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Severe acute respiratory syndrome coronavirus two (Sars-Cov-2) review

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Abstract
Covid-19 is a highly contagious zoonotic disease of humans and animals caused by SARS-CoV-2. The WHO has declared the enduring outbreak of COVID-19 is a global public health emergency and pandemic. SARS-CoV-2 is a spherical positive sense, single-stranded RNA viruses that belong to beta coronavirus, subgenus sarbecovirus. The genomes of the virus are mainly composed of four types of structural proteins that are spike glycoprotein, envelope protein, nucleocapsid protein, and membrane protein. The genetic sequence of the SARS-CoV-2 showed more than 80% identity to SARS-CoV and 50% to the MERS-CoV. RaTG13 is the only BatCoV that shows as high as 96% full genome homology with SARS-CoV-2. The virus is suggested to be originated from bats and most closely relating to beta coronavirus of bat origin since it has 87.9% and more nucleotide similarity.
The mutation is one of the driving factors for the evolution of the organisms. Community transmission, antiviral treatments, and ecological changes along with the genetic plasticity of RNA viruses facilitate the emergence of several new RNA viruses and the development of more virulent strains. The evolved strains may cause a high mortality rate and resistant to treatments. Therefore, systematic tracking of demographic, clinical patient information, and strain information is crucial to successfully combat COVID-19. Molecular methods, serology, and viral culture are used as diagnostic tools for isolation and identification of newly emerged viral disease. The RT-qPCR assay is regarded as the gold standard method for identification and surveillance of SARS-CoV-2 target sequences.

Keywords: SARS-CoV-2, COVID-19, genetic organization, phylogenetic analysis, and diagnosis

1. Introduction
Coronaviruses are zoonotic pathogens that infect both humans and animals (Sahin *et al*., 2020) [59]. Coronaviruses are known for their capacity to mutate quickly, change tissue tropism, traverse the species barrier, and adapt to a variety of epidemiological situations (Helmy *et al*, 2020) [32]. Since the 1960s, seven different kinds of human coronaviruses have been identified as respiratory pathogens. These are HCoV-229E, HCoV-NL63, HCoV-OC43, KHU1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). MERS-CoV, SARS-CoV, and SARS-CoV-2 belong to the genus Betacoronavirus (Ortiz-prado *et al*., 2020) [52]. Among these human coronaviruses, four of them cause mild illness which is similar to the common cold and gastrointestinal tract infection. Whereas severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV-2) are highly pathogenic and have significant public health concerns due to their zoonotic emergence and crossing of the species barrier (Wu *et al*., 2020) [69]. Coronaviruses are enveloped single-strand RNA viruses that can infect a wide range of hosts including avian, wild, domestic mammalian species, and humans.
The SARS-CoV-2 virus causes Coronavirus Disease 2019 (COVID-19), a newly emerging epidemic pathogen of humans and animals (Mason, 2020). Humans suffer a severe acute respiratory infection caused by the virus, which can be spreed from person to person. (Tang *et al*, 2020) [70]. SARS-CoV-2 was first detected in Wuhan, China, causing outbreaks of the coronavirus disease COVID-19 that has now spread globally (Jaimes, *et al*., 2020) [35]. The World Health Organization (WHO) temporarily named the newly emerged pathogenic virus like the 2019 novel coronavirus (2019-nCoV (Tahir *et al*., 2020) [63].

International Committee of Taxonomy of Viruses (ICTV) announced "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" as the name of the new virus on 11 February 2020 based on phylogeny, taxonomy, and established practice (Zu *et al*., 2019b) [75]. The virus was given this name because it is genetically linked to the coronavirus that responsibl the SARS pandemic in 2003 (Li *et al*., 2020).

COVID-19 was announced a Public Health Emergency of International Concern by the World Health Organization on January 31, 2020. (PHEIC). The WHO declared the COVID-19 outbreak a pandemic on March 11, 2020, since the virus is spreading globally (Ramphul and Mejias, 2020) [56]. The emergence of SARS-CoV-2 has been marked as the third introduction of a highly pathogenic coronavirus into the human population after the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) in the twenty-first century (Li *et al*., 2020) [45].

The newly emerged coronavirus is mainly caused respiratory and gastrointestinal tract infections which made similar to highly pathogenic human coronaviruses. Droplets and close contact are the most common routes of transmission of SARS-CoV-2 generated during coughing and sneezing by symptomatic and asymptomatic patients (Singhal, 2020) [62]. Source of infection, route of transmission, and susceptibility were reported as important factors in the viral spreading mechanism. Bats are considered to be the natural host of the virus and serve as the sources of infection. Pangolins, snakes, and other animals are thought to be intermediate hosts (Wang *et al*., 2020) [67].

SARS-CoV-2 forms a different lineage with Bats SARS-like CoV that is belonging to the Coronaviridae family, Betacoronavirus genus, and Sarbecovirus subgenus based on phylogenetic analysis. It shares 96.3%, 89%, and 82% nucleotide resemblance with bat CoV RaTG13, SARS-like CoV ZXC21, and SARS-CoV respectively. This genomic resemblance of the virus confirms its zoonotic origin (Helmy *et al*., 2020) [32].

Viral infections are the most frequent infectious diseases and are common triggers for constituting major biological, clinical, and socioeconomic problems worldwide. SARS-CoV-2, SARS-CoV, and MERS-CoV are the major global outbreaks infections with alarming health and economic concerns in their respective periods (Meo *et al*., 2020) [49]. The first outbreak of SARS-CoV occurred in Saudi Arabia in 2002, the second MERS-CoV in Asia in 2012, and followed by the novel coronavirus 2019 in Wuhan, China which has become the third coronavirus to appear in the human population and threatened the entire world (Helmy *et al*., 2020) [32].

These coronaviruses have zoonotic importance and infect different species of wild and domestic animals such as camels, cattle, cats, and bats. The viruses are transmitted from animal to animal, animal to human, and human to human. MERS-CoV transmission originated from bats to camels and then to humans.

Genetic sequence data suggested that the SARS-CoV-2 virus originated from an animal source, but currently, there is no sufficient proof to identify exactly either the source or the route of transmission from the original animal reservoir to a proposed intermediate host and then to humans (European Commission, 2020). But bats are considered as the possible origin of the newly emerged SARS-CoV-2 virus. Ones COVID-19 disease transmitted in either source to human in turn human to human transmission has been confirmed (Meo *et al*., 2020) [49]. All the sequences from United States patients are similar to the one that China initially posted which suggesting that the likely origin of the novel coronavirus is from the animal reservoir.

This is, therefore; the objective of this seminar is to systematically review newly emerged SARS-CoV-2 via focusing on genomic organization, phylogenic analysis, diagnosis, and other features based on recent research progress.

2. History and origin of Sars-COV-2

China notified the outbreak of COVID-19 disease to the World Health Organization on December 31st, 2019. China shuttered the Huanan seafood market on January 1st. The virus is originated from the Huanan seafood market since environmental samples taken from there show positive results (Singhal, 2020) [62]. The massive migration of Chinese during the Chinese New Year increases the epidemic. Cases of COVID-19 in countries outside china were reported with no history of travel to china suggesting that the occurrence of human to human transmission. SARS-CoV-2 has infected several thousands of people and has caused many fatal cases. The cases of the virus are increasing exponentially across the world.

Determining the origin and evolution of 2019-nCoV is important for the surveillance, drug and vaccine discovery, and prevention of the epidemic (Zhang *et al*., 2020) [71]. The zoonotic source of SARS-CoV-2 is not confirmed, however, the sequence-based analysis suggested bats are as the main reservoir (Hafeez *et al*., 2020) [29]. The studies showed that SARS-CoV-2 has an 80% resemblance to Rhinolophus sinicus bat and 96% resemblance with the Rhinolophus affinis bat based on the genomic comparison. Based on this evidence SARS-CoV-2 originated from bats. The novel coronavirus had 91.02% or more genomic similarity with pangolins as different findings indicated and suggested that the animal may be an intermediate host of the virus (Ramphul and Mejias, 2020) [56].

