the bat.

It is also abnormal in asymptomatic patients/ patients with no clinical evidence of lower respiratory tract involvement. In fact, abnormal CT scans have been used to diagnose COVID-19 in suspect cases with negative molecular diagnosis; many of these patients had positive molecular tests on repeat testing [22].

Differential Diagnosis [21]

The differential diagnosis includes all types of respiratory viral infections [influenza, parainfluenza, respiratory syncytial virus (RSV), adenovirus, human metapneumovirus, non COVID-19 coronavirus], atypical organisms (mycoplasma, chlamydia) and bacterial infections. It is not

possible to differentiate COVID-19 from these infections clinically or through routine lab tests. Therefore travel history becomes important.

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

In a randomized controlled phase I/II trial, it induced neutralizing antibodies against SARS-CoV-2 in all 1,077 participants after a second vaccine dose, while its safety profile was acceptable as well[163]. The NIAID and Moderna co-manufactured mRNA-1273, a lipid nanoparticle-formulated mRNA vaccine candidate that encodes the stabilized prefusion SARS-CoV-2 S protein. Its immunogenicity has been confirmed by a phase I trial in which robust neutralizing antibody responses were induced in a dose-dependent manner and increased after a second dose[164]. Regarding inactivated vaccines, a successful phase I/II trial involving 320 participants has been reported in China. The whole-virus COVID-19 vaccine had a low rate of adverse reactions and effectively induced neutralizing antibody production[165]. The verified safety and immunogenicity support advancement of these vaccine candidates to phase III clinical trials, which will evaluate their efficacy in protecting healthy populations from SARS-CoV-2 infection.

Future perspectives

COVID-19 is the third highly pathogenic human coronavirus disease to date. Although less deadly than SARS and MERS, the rapid spreading of this highly contagious disease has posed the severest threat to global health in this century.

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

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

These findings will not have any significance until a significant outbreak occurs due to a virus-like SARS-CoV-2.

There is a steady increase in the reports of COVID-19 in companion and wild animals around the world. Further studies are required to evaluate the potential of animals (especially companion animals) to serve as an efficient reservoir host that can further alter the dynamics of human-to-human transmission (330). To date, two pet dogs (Hong Kong) and four pet cats (one each from Belgium and Hong Kong, two from 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.

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).

Notably, the risk of disease was not higher for pregnant women. However, evidence of transplacental transmission of SARS-CoV-2 from an infected mother to a neonate was reported, although it was an isolated case[83,84]. On infection, the most common symptoms are fever, fatigue and dry cough[13,60,80,81]. Less common symptoms include sputum production, headache, haemoptysis, diarrhoea, anorexia, sore throat, chest pain, chills and nausea and vomiting in studies of patients in China[13,60,80,81]. Self-reported olfactory and taste disorders were also reported by patients in Italy[85]. Most people showed signs of diseases after an incubation period of 1-14 days (most commonly around 5 days), and dyspnoea and pneumonia developed within a median time of 8 days from illness onset[9].

In a report of 72,314 cases in China, 81% of the cases were classified as mild, 14% were severe cases that required ventilation in an intensive care unit (ICU) and a 5% were critical (that is, the patients had respiratory failure, septic shock and/or multiple organ dysfunction or failure)[9,86]. On admission, ground-glass opacity was the most common radiologic finding on chest computed tomography (CT)[13,60,80,81]. Most patients also developed marked lymphopenia, similar to what was observed in patients with SARS and MERS, and non-survivors developed severer lymphopenia over time[13,60,80,81].

Patients with SARI can be given conservative fluid therapy only when there is no evidence of shock. Empiric antimicrobial therapy must be started to manage SARI. For patients with sepsis, antimicrobials must be administered within 1 hour of initial assessments. The WHO and CDC recommend that glucocorticoids not be used in patients with COVID-19 pneumonia except where there are other indications (exacerbation of chronic obstructive pulmonary disease). [59]

Patients' clinical deterioration is closely observed with SARI; however, rapidly progressive respiratory failure and sepsis require immediate supportive care interventions comprising quick use of neuromuscular blockade and sedatives, hemodynamic management, nutritional support, maintenance of blood glucose levels, prompt assessment and treatment of nosocomial pneumonia, and prophylaxis against deep venous thrombosis (DVT) and gastrointestinal (GI) bleeding.[60] Generally, such patients give way to their primary illness to secondary complications like sepsis or multiorgan system failure.[48]

These abnormalities progress from the initial focal unilateral to diffuse bilateral ground-glass opacities and will further progress to or coexist with lung consolidation changes within 1 to 3 weeks (159). The role played by radiologists in the current scenario is very important. Radiologists can

help in the early diagnosis of lung abnormalities associated with COVID-19 pneumonia. They can also help in the evaluation of disease severity, identifying its progression to acute respiratory distress syndrome and the presence of secondary bacterial infections (160). Even though chest CT is considered an essential diagnostic tool for COVID-19, the extensive use of CT for screening purposes in the suspected individuals might be associated with a disproportionate risk-benefit ratio due to increased radiation exposure as well as increased risk of cross- infection. Hence, the use of CT for early diagnosis of SARS-CoV-2 infection in high-risk groups should be done with great caution (292).

More recently, other advanced diagnostics have been designed and developed for the detection of SARS-CoV-2 (345, 347, 350-352).

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.

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.

In addition, it is advisable that breast pumps are cleaned properly after each use and, if possible, a healthy individual is available to feed the expressed breast milk to the infant.[42]

7.2 Children and elderly population

On the basis of the available reports, COVID-19 among children accounted for 1-5% of the confirmed cases, and this population does not seem to be at higher risk for the disease than adults. There is no difference in the COVID-19 symptoms between adults and children. However, the available evidence indicated that children diagnosed with COVID-19 have milder symptoms than the adults, with a low mortality rate.[48, 49] On the contrary, older people who are above the age of 65 years are at higher risk for a severe course of disease. In the United Stated, approximately 31-59% of those with confirmed COVID-19 between the ages of 65 and 84 years old required hospitalisation, 11-31% of them required admission to the intensive care unit, and 4-11% died.[50]

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.

Cases continued to increase exponentially and modelling studies reported an epidemic doubling time of 1.8 d [10]. In fact on the 12th of February, China changed its definition of confirmed cases to include patients with negative/ pending molecular tests but with clinical, radiologic and epidemiologic features of COVID-19 leading to an increase in cases by 15,000 in a single day [6]. As of 05/03/2020 96,000 cases worldwide (80,000 in China) and 87 other countries and 1 international conveyance (696, in the cruise ship Diamond Princess parked off the coast of Japan) have been reported [2]. It is important to note that while the number of new cases has reduced in China lately, they have increased exponentially in other countries including South Korea, Italy and Iran.

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.

The in vitro and in vivo studies carried out on the isolated virus confirmed that there is a potential risk for the reemergence of SARS-CoV infection from the viruses that are currently circulating in the bat population (105).

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).

In comparison with the Guangdong strains, pangolin coronaviruses reported from Guangxi are less similar to SARS-CoV-2, with 85.5% genome sequence identity[39]. The repeated occurrence of SARS-CoV-2-related coronavirus infections in pangolins from different smuggling events suggests that these animals are possible hosts of the viruses. However, unlike bats, which carry coronaviruses healthily, the infected pangolins showed clinical signs and histopathological changes, including interstitial pneumonia and inflammatory cell infiltration in diverse organs[40]. These abnormalities suggest that pangolins are unlikely to be the reservoir of these coronaviruses but more likely acquired the viruses after spillover from the natural hosts.

An intermediate host usually plays an important role in the outbreak of bat-derived emerging coronaviruses; for example, palm civets for SARS-CoV and dromedary camels for MERS-CoV. The virus strains carried by these two intermediate hosts were almost genetically identical to the corresponding viruses in humans (more than 99% genome sequence identity)[1]. Despise an RBD that is virtually identical to that of SARS-CoV-2, the pangolin coronaviruses known to date have no more than 92% genome identity with SARS-CoV-2 (REF.[42]). The avail- able data are insufficient to interpret pangolins as the intermediate host of SARS-CoV-2. So far, no evidence has shown that pangolins were directly involved in the emergence of SARS-CoV-2.

