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Novel Coronavirus 2019: An Updated Overview

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ABSTRACT

The World Health Organization (WHO) officially confirmed COVID-19 as a viral pandemic caused via SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Novel coronavirus disease 2019 (COVID-19) is an extremely pathogenic and transmissible infection induced by a virus named SARS-CoV-2. SARS-CoV-2 is a single-stranded positive-sense ribonucleic acid (RNA) virus. Genomic analysis has shown that SARS-CoV-2 is phylogenetically analogous to SARS-like viruses. It primarily effects on both upper and lower respiratory tract system. Pneumonia is a typical feature of the SARS-CoV-2 infected individuals. Numerous therapeutic drugs are presently being used alone or in combination along with other drugs to overcome this disease. The purpose of this review is primarily to present an outline of the origin, classification, possible reservoirs and biological morphology of the SARS-CoV-2 virus. It also outlines the emerging diagnostic and therapeutic treatment approaches for COVID-19.

INTRODUCTION

At the beginning of December 2019, the World Health Organization (WHO) reported an unknown viral outburst in the seafood market in Wuhan City, China, in which animals for instance fish, birds, snakes, frogs and multiple species of rodents were mostly sold (T. Zhang *et al*., 2020b). Some of the market inhabitants and visitors developed signs of illness which was quite similar to viral pneumonia (WHO, 2020). The Chinese Center for Disease Control and Prevention (CCDC) flagged up it as a novel coronavirus (nCoV) infection on 7 January 2020. Following this, on 30 January 2020, the WHO office stated the nCoV epidemic a public health crisis of worldwide concern and declared a new term for it as 2019-novel Coronavirus (2019-nCoV) disease which presently termed as COVID-19 (Figure 1) [WHO (World Health Organization), 2020]. Consequently, the International Committee on Taxonomy of Viruses (ICTV) named 2019-nCoV as SARS-CoV-2 (Whitworth, 2020).

ORIGIN AND CLASSIFICATION

Coronaviruses are a versatile family of viruses which are primarily notorious to cause various types of diseases varying from common cold to more drastic ones like the severe acute respiratory syndrome (SARS) and the middle east respiratory syndrome (MARS) (Abd El-Aziz & Stockand, 2020). It was first isolated from a child's nasal washings by Tyrrell and Bynoe in 1965 (Tyrrell & Bynoe, 1965). Ever since their discovery in 1965, numbers of other different strains of coronaviruses have been identified. ICTV has classified coronaviruses under an order Nidovirales, which consist of *Coronaviridae*, *Mesoniviridae, Roniviridae* and *Arteriviridae*families. *Coronavirinae* is one of the two sub-families of the *Coronaviridae*family, the other one is *Torovirinae*family (Figure 3). The *Coronavirine* are taxonomically grouped into four different genera: Alpha-Coronavirus (alpha-CoV), Beta-coronavirus (beta-CoV), Delta-Coronavirus (delta-CoV) and Gamma-Coronavirus (gamma-CoV) (de Groot *et al*., 2013). Prominent features of the *Nidovirales* order include: (1) enveloped, non-segmented positive-sense RNA viruses; (2) wide RNA-virus genomes; up to 33.5 kilobase; (3) anextremely conserved genome

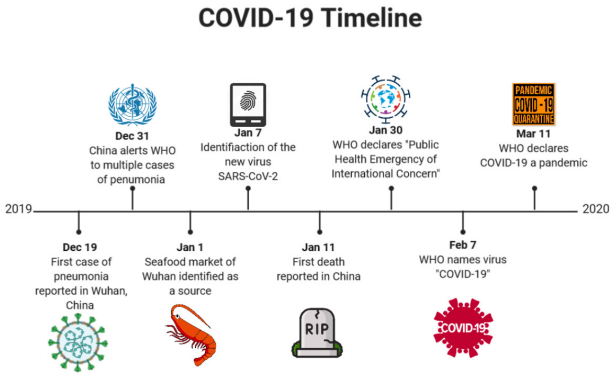

Figure 1. Key episodes in the initial phase of the SARS-CoV-2 pandemic *(Figure created with Biorender)*.

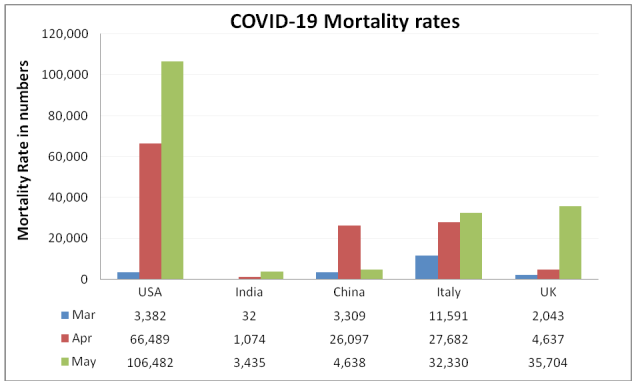

Figure 2: Mortality Rates due to COVID-19 in USA, India, China, Italy and UK

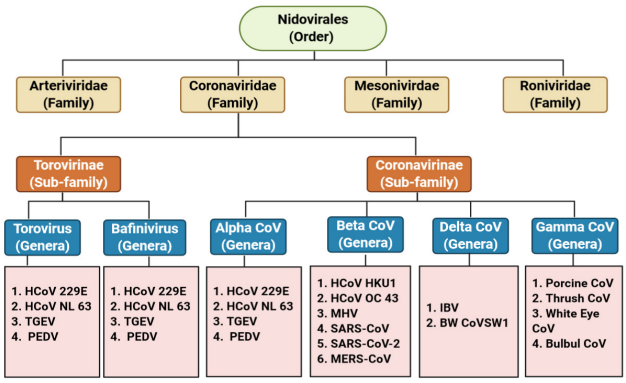

Figure 3: Taxonomic classification of the various coronaviruses. SARS-CoV-2 strain belongs to the beta coronavirus (beta-CoV) genus.