Generally, research evidence suggests that SARS-CoV-2, MERS-CoV, and the original SARS-CoV all originated from bats. SARS-CoV and MERS-CoV then spread from infected civets and dromedary camels to people respectively. Scientists are trying to determine how SARS-CoV-2 spread to people (Tahir *et al*., 2020) [63]. Extensive recombination among bat coronaviruses and strong purifying selection pressure among viruses from humans, bats, and pangolin may allow jumping between new hosts (Akram and Mannan, 2020a) [4].

3. Classification of sars-cov-2

The genus Coronaviruses are the largest group of viruses belongs to the order Nidovirales in the *coronaviridae* family (Sahin *et al*., 2020) [59]. The family *Coronaviridae* contains two

subfamilies: Orthocoronavirinae and Torovirinae. The subfa mily Orthocoronavirinae is further classified on basis of phy logenic analysis and genomic structure into four genera: *alp hacoronavirus, betacoronavirus, gamma coronavirus, and delta coronavirus* (Ashour *et al*, 2020) [7]. *Alpha and beta coronaviruses* circulate in mammals and bats. Gamma coronaviruses mostly infect avian species and a few

mammalian species, whereas delta coronaviruses infect birds and mammals. SARS-CoV-2 represents a distinct lineage in the subgenus *Sarbecovirus*. Corona in Latin means crown and gets its name due to the presence of spike projection from an enveloped virus. Nido refers to the ability of the order to make a nested set of subgenomic mRNA (Helmy *et al*., 2020) [32].

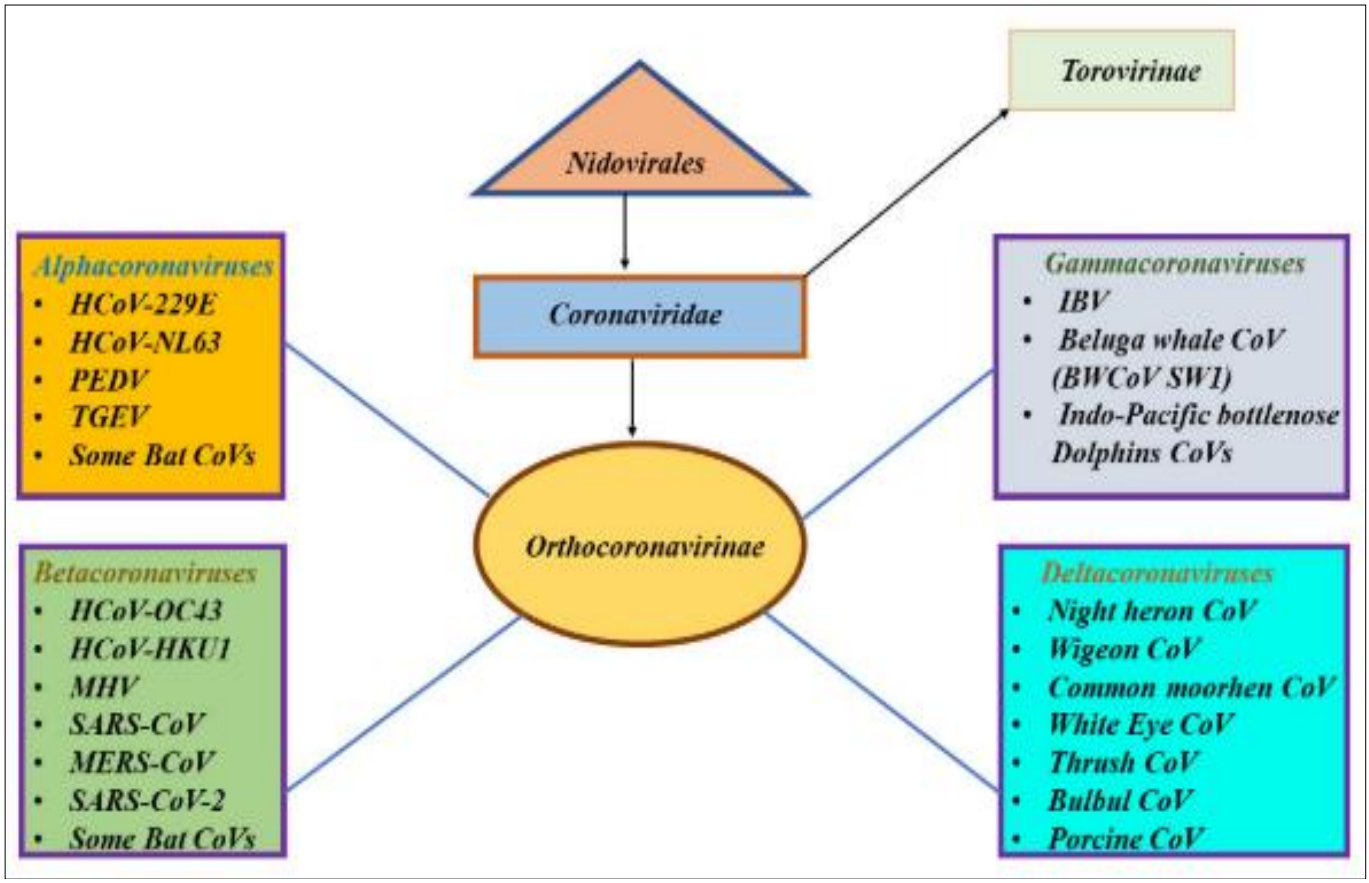


Fig 1: Classification of different types of coronaviruses within the family Coronaviridae, subfamily Orthocoronavirinae, and their respective genera (Ashour *et al*., 2020) [7]

4. Host diversity

There is a limited study on the investigation of SARS-CoV-2 in animals. SARS-CoV-2 can distinguish the host cells receptor ACE2 of pigs, ferrets, cats, and monkeys in similar efficiency with humans as different studies confirmed. Cats and ferrets are well susceptible to SARS-CoV-2 in an experimental study (Hossain *et al*., 2020) [33]. Identifying the host range of SARS-CoV-2 is important since some domestic species might harbor the virus and transmit it back to humans (Li *et al*., 2020) [45].

Cats, dogs, ferrets, lions, tigers, and other animals can contract the virus from a different source. Cat, dog, and ferrets are susceptible to SARS-CoV-2 according to natural infections history and experimental results. Whereas pigs, chickens, and ducks are not susceptible to the virus (Hossain *et al*., 2020) [33]. The susceptibility of the host to coronavirus infection is depending on the affinity between the viral receptor-binding domain and host ACE2 in the initial attachment step. The spike protein receptor-binding domain (RBD) is the critical determinant of viral tropism and infectivity. Infected cats and ferrets may transmit SARS-CoV-2 into the healthy groups lived together through direct or indirect contact and/or airborne routes. There is no current evidence of transmission from cats to humans. ACE2 molecules responsible for the binding of the SARS-CoV-2 spike are almost identical in pigs, ferrets, cats, orangutans, monkeys, and humans (Wan *et al*., 2020) [66]. Phylogenetic clustering and sequence alignment study showed that ACE2 of several domestic animals (cat, cow, buffalo, goat, sheep, and pigeon) could be used by SARS-CoV-2 (Qiu *et al*., 2020) [55]. Therefore, a wide range of domestic and wild animals may support the entry of the virus to humans.