Owing to these residue changes, interaction of SARS-CoV-2 with its receptor stabilizes the two virus-binding hotspots on the surface ofhACE2 (REF.[50]) (FIG. 3D). Moreover, a four-residue motif in the RBM of SARS-CoV-2 (amino acids 482-485: G-V-E-G) results in a more compact conformation of its hACE2-binding ridge than in SARS-CoV and enables better contact with the N-terminal helix of hACE2 (REF.[50]). Biochemical data confirmed that the structural features of the SARS-CoV-2 RBD has strengthened its hACE2 binding affinity compared with that of SARS-CoV[50,52,53].

Similarly to other coronaviruses, SARS-CoV-2 needs proteolytic processing of the S protein to activate the endocytic route. It has been shown that host proteases participate in the cleavage of the S protein and activate the entry of SARS-CoV-2, including transmembrane protease serine protease 2 (TMPRSS2), cathepsin L and furin[47,54,55]. Single-cell RNA sequencing data showed that TMPRSS2 is highly expressed in several tissues and body sites and is co-expressed with ACE2 in nasal epithelial cells, lungs and bronchial branches, which explains some of the tissue tropism of SARS-CoV-2 (REFS[56,57]). SARS-CoV-2 pseudovirus entry assays revealed that TMPRSS2 and cathepsin L have cumulative effects with furin on activating virus entry[55]. Analysis of the cryo-electron microscopy structure of SARS-CoV-2 S protein revealed that its RBD is mostly in the lying-down state, whereas the SARS-CoV S protein assumes equally standing-up and lying-down conformational states[50,51,58,59]. A lying-down conformation of the SARS-CoV-2 S protein may not be in favour of receptor binding but is helpful for immune evasion[55].

Introduction

The 2019 novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as it is now called, is rapidly spreading from its origin in Wuhan City of Hubei Province of China to the rest of the world [1]. Till 05/03/2020 around 96,000 cases of coronavirus disease 2019 (COVID-19) and 3300 deaths have been reported [2]. India has reported 29 cases till date. Fortunately so far, children have been infrequently affected with no deaths. But the future course of this virus is unknown.

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-CoVZC45 and bat-SL-CoVZXC21).

The median time from onset of symptoms to dyspnea was 5 d, hospitalization 7 d and acute respiratory distress syndrome (ARDS) 8 d. The need for intensive care admission was in 25- 30% of affected patients in published series. Complications witnessed included acute lung injury, ARDS, shock and acute kidney injury. Recovery started in the 2nd or 3rd wk. The median duration of hospital stay in those who recovered was 10 d. Adverse outcomes and death are more common in the elderly and those with underlying co-morbidities (50-75% of fatal cases). Fatality rate in hospitalized adult patients ranged from 4 to 11%. The overall case fatality rate is estimated to range between 2 and 3%

At the community level, people should be asked to avoid crowded areas and postpone non-essential travel to places with ongoing transmission. They should be asked to practice cough hygiene by coughing in sleeve/ tissue rather than hands and practice hand hygiene frequently every 15–20 min. Patients with respiratory symptoms should be asked to use surgical masks.

The use of mask by healthy people in public places has not shown to protect against respiratory viral infections and is currently not recommended by WHO. However, in China, the public has been asked to wear masks in public and especially in crowded places and large scale gatherings are prohibited (entertainment parks etc).

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).

And ritonavir had little therapeutic benefit in patients with COVID-19, but appeared more effective when used in combination with other drugs, including ribavirin and interferon beta-1b[143,144]. The Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial, a national clinical trial programme in the UK, has stopped treatment with lopinavir and ritonavir as no significant beneficial effect was observed in a randomized trial established in March 2020 with a total of 1,596 patients[145].

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 *Nidovirales*, 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).

Results of the clinical trial showed that the patients who were given chloroquine had a significant reduction in their body temperature. The clinical trial also showed better recovery among the patients who were given chloroquine and hydroxy chloroquine.[63-65] Hydroxychloroquine treatment is significantly associated with viral load reduction as well as disappearance in COVID-19 patients. Further, the outcome is reinforced by azithromycin. The role of lopinavir and ritonavir in the treatment of COVID-19 is uncertain. A potential benefit was suggested by preclinical data, but additional data has failed to confirm it. Tocilizumab is an immunomodulating agent used as adjunct therapy in some protocols based on a theoretical mechanism and limited preliminary data.[66]

15 HOME CARE

Home management may be appropriate for patients with mild infection who can be adequately isolated in the outpatient setting. Management of such patients should focus on prevention of transmission to others, and monitoring for clinical deterioration, which should prompt hospitalisation.

Princess, Celebrity Apex, and Ruby Princess. The number of confirmed COVID-19 cases around the world is on the rise. The success of preventive measures put forward by every country is mainly dependent upon their ability to anticipate the approaching waves of patients. This will help to properly prepare the health care workers and increase the intensive care unit (ICU) capacity (321). Instead of entirely relying on lockdown protocols, countries should focus mainly on alternative intervention strategies, such as large-scale testing, contract tracing, and localized quarantine of suspected cases for limiting the spread of this pandemic virus. Such intervention strategies will be useful either at the beginning of the pandemic or after lockdown relaxation (322). Lockdown should be imposed only to slow down disease progression among the population so that the health care system is not overloaded.

The reproduction number (R0) of COVID-19 infection was earlier estimated to be in the range of 1.4 to 2.5 (70); recently, it was estimated to be 2.24 to 3.58 (76). Compared to its coronavirus predecessors, COVID-19 has an Ro value that is greater than that of MERS (Ro < 1) (108) but less than that of SARS (Ro value of 2 to 5) (93).

Epidemiology and Pathogenesis [10, 11]

All ages are susceptible. Infection is transmitted through large droplets generated during coughing and sneezing by symptomatic patients but can also occur from asymptomatic people and before onset of symptoms [9]. Studies have shown higher viral loads in the nasal cavity as compared to the throat with no difference in viral burden between symptomatic and asymptomatic people [12]. Patients can be infectious for as long as the symptoms last and even on clinical recovery. Some people may act as super spreaders; a UK citizen who attended a conference in Singapore infected 11 other people while staying in a resort in the French Alps and upon return to the UK [6].

In the case series of children discussed earlier, all children recovered with basic treatment and did not need intensive care [17].

There is anecdotal experience with use of remdeswir, a broad spectrum anti RNA drug developed for Ebola in management of COVID-19 [27]. More evidence is needed before these drugs are recommended. Other drugs proposed for therapy are arbidol (an antiviral drug available in Russia and China), intravenous immunoglobulin, interferons, chloroquine and plasma of patients recovered from COVID-19 [21, 28, 29]. Additionally, recommendations about using traditional Chinese herbs find place in the Chinese guidelines [21].

However, this study did not include a control arm, and most of the trials of favilavir were based on a small sample size. For more reliable assessment of the effectiveness of favilavir for treating COVID-19, large-scale randomized controlled trials should be conducted.

Lopinavir and ritonavir were reported to have in vitro inhibitory activity against SARS-CoV and MERS-CoV[141,142].

Possible origin of SARS-CoV-2 and the first mode 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.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, 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. 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.

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).

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).

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).

In a historical control study in patients with SARS, patients treated with lopinavir- ritonavir with ribavirin had better outcomes as compared to those given ribavirin alone [15].

In the case series of 99 hospitalized patients with COVID-19 infection from Wuhan, oxygen was given to 76%, non- invasive ventilation in 13%, mechanical ventilation in 4%, extracorporeal membrane oxygenation (ECMO) in 3%, continuous renal replacement therapy (CRRT) in 9%, antibiotics in 71%, antifungals in 15%, glucocorticoids in 19% and intravenous immunoglobulin therapy in 27% [15]. Antiviral therapy consisting of oseltamivir, ganciclovir and lopinavir ritonavir was given to 75% of the patients.

