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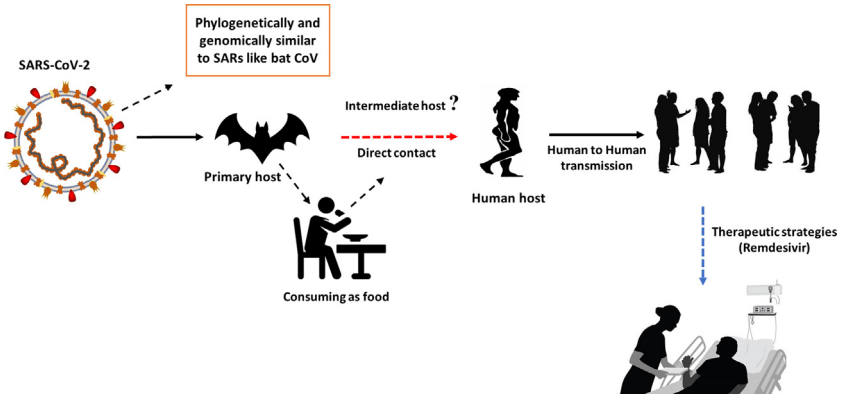
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COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses

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GRAPHICAL ABSTRACT



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ABSTRACT

The coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China and spread around the world. Genomic analysis revealed that SARS-CoV-2 is phylogenetically related to severe acute respiratory syndrome-like (SARS-like) bat viruses, therefore bats could be the possible primary reservoir. The intermediate source of origin and transfer to humans is not known, however, the rapid human to human transfer has been confirmed widely. There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. However, few broad-spectrum antiviral drugs have been evaluated against COVID-19 in clinical trials, resulted in clinical recovery. In the current review, we summarize and comparatively analyze the emergence and pathogenicity of COVID-19 infection and previous human coronaviruses severe acute respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory syndrome coronavirus (MERS-CoV). We also discuss the approaches for developing effective vaccines and therapeutic combinations to cope with this viral outbreak.
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Introduction

Coronaviruses belong to the Coronaviridae family in the Nidovirales order. Corona represents crown-like spikes on the outer surface of the virus; thus, it was named as a coronavirus.

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Coronaviruses are minute in size (65–125 nm in diameter) and contain a single-stranded RNA as a nucleic material, size ranging from 26 to 32kbs in length (Fig. 1). The subgroups of coronaviruses family are alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. The severe acute respiratory syndrome coronavirus (SARS-CoV), H5N1 influenza A, H1N1 2009 and Middle East respiratory syndrome coronavirus (MERS-CoV) cause acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) which leads to pulmonary failure and result in fatality. These viruses were thought to infect only animals until the world witnessed a severe acute respiratory syndrome (SARS) outbreak caused by SARS-CoV, 2002 in Guangdong, China [1]. Only a decade later, another pathogenic coronavirus, known as Middle East respiratory syndrome coronavirus (MERS-CoV) caused an endemic in Middle Eastern countries [2].

Recently at the end of 2019, Wuhan an emerging business hub of China experienced an outbreak of a novel coronavirus that killed more than eighteen hundred and infected over seventy thousand individuals within the first fifty days of the epidemic. This virus was reported to be a member of the β group of coronaviruses. The novel virus was named as Wuhan coronavirus or 2019 novel coronavirus (2019-nCov) by the Chinese researchers. The International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 and the disease as COVID-19 [3–5]. In the history, SRAS-CoV (2003) infected 8098 individuals with mortality rate of 9%, across 26 contries in the world, on the other hand, novel corona virus (2019) infected 120,000 induviduals with mortality rate of 2.9%, across 109 countries, till date of this writing. It shows that the transmission rate of SARS-CoV-2 is higher than SRAS-CoV and the reason could be genetic recombination event at S protein in the RBD region of SARS-CoV-2 may have enhanced its transmission ability. In this review article, we discuss the origination of human coronaviruses briefly. We further discuss the associated infectiousness and biological features of SARS and MERS with a special focus on COVID-19.