5. Molecular characterization of sar-CoV-2

The genomic sequence of SARS-CoV-2 is almost identical to more than 99.9% sequence identity among patients. The length of the genome is 29, 891bases. It has six major open reading frames and encodes accessory proteins (Artika *et al*., 2020) [6]. Some of the SARS-CoV-2 has less than 80% nucleotide sequence identity to the analogous genes of SARS-CoV. However, there is 94.4% sequence identity between SARS-CoV-2 and SARS-CoV when compared with the amino acid sequence of conserved replacase domain in ORF1ab. Therefore, SARS-CoV-2 and SARS-CoV are considered as the same species which are SARS-related coronavirus as different findings indicated (Zhou *et al*., 2020) [74].

5.1 Genetic structure of the SARS-CoV-2

SARS-CoV-2 are enveloped icosahedral, spherical, positive sense, single-stranded RNA viruses with non-segmented genome that are characterized by spike proteins projecting from the virion surface (Helmy *et al*., 2020) [32]. The name

coronavirus is given due to the appearance of the virus as a royal crown under an electron microscope. Spike glycoprotein on the envelope leads to the formation of a royal crown shape. The viral structure formed primarily from four structural proteins such as spike (S), membrane (M), envelope (E), matrix, and nucleocapsid (N) proteins (Ashour *et al*., 2020) [7]. Nucleocapsid protein holds the RNA genome. The remaining protein S, M, and E together create a viral envelope (Akram and Mannan, 2020b) [5]. COVID-19 differs from other coronaviruses by encoding an additional glycoprotein that has acetyl esterase and hemagglutination (HE) properties (Kannan *et al*, 2020) [37]. HE found on the surface of some beta coronavirus that binds to sialic acid on host cell surface glycoprotein and possesses acetyl-esterase activity. It enhances the entry and pathogenesis of coronavirus (Ashour *et al*., 2020) [7].

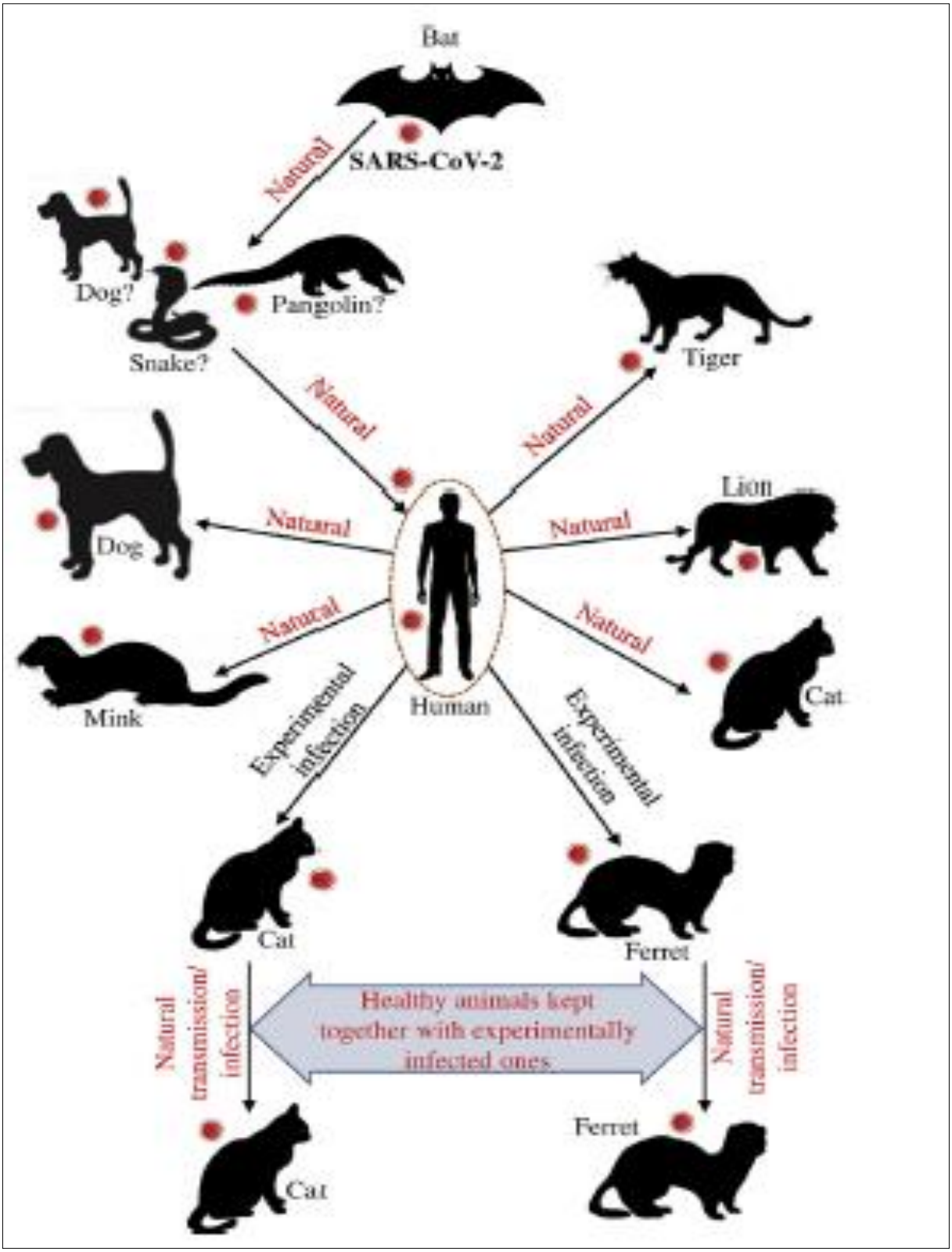


Fig 2: Host range of SARS-CoV-2.Different pets/animals are susceptible to SARS-CoV-2 might be occurred naturally and/or experimentally (Hossain *et al*., 2020) [33]

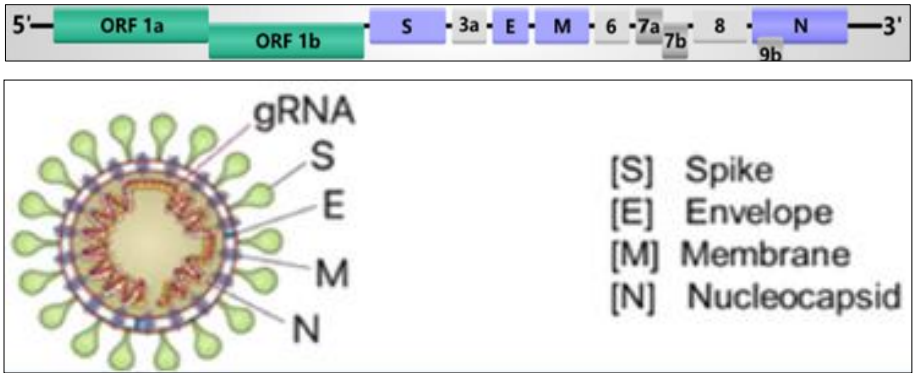


Fig 3: Genomic structure of SARS-CoV-2.The SARS-CoV-2 genome comprises of the 5′-untranslated region (5′-UTR), open reading frame (ORF)1a/b encoding non-structural proteins(nsp), structural proteins, accessory proteins, and the 3′-untranslated region (3′-UTR) (Wu *et al.*, 2020) [6]

5.1.1 Nucleocapsid protein (N)

Nucleocapsid protein is encoded via a conserved gene and used as a molecular marker. It binds viral RNA and is located in the core of viral particles while the Spike, membrane, and envelope proteins are all embedded in the viral envelope. The protein is used for the packing of viral RNA into viral particles during viral assembly (Artika *et al*., 2020) [6]. The nucleocapsid (N) protein of novel coronavirus has almost 90% amino acid sequence identity with SARS-CoV (Kannan *et al*., 2020) [37]. N protein contains two non-interacting structural domains that are the N-terminal RNA-binding domain (RBD) and the C-terminal dimerization domain (DD), surrounded by flexible linkers. The C-terminal domain presents completely as a dimer in solution. The flexible linkers are essentially interaction sites with other protein and protein-RNA partners (Wan *et al*., 2020) [66].

