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COVID-19 Pandemic: Current Scenario, Challenges and Future Perspectives

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Abstract: Background: The new public health emergency of COVID-19 caused by a novel Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which originated in Wuhan, Hubei province, China in December 2019, evolved into a pandemic in no time and is still in progression. The novel virus mainly targets the lower respiratory system, leading to viral pneumonia, with other associated complications of multi organ failure.

Discussion: The bats, in particular Rhinolophus affinis, is a natural host of SARS-CoV-2 and the virus is considered to have spread to humans through yet controversial intermediate host pangolins. The incubation period ranges from 2-14 days and mode of person-to-person transmission is primarily via the direct contact with the infected person or through the droplets generated by the infected person during coughing or sneezing. The initiation of the infection process by SARS-CoV-2 virus is the invasion of lung type II alveolar cells via a receptor protein called angiotensin-converting enzyme 2 (ACE2) present on the cell membrane with glycosylated spike (S) viral protein that mediates host cell invasion. The main diagnostic tools employed are molecular methods based on nucleic acid detection engaging real-time quantitative polymerase chain reaction (RT-qPCR) and a new immunoassays based on antibodies IgM/IgG.

Conclusion: Due to the lack of specific clinically approved anticovid-19 drugs or vaccines that could be used for its prevention or treatment, the current management approach is essentially supportive and symptomatic. The precautionary measures like, social distancing, cleaning hands with soap or sanitizers, using disinfectant solutions to decontaminate the surfaces of things and proper ventilation, wearing masks and other protective gears to curb transmission. The knowledge regarding COVID-19 therapies is still evolving and collaborative efforts are being put in to discover definitive therapies on different themes in the form of vaccines, repurposing drugs, RNA interference, docking studies, etc.

Keywords: SARS-CoV-2, pneumonia, drug repurposing, vaccines, microRNAs, pathogenic febrile respiratory infection.

1. INTRODUCTION

The outbreak of the highly pathogenic febrile respiratory infection termed as Novel Coronavirus Disease (COVID-19) is the most concerning public health emergency of the 21st century so far. The causative agent of COVID-19 pandemic was identified by the Chinese authorities on January 7, 2020, to be a new category of beta coronaviruses. The World Health Organization temporarily named this viral pneumonia like disease as 2019-nCoV on January 12, 2020 which later on was officially substituted with the name ‘COVID-19’. Based on the structure of virus and genetics, the International Committee on Taxonomy specified the name ‘severe acute respiratory syndrome coronavirus-2’ (SARS-CoV-2) for this newly identified coronavirus on February 11, 2020 [1].

Ever since the first reports of the outbreak of COVID-19 came in December 2019 in Wuhan city of China, the virus has been propagating all over the world, affecting more than 188 countries/regions. As of 17 July 2020, there are more than 13 million cases (13,818,963 of COVID-19 with 602,657 deaths having been reported [2] with continuous escalation of positive cases. The vulnerability to COVID-19 seems to be allied with age (poor immune function as in older people) and other health conditions like renal or hepatic dysfunction [3, 4].

Currently, there are no effective drugs targeting SARS-CoV-2, nor any vaccines for its prevention [5]. In such a situation, the medical interventions mainly rely on the isolation
of the COVID-19 affected individuals and providing symptomatic treatment for the patients representing a mild disease and increase in severity of the disease requiring ventilator support/oxygen therapy.

Since the knowledge about the SARS-CoV-2 is rapidly evolving and the future course of this virus is still unknown, this article provides the holistic overview of the current scenario of this pandemic disease with epidemiology, clinical characteristics, diagnosis and potent treatment options that may help to restrain the disease for global health and economic stability.

2. CORONAVIRUSES PATHOPHYSIOLOGY

Coronaviruses (CoVs) are pathogens posing serious health complications in the form of respiratory, hepatic, enteric, and central nervous diseases in humans and animals. The zoonotic coronaviruses, namely HCoV-HKU1, NL63, 229E and OC43, have historically been in circulation and infecting the respiratory tract of mammals, including humans leading to the mild common cold or severe respiratory tract infections [6, 7]. However, the past two decades have witnessed the emergence of the extremely pathogenic human coronaviruses (HCoVs), with high morbidity and mortality [8]. In 2002-2003, the occurrence of Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) affected more than 8,000 people worldwide with a death rate of 10%, while the Middle East respiratory syndrome coronavirus (MERS-CoV), emerging from animal pool in 2012, has a fatality rate of 36% with 2,500 confirmed cases till November 2019 according to the World Health Organization (WHO) [9]. In December 2019, a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), probably as zoonotic mediator (from animal to human), was reported from the Wuhan city of China named as SARS-CoV-2. This novel virus mainly targets the lower respiratory system leading to viral pneumonia, with other associated complications of multi organ failure with prime targets being kidney, liver, heart and central nervous system [6]. As per the current knowledge, the SARS-CoV-2 represents higher transmissibility, pandemic risk and contagious nature than SARS-CoV, and till date has created a havoc throughout the world affecting more than 188 countries/regions possibly due to human-to-human transmission [4, 10]. As of 17 July 2020, there are more than 13 million cases (13,818,963) of COVID-19 with 602,657 deaths having been reported [2] as represented by (Figs. 1a and b).