Comparative analysis of emergence and spreading of coronaviruses

In 2003, the Chinese population was infected with a virus causing Severe Acute Respiratory Syndrome (SARS) in Guangdong province. The virus was confirmed as a member of the Beta-coronavirus subgroup and was named SARS-CoV [6,7]. The infected patients exhibited pneumonia symptoms with a diffused alveolar injury which lead to acute respiratory distress syndrome (ARDS). SARS initially emerged in Guangdong, China and then spread rapidly around the globe with more than 8000 infected persons and 776 deceases. A decade later in 2012, a couple of Saudi Arabian nationals were diagnosed to be infected with another coronavirus. The detected virus was confirmed as a member of coronaviruses and named as the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The World health organization reported that MERS-coronavirus infected more than 2428 individuals and 838 deaths [8]. MERS-CoV is a member beta-coronavirus subgroup and phylogenetically diverse from other human-CoV. The infection of MERS-CoV initiates from a mild upper respiratory injury while progression leads to severe respiratory disease. Similar to SARS-coronavirus, patients infected with MERS-coronavirus suffer pneumonia, followed by ARDS and renal failure [9].

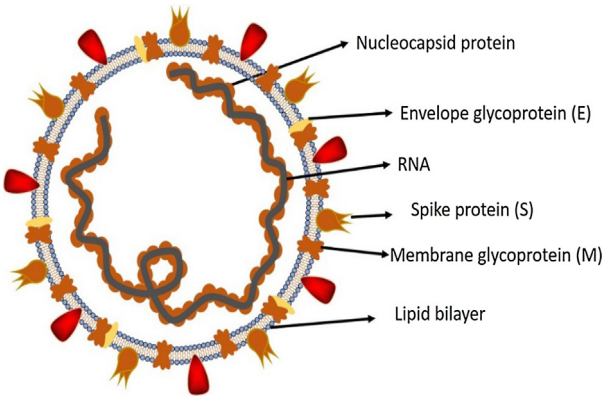


Fig. 1. Structure of respiratory syndrome causing human coronavirus.

Recently, by the end of 2019, WHO was informed by the Chinese government about several cases of pneumonia with unfamiliar etiology. The outbreak was initiated from the Hunan seafood market in Wuhan city of China and rapidly infected more than 50 peoples. The live animals are frequently sold at the Hunan seafood market such as bats, frogs, snakes, birds, marmots and rabbits [10]. On 12 January 2020, the National Health Commission of China released further details about the epidemic, suggested viral pneumonia [10]. From the sequence-based analysis of isolates from the patients, the virus was identified as a novel coronavirus. Moreover, the genetic sequence was also provided for the diagnosis of viral infection. Initially, it was suggested that the patients infected with Wuhan coronavirus induced pneumonia in China may have visited the seafood market where live animals were sold or may have used infected animals or birds as a source of food. However, further investigations revealed that some individuals contracted the infection even with no record of visiting the seafood market. These observations indicated a human to the human spreading capability of this virus, which was subsequently reported in more than 100 countries in the world. The human to the human spreading of the virus occurs due to close contact with an infected person, exposed to coughing, sneezing, respiratory droplets or aerosols. These aerosols can penetrate the human body (lungs) via inhalation through the nose or mouth (Fig. 2) [11–14].

Primary reservoirs and hosts of coronaviruses

The source of origination and transmission are important to be determined in order to develop preventive strategies to contain the infection. In the case of SARS-CoV, the researchers initially focused on raccoon dogs and palm civets as a key reservoir of infection. However, only the samples isolated from the civets at the food market showed positive results for viral RNA detection, suggesting that the civet palm might be secondary hosts [15]. In 2001 the samples were isolated from the healthy persons of Hongkong and the molecular assessment showed 2.5% frequency rate of antibodies against SARS-coronavirus. These indications suggested that SARS-coronavirus may be circulating in humans before causing the outbreak in 2003 [16]. Later on, Rhinolophus bats were also found to have anti-SARS-CoV antibodies suggesting the bats as a source of viral replication [17]. The Middle East respiratory syndrome (MERS) coronavirus first emerged in 2012 in Saudi Arabia [9]. MERS-coronavirus also pertains to beta-coronavirus and having camels as a zoonotic source or primary host [18]. In a recent study, MERS-coronavirus was also detected in Pipistrellus and Perimyotis bats [19], proffering that bats are the key host and transmitting medium of the virus [20,21]. Initially, a group of researchers suggested snakes be the possible host, however, after genomic similarity findings of novel coronavirus with SARS-like bat viruses supported the statement that not snakes but only bats could be the key reservoirs (Table 1) [22,23]. Further analysis of homologous recombination revealed that receptor binding spike glycoprotein of novel coronavirus is developed from a SARS-CoV (CoVZXC21 or CoVZC45) and a yet unknown Beta-CoV [24]. Nonetheless, to