5.1.2 Membrane protein (M)

The membrane protein is found in higher quantities than other proteins. It has a critical role in virion assembly via M-M, M-S, and M-N protein interactions. The M proteins have the same basic structure as other coronaviruses even though their amino acid contents are different (Artika *et al*., 2020) [6]. M protein has a triple-helix bundle which forms a single three-transmembrane domain(TM). It has also a short aminoterminal domain outside of the membrane and a long carboxy terminal domain inside the membrane (Thomas, 2019) [64]. A mutation may influence host cell attachment and entry of the virus when M protein cooperates with spike protein.

5.1.3 Spike surface glycoprotein (S)

Spike protein is critical for SARS-CoV-2 infection. Glycosylated spike protein coat the surface of SARS-CoV-2 and bind to host cell receptor ACE2 which is mediating the entry of the virus into the host cell. S protein consists of an extracellular N-terminus, a transmembrane (TM) domain attached in the viral membrane, and a short intracellular C-terminal segment (Berend *et al*., 2007) [9]. Transmembrane protease serine two (TMPSS2) is located on the host cell membrane which promotes entry of the virus when it binds to the spike surface glycoprotein of the virus. The SARS-CoV-2 S protein is highly conserved among all human coronaviruses (HCoVs) and is involved in receptor recognition, viral attachment, and entry into host cells (Huang *et al*., 2020) [34]. The Spike protein consists of receptor-binding S1 and membrane-fusion S2 subunits, which is responsible for attachment to the host cell receptor and fusion with the cell membrane (Wu *et al*., 2020) [69]. Therefore, it is one of the most important targets for the SARS-CoV-2 vaccine and therapeutic developments.

The spike surface glycoprotein and its RBD in the SARS-CoV-2 virus is a principal determinant of the host range, tissue tropism, and pathogenicity. It is the major antigens that stimulate neutralizing antibody and important targets of cytotoxic lymphocytes. Spike protein(S gene) is highly important for molecular characterization of SARS-CoV-2 (Artika *et al*., 2020) [6].

5.1.4 Envelope protein (E)

The envelope protein is present in a small amount within the virion that determines the shape of the virus jointly with membrane protein (Schoeman and Fielding, 2019) [60]. It is highly divergent which has N-terminal ectodomain and C-terminal endodomain. Envelope protein plays a significant role during the replication of coronavirus which facilitating assembly and release, budding, envelope formation, and pathogenesis aspects (Fehr and Perlman, 2015) [26]. The primary and secondary structure of envelope proteins indicates that as it has short hydrophobic N-terminus of 7-12 amino acids. The envelope protein of SARS-CoV-2 has long amino acid (76aa) and possesses three critical domains. They are N-terminus, transmembrane (25amino acid), and C-terminus. The long amino terminus has hydrophilic ends. The C-terminus domain of novel corona virus is essential for the establishment and maintenance of epithelial polarity in mammals (Hassan *et al*., 2020) [31].

5.2 Genomic organization of SARS-COV-2

Coronaviruses are viruses whose genome structure is best known among all RNA viruses. There are four major structural proteins encoded by the coronaviral genome on the envelope (Zu *et al*., 2019b) [75]. The structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N). The spike protein allows the virus to bind to the host's cell membrane (Ramphul and Mejias, 2020) [56]. The typical features of the genus coronavirus are a highly conserved genomic organization with 27-32kb positive polarity (Sahin *et al*., 2020) [59].

A SARS-CoV-2 virion is a monopartite, an enveloped, positive-sense, single-stranded RNA virus with a genome size of 29,903 nucleotides (Helmy *et al*., 2020) [31]. SARS-CoV-2 has a similar genomic organization to other beta-coronaviruses. The viral genome contains two untranslated regions (UTRs) that are 5'-untranslated region and

3'untranslated region or 3' poly (A) tail. It also contains 11 open reading frames (ORF) that encodes 27 proteins. The ORF1ab and ORF1a genes are located at the 5'-terminus of the genome that encodes the PP1ab and pp1a proteins respectively. The 3'-terminus of the genome holds four structural proteins and eight accessory proteins. The UTR acts as mRNA for the translation of replicase polyproteins (Wu *et al*., 2020) [6].

The two large open reading frame genes (ORF1ab and ORF1a) or replicase gene contain two-third of the genomes (20kb) which encodes 16 non-structural proteins.

The remaining one-third of viral genomes comprise a structural gene unit that encodes four structural proteins(S, E, M, and N) and eight accessory proteins. Eight accessory proteins are orf3a, orf3b, orf6, orf7a, orf7b, orf8, orf14, and orf10) (Helmy *et al*., 2020) [32].

The structural and accessory proteins contain only 10kb of the viral genome. The 5' cap of the genome contains a leader sequence and untranslated region (UTR) that contains multiple stem-loop structures required for RNA replication and transcription (Fehr and Perlman, 2015) [26]. The 3′UTR also contains RNA structures required for replication and synthesis of viral RNA. Some accessory proteins have a significant role in viral pathogenesis. But it is nonessential for replication in tissue culture. Nonstructural proteins are essential for virus replication and pathogenesis. The four structural proteins are important for virus subtyping and response to vaccines.

5.3 Phylogenetic Analysis

Phylogenetic analysis is a technique to elucidate the evolutionary narration and relationships among a group of organisms. It is usually demonstrated in the form of a phylogenic tree to decide the evolutionary relationship between species clearly (Dayu *et al*., 2020) [17]. Single-stranded RNA viruses demonstrate a faster biological mutation rate due to the lack of proofreading activity of viral RNA polymerases (Elena and Sanjua, 2005) [25].

Phylogenetic analyses of coronaviruses have confirmed that the immediate ancestor of SARS-CoV-2 is most likely originated in a bat species. Though, whether SARS-CoV-2 or the progenitor of this virus is transmitted directly to humans or via an intermediate host is not yet determined. Deep comparative genomic analysis, evolutionary understanding, and structural analysis of ACE2 play a significant role to identify candidate intermediate host species and species at risk for SARS-CoV-2 (Damas *et al*., 2020) [16].

SARS-CoV-2 is a beta coronavirus, subgenus sarbecovirus, lineage B, and genetically related to SARS related coronaviruses found in Chinese horseshoe bats (Chan *et al*., 2020) [11]. The genome sequence of COVID-19 isolates showed that 99.98 and 99.9% sequence identity among patients as different studies reported. The genetic sequence of the SARS-CoV-2 showed more than 80% identity to SARS-CoV and 50% to the MERS-CoV (Rothan and Byrareddy, 2020) [57]. MERS-CoV and human CoV HKU1 are very distant from SARS-CoV-2 (Wu *et al*., 2020) [69]. Based on the genetic sequence identity and the phylogenetic reports, SARS-CoV-2 is sufficiently different from SARS-CoV and it can be considered as a new beta coronavirus that infects humans. The amino acid sequence of the virus differs from other coronaviruses specifically in the regions of ORF1ab polyprotein and surface glycoprotein (Kannan *et al*., 2020) [37].