Thus, it has been suggested that CT scanning combined with repeated swab tests should be used for individuals with high clinical suspicion of COVID-19 but who test negative in initial nucleic acid screening[118]. Finally, SARS-CoV-2 serological tests detecting antibodies to N or S protein could complement molecular diagnosis, particularly in late phases after disease onset or for retrospective studies[116,120,121]. However, the extent and duration of immune responses are still unclear, and available serological tests differ in their sensitivity and specific- ity, all of which need to be taken into account when one is deciding on serological tests and interpreting their results or potentially in the future test for T cell responses.

Therapeutics

To date, there are no generally proven effective therapies for COVID-19 or antivirals against SARS-CoV-2, although some treatments have shown some benefits in certain subpopulations of patients or for certain end points (see later). Researchers and manufacturers are conducting large-scale clinical trials to evaluate various therapies for COVID-19. As of 2 October 2020, there were about 405 therapeutic drugs in development for COVID-19, and nearly 318 in human clinical trials (COVID-19 vaccine and therapeutics tracker). In the following sections, we summarize potential therapeutics against SARS-CoV-2 on the basis of published clinical data and experience.

The CRISPR-Cas9 gene-editing tool has been used for inserting genomic alterations in mice, making them susceptible to MERS-CoV infection (222). Efforts are under way to recognize suitable animal models for SARS-CoV2/COVID-19, identify the receptor affinity of this virus, study pathology in experimental animal models, and explore virus-specific immune responses and protection studies, which together would increase the pace of efforts being made for developing potent vaccines and drugs to counter this emerging virus. Cell lines, such as monkey epithelial cell lines (LLC-MK2 and Vero-B4), goat lung cells, alpaca kidney cells, dromedary umbilical cord cells, and advanced ex vivo three-dimensional tracheobronchial tissue, have been explored to study human CoVs (MERS-CoV) (223, 224). Vero and Huh-7 cells (human liver cancer cells) have been used for isolating SARS-CoV-2 (194).

Recently, an experimental study with rhesus monkeys as animal models revealed the absence of any viral loads in nasopharyngeal and anal swabs, and no viral replication was recorded in the primary tissues at a time interval of 5 days post-reinfection in reexposed monkeys (274).

It is advisable to distinguish COVID-19 from other pneumonias such as mycoplasma pneumonia, chlamydia pneumonia and bacterial pneumonia.[33] Several published pieces of literature based on the novel coronavirus reported in China declared that stool and blood samples can also collected from the suspected persons in order to detect the virus. However, respiratory samples show better viability in identifying the virus, in comparison with the other specimens.[34-36]

6.2 Nucleic acid amplification tests (NAAT) for COVID-19 virus

The gold standard method of confirming the suspected cases of COVID-19 is carried out by detecting the unique sequences of virus RNA through reverse transcription polymerase chain reaction (RT-PCR) along with nucleic acid sequencing if needed. The various genes of virus identified so far include N, E, S (N: nucleocapsid protein, E: envelope protein gene, S: spike protein gene) and RdRP genes (RNA- dependent RNA polymerase gene).[32]

We also predict the possibility of another outbreak, as predicted by Fan et al. (6). Indeed, the present outbreak caused by SARS-CoV-2 (COVID- 19) was expected. Similar to previous outbreaks, the current outbreak also will be contained shortly. However, the real issue is how we are planning to counter the next zoonotic CoV epidemic that is likely to occur within the next 5 to 10 years or even sooner.

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).

Subfamily and is entirely amerem hom the viruses responsible for MERS-CoV and SARS-CoV (3). The newly emerged SARS-CoV-2 is a group 2B coronavirus (2). The genome sequences of SARS-CoV-2 obtained from patients share 79.5% sequence similarity to the sequence of SARS-CoV (63).

As of 13 May 2020, a total of 4,170,424 confirmed cases of COVID-19 (with 287,399 deaths) have been reported in more than 210 affected countries worldwide.

Coronavirus S protein is a large, multifunctional 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, Sprotein 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 S2 (43). The first one, S1, helps in host receptor binding, while the second one, S2, accounts for fusion. The former (S1) is further divided into two subdomains, namely, the N-terminal domain (NTD) and C-terminal domain (CTD). Both of these subdomains

act as receptor-binding domains, interacting efficiently with various host receptors (45). The S1 CTD contains the receptor-binding motif (RBM). In each coronavirus spike protein, the trimeric S1 locates itself on top of the trimeric S2.

All clinicians should keep themselves updated about recent developments including global spread of the disease. Non-essential international travel should be avoided at this time. People should stop spreading myths and false information about the disease and try to allay panic and anxiety of the public.

Conclusions

This new virus outbreak has challenged the economic, medical and public health infrastructure of China and to some extent, of other countries especially, its neighbours. Time alone will tell how the virus will impact our lives here in India. More so, future outbreaks of viruses and pathogens of zoonotic origin are likely to continue.

This novel bat virus, denoted 'RmYN02', is 93.3% identical to SARS-CoV-2 across the genome. In the long lab gene, it exhibits 97.2% identity to SARS-CoV-2, which is even higher than for RaTG13 (REF. [28]). In addition to RaTG13 and RmYN02, phylogenetic analysis shows that bat coronaviruses ZC45 and ZXC21 previously detected in Rhinolophus pusillus bats from eastern China also fall into the SARS-CoV-2 lineage of the subgenus Sarbecovirus[36] (FIG. 2). The discovery of diverse bat coronaviruses closely related to SARS-CoV-2 suggests that bats are possible reservoirs of SARS-CoV-2 (REF.[37]). Nevertheless, on the basis of current findings, the divergence between SARS-CoV-2 and related bat coronaviruses likely represents more than 20 years of sequence evolution, suggesting that these bat coronaviruses can be regarded only as the likely evolutionary precursor of SARS-CoV-2 but not as the direct progenitor of SARS-CoV-2 (REF.[38]).

Beyond bats, pangolins are another wildlife host probably linked with SARS-CoV-2. Multiple SARS-CoV-2- related viruses have been identified in tissues of Malayan pangolins smuggled from Southeast Asia into southern China from 2017 to 2019. These viruses from pangolins independently seized by Guangxi and Guangdong provincial customs belong to two distinct sublineages[39-41]. The Guangdong strains, which were isolated or sequenced by different research groups from smuggled pangolins, have 99.8% sequence identity with each other[41]. They are very closely related to SARS-CoV-2, exhibiting 92.4% sequence similarity. Notably, the RBD of Guangdong pangolin coronaviruses is highly similar to that of SARS-CoV-2. The receptor-binding motif (RBM; which is part of the RBD) of these viruses has only one amino acid variation from SARS-CoV-2.

Environmental samples from the Huanan seafood market also tested positive, signifying that the virus originated from there [7]. The number of cases started increasing exponentially, some of which did not have exposure to the live animal market, suggestive of the fact that human-to-human transmission was occurring [8]. The first fatal case was reported on 11th Jan 2020. The massive migration of Chinese during the Chinese New Year fuelled the epidemic. Cases in other provinces of China, other countries (Thailand, Japan and South Korea in quick succession) were reported in people who were returning from Wuhan. Transmission to healthcare workers caring for patients was described on 20th Jan, 2020. By 23rd January, the 11 million population of Wuhan was placed under lock down with restrictions of entry and exit from the region.

Polymorphism at nucleotide position 28,144, which results in amino acid substitution of Ser for Lys at residue 84 of the ORF8 protein. Those variants with this mutation make up a single subclade labelled as 'clade S'[33,34]. Currently, however, the available sequence data are not sufficient to interpret the early global transmission history of the virus, and travel patterns, founder effects and public health measures also strongly influence the spread of particular lineages, irrespective of potential biological differences between different virus variants.

Animal host and spillover

Bats are important natural hosts of alphacoronaviruses and betacoronaviruses. The closest relative to SARS-CoV-2 known to date is a bat coronavirus detected in *Rhinolophus affinis* from Yunnan province, China, named 'RaTG13', whose full-length genome sequence is 96.2% identical to that of SARS-CoV-2 (REF.[11]). This bat virus shares more than 90% sequence identity with SARS-CoV-2 in all ORFs throughout the genome, including the highly variable S and ORF8 (REF.[11]). Phylogenetic analysis confirms that SARS-CoV-2 closely clusters with RaTG13 (FIG. 2). The high genetic similarity between SARS-CoV-2 and RaTG13 supports the hypothesis that SARS-CoV-2 likely originated from bats[35]. Another related coronavirus has been reported more recently in a *Rhinolophus malayanus* bat sampled in Yunnan.