Coronaviruses (*corona* in Latin means crown), belong to the family *Coronaviridae* in the order *Nidovirales* and are enveloped, spherical pleomorphic positive sense RNA viruses ranging in size from 80 to 160nm [11]. The viruses have spike projections on the surface, giving the appearance of having crowns and hence named so. These viruses have many natural hosts and are proficient in adapting to new environments through mutations. Due to their high prevalence, large genetic diversity, frequent recombination in their genomes, along with the increasing activity at the human-animal interface, these viruses represent an enduring threat to human health [12]. On the basis of serotype antigenic cross-reactivity and genome features, coronavirus subfamily is grouped into four genera: α, β, γ, and δ. The α, and β, coronaviruses infect mammals, γ coronaviruses infect birds, and δ elicit infection in both. SARS-CoV-2, responsible for COVID-19 disease, belongs to the β-coronavirus subfamily and is characterized by a spherical morphology having spike protrusions on the surface with a diameter of approximately 60–140nm. SARS-CoV-2 became the seventh member of the β family and prior to it, six coronaviruses were already known to infect humans, including SARS-CoV and MERS-CoV.

![Fig. 1](image-url)

Fig. (1). (a) Globally confirmed cases of the novel coronavirus (SARS-CoV-2) [4] and graphical representation of the confirmed case and deaths related to most affected countries (b). *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.
Recent studies have shown high sequence >96% homology of SARS-CoV-2 with the bat SARS-like coronavirus (S-L-CoV), >75% genetic similarity with that of SARS-CoV and about 50% sequence identity with MERS-CoV [13-15]. The genome of SARS-CoV-2 is approximately 30kB single-stranded RNA, packed inside the nucleocapsid protein (N) and is further bounded by an outer envelope. Associated with the viral envelope are membrane protein (M), the envelope protein (E), and the spike protein (S). The two-thirds of SARS-CoV-2 genes, designated as ORF1α-ORF1b, encode for the viral RNA-dependent RNA Polymerase (RdRP), RNA synthesis materials and all the non-structural polyproteins including coronavirus main protease (3CLpro), and papain-like protease (PLpro) [16, 17], that are not implicated in host response modulation [18]. The rest one-third of the viral genes encode for the four structural proteins, envelope (E), membrane (M), nucleocapsid (N) involved in virus assembly and spike (S) mediating virus entry into host cells. The spike glycoprotein is composed of two subunits (S1 and S2), which form homotrimers on the viral surface, guiding the link to host receptors and inducing host immune responses. Importantly, in SARS-CoV-2, the S2 subunit of spike glycoprotein is highly conserved and so could be a target for antiviral compounds [19]. The variability in the length of CoV genome for ORF1ab and the structural proteins is mostly associated with the size and number of accessory proteins [20].

The ORF1ab of CoV genome is employed for the production of polyproteins that are processed by proteolytic cleavage into 16 proteins entitled nsp1-16 performing their individual functions [21]. The viral genome duplication is ensured by RNA-dependent RNA polymerase (RdRp) and RNA helicase activity harbored in nsp12 protein and nsp13 protein, respectively, while nsp14 protein possesses the activities of exoribonuclease (exoN) and N7-methyltransferase [20, 22]. Nidoviral ribonuclease specific for U (uracil) is housed within the nsp15 protein while nsp16 protein has a SAM-dependent O-methyltransferase activity [18]. However, for all structural proteins along with accessory proteins, a discontinuous transcription is employed to produce a subset of 7-9 sub-genomic RNAs. The host immune response is further suppressed by the deubiquitination of certain host cell proteins, including NF-κB and interferon factor 3 by PLpro functioning as deubiquitinase [23].

As per the information available on NextStrain, multiple strains of the coronavirus SARS-CoV-2 are migrating the globe [24]. This proficiency of coronaviruses in adapting swiftly to new hosts through in vivo genetic recombination, and mutations can be attributed to RNA-dependent RNA 5 polymerase responsible for viral genome replication with an intrinsic error rate of 1,000,000 mutation/site/replication. The genomic analysis of SARS-CoV-2 has revealed the evolution of virus into two major well-defined types designated as L and S types, due to two different SNPs and so far, only an 11-base pair change has been identified in the virus's most divergent strains sequenced till date. S type is considered as the ancestral version of the virus, being less prevalent (~30%), as compared to the L type, which being more prevalent (~70%) is believed to be more aggressive due to human intervention [25].

Human coronaviruses (HCoVs) infection results in the Acute Respiratory Distress Syndrome (ARDS), leading to enduring diminution of lung function, cardiac dysrhythmia and ultimately death [26, 27]. The initiation of the infection process by SARS-CoV-2 virus is the invasion of lung type II alveolar cells via a receptor protein called angiotensin-converting enzyme 2 (ACE2) present on the cell membrane with a glycosylated spike (S) viral protein that mediates host cell invasion as schematically represented in Fig. (2) [16, 28-30]. As compared with the SARS-CoV S protein, the interaction of SARS-CoV-2 S protein to ACE2 is about 10–20 times higher, as revealed by the Cryo-EM structure analysis [30, 31]. The higher affinity of the SARS-CoV-2 S protein appears to aid in a more efficient spread of the virus enhancing its pandemic prospective and hence rendering it difficult to control. The viral genome is encapsulated and released into the host cell as single-stranded positive RNA, polyadenylated and encoding for the structural and non-structural polypeptides [16, 17].