Discovering of the possible intermediate hosts of SARS-CoV-2 has a significant role in blocking of its interspecies transmission. The full-length genome of SARS-CoV-2 shows that as there is a high similarity to the genome of bat coronavirus, detected in Rhinolophus affinis (horseshoe bats). It is 96.2% sequence identity with bat coronavirus (BatCoVRaTG13). RaTG13 is the only BatCoV that shows as high as 96% full genome homology with SARS-CoV-2. The phylogenic analysis of the spike gene and RdRp gene also shows RaTG13 is the first closest relative of the SARS-CoV-2 and they form a distinct lineage from the other SARS-related CoVs. It is also 98.7% nucleotide similarity to the partial RdRp gene of the bat coronavirus strain BtCoV/4991 and 87.9% nucleotide similarity to bat coronavirus strain bat-SL CoVZC45 and bat-SL-CoVZXC21 (Lai *et al*., 2020) [42]. These two bats SARS-like CoV are the second closest virus from bats to SARS-CoV-2. Therefore, based on sequence genome and phylogenic analysis, SARS-CoV-2 is suggested to be originated from bats and most closely relating to betacoronavirus of bat origin (Zhou *et al*., 2020; Zhang and Holmes, 2020) [74, 73]. The phylogenetic analysis indicates that the SARS-CoV-2 belongs to subgenus Sarbecovirus, genus Betacoronavirus, and family Coronaviridae, which includes SARS-CoV and MERS-CoV (Dayu *et al*., 2020) [17].

Pangolin-CoV isolated from Malayan pangolins demonstrates that 91.02% genomic distinctiveness to SARS-CoV-2 and 90.55% identity to the BatCoV RaTG13 (Zhang *et al*., 2020) [71] based on research findings. The pangolin coronavirus spike protein sequences shared five key amino acids in the RBD with that of SARS-CoV-2 whereas RaTG13 shared only one key amino acids in its RBD. And only SARS-CoV-2 has a G-C rich polybasic furin cleavage site at the S1/S2 junction in the spike protein. Polybasic cleavage site (RRAR) is the second distinct characteristic of SARS-CoV2 located at the junction of S1 and S2 subunit of the spike protein. RaTG13 and pangolin coronaviruses do not have G-C rich cleavage site (Lin *et al*., 2020) [46]. Pangolin-CoV may be the second closest relative of SARS-CoV-2 next to RaTG13. The subunit of the S1 protein of Pangolin-CoV more similar to SARS-CoV-2 than RaTG13 (Artika *et al*., 2020) [6].

Generally, Pangolins may be considered as possible hosts of SARS-CoV-2 and play critical roles in the emergence of novel coronavirus since they have many similar identities to novel coronavirus as different findings reported Snakes, birds, and other small mammals also suggested being the origin of the newly emerged novel coronavirus since there is no specific animal associated with SARS-CoV-2 identified (Prompetchara *et al*., 2020) [78].

The existence of a higher degree of homology of the ACE2 receptor from a diversity of animal species is indicating that animal species can be a possible intermediate host or animal model for COVID-19 infections (Rothan and Byrareddy, 2020) [57].

The S proteins of SARS-CoV-2 and SARS-CoV have an amino-acid sequence identity of around 77% as studies reported. The Spike gene of SARS-CoV-2 and RaTG13 is longer than SARS-related coronavirus. It has three short insertions in the N terminal domain and changes in four out of five key residues in receptor binding motif (RBM) compared with the S gene sequences of SARS-CoV (Wu *et al*., 2020) [69].

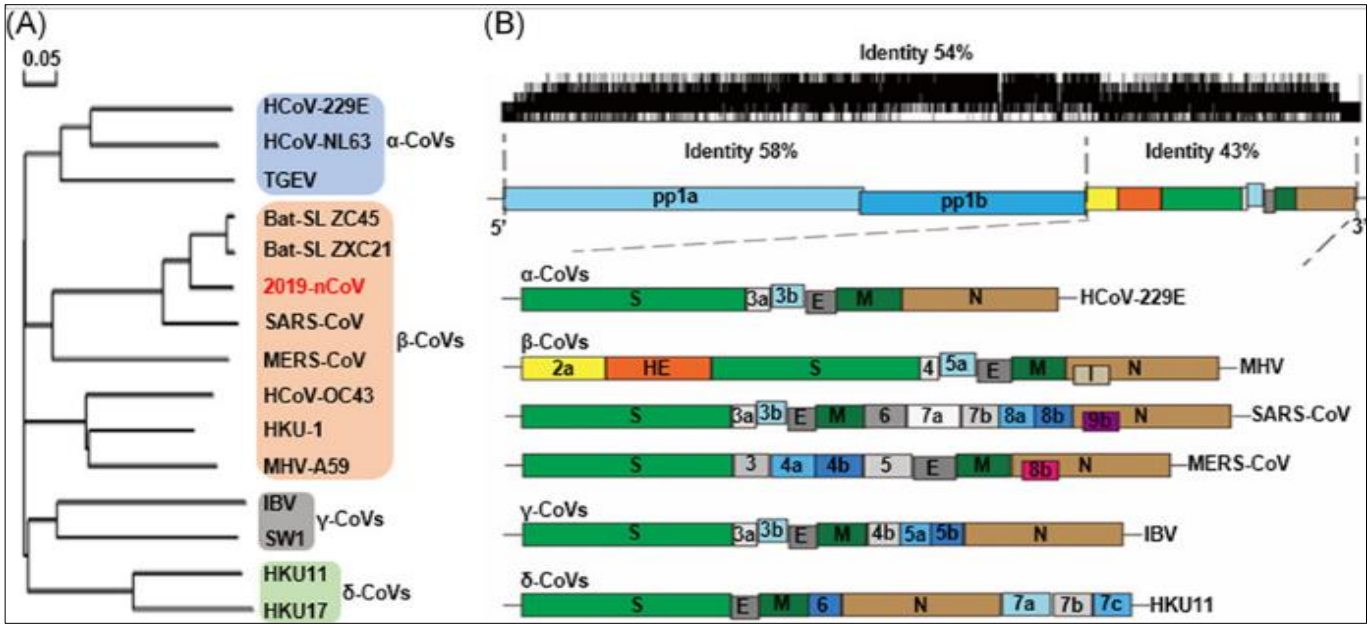


Fig 4: The genomic structure and phylogenetic tree of coronaviruses. B, The genome structure of four genera of coronaviruses. Pp1a and pp1b represent the two long polypeptides that are processed into 16 nonstructural proteins. HKU, coronaviruses identified by Hong Kong University; HCoV, human coronavirus; IBV, infectious bronchitis virus; MHV, murine hepatitis virus; TGEV, transmissible gastroenteritis virus (Mousavizadeh and Ghasemi, 2020) [50].

Table 1: Genomic comparison of SARS-CoV-2 with other CoV strains as a summary

SARS related CoV	Genomic identity with COVID-19
bat-SL-RaTG13	96.2%
bat-SL-CoVZC45	87.9%
bat-SL-ZXC21	87.9%
Pangolin-CoV	91.02%
SARS-CoV	>80%
MERS-Cov-2	50%

6. Genetic diversity and evaluation of sars-cov-2

Evolutionary changes in an organism occur as a result of mutations. The mutation is a modification of nucleotide sequences in the genome of organisms. Community transmission, antiviral treatments, and ecological changes along with the genetic plasticity of RNA viruses facilitate the emergence of many new RNA viruses. These results developments of new viral pathogens that affect potential hosts and stimulate host jumping (Kaur *et al*, 2020) [51]. In addition to the emergence of new pathogens, mutation of the viruses can result in the development of more virulent strains with a high mortality rate and appearance of resistant strains to treatment. Therefore, systematic tracking of demographic, clinical patient information, and strain information is crucial to successfully combat COVID-19 (Koyama and Parida, 2020) [38]. Mutations at S1 and S2 junction of the spike protein of SARS-CoV-2 are also responsible for evolutionary changes.