Heptad repeat 1 (HR1) and heptad repeat 2 (HR2) can interact and form a six-helix bundle that brings the viral and cellular membranes in close proximity, facilitating its fusion. The sequence alignment study conducted between COVID-19 and SARS-CoV identified that the S2 subunits are highly conserved in these CoVs. The HR1 and HR2 domains showed 92.6% and 100% overall identity, respectively (210). From these findings, we can confirm the significance of COVID-19 HR1 and HR2 and their vital role in host cell entry. Hence, fusion inhibitors target the HR1 domain of S protein, thereby preventing viral fusion and entry into the host cell. This is another potential therapeutic strategy that can be used in the management of COVID-19. Other than the specific therapy directed against COVID-19, general treatments play a vital role in the enhancement of host immune responses against the viral agent. Inadequate nutrition is linked to the weakening of the host immune response, making the individual more susceptible. The role played by nutrition in disease susceptibility should be measured by evaluating the nutritional status of patients with COVID-19 (205).

Personal protective equipment (PPE), like face masks, will help to prevent the spread of respiratory infections like COVID-19. Face masks not only protect from infectious aerosols but also prevent the transmission of disease to other susceptible individuals while traveling through public transport systems (313). Another critical practice that can reduce the transmission of respiratory diseases is the maintenance of hand hygiene. However, the efficacy of this practice in reducing the transmission of respiratory viruses like SARS-CoV-2 is much dependent upon the size of droplets produced. Hand hygiene will reduce disease transmission only if the virus is transmitted through the formation of large droplets (314). Hence, it is better not to overemphasize that hand hygiene will prevent the transmission of SARS-CoV-2, since it may produce a false sense of safety among the general public that further contributes to the spread of COVID-19. Even though airborne spread has not been reported in SARS-CoV-2 infection, transmission can occur through droplets and fomites, especially when there is close, unprotected contact between infected and susceptible individuals.

6.3 Serological testing

Serological surveys are also considered to be one of the most effective ones in facilitating outbreak investigation and it also helps us to derive a retrospective assessment of the disease by estimating the attack rate.[32]

According to the recent literature, paired serum samples can also help clinicians to diagnose COVID-19 in case of false negative results in NAAT essays.[37] The literature also declared that the commercial and non-commercial serological tests are under consideration in order to support the practising clinicians by assisting them in diagnosis. Similarly, there are studies published on COVID-19 which are comprised of the serological data on clinical samples.[38, 39]

6.4 Viral sequencing

Apart from confirming the presence of virus in the specimens, viral sequencing is also quite useful in monitoring the viral genomic mutations, which plays a very significant role in influencing the performance of the medical countermeasures inclusive of the diagnostic test. Genomic sequencing of the virus can also help further in developing several studies related to molecular epidemiology.[32]

Coronaviruses are a diverse group of viruses infecting many different animals, and they can cause mild to severe respiratory infections in humans. In 2002 and 2012, respectively, two highly pathogenic coronaviruses with zoonotic origin, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in humans and caused fatal respiratory illness, making emerging coronaviruses a new public health concern in the twenty-first century[1]. At the end of 2019, a novel coronavirus designated as SARS-CoV-2 emerged in the city of Wuhan, China, and caused an outbreak of unusual viral pneumonia. Being highly transmissible, this novel coronavirus disease, also known as coronavirus disease 2019 (COVID-19), has spread fast all over the world[2,3]. It has overwhelmingly surpassed SARS and MERS in terms of both the number of infected people and the spatial range of epidemic areas. The ongoing outbreak of COVID-19 has posed an extraordinary threat to global public health[4,5]. In this Review, we summarize the current understanding of the nature of SARS-CoV-2 and COVID-19. On the basis of recently published findings, this comprehensive Review covers the basic biology of SARS-CoV-2, including the genetic characteristics, the potential zoonotic origin and its receptor binding. Furthermore, we will discuss the clinical and epidemiological features, diagnosis of and countermeasures against COVID-19.

Emergence and spread

In late December 2019, several health facilities in Wuhan, in Hubei province in China, reported clusters of patients with pneumonia of unknown cause. Similarly to patients with SARS and MERS, these patients showed symptoms of viral pneumonia, including fever, cough.

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.

Suspected cases should be referred to government designated centres for isolation and testing (in Mumbai, at this time, it is Kasturba hospital). Commercial kits for testing are not yet available in India.

Patients admitted with severe pneumonia and acute respiratory distress syndrome should be evaluated for travel history and placed under contact and droplet isolation. Regular decontamination of surfaces should be done. They should be tested for etiology using multiplex PCR panels if logistics permit and if no pathogen is identified, refer the samples for testing for SARS- CoV-2.

Initially, the epicenter of the SARS-CoV-2 pandemic was China, which reported a significant number of deaths associated with COVID-19, with 84,458 laboratory-confirmed cases and 4,644 deaths as of 13 May 2020. As of 13 May 2020, SARS-CoV-2 confirmed cases have been reported in more than 210 countries apart from China (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.

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.

Hence, hand hygiene is equally as important as the use of appropriate PPE, like face masks, to break the transmission cycle of the virus; both hand hygiene and face masks help to lessen the risk of COVID-19 transmission (315).

Medical staff are in the group of individuals most at risk of getting COVID-19 infection. This is because they are exposed directly to infected patients. Hence, proper training must be given to all hospital staff on methods of prevention and protection so that they become competent enough to protect themselves and others from this deadly disease (316). As a preventive measure, health care workers caring for infected patients should take extreme precautions against both contact and airborne transmission. They should use PPE such as face masks (N95 or FFP3), eye protection (goggles), gowns, and gloves to nullify the risk of infection (299).

The human-to-human transmission reported in SARS-CoV-2 infection occurs mainly through droplet or direct contact. Due to this finding, frontline health care workers should follow stringent infection control and preventive measures, such as the use of PPE, to prevent infection (110).

10 RECOMBINANT SUBUNIT VACCINE

Clover Biopharmaceuticals is producing a recombinant subunit vaccine based on the trimeric S-protein of COVID-19.[55] The oral recombinant vaccine is being expanded by Vaxart in tablet formulation, using its proprietary oral vaccine platform.

11 CLINICAL MANAGEMENT AND TREATMENT

In severe COVID-19 cases, treatment should be given to support vital organ functions. People who think they may have been exposed to COVID-19 should contact their healthcare provider immediately. Healthcare personnel should care for patients in an Airborne Infection Isolation Room (AIIR). Precautions must be taken by the healthcare professional, such as contact precautions and airborne precautions with eye protection.[56]

Individuals with a mild clinical presentation may not require primary hospitalisation. Close monitoring is needed for the persons infected with COVID-19.

The number of confirmed cases suddenly increased, with thousands of new cases diagnosed daily during late January[15]. On 30 January, the WHO declared the novel coronavirus outbreak a public health emergency of inter- national concern[16]. On 11 February, the International Committee on

Taxonomy of Viruses named the novel coronavirus 'SARS-CoV-2, and the WHO named the disease 'COVID-19' (REF. [17]).

The outbreak of COVID-19 in China reached an epidemic peak in February. According to the National Health Commission of China, the total number of cases continued to rise sharply in early February at an average rate of more than 3,000 newly confirmed cases per day. To control COVID-19, China implemented unprecedentedly strict public health measures. The city of Wuhan was shut down on 23 January, and all travel and transportation connecting the city was blocked. In the following couple of weeks, all outdoor activities and gatherings were restricted, and public facilities were closed in most cities as well as in countryside[18]. Owing to these measures, the daily number of new cases in China started to decrease steadily[19].

However, despite the declining trend in China, the international spread of COVID-19 accelerated from late February. Large clusters of infection have been reported from an increasing number of countries[18]. The high transmission efficiency of SARS-CoV-2 and the abundance of international travel enabled rapid worldwide spread of COVID-19. On 11 March 2020, the WHO officially characterized the global COVID-19 out- break as a pandemic[20]. Since March, while COVID-19 in China has become effectively controlled, the case numbers in Europe, the USA and other regions have jumped sharply. According to the COVID-19 dash- board of the Center for System Science and Engineering at Johns Hopkins University, as of 11 August 2020.