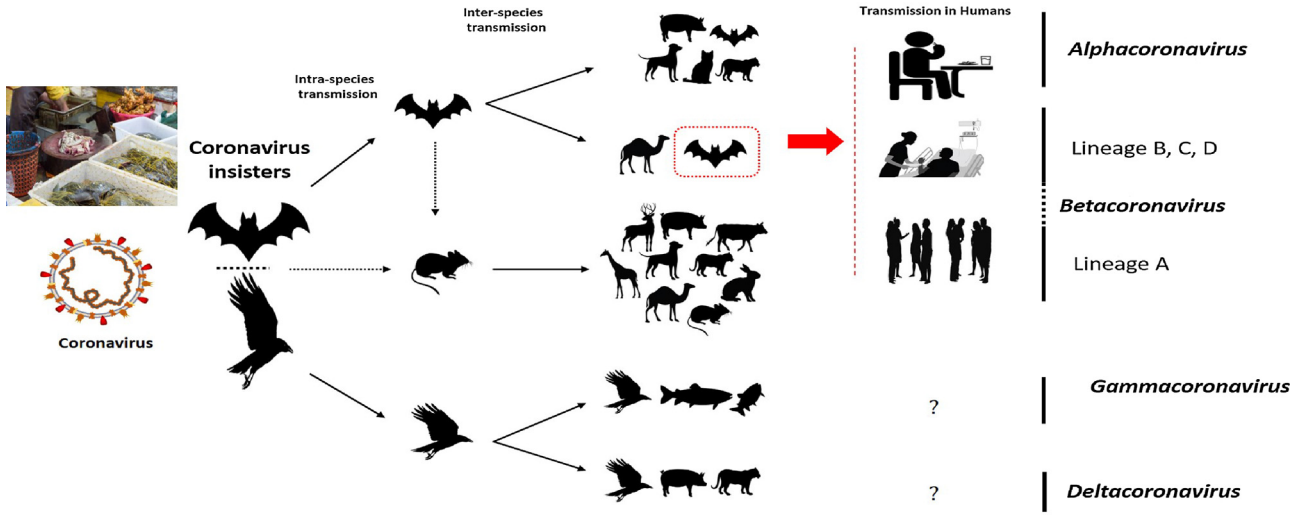


Fig. 2. The key reservoirs and mode of transmission of coronaviruses (suspected reservoirs of SARS-CoV-2 are red encircled); only α and β coronaviruses have the ability to infect humans, the consumption of infected animal as a source of food is the major cause of animal to human transmission of the virus and due to close contact with an infected person, the virus is further transmitted to healthy persons. Dotted black arrow shows the possibility of viral transfer from bat whereas the solid black arrow represent the confirmed transfer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparative analysis of biological features of SARS-CoV and SARS-CoV-2.

Features	SARS-CoV	SARS-CoV-2	Reference
Emergence date	November 2002	December 2019	[37,79–81]
Area of emergence	Guangdong, China	Wuhan, China	
Date of fully controlled	July 2003	Not controlled yet	
Key hosts	Bat, palm civets and Raccon dogs	Bat	[22,82,83]
Number of countries infected	26	109	[84]
Entry receptor in humans	ACE2 receptor	ACE2 receptor	[22,55,85]
Sign and symptoms	fever, malaise, myalgia, headache, diarrhoea, shivering, cough and shortness of breath	Cough, fever and shortness of breath	[12,23,85]
Disease caused	SARS, ARDS	SARS, COVID-19	[85,86]
Total infected patients	8098	123882	[84]
Total recovered patients	7322	67051	
Total died patients	776 (9.6% mortality rate)	4473 (3.61% mortality rate)	

eradicate the virus, more work is required to be done in the aspects of the identification of the intermediate zoonotic source that caused the transmission of the virus to humans.