RNA viruses usually have a relatively high mutation rate than DNA viruses and a million times greater than their hosts. This is due to the absence of proofreading RNA polymerase enzymes in RNA viruses. The advantages of high mutation rates are for better viral adaptability and evolvability of RNA viruses (Duffy, 2018) [22]. On the other hand, Coronaviruses which have the largest known genome size show relatively low rates of mutations than other RNA viruses. The slower mutations rates or conservation of genome associated with especial characteristics of the coronavirus replication-transcription complex which contains 3'-5'exonuclease activity. The exonuclease enzymes provide proofreading functions to the viruses (Chen *et al*., 2020) [12].

Annotation of the genome is the first step to explain the genetic dissimilarity of the organism since genetic information of any life is preserved in the genome (Junejo *et al*., 2020) [36]. Genetic variation can be assessed by retrieving complete or nearly complete genomes of SARS-CoV-2 from GISAID. All SARS-CoV-2, SARS-CoV, and MERS-CoV have high mutation rates that result in viral genetic diversity, flexibility, and adaptability to invade a wide range of hosts. SARS-CoV-2 genome has 80% similarity to the previous human coronavirus (SARS-like bat CoV) as different studies reported (Adnan *et al*., 2020) [3]. Nucleotide substitution is one of the driving forces for the occurrence of viral evolution in nature. The rapid spread of SARS-CoV-2 raises stimulating questions such as whether its evaluation is driven by mutation (Phan, 2020) [54]. Open reading frame 1ab, spike surface glycoprotein, matrix, and nucleoside protein are considered as a site of mutation.

The spike surface glycoprotein plays an essential role in binding to receptors on the host cell, which determines host tropism and major targets of neutralizing antibodies (Fei *et al*., 2020) [27]. Mutations in the spike surface glycoprotein might encourage its conformational changes, which probably led to the changing antigenicity (Phan, 2020) [5].

6.1 Variants of SARS-COV-2

Different types of mutations such as missense mutations, synonymous mutations, and deletion mutation in the non-coding regions are major causes for the occurrences of genetic variants. As different studies indicate that there are many variants of SAS-CoV-2 found across different parts of the world. Currently, there are six stains of SARS-Cov-2. These are L, G, V, S, GR, and GH

(https://www.sciencedaily.com/releases/2020/08/200803105246.htm).

The D614G strain has occurred where the aspartic acid residue at position 614 is replaced by glycine. This is done at the carboxy-terminal end of the S1 domain of the SARS-CoV-2 spike protein. The strain was first found in Europe in January 2020. D614G stain is the most widespread strain across the world and mutated into GR and GH after a while. It has five sublineages which are located in B-cell epitopes. D614G may influence the effectiveness of the vaccine. The other major lineage/strain is the L84S lineage with two subclades which is frequently observed in the United States. D448del and G392D lineages are small and they are without any significant sub lineages (Koyama *et al*., 2020) [38].

Mutations in the receptor-binding domain (RBD) of the spike protein suggest that variants are unlikely to reduce binding affinity with ACE2 because that would decrease the fitness of the virus. The V367F and D364Y variants increase the structural stability of spike protein that facilitating efficient binding to the ACE2 receptor as reported in studies (Ou *et al*., 2020) [53].

In general, the genomic analysis showed that the presence of a high rate of non-synonymous mutation which can result in non-conservative substitution. This is indirect evidence of the intensive circulation of the virus in the human population, increased transmissibility of the virus, and its adaptation process to a new host (Kozlovskaya *et al*., 2020) [40].

7. Pathogenic mechanism of sars-cov-2

The pathogenesis of the virus is poorly understood. The pathogenic mechanisms of SARS-CoV and MERS-CoV can give us a lot of information on the pathogenesis of SARS-CoV-2 infection to facilitate our recognition of COVID-19 (Li *et al*., 2020) [4]. SARS-CoV-2 uses ACE2 as its receptor which is similar to SARS-CoV.

7.1 Viral entry and spread

Epithelial cells in the respiratory and gastrointestinal tract are the primary target cells (Khan *et al*., 2020) [48]. Viral replication primarily takes place in the mucosal epithelium of the upper respiratory tract (nasal cavity and pharynx), and further multiplication in the lower respiratory tract and gastrointestinal mucosa (Duan, 2020b) [21]. The spike protein of SARS-CoV-2 exploits the ACE2 receptor that is broadly expressed in epithelial cells of the nasal mucosa, bronchus, lung, heart, esophagus, kidney, stomach, bladder, and ileum. The process of virus infection is the interaction of sensitive human cell receptors with the spike protein. The spike (S) proteins are heavily glycosylated which binds to ACE2 receptor and mediates subsequent fusion between the envelopes and host cell membranes. It then undergoes structural changes to fuse with the host and eventually allows viral genes entry into the host cell. The spike protein of coronavirus contains two subunits namely S1 and S2. The S1 subunit binds to the receptor on the surface of the host cell whereas the S2 subunit mediates the cell membrane fusion (Harapan *et al*., 2020) [30]. Protease enzymes, TMPRS2 help to complete the entry of the virus into the cells (Mousavizadeh and Ghasemi, 2020) [50]. The virus encodes genomes to facilitate the expression of genes that encode useful accessory proteins following the entering of the virus in the cell. Then the virus advances the adaptation of host cells. SARS-CoV-2 should be able to inhibit or evade host innate immune signaling to infect new host productively (Harapan *et al*., 2020) [30].

A structure model analysis shows that SARS-CoV-2 binds to ACE2 with more than a 10-fold higher affinity than SARS-CoV. There are faster contagious capabilities, a higher number of confirmed cases, and a higher affinity of SARS-CoV-2 binding to ACE2 compared to SARS-CoV infection (Wang *et al*., 2020) [67].

7.2 Acute Respiratory Distress Syndrome (ARDS)

ARDS is a life-threatening lung condition that prevents enough oxygen from getting to the lungs and into the circulation, accounting for mortality of most respiratory disorders and acute lung injury. Genetic susceptibility and inflammatory cytokines are closely related to the occurrence of ARDS as different studies reported previously. ACE2, interleukin 10 (IL-10), tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF) are associated with the development of ARDS (Wu *et al*., 2020) [69].

8. Spectrum of clinical symptom

Clinical manifestations of SARS-CoV-2 have similarities with SARS-CoV and MERS-CoV infections. The clinical picture of the virus ranges from very mild to life-threatening (Chen *et al.*, 2020) [12]. Most cases of the virus are mild as the report suggests in china. Common symptoms are fever, nonproductive cough, dyspnea, and fatigue. Extra-pulmonary feature of the virus includes nausea, vomiting, diarrhea, and acute renal failure (Meo *et al*., 2020) [49]. Fever is the dominant symptom of infection. The other clinical findings are increase white blood cell counts (neutrophils), decrease in lymphocytes, platelets, and red blood cells. Pneumonia, metabolic acidosis, septic shock, and bleeding are recognized in severe cases of the disease (Helmy *et al*., 2020) [32]. The mortality rate of SARS-CoV-2 is lower compared to SARS and MERS coronavirus. The mortality rate of COVID-19 is 3.4% to 6.6% while SARS or MERS CoV mortality rate is 9.6% and 34.3% respectively.

9. Epidemiology

The first cases of the acute respiratory syndrome of unknown etiology were reported in Wuhan City, Hubei Province, China among people linked to a local seafood market on 29 December 2019. Most of the early cases had some sort of contact history with the original seafood market. A secondary source of infection was found to be human to human transmission via close contact with no history of exposure to wildlife or visiting Wuhan (Adhikari *et al*., 2020) [1]. The virus rapidly spread in china and other countries around the world (Zu *et al.*, 2019a) [75]. The novel coronavirus is suggested to be originated from wild bats and belongs to beta coronavirus.