This emerging virus will establish a niche in humans and coexist with us for a long time[166]. Before clinically approved vaccines are widely available, there is no better way to protect us from SARS-CoV-2 than personal preventive behaviours such as social distancing and wearing masks, and public health measures, including active testing, case tracing and restrictions on social gatherings. Despite a flood of SARS-CoV-2 research published every week, current knowledge of this novel coronavirus is just the tip of the iceberg. The animal origin and cross-species infection route of SARS-CoV-2 are yet to be uncovered.

Caregivers should be asked to wear a surgical mask when in the same room as patient and use hand hygiene every 15-20 min.

The greatest risk in COVID-19 is transmission to healthcare workers. In the SARS outbreak of 2002, 21% of those affected were healthcare workers [31]. Till date, almost 1500 health care workers in China have been infected with 6 deaths. The doctor who first warned about the virus has died too. It is important to protect healthcare workers to ensure continuity of care and to prevent transmission of infection to other patients. While COVID-19 transmits as a droplet pathogen and is placed in Category B of infectious agents (highly pathogenic H5N1 and SARS).

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.

Inhibition of virus entry. SARS-CoV-2 uses ACE2 as the receptor and human proteases as entry activators; subsequently it fuses the viral membrane with the cell membrane and achieves invasion. Thus, drugs that interfere with entry may be a potential treatment for COVID-19. Umifenovir (Arbidol) is a drug approved in Russia and China for the treatment of influenza and other

respiratory viral infections. It can target the interaction between the S protein and ACE2 and inhibit membrane fusion (FIG. 5). In vitro experiments showed that it has activity against SARS-CoV-2, and current clinical data revealed it may be more effective than lopinavir and ritonavir in treating COVID-19 (REFS[122,123]). However, other clinical studies showed umifenovir might not improve the prognosis of or accelerate SARS-CoV-2 clearance in patients with mild to moderate COVID-19 (REFS[124,125]). Yet some ongoing clinical trials are evaluating its efficacy for COVID-19 treatment. Camostat mesylate is approved in Japan for the treatment of pancreatitis and postoperative reflux oesophagitis. Previous studies showed that it can prevent SARS-CoV from entering cells by blocking TMPRSS2 activity and protect mice from lethal infection with SARS-CoV in a pathogenic mouse model (wild-type mice infected with a mouse-adapted SARS-CoVstrain)[126,127]. Recently, a study revealed that camostat mesylate blocks the entry of SARS-CoV-2 into human lung cells[47]. Thus, it can be a potential antiviral drug against SARS-CoV-2 infection, although so far there are not sufficient clinical data to support its efficacy.

8 PREVENTION

The WHO and other agencies such as the CDC have published protective measures to mitigate the spread of COVID-19. This involves frequent hand washing with handwash containing 60% of alcohol and soap for at least 20 seconds. Another important measure is avoiding close contact with sick people and keeping a social distance of 1 metre always to everyone who is coughing and sneezing. Not touching the nose, eyes and mouth was also suggested. While coughing or sneezing, covering the mouth and nose with a cloth/tissue or the bent elbow is advised. Staying at home is recommended for those who are sick, and wearing a facial mask is advised when going out among people. Furthermore, it is recommended to clean and sterilise frequently touched surfaces such as phones and doorknobs on a daily basis.[51, 52] Staying at home as much as possible is advisable for those who are at higher risk for severe illness, to minimise the risk of exposure to COVID-19 during outbreaks.[53]

14 ANTIVIRAL THERAPY

COVID-19 is an infectious disease caused by SARS-CoV-2, which is also termed the novel coronavirus and is diligently associated with the SARS virus. The Ministry of Science and Technology from the People's Republic of China declared three potential antiviral medicines suitable for treating COVID-19. Those three medicines are, namely, Favilavir, chloroquine phosphate and remdesivir. A clinical trial was conducted to test the efficacy of those three drugs, and the results proved that out of the three medicines above only Favilavir is effective in treating the patients with novel coronavirus. The remaining two drugs were effective in treating malaria.[62]

Likewise a study carried out in the United States by the National Institute of Health proved that remdesivir is effective in treating the Middle East respiratory syndrome coronavirus (MERS- CoV), which is also a type of coronavirus that was transmitted from monkeys. The drug remdesivir was also used in the United States for treating the patients with COVID-19. There has been a proposal to use the combination of protease inhibitors lopinavir-ritonavir for treating the patients affected by COVID-19.[62]

It is also evident that remdesivir was effective in treating the patients who were infected with Ebola virus. Per this evidence, China has already started testing the efficacy of remdesivir in treating the patients with COVID-19, especially in Wuhan, where the outbreak occurred. Chloroquine, which is an existing drug which is currently used in treating malaria cases, was given to more than 100 patients who were affected with novel coronavirus to test its efficacy.[62]

A multicentric study was conducted in China to test the effectiveness of remdesivir in treating the patients with COVID-19. Thus, the results of the clinical trial proved that remdesivir has a considerably acceptable level of efficacy for treating the patients with COVID-19. Therefore, the National Health Commission of the People's Republic of China decided to include remdesivir in the Guidelines for the Prevention, Diagnosis and Treatment of Pneumonia Caused by COVID- 19.[62]

Chloroquine and hydroxychloroquine are existing anti-malaria drugs also given to more than 30 patients infected with COVID-19 in Guangdong province and Hunan province to test their effectiveness and efficacy.

Absence of this protein is related to the altered virulence of coronaviruses due to changes in morphology and tropism (54). The E protein consists of three domains, namely, a short hydrophilic amino terminal, a large hydrophobic transmembrane domain, and an efficient C-terminal domain (51). The SARS-CoV-2 E protein reveals a similar amino acid constitution without any substitution (16).

N Protein

The N protein of coronavirus is multipurpose. Among several functions, it plays a role in complex formation with the viral genome, facilitates M protein interaction needed during virion assembly, and enhances the transcription efficiency of the virus (55, 56). It contains three highly conserved and distinct domains, namely, an NTD, an RNA-binding domain or a linker region (LKR), and a CTD (57). The NTD binds with the 3' end of the viral genome, perhaps via electrostatic interactions, and is highly diverged both in length and sequence (58). The charged LKR is serine and arginine rich and is also known as the SR (serine and arginine) domain (59). The LKR is capable of direct interaction with *in vitro* RNA interaction and is responsible for cell signaling (60, 61). It also modulates the antiviral response of the host by working as an antagonist for interferon.

Acute viral interstitial pneumonia and humoral and cellular immune responses were observed[48,75]. Moreover, prolonged virus shedding peaked early in the course of infection in asymptomatic macaques[69], and old monkeys showed severer interstitial pneumonia than young monkeys, which is similar to what is seen in patients with COVID-19. In human ACE2-transgenic mice infected with SARS-CoV-2, typical interstitial pneumonia was present, and viral anti- gens were observed mainly in the bronchial epithelial cells, macrophages and alveolar epithelia. Some human ACE2-transgenic mice even died after infection[70,71]. In wide-type mice, a SARS-CoV-2 mouse-adapted strain with the N501Y alteration in the RBD of the S protein was generated at passage 6. Interstitial pneumonia and inflammatory responses were found in both young and aged mice after infection with the mouse-adapted strain[74]. Golden hamsters also showed typical symptoms after being infected with SARS-CoV-2 (REF.[77]). In other animal models, including cats and ferrets, SARS-CoV-2 could efficiently replicate in the upper respiratory tract but did not induce severe clinical symptoms[43,78]. As transmission by direct contact and air was observed in infected ferrets and hamsters, these animals could be used to model different transmission modes of COVID-19 (REFS[77-79]). Animal models offer important information for understanding the pathogenesis of SARS-CoV-2 infection and the transmission dynamics of SARS- CoV-2, and are important to evaluate the efficacy of antiviral therapeutics and vaccines.