Key features and entry mechanism of human coronaviruses

All coronaviruses contain specific genes in ORF1 downstream regions that encode proteins for viral replication, nucleocapsid and spikes formation [25]. The glycoprotein spikes on the outer surface of coronaviruses are responsible for the attachment and entry of the virus to host cells (Fig. 1). The receptor-binding domain (RBD) is loosely attached among virus, therefore, the virus may infect multiple hosts [26,27]. Other coronaviruses mostly recognize aminopeptidases or carbohydrates as a key receptor for entry to human cells while SARS-CoV and MERS-CoV recognize exopeptidases [2]. The entry mechanism of a coronavirus depends upon cellular proteases which include, human airway trypsin-like protease (HAT), cathepsins and transmembrane protease serine 2 (TMPRSS2) that split the spike protein and establish further penetration changes [28,29]. MERS-coronavirus employs dipeptidyl peptidase 4 (DPP4), while HCoV-NL63 and SARS-coronavirus require angiotensin-converting enzyme 2 (ACE2) as a key receptor [2,26].

SARS-CoV-2 possesses the typical coronavirus structure with spike protein and also expressed other polyproteins, nucleoproteins, and membrane proteins, such as RNA polymerase, 3-chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and accessory proteins [30,31]. The spike protein of SARS-CoV-2 contains a 3-D structure in the RBD region to maintain the van der Waals forces [32]. The 394 glutamine residue in the RBD region of SARS-CoV-2 is recognized by the critical lysine 31 residue on the human ACE2 receptor [33]. The entire mechanism of pathogenicity of SARS-CoV-2, from attachment to replication is well mentioned in Fig. 3.

Genomic variations in SARS-CoV-2

The genome of the SARS-CoV-2 has been reported over 80% identical to the previous human coronavirus (SARS-like bat CoV) [34]. The Structural proteins are encoded by the four structural genes, including spike (S), envelope (E), membrane (M) and nucleocapsid (N) genes. The *orf1ab* is the largest gene in SARS-CoV-2 which encodes the pp1ab protein and 15 nsps. The *orf1a* gene encodes for pp1a protein which also contains 10 nsps [34–36]. According to the evolutionary tree, SARS-CoV-2 lies close to the group of SARS-coronaviruses [37,38] (Fig. 5). Recent studies have indicated notable variations in SARS-CoV and SARS-CoV-2 such as the absence of 8a protein and fluctuation in the number of amino acids in 8b and 3c protein in SARS-CoV-2 [34] (Fig. 4). It is also reported that Spike glycoprotein of the Wuhan coronavirus