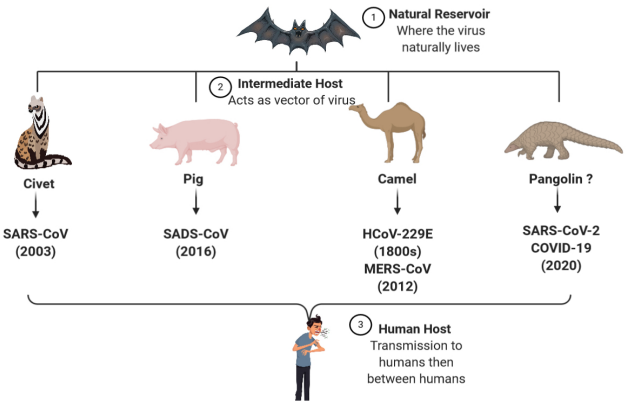

Figure 4: Routes of potential cross-species transmission of different bat-origin coronaviruses. Question mark indicates potential, but unknown, transmission from pangolin to human *(Figure created with Biorender)*.

organization along with a large replicase gene; (4) a number of distinctive or irregular enzyme-mediatedactions encrypted within the large replicase–transcriptase polyprotein; (5) non-structural genes expression via ribosomal frame-shifting process; (6) downstream gene expression by synthesis of 3' nested sub-genomic mRNAs (Maier *et al*., 2015). Alpha-CoVs and beta-CoVs circulate in human and other mammals, whereas gamma- and delta-CoVs primarily infect the birds (Wertheim *et al*., 2013). Till now, there are total seven strains of coronaviruses were documented in humans, including HCoV-229E, HCoV-HKU1, HCoVNL63, HCoV-OC43, SARS-CoV, SARS-CoV-2 and MERS-CoV (Chen *et al*., 2020). HCoV-43 and HCoV-HKU1 belongs to alpha-CoV, HCoV-NL63and HCoV-229E belongs to beta-CoV, and the three extremely pathogenic ones SARS-CoV, SARS-CoV-2 and MERS-CoV are also member of the beta-CoV genera (Bauch *et al*., 2005), (Benvenuto *et al*., 2020). Over the last few decades, these coronaviruses have caused two major pandemics: SARSand MERS (Peiris et al., 2004) and now this SARS-CoV-2. Coronaviruses have been found in both domestic and wild-type animals, as well as in birds. Studies have found that bats and rodents are natural reservoirs for alpha-CoV and beta-CoVs (Wendong Li *et al*., 2005). So, it is assumed that the novel coronavirus 2019 (nCoV-19) is a zoonotic type of RNA virus, means the first person who was infected acquired these viruses originally from animals. SARS-CoV-2 virus represents a new form ofSARS-CoV strain not previously identifiedin humans. However, phylogenetic relationships and several genetic research of SARS-CoV-2 have revealed that it falls within the *Sarbecoronavirus* subgenus of the beta-CoVs gene, which has high resemblance of the SARS-CoV patterns, although there are some variations in key amino acid sequences and the ability of this virus to bind to the receptors in humans (Weiss & Navas-Martin, 2005), (Woo *et al*., 2012).

RESERVOIRS FOR EMERGING COVS

Are bats are special reservoirs for emerging zoonotic diseases? Most scientists have estimated that SARS-CoV-2 came from a bat. They verified that the bats have much more zoonotic viruses per species than rodents. Calisher et al. explored the relationship among bats and emerging viruses in a review paper highlighting different aspects of the life, history and ecology of bats that are likely to affect the role of bats as a natural viral reservoir (Calisher *et al*., 2006). Some research findings have demonstrated that the bat is a potential species of the COVID-19 origin because it exhibits approximately 96% whole-genome sequence identity with the bat CoV (Bat CoV RaTG13) from *Rhinolophusaffinis* (P. Zhou, Yang, Wang, Hu, Zhang, Zhang, Si, Zhu, Li, Huang, Chen, Chen, Luo, Guo, Jiang, Liu, Chen, Shen, Wang, *et al*., 2020). This virus showed 96.1% nucleotide identity and 92.9% S gene identity, suggesting that bats play a crucial role as coronavirus reservoirs. *Rhinolophusaffinis* is considered the natural host of SARS-CoV-2 (P. Zhou, Yang, Wang, Hu, Zhang, Zhang, Si, Zhu, Li, Huang, Chen, Chen, Luo, Guo, Jiang, Liu, Chen, Shen, Zheng, *et al*., 2020). Now there is another potential question, which is "*What will be the intermediate host of the SARS-CoV-2?*" Numerous studies have speculated that Malayan pangolin *(Manisjavanica)* is a possible 2019 nCoV intermediate host (Figure 4)

(T. Zhang *et al*., 2020a). Although it is still likely that other types of animals could also be an intermediate host (Guan *et al*., 2003). Additional investigations for other possible intermediate hosts must therefore be warranted.