3. TRANSMISSION, SOURCES AND SURVIVAL OF COVID-19

The COVID-19 pandemic, as declared by WHO on March 11 2020, is believed to have originated from the animal source as the first cases of the disease were linked to direct exposure of people to the wet animal market of Wuhan City in China, which is regarded as the epicenter of this disease. The bats, in particular species of the genus *Rhinolophus*, are considered as natural reservoir hosts for SARS-CoV and SARS-CoV-2 viruses [32]. *Rhinolophus affinis* is a natural host of SARS-CoV-2. Initially, evidence proposed that the two snake species, Chinese krait and Chinese cobra, might be the possible reservoirs, with the data supporting that civet cats or raccoon dogs may be the possible intermediate sources SARS-CoV [12]. But, as efforts are being continuously put in to identify the reservoir host or intermediate carriers associated with the spillover dynamics, these previous assumptions stand discarded [31, 33].

A recent study suggests that pangolins (scaly, anteater-like animals) are the prime suspected missing link for SARS-CoV-2 spread between bats and humans. The pangolin coronavirus has just five dissimilar amino acids from SARS-CoV-2 on the spike protein, yet existence of some additional intermediate hosts is also expected [34]. Even though animal-to-human transmission is presumed as the primary mechanism of spread, subsequent cases are linked with different exposure means [35-37].

Studies have concluded that the virus transmission from infected human, both symptomatic as well as asymptomatic, is the main source of COVID-19 spread as positive cases were found that did not visit the Wuhan wet market [38, 39]. The mode of person-to-person transmission is primarily via the direct contact with the infected person or through the droplets generated by the infected person during coughing or sneezing that may even occur before the infection of sym-
Fig. (2). The life cycle of SARS-CoV-2. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Symptoms from an asymptomatic individual. The transmission of the infection from an asymptomatic person during the prodromal period of COVID-19 has been reported [40], and studies have shown no difference between symptomatic and asymptomatic persons in the viral burdens except that the nasal cavity having higher viral loads compared to the throat [41]. The first non-Chinese case was reported on January 13, 2020 from Thailand having no epidemiologic connection with Wuhan wet market [42], with cases from other overseas countries pouring in [43]. A single asymptomatic UK citizen transmitted the infection to 11 healthy individuals while staying in a resort in the French Alps and had returned from a conference in Singapore [44].

The incubation period for COVID-19 varies from 2 to 14 days [median 5 days], with Basic Case Reproduction rate (BCR) to be estimated around 2 to 6.47 as per various modelling studies [45], compared to 2 and 1.3 in the case of SARS and pandemic flu H1N12009, respectively [46]. COVID-19 has affected all age groups from infants of 112 days old to elderly people above 100 years of age [47].

Since pregnant women are susceptible to respiratory infections, the trans-placental transmission is a concern, however, as per the currently available information, no mother to fetal transmission has been reported [48]. According to the study conducted on COVID-19 infected women in their third trimester show no evidence of mother to child transmission in case of cesarean sections, keeping the question about the possibility of transmission during vaginal birth [49].

Now evidence is pouring in that the actual elements responsible for the carriage of virus from one human to another are the respiratory droplets produced by the infected person which are also as per WHO, the mode of transmission of SARS viruses and virtually all upper respiratory tract pathogens [50]. During sneezing or coughing, respiratory droplets produced can spread up to 1-2 meters as represented in Fig. (3), and may deposit on the surfaces which, when touched, spread COVID-19 [51]. A recent investigation of air samples of COVID-19 hospital wards suggested the presence of virus-laden aerosols up to four meters [52]. The transmission appears to occur when viral excretion is at peak usually during the second week of the illness, supporting the airborne spread due to the small particle aerosols that can settle in the mouth or nasal mucosa and lungs after inhalation as represented in Fig. (4) [12, 53].

Several studies with the human coronaviruses (HCoVs), like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) and Middle East Respiratory Syndrome have revealed that Human-to-human transmission is facilitated by the contaminated
hands or surfaces by droplets and small particle aerosols that an infected person produces during sneezing or coughing. SARS-CoV-2 like other human coronaviruses (HCoVs), can remain viable for up to 3hrs in air as micro droplets (aerosols) [54] and from hours to days (up to 9 days) on inanimate surfaces ranging from 24hrs on porous surfaces like fabric, cardboard and sponge, up to 72hrs on non-porous hard and shiny surfaces like glass, stainless steel, aluminum, copper and varnished wood under favorable atmospheric conditions [54-56]. The transmission via feco-oral route is also hypothesized since the presence of the virus in stool has been corroborated [57]. However, the coronaviruses contaminated surfaces can be efficiently decontaminated in less than minutes by the commonly used disinfects like 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite, while other biocidal agents such as 0.05-0.2% benzalkonium chloride, 0.02% chlorhexidinedigluconate and 0.55% orthophtalaldehyde are effective to a lesser extent [55].
4. CLINICAL PRESENTATION

The coronaviruses target the respiratory tract of humans leading to a spectrum of illness with diverse severity profiles oscillating from a mild infection to severe pneumonia. The SARS-CoV-2 primarily invades alveolar epithelial cells of lungs, stimulating robust innate immune response, resulting in respiratory features parallel to the clinical aspects of infections inflicted by SARS CoV and MERS CoV [42].