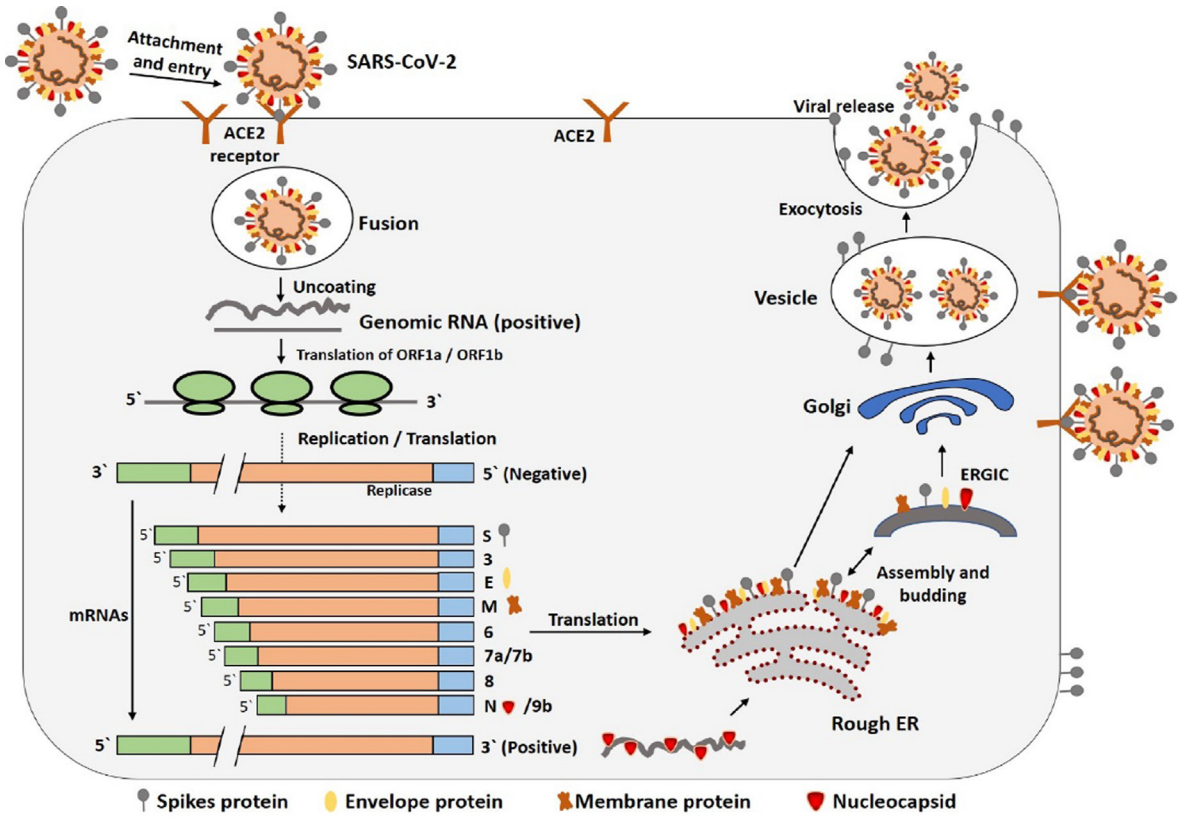


Fig. 3. The life cycle of SARS-CoV-2 in host cells; begins its life cycle when S protein binds to the cellular receptor ACE2. After receptor binding, the conformation change in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway. Then SARS-CoV-2 releases RNA into the host cell. Genome RNA is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. The polymerase produces a series of subgenomic mRNAs by discontinuous transcription and finally translated into relevant viral proteins. Viral proteins and genome RNA are subsequently assembled into virions in the ER and Golgi and then transported via vesicles and released out of the cell. ACE2, angiotensin-converting enzyme 2; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment.

is modified via homologous recombination. The spike glycoprotein of SARS-CoV-2 is the mixture of bat SARS-CoV and a not known Beta-CoV [38]. In a fluorescent study, it was confirmed that the SARS-CoV-2 also uses the same ACE2 (angiotensin-converting enzyme 2) cell receptor and mechanism for the entry to host cell which is previously used by the SARS-CoV [39,40]. The single N501T mutation in SARS-CoV-2's Spike protein may have significantly enhanced its binding affinity for ACE2 [33].

The major obstacle in research progress

Animal models play a vital role to uncover the mechanisms of viral pathogenicity from the entrance to the transmission and designing therapeutic strategies. Previously, to examine the replication of SARS-CoV, various animal models were used which showed the symptoms of severe infection [43]. In contrast to SARS-CoV, no MERS-CoV pathogenesis was observed in small animals. Mice are not vulnerable to infection by MERS-coronavirus due to the non-compatibility of the DPP4 receptor [44]. As the entire genome of the 2019-novel coronavirus is more than 80% similar to the previous human SARS-like bat CoV, previously used animal models for SARS-CoV can be utilized to study the infectious pathogenicity of SARS-CoV-2. The human ACE2 cell receptor is recognized by both SARS and Novel coronaviruses. Conclusively, TALEN or CRISPR-mediated genetically modified hamsters or other small animals can be utilized for the study of the pathogenicity of novel coronaviruses. SARS-CoV has been reported to replicate and cause severe disease in Rats (F344), where the sequence analysis revealed a mutation at spike glycoprotein [45]. Thus, it could be another suitable option to develop spike glycoprotein targeting therapeutics against novel coronaviruses. Recently, mice models and clinical isolates were used to develop any therapeutic strategy against SARS-CoV-2 induced COVID-19 [46,47]. In a similar study, artificial intelligence prediction was used to investigate the inhibitory role of the drug against SARS-CoV-2 [48]. SARS-CoV-2 infected patients were also used to conduct randomized clinical trials [46,49,50]. It is now important that the scientists worldwide collaborate the design a suitable model and investigate the in vivo mechanisms associated with pathogenesis of SARS-CoV-2.