MODE OF TRANSMISSION

Coronavirus disease is a highly pathogenic viral infection that primarily affects the respiratory system of mammals and birds. SARS-CoV-2 attacks internal organs including the lungs, intestines and vessels. Throughout the lungs, virus attacks cells present in the lining of the lungs named *pneumocytes* resulting in respiratory distress. This results to a reduction in the amount of oxygen in the blood and eventually leads to death. As a new coronavirus, the transmission route of SARS-CoV-2 remains an open question. Nevertheless, evidence strongly suggests that pathways for possible transmission of viruses are because of close contact with infected persons, respiratory and airborne droplets which are analogous to SARS-CoV and MERS-CoV (Chan *et al*., 2020), (Q. Li *et al*., 2020). Furthermore, there are several other ways of transmissions by which this virus can be spread, such as contaminated surfaces and things that have been touched by the infected individuals and feces transmission, although the later route of transmission is quite limited [5].

BIOLOGICAL MORPHOLOGY OF SARS-COV-2

Structure of the Virion

Coronavirus virions are sphere-shaped with a size of around 125 nanometer (nm). The most significant characteristic of coronaviruses is their club-like shape spike projections emerging from the surface of virion. Spike gives them the solar corona-like appearance, therefore the name coronavirus (R. *et al*., 2020). Virions of coronaviruses comprise four different structural proteins (Figure 5). These are spike (S) glycoprotein, it directs binding to host cell receptors, and allows entry of the virus into the cells; envelope (E) and membrane (M) mediates virion budding; and the nucleocapsid (N) protein. Studies have shown that E protein is a viroporinwhichconstitutes an ion channels. It plays a vital role, but, it is still in infancy in the context of viral morphogenesis and budding. Several research studies on coronaviruses, along with SARS-CoV, have reported that the reduction of E gene from the coronavirus genome significantly reduces the growth and development of the virus. In addition, there is another structural protein called hemagglutinin-esterase (HE), although it present only in a subset of beta-CoVs. It enhances the S-protein which promotes cell entry and spread of the virus through the mucosa. Once a sufficient amount of new genomic RNAs and all these structural proteins were generated, formation of viral particles take place which constitutes the helical nucleocapsid structure (Versalovick *et al*., 2015), (de Haan *et al*., 1998).

Genomic Organization

Coronaviruses are non-segmented, positive-sense RNA of around 26-32 kilobase (Kb) in length, with a 5' capping and a 3' polyadenylate tail; this enables it to function like an mRNA for replicase polyproteins translation. The 5 'end of the RNA genome comprises a leader sequence and an untranslated region (UTR) containing seven loop structures vital for transcription and replication of the RNA (Yang & Leibowitz, 2015). At the 5' terminus two third part of the RNA genome comprises two open reading frames (ORFs), rep1a and rep1b that encode viral replicase-transcriptase polyproteins. ORF1ab, a principal gene in SARS-CoV-2, encodes pp1ab polyprotein and 15 non-structural proteins (nsps), while ORF1a encodes pp1a polyprotein and contains 10 nsps. The nsps are assembled into the replicase transcriptase complex (RTC) to construct surroundings appropriate for synthesis of RNA and are typically accountable for the replication and transcription. The down-stream end of the RNA genome comprises genes for the four chief structural proteins in

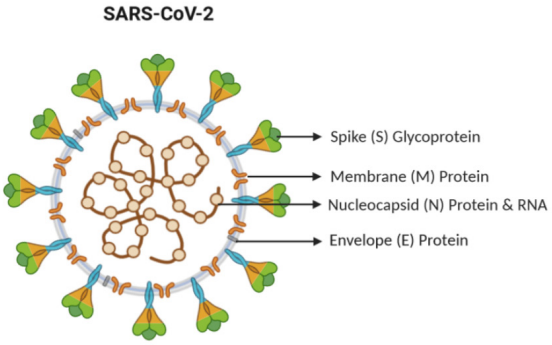

Figure 5. Diagrammatic illustration of SARS-CoV-2 Virion. Virus have spike (S) glycoprotein, an integral membrane (M) protein, a small number of molecule of an envelope (E) protein and a nucleocapsid (N) protein along with genomic RNA (gRNA) *(Figure created with Biorender)*.

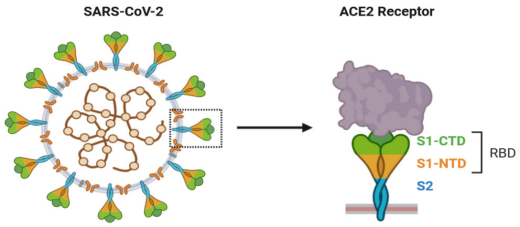

Figure 6. Schematic of spike-receptor binding mechanism of SARS-CoV-2.S glycoprotein makes up the spike like-structure of the virus. The trimeric S glycoprotein, a class I fusion protein, facilitates attachment to the host receptor. S is hewed off by a host cell furin-like protease into two different polypeptides, S1 and S2. S1 constitutes the large receptor binding domain (RBD) of the S glycoprotein, whereas S2 forms the stalk of the spike molecule. *(Figure created with Biorender)*.

the ever-changing sequence of S, E, M, and N, along with interspersed accessory genes (Figure 7). These structural proteins are encoded by ORFs 10 and 11 on one third part of the RNA genome adjacent to 3' terminus (van Boheemen *et al*., 2012).