SARS-CoV-2 infection elicits both innate and adaptive immune response, with hallmarks of lymphocytopenia and an alteration in total neutrophils. In comparison to healthy controls, a striking upsurge of at least 14 cytokines in COVID-19 patients has been elucidated compared with healthy controls. The dysregulated pro-inflammatory host response as reflected by massive cytokine (IL-6, IL-1β, IL-2, IL-8, IL-17, GM-CSF, TNFα etc.) and chemokines release (cytokine storm), has been postulated to instigate an immune pathology resulting in the rapid course of acute lung injury and ARDS, thus specifying that the cytokine storm might be allied with disease severity. Elevated levels of IL-6 have been consistently reported in plasma of COVID-19 patients and are presumed to be allied with poor prognosis. B cells and Natural Killers (NK) cells have been reported in COVID-19 patients. Talking about humoral immunity, IgM and IgA antibodies have been detected during early phase of infection, whereas IgG can be detected about 14 days after the occurrence of initiation of symptoms. Yet, the question of how long the shielding levels of these antibodies will prevent disease recurrence, is still debatable and a topic of more research [58].

When the clinical course manifests, patients infected with COVID-19 demonstrate a protean nature of clinical indicators ranging from fever, headache, cough, hemoptysis, dyspnea, sore throat, anosmia and dysgeusia [59]. Other associated complications like myalgia, breathlessness, Acute Respiratory Distress Syndrome (ARDS) and multi organ dysfunction leading to death, have also been identified [60]. The patients hospitalized with COVID-19 also displayed convulsions, confusion, nausea, vomiting, loss of smell and chest pain. Mild COVID-19 infections have been managed with the available drugs whereas cases with severe pneumonia mandate intensive care with oxygen therapy and in more critical cases progressing to Acute Respiratory Distress Syndrome (ARDS), mechanical ventilation is compulsory [60-63]. SARS-CoV-2 Infection is also associated with blood clot formation thereby causing local rupture and haemorrhage into tissues by weakened blood vessel walls [64].

As per the study published in American Journal of Gastroenterology, diarrhea with other intestinal symptoms is present in COVID-19 patients, even before the respiratory symptoms emerge. This also points to the fact that COVID-19 infection could be suspected even in the absence of respiratory symptoms suggesting that complete clinical manifestation of COVID-19 may have more to it [65].

The impact of this disease has been found to be significantly placid in children with infants being either asymptomatic or having mild representation with fever and cough [66]. No severe or critically ill neonates affected with COVID-19 has been reported, signifying it to be milder on them as compared to their adult counterparts [67].

As per the studies based on hospitalized patients with SARS-CoV-2 the median incubation period was 4 days (interquartile range, 2 to 7). The fever came up to be as the initial symptom with the most common symptoms being sore throat, cough, dyspnea, myalgia, headache pharyngalgia [60], rhinorrhea 4.0% and diarrhea [68].

In some cases, the onset of complications encompassing pneumonia, Acute Respiratory Distress Syndrome (ARDS), pneumothorax, acute heart and kidney damage and secondary infections have been seen to appear after a week of infection having already passed over [60] requiring intensive care unit admission [61].

The more sternness of COVID-19 in patients with heart or hypertension problems might be allied with augmented secretion of ACE2 (functional binding receptor of SARS-CoV-2) compared to healthy individuals. At the same time, ACE2 levels can get increased by the use of anti-hypertension therapy of renin–angiotensin–aldosterone system inhibitors putting a question mark on use of these in patients with COVID-19 [69].

Nevertheless, there have been also the reports of patients infected with SARS-CoV-2 being initially asymptomatic till the incubation period ranging between 2 to 14 days (median 4 days), or can remain asymptomatic all the way through the progression of the disease [70-72]. The first asymptomatic COVID-19 positive cases were diagnosed on the basis of positive viral nucleic acid test results, while being neutral for COVID-19 symptoms such as fever, cough, diarrhea and respiratory symptoms, with no prominent aberration on chest radiograph [73, 74].

5. DIAGNOSIS OF SARS-COV-2

The diagnosis of COVID-19 like other coronavirus infections (SARS and MERS), is centered on a detailed epidemiological history, contact, travel and clinical manifestations such as fever, dyspnea, cough and respiratory symptoms and viral pneumonia [72]. The main diagnostic tools employed, are molecular methods based on nucleic acid detection engaging real-time quantitative polymerase chain reaction (RT-qPCR) and high-throughput sequencing demanding BS-L-2 facilities, and serological or biochemical analysis of coronavirus in blood/viral cultures requiring minimum BS-L-3 facilities [75].

Presently, the commercial tests are not available, so WHO has ratified the nucleic acid amplification tests (NAAT) carried out by real-time quantitative polymerase chain reaction (RT-qPCR) or RT-PCR (reverse transcription) as the most efficient and straightforward technique for identification of SARS-CoV-2 infection [76]. This test employs the set of available gene targets for the SARS-CoV-2 genome as described in Table 1. [77]. The analysis can be done on symptomatic patients or the persons who have estab-
lished contact with a COVID-19 positive case so as to curb the spread of the virus. PCR is executed on the samples, collected in viral transport media, from the nasopharyngeal swab, oropharyngeal swabs, sputum, deep tracheal aspirate and bronchoalveolar lavage of the subjects [78] as described in Table 2, [77].

Table 1. The target gene (s) locations employed for the detection of SARS-CoV-2 by PCR [80].