Potential therapeutic strategies against COVID-19

Initially, interferons-α nebulization, broad-spectrum antibiotics, and anti-viral drugs were used to reduce the viral load [49,51,52], however, only remdesivir has shown promising impact against the virus [53]. Remdesivir only and in combination with chloroquine or interferon beta significantly blocked the SARS-CoV-2 replication and patients were declared as clinically recovered [46,50,52]. Various other anti-virals are currently being evaluated against infection. Nafamostat, Nitazoxanide, Ribavirin, Penciclovir, Favipiravir, Ritonavir, AAK1, Baricitinib, and Arbidol exhibited moderate results when tested against infection in patients and *in-vitro* clinical isolates [46,48,50,52]. Several other combinations, such as combining the antiviral or antibiotics with traditional Chinese medicines were also evaluated against SARS-CoV-2 induced infection in humans and mice [46]. Recently in Shanghai, doctors isolated the blood plasma from clinically recovered patients of COVID-19 and injected it in the infected patients

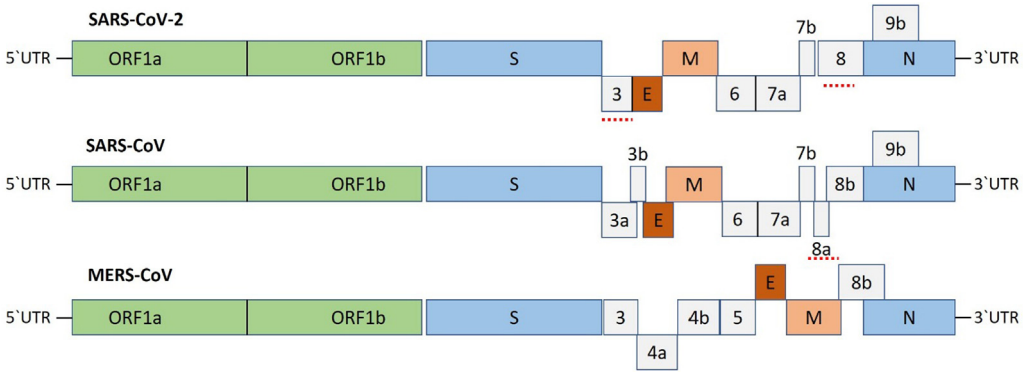


Fig. 4. Betacoronaviruses genome organization; The Betacoronavirus for human (SARS-CoV-2, SARS-CoV and MERS-CoV) genome comprises of the 5′-untranslated region (5′-UTR), open reading frame (orf) 1a/b (green box) encoding non-structural proteins (nsp) for replication, structural proteins including spike (blue box), envelop (maroon box), membrane (pink box), and nucleocapsid (cyan box) proteins, accessory proteins (light gray boxes) such as orf 3, 6, 7a, 7b, 8 and 9b in the SARS-CoV-2 genome, and the 3′-untranslated region (3′-UTR). The doted underlined in red are the protein which shows key variation between SARS-CoV-2 and SARS-CoV. The length of nsps and orfs are not drawn in scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