LIFE CYCLE OF SARS-COV-2

Virion binding to the host cell is triggered by direct interaction among both the angiotensin-converting enzyme 2 (ACE2) receptor and S glycoprotein. SARS-CoV-2 recognizes ACE2 receptor via the receptor binding domain (RBD) (Wenhui Li *et al*., 2003). After attachment of the RBD at the C-terminus of S1 to the ACE2 receptor, a conformational alteration of S2 is triggered that allows the ACE2-Virus complexfusion with the cellular membrane (Figure 6). Following receptor binding, the virus will then gain an entry to the cytoplasm of the host cell, where the S protein is chopped off by endosomal acid protease enzymes, such as cathepsin L and transmembrane protease serine 2 (TMPRRS2) to trigger the fusion activities of the virus. Cleavage of S protein take place at two specific sites within the protein's S2 portion; first cleavage is necessary to differentiate the S protein's RBD and fusion domains, and second cleavage to reveal the fusion peptide (Glowacka *et al*., 2011), (Matsuyama *et al*., 2010). Cleavage at the S2 site reveals a fusion peptide that integrates into the cellular membrane, that then continues via linking of two heptad repeats in S2 constructing an anti-parallel six-helix bundle core structure. This six-helix bundle allows the integration of the virus and cell membranes, which results in the fusion and gradual discharge of the viral RNA contents into the cytoplasm region (Bosch *et al*., 2003). The genomic RNA, ORF1a and ORF1b are then translated into viral replicase

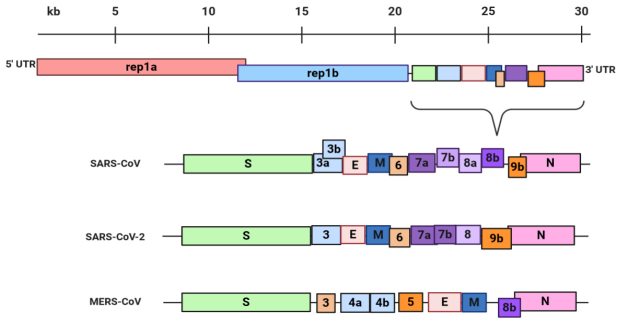

Figure 7. Genomic sequence of SARS-CoV, SARS-CoV-2 and MERS-CoV strains. Size of coronavirus genome ranges from around 26-32 kb in length and contains 6-11 ORFs. The first ORF, ORF 1ab which consist of around 67% of the genomic sequence encodes 16 nsps. The 5' end encompasses rep1a and rep1b genes which encode the viral replicase-transcriptase. The expanded region below show the 3'end comprises the different structural protein genes, including S, E, M and N. Also at the 3' end of the genome are auxiliary genes such as 3a, 3b, 6, 7a, 7b, 8a, 8b and 9b in SARS-CoV, 3, 6, 7a, 7b, 8 and 9b in SARS-CoV-2, and 3, 4a, 4b, 5, 8b in MERS-CoV. *(Figure created with Biorender).*

polyproteins pp1a and pp1ab, respectively. These polyproteins pp1a and pp1ab contain the 1-11 and 1-16 nsps, respectively, which are cleaved off by viral proteinase such as papain-like protease and serine-like proteases into small products (Ziebuhr *et al*., 2000). Replication of the viral RNA yields both genomic and subgenomic RNAs, which precedes the translation and assembly of the viral replicase complexes. The positive strand of the genomic RNA formed via replication becomes the genome of the new virus particle, whereas subgenomic RNAs serve as mRNAs and they are then translated into multiple structural proteins, such as S, E, M and N that comprise a virus (Figure 8) (Wu *et al*., 2020).

These viral structural proteins (S, E, and M) are subsequently encoded and inserted into the endoplasmic reticulum preceding transcription and subgenomic synthesis of the RNA. All these proteins traverse into the endoplasmic reticulum-golgi intermediate compartment (ERGIC) (Krijnse-Locker *et al*., 1994), (Masters, 2006). There they encapsulated by N protein bud into membranes of the ERGIC enclosing viral structural proteins, comprising a fully developed virions. The vesicle eventually fuses with the cell membrane and releases mature particles of the SARS-CoV-2 out from the infected cell via exocytosis. This process might be induced by the S-protein (Garoff *et al*., 1998).