Clinical and epidemiological features

It appears that all ages of the population are susceptible to SARS-CoV-2 infection, and the median age of infection is around 50 years [9,13,60,80,81]. However, clinical manifestations differ with age. In general, older men (>60 years old) with co-morbidities are more likely to develop severe respiratory disease that requires hospitalization.

Virological, radiological, and pathological observations indicated that the monkeys with reexposure had no recurrence of COVID-19, like the SARS-CoV-2-infected monkeys without rechallenge. These findings suggest that primary infection with SARS-CoV-2 could protect from later exposures to the virus, which could help in defining disease prognosis and crucial inferences for designing and developing potent vaccines against COVID-19 (274).

PREVENTION, CONTROL, AND MANAGEMENT

In contrast to their response to the 2002 SARS outbreak, China has shown immense political openness in reporting the COVID-19 outbreak promptly. They have also performed rapid sequencing of COVID-19 at multiple levels and shared the findings globally within days of identifying the novel virus (225). The move made by China opened a new chapter in global health security and diplomacy. Even though complete lockdown was declared following the COVID-19 outbreak in Wuhan, the large-scale movement of people has resulted in a radiating spread of infections in the surrounding provinces as well as to several other countries.

The initial stages of the outbreak, only mild symptoms were noticed in those patients that are infected by human-to-human transmission (14). The initial trends suggested that the mortality associated with COVID-19 was less than that of previous outbreaks of SARS (101). The updates obtained from countries like China, Japan, Thailand, and South Korea indicated that the COVID-19 patients had relatively mild manifestations compared to those with SARS and MERS (4). Regardless of the coronavirus type, immune cells, like mast cells, that are present in the submucosa of the respiratory tract and nasal cavity are considered the primary barrier against this virus (92). Advanced in-depth analysis of the genome has identified 380 amino acid substitutions between the amino acid sequences of SARS-CoV-2 and the SARS/SARS-like coronaviruses. These differences in the amino acid sequences might have contributed to the difference in the pathogenic divergence of SARS-CoV-2 (16). Further research is required to evaluate the possible differences in tropism, pathogenesis, and transmission of this novel agent associated with this change in the amino acid sequence. With the current outbreak of COVID-19, there is an expectancy of a significant increase in the number of published studies about this emerging coronavirus.

Of the four structural genes, SARS-CoV-2 shares more than 90% amino acid identity with SARS-CoV except for the S gene, which diverges[11,24]. The replicase gene covers two thirds of the 5' genome, and encodes a large polyprotein (pplab), which is proteolytically cleaved into 16 non-structural proteins that are involved in transcription and virus replication. Most of these SARS-CoV-2 non-structural proteins have greater than 85% amino acid sequence identity with SARS-CoV[25].

The phylogenetic analysis for the whole genome shows that SARS-CoV-2 is clustered with SARS-CoV and SARS-related coronaviruses (SARSr-CoVs) found in bats, placing it in the subgenus Sarbecovirus of the genus Betacoronavirus. Within this clade, SARS-CoV-2 is grouped in a distinct lineage together with four horse-shoe bat coronavirus isolates (RaTG13, RmYN02, ZC45 and ZXC21) as well as novel coronaviruses recently identified in pangolins, which group parallel to SARS-CoV.

The RBD of S protein 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).

Furthermore, SARS-CoV-2 is genetically distinct from SARS-CoV (79% similarity) and MERS-CoV (nearly 50%) (17). COVID-19 is associated with afflictions of the lungs in all cases and

generated 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).

Cases of COVID-19 in countries outside China were reported in those with no history of travel to China suggesting that local human-to- human transmission was occurring in these countries [9]. Airports in different countries including India put in screening mechanisms to detect symptomatic people returning from China and placed them in isolation and testing them for COVID-19. Soon it was apparent that the infection could be transmitted from asymptomatic people and also before onset of symptoms. Therefore, countries including India who evacuated their citizens from Wuhan through special flights or had travellers returning from China, placed all people symptomatic or otherwise in isolation for 14 d and tested them for the virus.

Cases continued to increase exponentially and modelling studies

Other laboratory investigations are usually non specific. The white cell count is usually normal or low. There may be lymphopenia; a lymphocyte count <1000 has been associated with severe disease. The platelet count is usually normal or mildly low. The CRP and ESR are generally elevated but procalcitonin levels are usually normal. A high procalcitonin level may indicate a bacterial co-infection. The ALT/AST, prothrombin time, creatinine, D-dimer, CPK and LDH may be elevated and high levels are associated with severe disease.

The chest X-ray (CXR) usually shows bilateral infiltrates but may be normal in early disease. The CT is more sensitive and specific. CT imaging generally shows infiltrates, ground glass opacities and sub segmental.

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.

9 VACCINES

The strange coronavirus outbreak in the Chinese city of Wuhan, now termed COVID-19, and its rapid transmission, threatens people around the world. Because of its pandemic nature, the National Institutes of Health (NIH) and pharmaceutical companies are involved in the development of COVID-19 vaccines. Xu Nanping, China's vice-minister of science and technology, announced that the first vaccine is expected to be ready for clinical trials in China at the end of April 2020.[54] There is no approved vaccine and treatment for COVID-19 infections.

Vaccine development is sponsored and supported by the Biomedical Advanced Research and Development Authority (BARDA), a component of the Office of the Assistant Secretary for Preparedness and Response (ASPR). Sanofi will use its egg-free, recombinant DNA technology to produce an exact genetic match to proteins of the virus.[55]

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. As of 13 May 2020, SARS-CoV-2 confirmed cases have been reported in more than 210 countries apart from China (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.

Assessed intrauterine vertical transmission of COVID-19 infection in nine infants born to infected mothers, found that none of the infants tested positive for the virus.[45] Likewise, there was no evidence of intrauterine infection caused by vertical transmission in the SARS and MERS epidemics.[43]

The CDC asserts that infants born to mothers with confirmed COVID-19 are considered persons under investigation (PUI) and should be temporarily separated from the mother and isolated.[46]

7.1 Breastfeeding and infant care

The data available to date is limited and cannot confirm whether or not COVID-19 can be transmitted through breast milk.[40] Assessing the presence of COVID-19 in breast milk samples from six patients showed negative result.[45] The CDC points out that in case of a confirmed or suspected COVID-19 infection, the decision of whether or how to start or continue breastfeeding should be made by the mother in collaboration with the family and healthcare practitioners.[47] Careful precautions need to be taken by the mother to prevent transmitting the disease to her infant through respiratory droplets during breastfeeding.

RBD, indicating its potential as a therapeutic agent in the management of COVID-19. It can be used alone or in combination with other effective neutralizing antibodies for the treatment and prevention of COVID-19 (202). Furthermore, SARS- CoV-specific neutralizing antibodies, like m396 and CR3014, failed to bind the S protein of SARS-CoV- 2, indicating that a particular level of similarity is mandatory between the RBDs of SARS-CoV and SARS-CoV-2 for the cross-reactivity to occur.

Further assessment is necessary before confirming the effectiveness of such combination therapy. In addition, to prevent further community and nosocomial spread of COVID-19, the postprocedure risk management program should not be neglected (309). Development of broad-spectrum inhibitors against the human coronaviral pathogens will help to facilitate clinical trials on the effectiveness of such inhibitors against endemic and emerging coronaviruses (203). A promising animal study revealed the protective effect of passive immunotherapy with immune serum from MERS-immune camels on mice infected with MERS-CoV (204). Passive immunotherapy using convalescent plasma is another strategy that can be used for treating COVID-19-infected, critically ill patients (205).

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).

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).

At present, treatment for sepsis and ARDS mainly involves antimicrobial therapy, source control, and supportive care. Hence, the use of therapeutic plasma exchange can be considered an option in managing such severe conditions. Further randomized trials can be designed to investigate its efficacy (311).

Potential Therapeutic Agents

Potent therapeutics to combat SARS-CoV-2 infection include virus binding molecules, molecules or inhibitors targeting particular enzymes implicated in replication and transcription process of the virus, helicase inhibitors, vital viral proteases and proteins, protease inhibitors of host cells, endocytosis inhibitors, short interfering RNA (siRNA), neutralizing antibodies, MAbs against the host receptor, MAbs interfering with the S1 RBD, antiviral peptide aimed at S2, and natural drugs/medicines (7, 166, 186). The S protein acts as the critical target for developing CoV antivirals, like inhibitors of S protein and S cleavage, neutralizing antibodies, RBD-ACE2 blockers, siRNAs, blockers of the fusion core, and proteases (168).