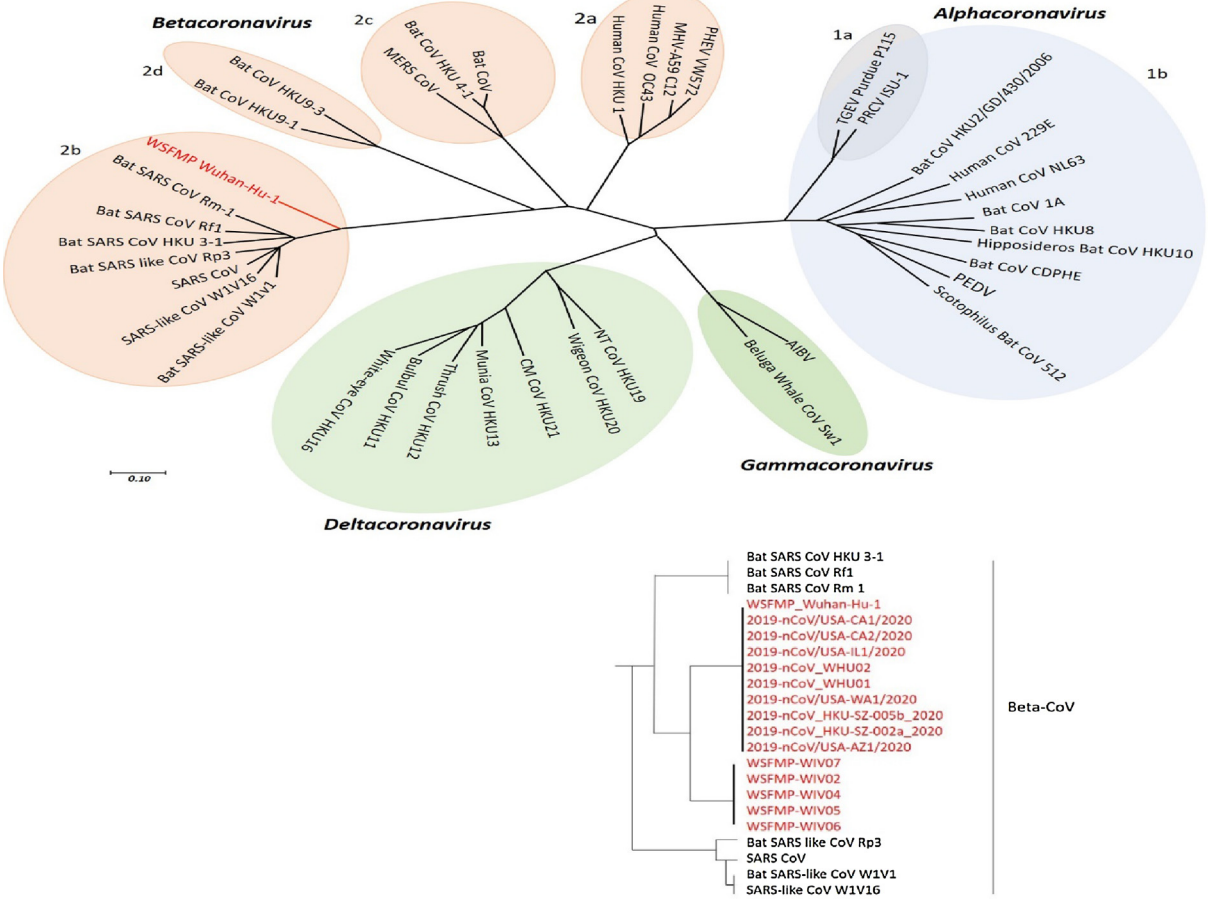


Fig. 5. Phylogenetic tree of coronaviruses (content in red is the latest addition of newly emerged SARS-CoV-2 and WSFMP Wuhan-Hu-1 is used as a reference in the tree); The phylogenetic tree showing the relationship of Wuhan-Hu-1 (denoted as red) to selected coronavirus is based on nucleotide sequences of the complete genome. The viruses are grouped into four genera (prototype shown): Alphacoronavirus (sky blue), Betacoronavirus (pink), Gammacoronavirus (green) and Deltacoronavirus (light blue). Subgroup clusters are labeled as 1a and 1b for the Alphacoronavirus and 2a, 2b, 2c, and 2d for the Betacoronavirus. This tree is based on the published trees of Coronavirinae [3,41] and reconstructed with sequences of the complete RNA- dependent RNA polymerase- coding region of the representative novel coronaviruses (maximum likelihood method using MEGA 7.2 software). severe acute respiratory syndrome coronavirus (SARS- CoV); SARS- related coronavirus (SARSr- CoV); the Middle East respiratory syndrome coronavirus (MERS- CoV); porcine enteric diarrhea virus (PEDV); Wuhan seafood market pneumonia (Wuhan-Hu-1). Bat CoV RaTG13 Showed high sequence identity to SARS-CoV-2 [42]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

who showed positive results with rapid recovery [54]. In a recent study, it was identified that monoclonal antibody (CR3022) binds - with the spike RBD of SARS-CoV-2. This is likely due to the antibody's epitope not overlapping with the divergent ACE2 receptor-binding motif. CR3022 has the potential to be developed as a therapeutic candidate, alone or in combination with other neutralizing antibodies for the prevention and treatment of - COVID-19 infection [55].