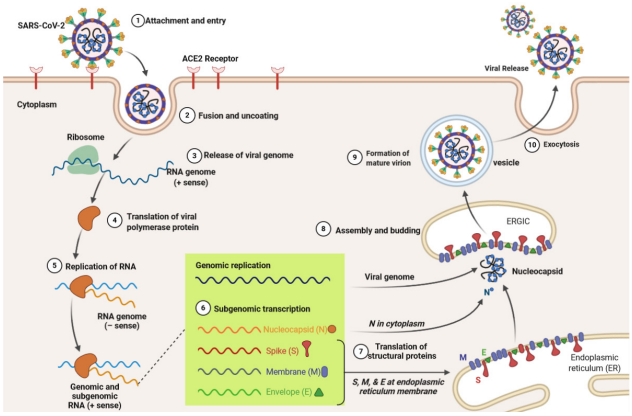

Figure 8. Diagrammatic representation of SARS-CoV-2 life cycle in a host cell.Following binding of S proteinto the ACE2 receptor, the conformational modification in the S glycoprotein promotes viral envelope fusion with the host cell membrane via the endosomal pathway, and, thereafter the envelope is peeled off. SARS-CoV-2 then releases its genomic RNA into the cytoplasm of the host cell. Genomic RNA is then translated into viral replicase polyproteins (pp1a and pp1ab), which are then cleave off by protease into small nsps. Some nsps construct a replication-transcription complex (RNA-dependent RNA polymerase, RdRp), that uses the positive-strand of RNA as a template. The positive-strand of RNA formed via replication becomes the genome of the new virus. Sub-genomic RNAs produced via transcription are then translated into structural viral protein (S, E, M and N) which forms a viral particle. Viral proteins and newly formed RNA genome are successively assembled in the ER and golgi. They all merge into the complete virus particle in the ERGIC and then conveyed via vesicles and released out of the cell *(Figure created with Biorender).*

SIGNS AND SYMPTOMS

Prior to the SARS-CoV outbreak, these types of viral infections have been believed to induce mild and limited respiratory infections in humans (Maier *et al*., 2015). Clinical findings imply that when someone is infected with SARS-CoV-2, the symptoms typically started to emerge afterwards an incubation time period of almost 5 to 7 days (Q. Li *et al*., 2020). According to WHO, this range is somewhat similar to SARS (Bauch *et al*., 2005). Nevertheless, it has been observed in many individual cases that the incubation time period of the virus will prolong up to 40 days with anaverage incubation period of approximately 14 days. This incubation time period varies in severe and mild COVID-19 cases and also relies on the individuals' age, health and immune responses. Individuals over 70 years had a shorter incubation time period of around 11.5 days than those less than 70 years, around 20 days (W. Wang *et al*., 2020). Pneumonia is a typical feature of the SARS-CoV-2 infected individuals. Across most of the population, the most common sign and symptoms of COVID-19 at the beginning of infection are usually cold, cough, fatigue, fever and influenza, whereas further clinical signs include headache, lymphopenia, diarrhea, sputum production and haemoptysis (Lei *et al*., 2020). Even so, it is different for those who suffer from some other kind of illnesses, such as diabetes, kidneys, lung or heart problems and perhaps something else. In such cases, the virus has an additional benefit, which can often lead to death. In certain cases, there are some other types of complications occurs like heart failure, respiratory distress syndrome (RDS), and secondary bacterial infection (N. *et al*., 2020), (Huang *et al*., 2020). It is become evident that infection with COVID-19 primarily occurs after exposure to the virus, and both healthy and immunocompromised individual appear to be vulnerable to it. And the majority of patients were around 35 and 55 yrs old with marginal cases in both children and infants (Wang, C., & Wang, 2020). SARS-CoV-2 can also affect the gastrointestinal system. Fang et al. showed that 79.1% of Wuhan patients had gastrointestinal symptoms (Fang *et al*., 2020). In another study, it was reported that 32.5% of COVID-19 infected individuals with gastrointestinal signs mainly had loss of appetite, diarrhea, and nausea, whereas abdominal pain and vomiting were rare (H. Zhang *et al*., 2020). SARS-CoV-2 infection manifested as gastrointestinal symptoms may pose diagnostic and treatment challenges and could also poses a serious threat to health-care individuals who are inadvertently exposed to the virus.

DIAGNOSIS

In present, the most extensively used *in vitro* diagnostic (IVD) technique for the recognition of COVID-19 in patients suspected of developing an infection is called Real time Reverse Transcription Polymerase Chain Reaction (real time RT-PCR) (Figure 9). The real-time RT-PCR assay is highly specific to SARS-CoV-2 RNA (Corman *et al*., 2020). It is capable of assessing all the four major human respiratory coronaviruses and may also be further suited to the identification of new coronaviruses (Emery *et al*., 2004), (Gaunt *et al*., 2010). It is a nuclear-derived technique for assessing the presence of particular genomic material from any pathogen, including viruses. This technique uses isotopic labeling with distinctive markers, most commonly fluorescent dyes (VanGuilder *et al*., 2008). Through the use of RT-PCR we can detect SARS-CoV-2 positive nuclei in saliva, nasal wash, nasopharyngeal swab, and lower respiratory tract infections (Version, 2020). The RT-PCR test identifies the RNA of SARS-CoV-2 through envelope (E) and RNA-dependent RNA polymerase (RdRp) gene assays. The E-gene assay method has been used for first line screening, whereas the RdRp gene assay method has been used for confirmatory testing. ID NOW™ COVID-19 is a latest and rapid IVD assay test that delivers lab-quality molecular results in just 10 minutes or less targeting the COVID-19 RdRp gene. It employs the isothermal nucleic acid amplification technology for the qualitative recognition of SARS-CoV-2 RNA (Abbott, 2020). Serological assays are yet another preferred method for human coronavirus diagnostic evaluations. This one is generally applicable in situations where it is difficult to isolate the RNA or when it is no longer accessible to detect. In comparison to viral RNA, which is not detectable after recovery from infection,