As different studies reported that the virus mainly occurred among old aged people with a median age of 59 years. The gender-based analysis demonstrates that the cases consisted mostly of men with a median age range of 50-65 years (Meo *et al*., 2020) [49]. SARS-CoV-2 has a greater level of transmissibility and pandemic risk than SARS-CoV. Since the reproductive number of (R) of SARS-CoV-2(2.9) which is higher than the reported effective reproduction number of

SARS-CoV (1.77). The mean incubation period of the virus is 7 days, ranging from 2 to 14days as Chinese health authorities stated (Adhikari *et al*., 2020) [1].

There is seasonal variation patterns of 2019-nCoV, SARS-CoV, and MERS-CoV infections. MERS-CoV infection outbreak has occurred in the summer season. 2019-nCoV and SARS-CoV infection outbreak took place in the winter season in contrast to the outbreaks of MERS-CoV (Dean, 2020) [18]. The discharged droplets of the virus can spread until 1-2meter, and remain viable on the surface for a day in a favorable atmospheric environment. The virus can be destroyed within a minute via common disinfectants like sodium hypochlorite and hydrogen peroxide (Singhal, 2020) [62].

COVID-19 disease spread across many geographical regions of the globe. By September 30, 2020, there were 33,852,048 confirmed cases and more than 1,012,743 deaths of people worldwide with the novel coronavirus. Every hour the number of cases and deaths is increasing across the world. The United States of America is recording the maximum number of positive cases and deaths. Italy, Spain, Germany, and France in Europe continue to be the most affected ones (Kumar *et al*., 2020) [41].

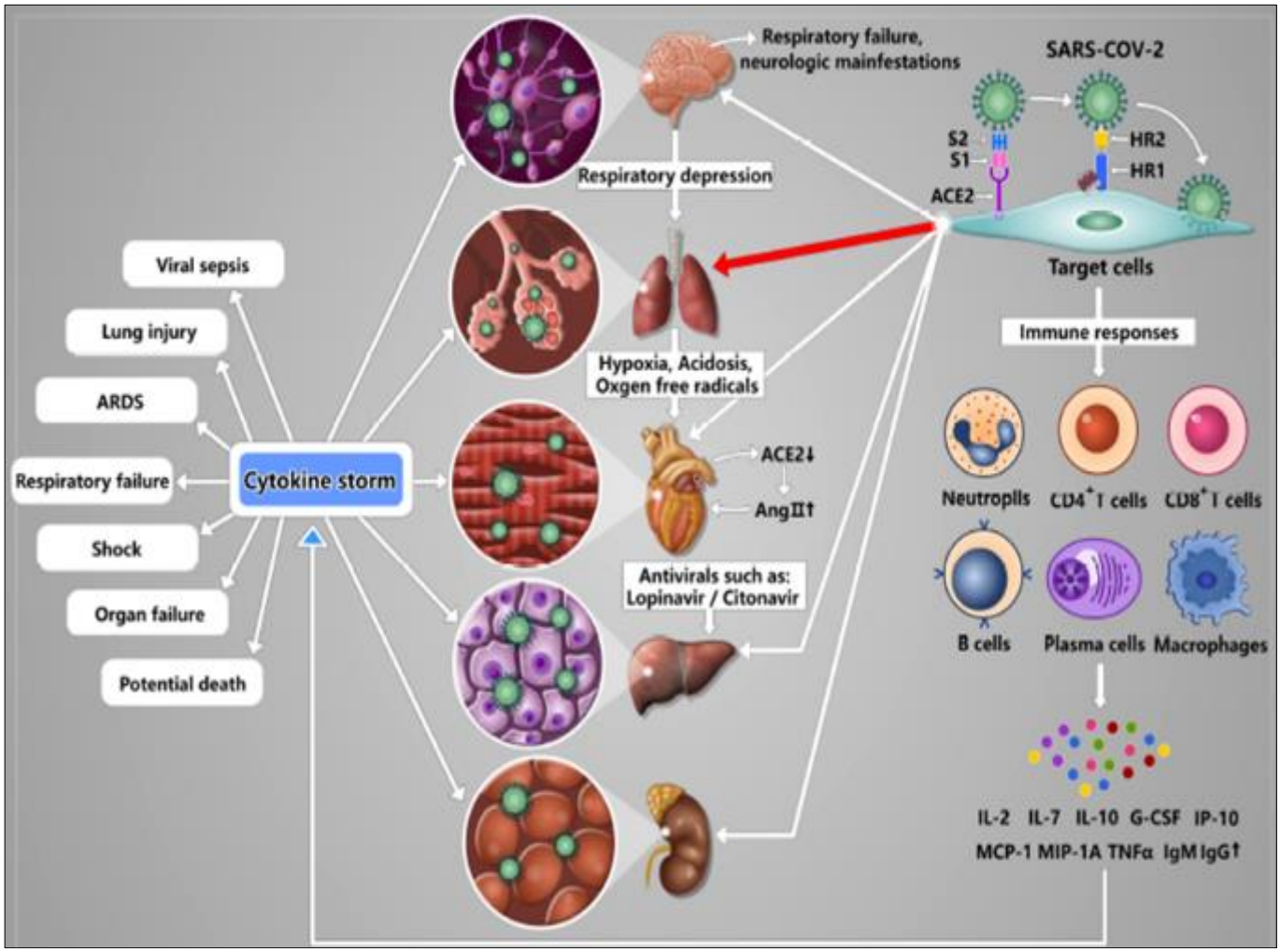
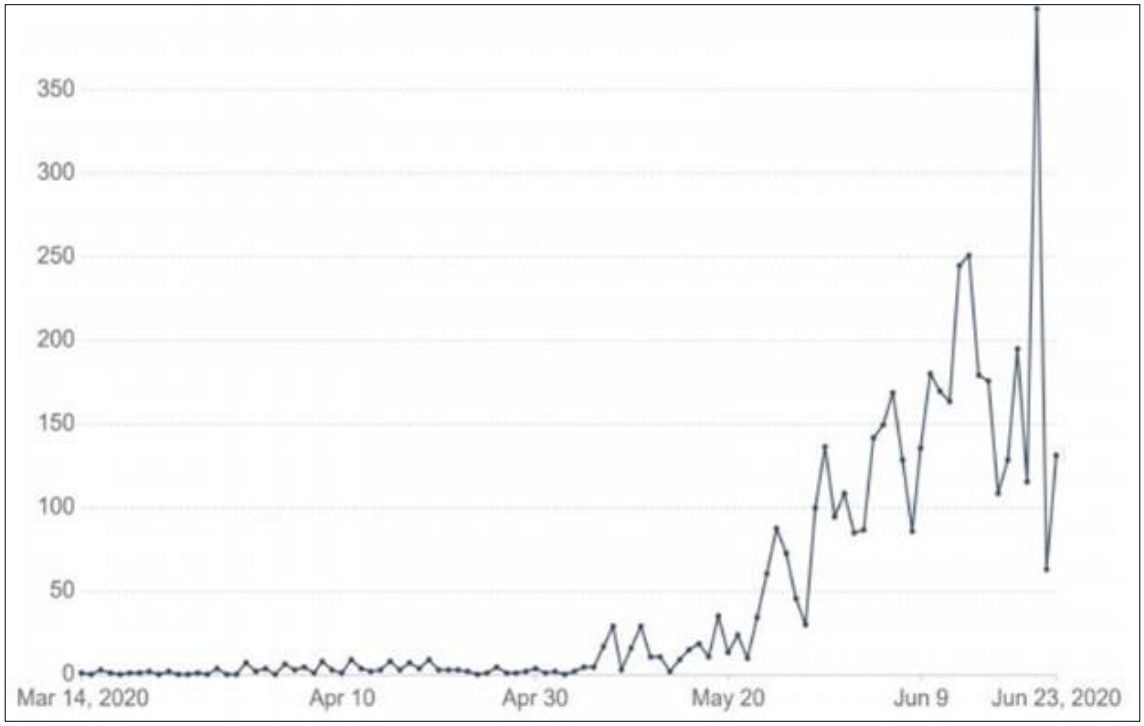