<table>
<thead>
<tr>
<th>Institute</th>
<th>Gene Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>China CDC, China</td>
<td>ORF1ab and N</td>
</tr>
<tr>
<td>Charité, Germany</td>
<td>RdRP, E, N</td>
</tr>
<tr>
<td>HKU, Hong Kong SAR</td>
<td>ORF1b-nsp14, N</td>
</tr>
<tr>
<td>Institut Pasteur, Paris, France</td>
<td>Two targets in RdRP</td>
</tr>
<tr>
<td>National Institute of Infectious Diseases, Japan</td>
<td>Pancorona and multiple targets, Spike protein</td>
</tr>
<tr>
<td>National Institute of Health, Thailand</td>
<td>N</td>
</tr>
<tr>
<td>US CDC, USA</td>
<td>Three targets in N gene</td>
</tr>
</tbody>
</table>

Enteric involvement has also been reported in patients as electron microscopy has revealed presence of SARS coronavirus inside enterocytes. So stool samples, currently not well studied, also require monitoring [79].

The specimen for analysis are to be stored at 2-8°C and shipped overnight to testing center on an ice pack with proper labels possessing unique specimen ID, specimen type, date of the sample collection, etc. The test samples from lower respiratory tract, contain significantly higher viral load and genome fraction as compared to the samples from upper respiratory tract [80]. The Chinese Center for Disease Control and Prevention (China CDC) recommends the detection of two gene regions ORF1ab and N for SARS-CoV-2 by RT-qPCR [77]. In the United States, the CDC endorses two nucleocapsid protein targets [N1 and N2], and a patient is confirmed as positive case when both the targets are detected. The first line confirmatory assay recommendations from WHO involves finding of the envelope (E) gene and RNA-dependent RNA polymerase (RdRp) gene [79]. Depending on the type of clinical sample and protocol employed, the detection of COVID-19 using RT-qPCR, a sensitivity of only 50%-79% is achievable [81]. The current enormous screening of people worldwide every day for the SARS-CoV-2, is cumbersome with lengthy waiting time for results, along with the safety alarms. This has raised the need for development of broad screening, easy and reliable detection tests such as identification by Nucleic Acid Amplification Tests (NAAT).

Table 2. Sources of specimens to be collected from symptomatic patients and contacts [80].

<table>
<thead>
<tr>
<th>Test</th>
<th>Target</th>
<th>Type of Sample</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact COVID-19 case</td>
<td>Nasopharyngeal and oropharyngeal swabs.</td>
<td>Within incubation period of last documented contact.</td>
</tr>
<tr>
<td>Serology</td>
<td>Patient</td>
<td>Consider stools, whole blood, urine, and if diseased, material from autopsy. Serum for serological testing once validated and available.</td>
<td>Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be established).</td>
</tr>
<tr>
<td></td>
<td>Contact COVID-19 case</td>
<td>Serum for serological testing once validated and available.</td>
<td>Baseline serum taken as early as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last contact (optimal timing for convalescent sample needs to be established).</td>
</tr>
</tbody>
</table>
The Abbott industries launched the rapid testing platform based on isothermal nucleic acid amplification technology named as ID NOW™ COVID-19 test, within just a week after the launch of Abbott m2000™ RealTime SARS-CoV-2 EUA test, which runs on the m2000™ RealTime System, delivering positive results within five minutes and negative results in 13 minutes. These kits may reach the market soon and are expected to ramp up testing rate [82].

The scientists are pushing limits to make expression of the viral protein in the native structure possible in non-native systems so that the precise antibodies recognizing these could be generated [83]. A new diagnostic test for COVID-19 infection has been developed based on immunosassays utilizing antibodies IgM/IgG that are raised by the immune system in response to the viral infection. The efficacy of this rapid test kit is still questionable as there might be false positive results due to a possibility of cross-reactivity with other coronaviruses. However, these COVID-19 IgG/IgM Rapid Test Cassette/kits that have been currently approved in US by US-FDA/CE-IVD requiring finger prick, whole blood, plasma or serum samples could help in screening large groups having the detection window of 3-5 days and 7 days for symptomatic and asymptomatic individuals respectively [84]. The results could be further authenticated by Nucleic Acid Amplification Tests (NAAT) in case of false positive or negative results. The evaluation by radiological technique like, CT scans and High-Resolution CT (HRC-T) along with Chest X-ray (CXR) is vital for early on diagnosis and appraisal of disease severity of SARS-CoV-2 infected patients [85, 86]. Eventhough Chest X-ray (CXR) may appear normal in the early disease but evident bilateral infiltrates in the later stages are visible [87]. However, being more sensitive and specific, the CT imaging of SARS-CoV-2 infected patients [88] demonstrates infiltrates, bilateral pulmonary ground-glass, consolidative pulmonary opacities, and sometimes a rounded morphology along with peripheral lung distribution being comparable to SARS-CoV and MERS-CoV infections [89, 90].

The CT scans have proven very beneficial in identifying COVID-19 infection in suspected cases with no or mild disease symptoms or having negative molecular diagnosis which later on come up with positive results upon repeated molecular tests. Other laboratory examinations like biochemical parameters and immunological investigations have also been found to be atypical in SARS-CoV-2 patients. The elevated C-Reactive Protein (CRP), high erythrocyte sedimentation rate, enhanced prothrombin time [87], elevated levels of alanine aminotransferase, aspartate aminotransferase, creatinine kinase and D-dimer have also been reported along with lymphocytopenia, thrombocytopenia and leucopenia in COVID-19 patients [78]. The disease progression has been found to be also associated with the enhanced levels of both inflammatory and pro-inflammatory cytokines and chemokines including IL-1β, IL7, IL8, IL9, IL10, IL1RA, FGF2, GCSF, GMCSF, IP10, IFNγ, MCP1, MIP1α, MIP1β, PDGF and VEGFA [60, 61]. Studies have also pointed out association of severity of COVID-19 with that of serum levels of interleukin-6 suggesting anti-IL-6 receptor drugs to be effective [61, 91].