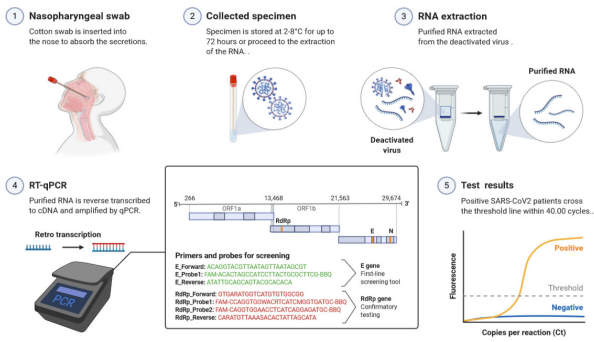

Figure 9. Schematic of COVID-19 diagnostic test.A sample is collected from a person's nose or throat regions. Then the sample was processed with different chemicals that eradicate the auxiliary constituents and extracts only the RNA that is present in a test sample. RNA is transcribed back to DNA using a particular enzyme. Thereafter,additional small DNA fragments were added that are complementary to specific parts of the transcribed viral DNA. These small fragments then attach themselves to target segments of the viral DNA if the virus is present in a test sample. The sample mixture is then positioned in a RT-PCR machine to be analyzed for virus identification. The marker tags the DNA strand and then releases a fluorescent dye. The computer analyze the level of fluorescence in the test sample after each cycle, if the level goes over a certain extent it means the virus is present. The shorter the cycle interval, the more critical the infection *(Figure created with Biorender)*.

SARS-CoV-2 antibodies may remain in the blood indefinitely and can be detected (Krammer & Simon, 2020). Serology testing is entirely based upon targeted antibodies that bind to specific SARS-CoV-2 antigens. The major purpose of serological test is to identify those people who have formerly been infected with SARS-CoV-2. The platforms for the COVID-19 serology tests includes the rapid diagnostic test (RDT), the enzyme-linked Immunosorbent assay (ELISA), the neutralization assay and the chemiluminescent assay (Center for Health Security, 2020). Another unique approach of diagnosis is a CRISPR based assay. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a family of repeated DNA sequences that can be found in prokaryotic organisms, for example bacteria and archaea (Wang, C., & Wang, 2020). Two individual firms, Sherlock Biosciences and Mammoth Biosciences are investigating the potentials of CRISPR technology for SARS-CoV-2 diagnosis. SHERLOCK method uses the Cas13 system, which is responsible for extracting reporter RNA sequences in response to SARS-CoV-2-specific guide RNA activation (F. Zhang *et al*., 2020) whereas the DETECTR assay developed by Mammoth Biosciences depends on excision of reporter RNA by Cas12a system to precisely identify viral RNA sequences of the E and N genes, followed by isothermal amplification of the target, resulting in visible fluorophore analysis. (Broughton *et al*., 2020). CRISPR-based methods do not entail intricate instrumentation setup and can be analyze by means of paper strips to confirm the occurrence of SARS-CoV-2 virus lacking absence of specificity or sensitivity. Both of these tests are relatively inexpensive and can be achieved in less than 1 hour.

VACCINATION

Vaccines activate an individual's immune response to deter a potential pathogen attack. This is done by pre-exposing the person whether to kill or weakened pathogens, or to some of the structural parts of the pathogen, which leads the body to mount a defensive response against them. Unfortunately, no clinical vaccine against COVID-19 infection has yet been reported. While many possible vaccines are available on the market, none of them have been successful in inhibiting viral replication. Numerous studies and clinical trials have highlighted a significant number of appropriate anti-viral targets, like viral proteolytic enzymes, polypetides and entry proteins, which were analyzed against COVID-19 infection and have resulted in effective therapeutic interventions. These vaccines include live virus vectors, recombinant attenuated viruses and individual viral proteins expressed as DNA plasmids. In general, live attenuated vaccines are believed to be the most effective in targeting coronaviruses. Du *et al*. have demonstrated that S protein-based vaccines, including S-cleavage inhibitors, neutralizing antibodies, ACE2 blockers, S protein inhibitors, small interfering RNAs and protease inhibitors (Du *et al*., 2009) are probably the best anti-viral treatments in the setting of COVID-19. Recombinant DNA from Urbani strain SARS-CoV (AY278741) injected to mice and hamsters resulted in the formation of neutralizing antibodies and suppression of the entire virus (Kam *et al*., 2007), (Bisht *et al*., 2005) and thereby it significantly reduced the viral load in the animals (Kapadia *et al*., 2005). Numerous other different strains of SARS-CoV can also be utilized, including FRA (AY310120), Utah (AY714217), NSI (AY508724), Tor2 (AY274119) and GZ50 (AY304495), to produce inactivated or live attenuated vaccines that significantly decrease viral load (Stadler *et al*., 2005),(Spruth *et al*., 2006),(J. Zhou *et al*., 2005),(Weingartl *et al*., 2004),(Qu *et al*., 2005). The natural tendency of the virus recombination may present a problem by rendering the vaccine ineffective. This would also significantly raise the progression and diversification of viruses in the wild. Through understanding the nature of these viruses, it can allow us to anticipate the next outbreak (L. Wang *et al*., 1993). To reduce the incidence of recombination, a number of vaccination strategies are being developed, such as large deletions in nsp1 (Züst *et al*., 2007) or E protein (Netland *et al*., 2010), TRS sequence modification (Yount *et al*., 2006), 3' end terminus rearrangement (de Haan *et al*., 2002) and use of mutated viruses with excessively high mutation rates that substantially neutralize the virus (Graham *et al*., 2012). In a recent vaccine study, Grifoni et al. used Immune epitope database and analysis resource (IEDB) data containing more than 600,000 documented epitopes from some 3,600 distinct virus species. They summarized all known SARS-CoV epitopes and documented the analogous SARS-CoV-2 regions. With all of this, they were able to trace some ten B- and T-cell epitopes to the new coronavirus. The assumption that several B- and T-cell epitopes have been found to be strongly conserved between SARS-CoV and SARS-CoV-2 provides a good reference point for vaccine production (Grifoni *et al*., 2020). Defining the coronavirus mechanism and understanding the immune-pathological function of the host would greatly enhance our ability to develop vaccines. However, for the time being, due to lack of effective therapeutic interventions, the best way to manage it is to avoid close contact, keep a safe distance, wash hands regularly and follow the guidelines of the health authorities.