Vaccines for SARS-CoV-2

There is no available vaccine against COVID-19, while previous vaccines or strategies used to develop a vaccine against SARS-CoV can be effective. Recombinant protein from the Urbani (AY278741) strain of SARS-CoV was administered to mice and hamsters, resulted in the production of neutralizing antibodies and protection against SARS-CoV [56,57]. The DNA fragment, inactivated whole virus or live-vectored strain of SARS-CoV (AY278741), significantly reduced the viral infection in various animal models [58–63]. Different other strains of SARS-CoV were also used to produce inactivated or live-vectored vaccines which efficiently reduced the viral load in animal models. These strains include, Tor2 (AY274119) [64,65], Utah (AY714217) [66], FRA (AY310120) [59], HKU-39849 (AY278491) [57,67], BJ01 (AY278488) [68,69], NS1 (AY508724) [70], ZJ01 (AY297028) [70], GD01 (AY278489) [69] and GZ50 (AY304495) [71]. However, there are few vaccines in the pipeline against SARS-CoV-2. The mRNA based vaccine prepared by the US National Institute of Allergy and Infectious Diseases against SARS-CoV-2 is under phase 1 trial [72]. INO-4800-DNA based vaccine will be soon available for human testing [73]. Chinese Centre for Disease Control and Prevention (CDC) working on the development of an inactivated virus vaccine [74,75]. Soon mRNA based vaccine's sample (prepared by Stermirna Therapeutics) will be available [76]. GeoVax-BravoVax is working to develop a Modified Vaccina Ankara (MVA) based vaccine [77]. While Clover Biopharmaceuticals is developing a recombinant 2019-nCoV S protein subunit-trimer based vaccine [78].

Although research teams all over the world are working to investigate the key features, pathogenesis and treatment options, it is deemed necessary to focus on competitive therapeutic options and cross-resistance of other vaccines. For instance, there is a possibility that vaccines for other diseases such as rubella or measles can create cross-resistance for SARS-CoV-2. This statement of cross-resistance is based on the observations that children in china were found less vulnerable to infection as compared to the elder population, while children are being largely vaccinated for measles in China.

Conclusion and perspective

The novel coronavirus originated from the Hunan seafood market at Wuhan, China where bats, snakes, raccoon dogs, palm civets, and other animals are sold, and rapidly spread up to 109 countries. The zoonotic source of SARS-CoV-2 is not confirmed, however, sequence-based analysis suggested bats as the key reservoir. DNA recombination was found to be involved at spike glycoprotein which assorted SARS-CoV (CoVZXC21 or CoVZC45) with the RBD of another Beta CoV, thus could be the reason for cross-species transmission and rapid infection. According to phylogenetic trees, SARS-CoV is closer to SARS-like bat CoVs. Until now, no promising clinical treatments or prevention strategies have been developed against human coronaviruses. However, the researchers are working to develop efficient therapeutic strategies to cope with the novel coronaviruses. Various broad-spectrum antivirals previously used against influenza, SARS and MERS coronaviruses have been evaluated either alone or in combinations to treat COVID-19 patients, mice models, and clinical isolates. Remdesivir, Lopinavir, Ritonavir, and Oseltamivir significantly blocked the COVID-19 infection in infected patients. It can be cocluded that the homologus recombination event at the S protein of RBD region enhanced the transmission ability of the virus. While the decision of bring back the nationals from infected area by various countries and poor screening of passengers, become the leading cause of spreading virus in others countries.

Most importantly, human coronaviruses targeting vaccines and antiviral drugs should be designed that could be used against the current as well as future epidemics. There are many companies working for the development of effective SARS-CoV-2 vaccines, such as Moderna Therapeutics, Inovio Pharmaceuticals, Novavax, Vir Biotechnology, Stermirna Therapeutics, Johnson & Johnson, VIDO-InterVac, GeoVax-BravoVax, Clover Biopharmaceuticals, CureVac, and Codagenix. But there is a need for rapid human and animal-based trails as these vaccines still require 3–10 months for commercialization. There must be a complete ban on utilizing wild animals and birds as a source of food. Beside the development of most efficient drug, a strategy to rapidly diagnose SARS-CoV-2 in suspected patient is also required. The signs and symptoms of SARS-CoV-2 induced COVID-19 are a bit similar to influenza and seasonal allergies (pollen allergies). Person suffering from influenza or seasonal allergy may also exhibit temprature which can be detected by thermo-scanners, hence the person will become suspected. Therefore, an accurate and rapid diagnostic kit or meter for detection of SARS-CoV-2 in suspected patients is required, as the PCR based testing is expensive and time consuming. Different teams of Chinese doctors should immediately sent to Eurpean and other countries, especially spain and Italy to control the over spread of COVID-19, because Chinese doctors have efficiently controlled the outbreak in china and limited the mortality rate to less than 3% only. The therapeutic strategies used by Chinese, should also be followed by other countries.

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Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest.

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