6. TREATMENT AND MANAGEMENT OF COVID-19

The COVID-19 global outbreak has intensely challenged all healthcare systems due to lack of specific clinically approved antiviral drugs or vaccines that could be used as potential therapy for its prevention or treatment in humans [9]. As such, the current treatment approach is essentially supportive and symptomatic based on the strategy used for other coronaviruses like SARS-CoV and MERS-CoV [7], which includes the use of broad-spectrum antivirals, conservative intravenous fluid management and supplemental oxygen. For critical care management, the WHO has also issued detailed guidelines to be followed till COVID-19 specific standardized therapeutics are established [61, 92]. On the basis of genomic organization and the molecular mechanisms of SARS-CoV-2 infection [31, 93] several existing antiviral agents like lopinavir/ritonavir, remdesivir, nucleoside analogs, neuraminidase inhibitors, umifenovir, peptide (EK1), chloroquine, ACE2-based peptides, 3C-like protease (3CLpro) inhibitors, novel vinylsulfone protease inhibitor [94], teicoplanin and RNA synthesis inhibitor [43, 94-100], do provide alternative treatment options for COVID-19 infection as these are safe for human use [95].

An attempt was made to treat a group of 75 patients with existing antiviral drugs encompassing 500mg lopinavir, 500mg ritonavir and the intravenous administration of 0.25g ganciclovir twice a day orally for 3–14 days along with oxygen therapy and mechanical ventilation in severe cases since there were reports that the use of the combination of Lopinavir/Ritonavir (LPV/RTV), ribavirin and pegylated interferon resulted in successful viral clearance in MERS-CoV case from South Korea [60]. So far, the use of Lopinavir/Ritonavir, anti-HIV drugs, for the treatment or prevention of COVID-19 have been inconclusive. Another antiviral drug remdesivir targeting RNA dependent RNA polymerase blocking viral RNA synthesis has shown promising results against SARS-CoV-2 [101] and in vitro studies in Vero E6 against nCoV-2019/BetaCoV/ Wuhan/WIV04/2019 have shown 90% effective concentration (EC90) of 1.76 mM [95]. The Washington Department of Health administration for the first time used-remdesivir intravenously and concluded that it might offer protection against SARS-CoV-2 infection [43].

The in vitro experiments in Vero E6 cells with chloroquine, an anti-malarial drug, have demonstrated that the drug functioned at both entry and at post-entry stages of COVID-19 having EC50 value of 6.90 mM [95] and some preliminary studies from China and France, have provided positive confirmations that the drug chloroquine phosphate was effective against COVID-19 related pneumonia [102]. The replication of SARS-CoV-2 was successfully blocked by remdesivir and in combination with chloroquine or interferon beta, and the treated patients were confirmed as clinically recovered [103, 104]. Both remdesivir and chloroquine have been found to be effective in controlling 2019-nCoV in vitro [95].

Albeit, clinical trials with some of these antiviral drugs evaluated against COVID-19, diminished the viral load to
bring about clinical recovery, yet their efficacy is still being investigated [104, 105]. Comprehensive studies, in controlled manner are essential to verify the therapeutic worth of these drugs and WHO has mentioned “Solidarity” clinical trial for confirmed COVID-19 cases (age ≥18 years). The treatment is supposed to be allocated between local standard of care and one of the drug(s) combination of Remdesivir, Chloroquine or Hydroxychloroquine, Lopinavir with Ritonavir or Lopinavir with Ritonavir plus Interferon beta-1a [102]. The other anti-virals like Nafamostat, galidesivir, Ritonavir or Lopinavir with Ritonavir has also revealed promising preliminary positive results during in vitro testing on clinical isolates and patients with SARS-CoV-2 infection [7, 103, 105-108]. At the same time, use of corticosteroids for the treatment of COVID-19 has not been recommended till now [109-111]. The use of passive immunization therapy using the convalescent plasma from the clinically recovered COVID-19 patients has been evaluated in Shanghai on a small number of patients and has also revealed promising preliminary positive results [112]. The information pertaining to potential COVID-19 therapies is still evolving and collaborative efforts are being put in to discover definitive therapies and therefore till then preventive methods are strictly to be followed.

Preventive measures ensure a halt of person-to-person transmission of the virus, especially in case of healthcare staff, which involves ample isolation of the patients or persons who have come in contact with the infected cases. The healthcare workers and managing persons are required to implement the protective measures such as the use of gloves, eye masks and N95 masks and other protective gears during the investigation and examination of the patients or suspected COVID-19 contact [36, 92, 113]. For general public, the simple precautionary measures like cleaning hands with soap or sanitizers, using disinfectant solutions to decontaminate the surfaces of things and proper ventilation are prescribed to curb the droplet transmission of the virus. Masks (including homemade masks to some extent) act as a physical barrier to pathogenic particulates and their use is highly recommended universally, as it has been found to minimize the respiratory aerosol transmission, the dominant route for the spread of COVID-19. Masks prevent community transmission by reducing area travelled by the exhaled breath that can spread the virus up to 6 feet away in a room, and also prevents the virus from getting into nose or mouth from contaminated hands. Infections are sometimes unknowingly spread by coughing or touching others, hence strict social distancing is also advised to decrease the chances of contracting COVID-19 [114].