THERAPEUTIC STRATEGIES

At present, the most effective therapeutic approach is to relieve symptoms in patients who represent pneumonia, while researchers are trying to find the best stable treatments. One approach to design the therapeutic interventions is to discover the genomic and molecular biology of the virus. Right now, broad-spectrum antibiotics, antiviral medications and various other therapeutic

Table 1: Common anti-viral drugs proposed against SARS-CoV-2 (COVID-19)

Drug	*Class*	*Target*	*Dosing*	*Adverse Effects*	*References*
Remdesivir (GS-5734)	Anti-viral drug	Viral RNA polymerase	200 mg on IV 1st day, then 100 mg IV daily for 5 to 10 days	Reports of increased Liver function tests (LFTs), nausea vomiting, gastro paresis, rectal bleeding	(Bethesda (MD), 2022), (Bethesda (MD), 2020), (M. Wang *et al*., 2020)
Chloroquine	Anti-malarial drug	ACE 2	500 mg by mouth twice daily for 10 days	Nausea, vomiting, abdominal cramping, QTc prolongation, skin eruptions	(Multicenter collaboration group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus pneumonia, 2020), (Sanofi-Aventis US LLC, 2017)
Hydroxychloroquine	Anti-malarial drug	Endosome, pH elevation	400 mg by mouth twice daily for one day, then 200 mg twice daily for 4 days	Nausea, vomiting, abdominal cramping, QTc prolongation, skin eruptions	(Keyaerts *et al*., 2004), (Concordia Pharmaceuticals Inc, 2017)
Ribavirin	Anti-viral drug	Viral RNA synthesis	Not established in clinical trials, 400mg by mouth twice daily for 14 days	Hemolytic anemia, hypocalcemia, hypomagnesemia, nausea, dry cough, rashes, teratogenic	(Crotty *et al*., 2002), (Merck & Co., 2013)
Lopinavir/Ritonavir	Anti-viral drug	Viral protease	400 mg/100 mg by mouth twice daily for 14 days	Diarrhea, nausea, vomiting, increased serum amylase	(Chu *et al*., 2004), (Yueping Li, 2020), ("Lopinavir/ Ritonavir, Ribavirin and IFN-Beta Combination for NCoV Treatment," 2020)
Nitazoxanide	Anti-protozoal drug	Pyruvate ferredoxinoxidoreductase enzyme	500 mg by mouth twice daily. Duration not defined	Abdominal pain, nausea, headache, urine discoloration, diarrhea, dizziness	(Romark Pharmaceuticals, 2005)
Nelfinavir	Anti-viral drug	HIV-1 protease	Effective dose for treatment of COVID-19 is unknown	Diarrhea, nausea, flatulence	(Agouron Pharmaceuticals Inc, 2005)

approaches are being used as an alternative to reduce the viral loads (Table 1).

Ciclesonide

The National Institute of Infectious Diseases (NIID), Japan, has demonstrated that the use of ciclesonide (marketed as Alvesco), an inhaled corticosteroid typically used to treat asthma (Kralimarkova *et al*., 2011) has helped relieve the symptoms of individuals with COVID-19. The drug is presently in its phase 2 stage of drug evaluation process. Researchers are looking to investigate whether ciclesonide alone or in conjunction with hydroxychloroquine can suppress SARS-CoV-2 (Woo joo kim, 2020).

Lopinavir/Ritonavir

Lopinavir/ritonavir act as a protease inhibitor and it is widely employed for HIV infections. Lopinavir inhibits viral protease resulting in non-infectious virus particles; ritonavir inhibits the degradation of lopinavir in the body and prolongs its half-life. Lopinavir/ritonavir was demonstrated to block the replication of MERS and SARS (Momattin *et al*., 2019). Future

research on this may help to elucidate the potential for effective treatment of lopinavir/ritonavir. Additionally, darunavir, another protease inhibitor used in HIV infection, may also act as a possible intervention in COVID-19 in combination with ritonavir as a booster (Zhu *et al*., 2020).

Chloroquine and Hydroxychloroquine

Chloroquine is a 9-aminoquinoline derivative anti-malarial drug. It exhibits broad-spectrum anti-viral effects (Y. Yan *et al*., 2013). It inhibits the virus-host interaction and virus replication by increasing the pH of the nucleus (Vincent *et al*., 2005). Wang et al. have revealed that chloroquine is a highly effective medication for infection with COVID-19 *in vitro* (Ma *et al*., 2017).Another antimalarial drug, 4-aminoquinoline derivative, hydroxychloroquine, was shown to be effective in *in vitro* tests. Clinical trials of hydroxychloroquine are currently in progress (Study & Study, 2020). Both of these drugs, however, chloroquine and hydroxychloroquine might be associated with cardiovascular toxicity. In a newly published report in *The Lancet*, Mehra et al. reported that treatment with antimalarial drug chloroquine and hydroxychloroquine, with or without azithromycin, offers no benefit and could be detrimental in COVID-19 patients (Mandeep R Mehra, Sapan S Desai, Frank Ruschitzka, 2020).