The receptor-binding gene region appears to be very similar to that of the SARS- CoV and it is believed that the same receptor would be used for cell entry.[17]

4.1 Virion structure and its genome

Coronaviruses are structurally enveloped, belonging to the positive-strand RNA viruses category that has the largest known genomes of RNA. The structures of the coronavirus are more spherical in shape, but their structure has the potential to modify their morphology in response to environmental conditions, being pleomorphic. The capsular membrane which represents the outer envelope usually has glycoprotein projection and covers the nucleus, comprising a matrix protein containing a positive-strand RNA. Since the structure possesses 5'-capped and 3'-polyadenylated ends, it remains identical to the cellular mRNAs.[18] The structure is comprised of hemagglutinin esterase (HE) (present only in some beta-coronaviruses), spike (S), small membrane (E), membrane (M) and nucleocapsid (N).

Vitro antiviral potential of FAD-approved drugs, viz., ribavirin, penciclovir, nitazoxanide, nafamostat, and chloroquine, tested in comparison to remdesivir and favipiravir (broad-spectrum antiviral drugs) revealed remdesivir and chloroquine to be highly effective against SARS-CoV-2 infection *in vitro* (194). Ribavirin, penciclovir, and favipiravir might not possess noteworthy *in vivo* antiviral actions for SARS-CoV-2, since higher concentrations of these nucleoside analogs are

needed *in vitro* to lessen the viral infection. Both remdesivir and chloroquine are being used in humans to treat other diseases, and such safer drugs can be explored for assessing their effectiveness in COVID-19 patients.

Several therapeutic agents, such as lopinavir/ritonavir, chloroquine, and hydroxychloroquine, have been proposed for the clinical management of COVID-19 (299). A molecular docking study, conducted in the RNA- dependent RNA polymerase (RdRp) of SARS-CoV-2 using different commercially available 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).

CONCLUDING REMARKS

Several years after the global SARS epidemic, the current SARS-CoV-2/COVID-19 pandemic has served as a reminder of how novel pathogens can rapidly emerge and spread through the human population and eventually cause severe public health crises. Further research should be conducted to establish animal models for SARS-CoV-2 to investigate replication, transmission dynamics, and pathogenesis in humans. This may help develop and evaluate potential therapeutic strategies against zoonotic CoV epidemics. Present trends suggest the occurrence of future outbreaks of CoVs due to changes in the climate, and ecological conditions may be associated with human-animal contact. Live-animal markets, such as the Huanan South China Seafood Market, represent ideal conditions for interspecies contact of wildlife with domestic birds, pigs, and mammals, which substantially increases the probability of interspecies transmission of CoV infections and could result in high risks to humans due to adaptive genetic recombination in these viruses (323-325). The COVID-19-associated symptoms are fever, cough, expectoration, headache, and myalgia or fatigue.

SARS-CoV-2 invades the lung parenchyma, esulting 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).

216 countries and regions from all six continents had reported more than 20 million cases of COVID-19, and more than 733,000 patients had died[21]. High mortality occurred especially when health-care resources were overwhelmed. The USA is the country with the largest number of cases so far.

Although genetic evidence suggests that SARS-CoV-2 is a natural virus that likely originated in animals, there is no conclusion yet about when and where the virus first entered humans. As some of the first reported cases in Wuhan had no epidemiological link to the seafood market[22], it has been suggested that the market may not be the initial source of human infection with SARS-CoV-2. One study from France detected SARS-CoV-2 by PCR in a stored sample from a patient who had pneumonia at the end of 2019, suggesting SARS-CoV-2 might have spread there much earlier than the generally known starting time of the outbreak in France[23]. However, this individual early report cannot give a solid answer to the origin of SARS-CoV-2 and contamination, and thus a false positive result cannot be excluded. To address thishighly controversial issue, further retrospective

investigations involving a larger number of banked samples from patients, animals and environments need to be conducted worldwide with well-validated assays.

Genomics, phylogeny and taxonomy

As a novel betacoronavirus, SARS-CoV-2 shares 79% genome sequence identity with SARS-CoV and 50% with MERS-CoV[24]. Its genome organization is shared with other betacoronaviruses. The six functional open reading frames (ORFs) are arranged in order from 5' to 3': replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N). In addition, seven putative ORFs encoding accessory proteins are interspersed between the structural genes[25].

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.

All the patients were either asymptomatic (9%) or had mild disease. No severe or critical cases were seen. The most common symptoms were fever (50%) and cough (38%). All patients recovered with symptomatic therapy and there were no deaths. One case of severe pneumonia and multiorgan dysfunction in a child has also been reported [19]. Similarly the neonatal cases that have been reported have been mild [20].

Diagnosis [21]

A suspect case is defined as one with fever, sore throat and cough who has history of travel to China or other areas of persistent local transmission or contact with patients with similar travel history.

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).

Considering the zoonotic links associated with SARS-CoV-2, the One Health approach may play a vital role in the prevention and control measures being followed to restrain this pandemic virus (317-319). The substantial importation of COVID-19 presymptomatic cases from Wuhan has resulted in independent, self- sustaining outbreaks across major cities both within the country and across the globe. The majority of Chinese cities are now facing localized outbreaks of COVID-19

(231). Hence, deploying efficient public health interventions might help to cut the spread of this virus globally.

The occurrence of COVID-19 infection on several cruise ships gave us a preliminary idea regarding the transmission pattern of the disease. Cruise ships act as a closed environment and provide an ideal setting for the occurrence of respiratory disease outbreaks. Such a situation poses a significant threat to travelers, since people from different countries are on board, which favors the introduction of the pathogen (320). Although nearly 30 cruise ships from different countries have been found harboring COVID-19 infection, the major cruise ships that were involved in the COVID-19 outbreaks are the *Diamond Princess*, *Grand Princess*, *Celebrity Apex*, and *Ruby Princess*.

The COVID-19 outbreak has also been associated with severe economic impacts globally due to the sudden interruption of global trade and supply chains that forced multinational companies to make decisions that led to significant economic losses (66). The recent increase in the number of confirmed critically ill patients with COVID-19 has already surpassed the intensive care supplies, limiting intensive 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 1 month (28 days) of the outbreak. This is impressively fast compared to the time taken to identify SARS-CoV reported in Foshan, Guangdong Province, China (125 days) (68). Immediately after the confirmation of viral etiology, the Chinese 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).

Suffering from novel SARS-CoV-2, with more than 4,170,424 cases and 287,399 deaths across the globe. There is an urgent need for a rational international campaign against the unhealthy food practices of China to encourage the sellers to increase hygienic food practices or close the crude live-dead animal wet markets. There is a need to modify food policies at national and international levels to avoid further life threats and economic consequences from any emerging or reemerging pandemic due to close animal-human interaction (285).

Even though individuals of all ages and sexes are susceptible to COVID-19, older people with an underlying chronic disease are more likely to become severely infected (80). Recently, individuals with asymptomatic infection were also found to act as a source of infection to susceptible individuals (81). Both the asymptomatic and symptomatic patients secrete similar viral loads, which indicates that the transmission capacity of asymptomatic or minimally symptomatic patients is very high. Thus, SARS-CoV-2 transmission can happen early in the course of infection (82). Atypical clinical manifestations have also been reported in COVID-19 in which the only reporting symptom was fatigue. Such patients may lack respiratory signs, such as fever, cough, and sputum (83).

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 × 105 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.

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

Notably, the reanalysis of the COVID-19 pandemic curve from the initial cluster of cases pointed to considerable human-to-human transmission. It is opined that the exposure history of SARS-CoV-2 at the Wuhan seafood market originated from human-to-human transmission rather than animal-to-human transmission (74); however, in light of the zoonotic spillover 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).