7. DRUG REPURPOSING TO TACKLE COVID-19

The high transmission and death toll associated with COVID-19 along with the number of positive cases escalating daily, demands drugs with potentially shorter development timelines, therefore drug repurposing, an approach for identifying new uses for already approved or trial compounds, seems to be resourceful. For this approach to work, identification of potential target entities and their roles in SARS-CoV-2 infection is vital. Since, there is little knowledge currently available about the molecular mechanisms of the SARS-CoV-2 infection; the development of the specific therapy seems a cumbersome task, however, bioinformatics, in silico methods, artificial intelligence and supercomputer has been put to use to find the possible candidate drugs that could help in controlling this pandemic. Drug repurposing studies are mostly targeted at the interaction of the virus with the host cell receptors, its replication mechanism and disease-related molecular networks [115]. The genome sequencing of SARS-CoV-2 has led to the identification of a number of key proteins like coronavirus main protease (3CLpro), RNA-dependent RNA polymerase (RdRp), papain-like protease (PLpro), transmembrane protease serine 2 (TMPRSS2), viral spike glycoprotein (S protein), angiotensin-converting enzyme 2 (ACE2), and angiotensin receptor (AT2) as the major targets for drug development so far [116]. The network bioinformatics approach has revealed about 34 genes that could be potentially aimed at by the available drugs. In a study about 30 repurposable drugs were predicted among 78 screened available drugs with known preclinical, pharmacokinetic, pharmacodynamic, and toxicity profiles [117]. In another study utilizing the power of super computer more than 100,000 docking studies were executed to study the interaction of SARS-CoV-2 Viral Spike Protein with Human ACE2 which led to the identification of repurposable small molecules to limit SARS-CoV-2 recognition by host cells [118]. The studies related to genomic sequence analysis together with protein structure modeling, have proposed a list of potential anti-COVID-19 drugs with more focus on viral protease (3CLpro) and RNA-dependent-RNA polymerase (RdRp), as these proteins share similarities with SARS-CoV. A fixed blend of anti-HIV lopinavir–ritonavir drugs, anti-parasitic Ivermectin [119], remdesivir, previously tested with ebola virus disease, arbidol or ribavirin [120], an antimalarial chloroquine with the macrolide antibiotic azithromycin [95], viral RNA synthesis interfering favipiravir, anti-inflammatory Baricitinib [106], antiprotozoal drug emetine and hydroxychloroquine are being evaluated for their efficacy in different clinical trials to treat COVID-19. Ivermectin has been demonstrated to impede replication of SARS-CoV-2 clinical isolates perhaps through inhibition of IMPα/β1-mediated nuclear import of viral proteins [119]. Currently, WHO has selected a group of four most promising drugs as a priority for study which encompasses remdesivir; lopinavir plus ritonavir, chloroquine and hydroxychloroquine; and along with this, the additional component interferon-beta.

Remdesivir, primarily developed to fight Ebola, inhibits viral replication by targeting RNA-dependent RNA polymerase. Chloroquine and hydroxychloroquine diminish the acidity in endosomes that certain viruses use to enter a cell. Albeit, SARS-CoV-2 path of entry into a cell is different one, but studies have suggested high doses of chloroquines are potent against SARS-CoV-2 cultures. Ritonavir and lopinavir inhibit the HIV protease, responsible for cleavage of a long protein chain into peptides for assembly of new viruses. Interferon-beta, is being added for regulation of inflam-
mation associated with COVID-19 in the body [121]. The
drug APN01 (human recombinant soluble angiotensin-con
verting enzyme 2—hrsACE2) has been found to significant-
ly reduce the recovery time of SARS-CoV-2 in Vero cells,
suggesting the drug blocking the early stages of SARS-CoV-
2 infections [122]. Apart from these some other, broad-spect
rum antiviral agents, (Nitazoxanide, mycophenolic acid,
Ribavirin, cepharainthine, melfoqine, nicosamide, luteolin,
emodin, amiodarone, dasatinib, nelfinavir, ritavancin, dal-
bavancin, teicoplanin, alisporivir, imatinib, cloromazine, ca-
mostat, indomethacin, galdecevir, ganciclovir and glicyrrhi-
zin) have also been recommended as potential candidate
for repurposing against SARS-CoV-2 infection [123]. Methylprednisolone, a corticosteroid, in combination with
oseltamivir, and oxygen therapy is under study against
COVID-19 [124]. Apart from these, some of the phytothera-
peutic formulations previously used as co-adjuvant therapy
in SARS-CoV infection in 2002, are also being verified
[125].