Remdesivir (GS-5734)

It is a nucleotide analog that was first established by Gilead as a successful anti-Ebola virus drug (Mulangu *et al*., 2019). Remdesivir inhibits viral replication and advances the lung tissue destruction by incorporating itself into nascent viral RNA chains, which results in pre-mature termination (Gordon *et al*., 2020). At present, Remdesivir seems to be the most promising medication for COVID-19.

Mesenchymal Stem Cell (MSC)

Mesenchymal stem cells (MSCs) were shown to display extensive and robust immunomodulatory functions. MSC transplantation can cure or substantially enhance the prognosis of treatment with COVID-19. Leng et al. conducted an MSC transplantation pilot study. They revealed that the administration of MSCs in severe COVID-19 individuals significantly improved the inflammatory condition. In their study, it was confirmed that serum levels of pro-inflammatory chemokines and cytokines had declined, thereby attracting fewer macrophages to the lungs, while inducing more regulatory dendritic cells to the inflammatory tissues. Moreover, elevated interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF) triggered lung repair (Leng *et al*., 2020). The therapeutic application of MSC therapy for the management of COVID-19 is still in its infancy, but it is a viable candidate and worth considering.

Convalescent Plasma Therapy

Convalescent plasma (CP) therapy is a standard adaptive immunotherapy for the inhibition and treatment of various infectious diseases, such as SARS, MERS and H5N1 pandemics (Cheng *et al*., 2005). Convalescent plasma therapy involves plasma extraction from a treated COVID-19 patient who has just recovered from the infection, and then passing his convalescent sera to the infected patient. Past findings have shown that CP therapy reduces the mortality rates in patients with SARS (Bloch *et al*., 2020). Duan et al. performed a pilot study to discover the viability of CP therapy in 10 severe COVID-19 patients. The results showed that all the examined individuals attained serum SARS-CoV-2 RNA negativity after CP transfusion, followed by decreased lymphocyte counts and oxygen saturation, and also enhanced liver and C-reactive protein (CRP) activity (Duan *et al*., 2020). No harmful side effects were observed with the transfusion of CP therapy (B. Zhang *et al*., 2020). Therefore, CP therapy might be a potential therapeutic approach in COVID-19.

CRISPR Based Therapy

CRISPR-Cas13 system is a potent anti-viral method capable of utilizing sequence-specific crRNAs (CRISPR RNA) to safeguard host bacteria from bacteriophage infections (W. X. Yan *et al*., 2018). Cas13d PAC-MAC (prophylactic antiviral CRISPR in human cells) is a CRISPR-based method that is highly operative at targeting and slicing SARS-CoV-2 sequences. Abbott et al. have confirmed that the Cas13d system can efficiently target out and cleave specific RNA sequences of SARS-CoV-2 with properly engineered crRNAs in lung epithelial cells. Bioinformatic analysis identified a group of six circular RNAs (circRNAs) capable of targeting 91% of sequential coronaviruses as well as a group of 22 circNAs capable of targeting all sequential coronaviruses. However, the main problem to clinical implementation of PAC-MAN is the development of an efficient and prudent *in vivo* delivery systems (Abbott *et al*., 2020). This remarkable work indicates that the CRISPR/Cas13d system can be utilized to precisely digest the RNA genome of SARS-CoV-2, thereby reducing its property to replicate (Nguyen *et al*., 2020). Through the development of a safe and effective respiratory tract delivery method, PAC-MAN system has the capability to turn into an important therapeutic technique.

CONCLUSION

COVID-19 pandemic poses a serious threat around the globe. No promising clinical strategies against SARS-CoV-2 were developed so far. Presently, prevention is

the only viable option for saving lives and to restrict the spread of infection. Despite our understanding of SARS-CoV-2, there are many other factors of SARS-CoV-2 that are not yet completely known, including interspecies transmission dynamics, host immunopathological reactions, and the full spectrum of viral replication and pathogenicity. An extensive research for finding the experiment models for the evolving zoonotic viral infections among animals and humans are absolutely necessary. Coronaviruses emphasizes the significance of bats as a natural vector for novel viruses that cause infectious diseases in humans, and can also be seen as an ideal study model for designing experimental trials and interventions to prevent such forms of viruses from emerging in the future. In addition, genomic sequencing analysis allows us to precisely discern the mechanism of SARS-CoV-2 replication process which might significantly improve our ability to develop vaccines and therapeutic against the virus. Nevertheless, scientists are attempting to create effective medicines to deal with this novel coronavirus. Several broad-spectrum antiviral agents which are commonly used in the management of influenza, MERS and SARS coronaviruses were tested either alone or in conjunction with others for the treatment of COVID-19. Many viral strains have been present in nature for eons without upsetting much of the inhabitants. But now their frequent spillover to several animal species and humans is a result of our own hazardous activities against nature. And thus, the best possible way to hold such types of threats at bay is to draw a barricade between their natural pools and reservoirs, and ourselves.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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