Interestingly, disease in patients outside Hubei province has been reported to be milder than those from Wuhan [17]. Similarly, the severity and case fatality rate in patients outside China has been reported to be milder [6]. This may either be due to selection bias wherein the cases reporting from Wuhan included only the severe cases or due to predisposition of the Asian population to the virus due to higher expression of ACE2 receptors on the respiratory mucosa [11].

Disease in neonates, infants and children has been also reported to be significantly milder than their adult counterparts. In a series of 34 children admitted to a hospital in Shenzhen, China between January 19th and February 7th, there were 14 males and 20 females. The median age was 8 y 11 mo and in 28 children the infection was linked to a family member.

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. 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. 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 others viruses.

The later stages of coronavirus-induced inflammatory cascades are characterized by the release of proinflammatory interleukin-1 (IL-1) family members, such as IL-1 and IL-33. Hence, there exists a possibility that the inflammation associated with coronavirus can be inhibited by utilizing anti-inflammatory cytokines that belong to the IL-1 family (92). It has also been suggested that the actin protein is the host factor that is involved in cell entry and pathogenesis of SARS-CoV-2. Hence, those drugs that modulate the biological activity of this protein, like ibuprofen, might have some

therapeutic application in managing the disease (174). The plasma angiotensin 2 level was found to be markedly elevated in COVID-19 infection and was correlated with viral load and lung injury. Hence, drugs that block angiotensin receptors may have potential for treating COVID-19 infection (121). A scientist from Germany, named Rolf Hilgenfeld, has been working on the identification of drugs for the treatment of coronaviral infection since the time of the first SARS outbreak (19).

The SARS-CoV S2 subunit has a significant function in mediating virus fusion that provides entry into the host cell.

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 (258, 259).

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.

The viral loads of SARS-CoV-2 were measured using N-gene- specific quantitative RT-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 10^4 to 10^7 copies/ml during this time (151). In another study, the viral load was found to be higher in the nasal swabs than the throat swabs obtained from COVID-19 symptomatic patients (82). Although initially it was thought that viral load would be associated with poor outcomes, some case reports have shown asymptomatic individuals with high viral loads (247). Recently, the viral load in nasal and throat swabs of 17 symptomatic patients was determined, and higher viral loads were 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).

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).

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

China is also considering introducing legislation to prohibit selling and trading of wild animals [32].

The international response has been dramatic. Initially, there were massive travel restrictions to China and people returning from China/ evacuated from China are being evaluated for clinical symptoms, isolated and tested for COVID-19 for 2 wks even if asymptomatic. However, now with rapid world wide spread of the virus these travel restrictions have extended to other countries. Whether these efforts will lead to slowing of viral spread is not known.

A candidate vaccine is under development.

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.

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);

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.

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 *Rhinolophus* bat family (86). in the

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).

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).

13 CONVALESCENT PLASMA THERAPY

Guo Yanhong, an official with the National Health Commission (NHC), stated that convalescent plasma therapy is a significant method for treating severe COVID-19 patients. Among the COVID-19 patients currently receiving convalescent plasma therapy in the virus-hit Wuhan, one has been discharged from hospital, as reported by Chinese science authorities on Monday, 17th February 2020 in Beijing. The first dose of convalescent plasma from a COVID-19 patient was collected on 1st and 9th February 2020 from a severely ill patient who was given treatment at a hospital in Jiangxia District in Wuhan. The presence of the virus in patients is minimised by the antibodies in the convalescent plasma. Guiqiang stated that donating plasma may cause minimal harm to the donor and that there is nothing to be worried about. Plasma donors must be cured patients and discharged from hospital. Only plasma is used, whereas red blood cells (RBC), white blood cells (WBC) and blood platelets are transfused back into the donor's body. Wang alleged that donor's plasma will totally improve to its initial state after one or 2 weeks from the day of plasma donation of around 200 to 300 millilitres.[61]

Such instance was in 2002-2003 when a new coronavirus of the ß genera and with origin in bats crossed over to humans via the intermediary host of palm civet cats in the Guangdong province of China. This virus, designated as severe acute respiratory syndrome coronavirus affected 8422 people mostly in China and Hong Kong and caused 916 deaths (mortality rate 11%) before being contained [4]. Almost a decade later in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV), also of bat origin, emerged in Saudi Arabia with dromedary camels as the intermediate host and affected 2494 people and caused 858 deaths (fatality rate 34%) [5].

Inhibition of virus replication. Replication inhibitors include remdesivir (GS-5734), favilavir (T-705), ribavirin, lopinavir and ritonavir. Except for lopinavir and ritonavir, which inhibit 3CLpro, the other three all target RdRp[128,135] (FIG. 5). Remdesivir has shown activity against SARS-CoV-2 in vitro and in vivo[128,136]. A clinical study revealed a lower need for oxygen support in patients with COVID-19 (REF.[137]). Preliminary results of the Adaptive COVID-19 Treatment Trial (ACTT) clinical trial by the National Institute of Allergy and Infectious Diseases (NIAID) reported that remdesivir can shorten the recovery time in hospitalized adults with COVID-19 by a couple days compared with placebo, but the difference in mortality was not statistically significant[138]. The FDA has issued an emergency use authorization for rem- desivir for the treatment of hospitalized patients with severe COVID-19. It is also the first approved option by the European Union for treatment of adults and adoles- cents with pneumonia requiring supplemental oxygen. Several international phase III clinical trials are contin- uing to evaluate the safety and efficacy of remdesivir for the treatment of COVID-19.

Favilavir (T-705), which is an antiviral drug devel- oped in Japan to treat influenza, has been approved in China, Russia and India for the treatment of COVID-19. A clinical study in China showed that favilavir signif- icantly reduced the signs of improved disease signs on chest imaging and shortened the time to viral clearance[139]. A preliminary report in Japan showed rates of clinical improvement of 73.8% and 87.8% from the start of favilavir therapy in patients with mild COVID-19 at 7 and 14 days, respectively, and 40.1% and 60.3% in patients with severe COVID-19 at 7 and 14 days.

In this cohort of patient, around 2.3% people died within a median time of 16 days from disease onset[9,86]. Men older than 68 years had a higher risk of respiratory failure, acute cardiac injury and heart failure that led to death, regardless of a history of cardiovascular disease6 (FIG. 4). Most patients recovered enough to be released from hospital in 2 weeks[9,80] (FIG. 4).

Early transmission of SARS-CoV-2 in Wuhan in December 2019 was initially linked to the Huanan Seafood Wholesale Market, and it was suggested as the source of the outbreak[9,22,60]. However, community transmission might have happened before that[88]. Later, ongoing human-to-human transmission propagated the outbreak[9]. It is generally accepted that SARS-CoV-2 is more transmissible than SARS-CoV and MERS-CoV; however, determination of an accurate

reproduction number (R0) for COVID-19 is not possible yet, as many asymptomatic infections cannot be accurately accounted for at this stage[89]. An estimated R0 of 2.5 (ranging from 1.8 to 3.6) has been proposed for SARS-CoV-2 recently, compared with 2.0-3.0 for SARS-CoV20. Notably, most of the SARS-CoV-2 human-to-human transmission early in China occurred in family clusters, and in other countries large outbreaks also happened in other settings, such as migrant worker communities, slaughter-houses and meat packing plants, indicating the necessity of isolating infected people[9,12,91-93]. Nosocomial transmission was not the main source of transmission in China because of the implementation of infection control measures in clinical settings[9].

Effective management of COVID-19 would be difficult for low-income countries due to their inability to respond rapidly due to the lack of an efficient health care system (65). Controlling the imported cases is critical in preventing the spread of COVID-19 to other countries that have not reported the disease until now. The possibility of an imported case of COVID-19 leading to sustained human-to-human transmission was estimated to be 0.41. This can be reduced to a value of 0.012 by decreasing the mean time from the onset of symptoms to hospitalization and can only be made possible by using intense disease surveillance systems (235). The silent importations of infected individuals (before the manifestation of clinical signs) also contributed significantly to the spread of disease across the major cities of the world. Even though the travel ban was implemented in Wuhan (89), infected persons who traveled out of the city just before the imposition of the ban might have remained undetected and resulted in local outbreaks (236). Emerging novel diseases like COVID-19 are difficult to contain within the country of origin, since globalization has led to a world without borders.

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.