In a novel study, the physical interaction of the 26
SARS-CoV-2 viral proteins cloned and expressed in human
cells, has led to the identification of 332 high confidences
SARS-CoV-2-human Protein-Protein Interactions (PPIs)
with 67 druggable human proteins/host factors that could
be targeted by the available drugs [126]. As the crystal struc-
ture of SARS-CoV-2 main protease Mpro became available,
the development of the inhibitors has been initiated. The de-
velopment of the potent α-ketoamide based inhibitor from
the previously available inhibitor has been reported with pos-
sible administration route being inhalative [127]. The screen-
ing with the homology protein models like 3CLpro and
RNA-dependent-RNA polymerase (RdRP) have also led to
the identification of suggested molecules that could be used
as drugs. The screening of the about 32,297 potential anti-vi-
ral phytochemicals/traditional Chinese medicinal com-
pounds on 3CLpro protease 3D homology model has identi-
fied nine hits that might serve as lead molecules for the
drug development against COVID-19 [128]. The molecular dock-
ing of nsp12 homology model has identified that methyl-
cobalamin (B12) may bind to the active site of nsp12 and
possibly serve as effective inhibitor blocking its RNA-depen-
dent-RNA polymerase (RdRP) activity [129].

Most of the above mentioned drugs are potent inhibitors
of vital mechanisms of the Coronavirus infection lifecycle.
Even though, considering the scenario of COVID-19, this re-
purposing strategy holds a promise of important leads but
we cannot deny the fact that some limitations like funding
opportunities, patent barriers etc., need to be taken care of.

8. VACCINES FOR SARS-COV-2

Ever since the first successful genomic sequencing of
SARS-CoV-2, submitted to GenBank on January 5, 2020, a
race against time has started to develop virus-based vaccines
valuable in fighting COVID-19 [130]. Due to the approxi-
mate 90% homology of SARS-CoV-2 with other coronoviruses (SARS and MERS) and the S protein of
SARS-CoV-2 being only 12.8% different from SARS-CoV,
the previously employed strategies to develop a vaccine
against SARS-CoV are being revisited for a successful an-
ti-SARS-CoV-2 vaccine design [131].

The vaccine against SARS-CoV-2 will be based on any
of the following platforms utilized for the development of an-
tiviral vaccine like inactive or live-attenuated viruses, virus-
like particle (VLP), viral vectors, protein-based, DNA and
mRNA-based vaccines. New vaccine designs centered on
the putative protective antigen or peptides obtained from
SARS-CoV-2 can be also be explored. The vaccine based on
inactivated or live-attenuated strains (AY278741, Tor2,
HKU-39849, Utah, FRA, BJ01, NS1, ZJ01, GD01 and
GZ50) in case of SARS-CoV significantly reduced the viral
infection in a variety of animal models [132-140]. The Chi-
nese Centre for Disease Control and Prevention (CDC) are
developing the vaccine based on inactivated virus, compara-
тивely with diminished pathogenesis, lung injury, neutrophil
influx, etc. [141, 142]. China has approved two experi-
tental vaccines developed by Sinovac Biotech and Wuhan Insti-
tute of Biological Products for human trials [143].

The SARS-CoV-2 has four vital structural proteins (enve-
lope, spike, membrane and nucleocapsid), which may serve
as antigens to stimulate the appropriate immune response.
However, previous studies with other viruses have shown
that subunit vaccines require appropriate adjuvants or sever-
ral booster shots to elicit an immune response [130].

A number of vaccines constructed on different themes
are currently in trials. The protein-based version, targeting
the S1 spike protein from the SARS-CoV-2 virus, developed
by the University of Pittsburgh deliverable through micr-
oneedles, has proven effective in eliciting antibody re-
sponses in mice, quite evident after two weeks of immuniza-
[144]. The vaccine based on Virus-Like Particle (VLP)
has been developed by the University of Bristol utilizing
Imphoroni's pre-clinical program platform, the ADDOmer
[145]. The protein vaccine against MERS based on para-
fluenza virus (PIV5) carrying the “spike” protein has suc-
cessfully protected mice against MERS lethal dose, and
could be employed to advance in vaccine assembly against
COVID-19 [146]. The vaccines based on T-cell epitopes
have been designed and about 57 epitopes with no similari-
ty/identity with human proteome have been identified and
could possibly be tested as candidate vaccines [147]. The
mRNA based vaccines against SARS-CoV-2 are being pre-
pared by Sterimira Therapeutics [148] and one such vaccine
prepared by the National Institute of Allergy and Infectious
Diseases, USA and another candidate vaccine mRNA-1273
by Moderna are under phase 1 trial [149, 150].

The vaccines based on recombinant SARS-CoV-2 S pro-
tein subunit-trimer are also in pipeline of development [151]
and DNA based vaccine INO-4800 developed by Inovio
Pharmaceuticals has entered Phase I trial in the US [150].
The six other vaccines, based on the SARS-CoV-2 antigens
in the phase I trials, include adenoviral vector 5 (NC-
T04313127), lentiviral vector (NCT04276896), artificial anti-
gen-presenting cells or aAPC (NCT04299724), mRNA (NC-
T04283461) and chimpanzee adenoviral vector ChAdOx1
CONCLUSION

The COVID-19 outbreak has become a threat both to the general population health and economy throughout the world. Effective control measures should be applied to reduce the person-to-person transmission in susceptible populations and provide healthcare systems with an opportunity to diminish the global spread until effective treatments/ vaccine(s) become available. Given the urgency of the situation, a further speculative area encompasses identification of vital targets in host as well as virus that may be crucial for development of successful treatments in any form ranging from repurposing medicines to vaccines or new chemical entities. The development of possible model organisms that will aid in the evaluation of such antiviral therapy and vaccine(s) with minimal impact on host is an area worth exploring with utmost urgency.

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CONFLICT OF INTEREST

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