Fig 5: Pathogenesis of SARS-CoV-2. Once SARS-CoV-2 enters into the lung by airway, the S1 subunit of S protein can bind to the receptor ACE2 expressing on II alveolar epithelial cells, and induce a conformational change of the S2 subunit, triggering the association between the heptad repeat (HR)1 and HR2 domains to form 6-HB, thus bring the viral and cellular membranes nearby for fusion, resulting in lung damage that is the main infection site (Wu *et al*., 2020) [69]

10. Status of covid-19 in Ethiopia

On February 14, 2020, COVID-19 was reported in Africa for the first time in Egypt. The first case of COVID-19 was reported in Ethiopia on March 13, 2020. A range of control strategies was implemented to compact the advance spread of newly emerged pandemic disease. The strategies were quarantine people for at least 14days coming from abroad, closure of schools, suspending public gathering, closures of charges, and mosques. A state of emergency was also declared and peoples were advised to avoid unnecessary travel. Although all of the above and other prevention and control measures are taken, the number of cases is not decreasing rather it increases exponentially day over a period of time (Baye, 2020) [8]. The number of case increase exponentially and high cases are reported in Addis Ababa. Ethiopia reported a total of 74,584 COVID-19 cases and 1,191 deaths on September 30, 2020 (Biadgilign and Yigzaw, 2020) [10].



Source: (https://rstmh.org/news-blog/news/covid-19-in-ethiopia-status-and-responses)

Fig 6: Daily confirmed number of COVID-19 cases in Ethiopia

11. Transmission of sars-cov-2

11.1 Route of transmission

Droplets and close contact are the most common routes of transmission of SARS-CoV-2 generated during coughing and sneezing by symptomatic and asymptomatic patients (Singhal, 2020) [62]. Researchers have also detected SARS-CoV-2 in samples of stool, gastrointestinal tract, saliva and urine, tears, and conjunctival secretion of patients with COVID-19. The cluster of infected family members and medical workers confirmed the presence of human to human transmission by droplets, contacts, and fomite (Zu *et al*., 2019a) [75]. A retrospective study of pregnant women with COVID-19 indicated that there is no possibility of intrauterine vertical transmission between mothers and infants during late pregnancy (Wang *et al*., 2020) [67]. And also it is not certain that the consumption of virus-contaminated foods will cause infection and transmission (Duan, 2020a) [20].

11.2 Susceptible population and viral latency

Susceptibility of the host to the virus is associated with age, biological sex, and other health conditions (Adhikari *et al*., 2020) [1]. All ages are susceptible to the virus. Peoples of old age and all ages groups with heart disease, cellular immune deficiency, hepatic and kidney problems, lung disease, and diabetes of peoples more susceptible and prone to death (Duan, 2020a) [20]. Higher viral loads are present in the nasal cavity as compared to the throat with no difference in viral burden between symptomatic and asymptomatic people (Singhal, 2020) [62]. The incubation period of the COVID-19 virus ranges from 2 to 14days. The median incubation period of COVID-19 is shorter than that of SARS and MERS CoV. The maximum latency of SARS-CoV-2 (24days) increases the risk of virus transmission. Disease progression is more rapid in elderly people compared with younger people.

12. Diagnosis of sars-cov-2

Rapid and accurate identification of pathogenic viruses plays a vital role in selecting appropriate treatments, saving people's lives, understanding epidemiology, suppression transmission, and preventing epidemics (Hafeez *et al*., 2020) [29]. A quick standard diagnostic test for the detection of infectious diseases (COVID-19) is important to prevent subsequent secondary spread (Shen *et al*., 2020) [61]. Diagnosis of SARS-CoV-2 infection is based on a history of detail contact, travel, and precise laboratory test. Molecular methods, serology, and viral culture are used as diagnostic tools for isolation and identification of newly emerged viral disease. Real-time reverse transcription Polymerase chain reaction and isothermal nucleic acid amplification are the golden standard molecular method for the diagnosis of COVID-19 with high sensitivity and specificity (Sahin *et al*., 2020) [59].

12.1 Specimens

The precise samples for the detection of the virus are taken from upper and lower respiratory tracts. Nasopharyngeal and oropharyngeal swab or wash in ambulatory patients are taken as upper respiratory specimens. Sputum, endotracheal aspirates, and bronchoalveolar lavage or deep tracheal aspirate from patients are considered as a lower respiratory specimen with more severe respiratory disease (East, 2020) [23]. There are significantly higher viral load and genome fraction in the lower respiratory tract than upper respiratory tract samples (Sahin *et al*., 2020) [59]. The virus may also be detected in the stool and blood as an additional clinical specimen. Specimens should be kept refrigerated at 4-8 °C and sent to the laboratory where they will be processed within 24-72hrs of collection. The samples should be refrigerated at-70°C if not send within this period until shipment to the reference laboratory (East and States, 2020) [23].

12.2 Diagnosis based on clinical symptoms

Diagnosis of COVID-19 is mainly based on epidemiological history and clinical manifestations such as coughing, fever, dyspnea, and history of travel from an epidemiological area. However; diagnosis based on clinical symptoms is atypical. Therefore; the auxiliary examination is necessary for the diagnosis of COVID-19 (Li *et al*., 2020) [45].

12.3 Cultural isolation and identification of SARS-CoV-2

Viral culture is a more time-consuming method compare to other methods. The virus can grow in Vero E6 cells and observe the cytopathic effect (Kozlovskaya *et al*., 2020) [40]. Cultures used in *in vitro* and *in vivo* antiviral treatment and vaccine evaluation trials.

12.4 Molecular diagnostic methods

12.4.1 RT-qPCR

Detection of SARS-CoV-2, SARS-CoV, and MERS-CoV is based on the amplification of nucleic acid because of its high sensitivity and specificity, mainly in the acute phase of infection. The RT-qPCR assay is regarded as the gold standard method for identification and surveillance of SARS-CoV-2 target sequences. Target sequence-based detection of the novel coronavirus is important due to the genomic similarity of SARS-CoV-2 with SARS-CoV (around 82% nucleotide identity (Chan *et al*., 2020b) [11]. Routine confirmations of the cases are based on the recognition of unique sequences of the virus via nucleic acid amplification technique.

Transcribing and amplifying specific SARS-CoV-2 genomic sequences are the basic principles of RT-qPCR. RNA extraction should be done in biosafety level two cabinets. Heat treatment of samples before RNA extraction is not recommended. The viral RNA is first extracted from the biological specimen collected from the nasal or nasopharyngeal swabs and is purified. The purified RNA template is transformed into a cDNA (complementary DNA) by reverse transcriptase (an RNA-dependent DNA polymerase enzyme). The cDNA is subsequently amplified by the PCR (Afzal, 2020) [2]. Sequence-specific forward and reverse primers and labeled probes are used in RT-qPCR diagnostic techniques according to the target genes of interest.

The viral genes that are conserved or plentifully expressed genes such as N, S, E, nonstructural RdRp, and ORF1a/b genes are preferred targets for SARS-CoV-2 RT-qPCR assay. The full genome directly amplified from RNA extracted from specimens using gene-specific primers for open reading frame 1b and N to produce overlapping PCR products (Sah, 2020) [58].

The positive result of RT-qPCR for the presence of the SARS-Co V-2 is further confirmed via partial or whole-genome sequencing of the virus. Sequencing can be done using the Illumina MiSeq sequence method (East *et al*., 2020) [23].

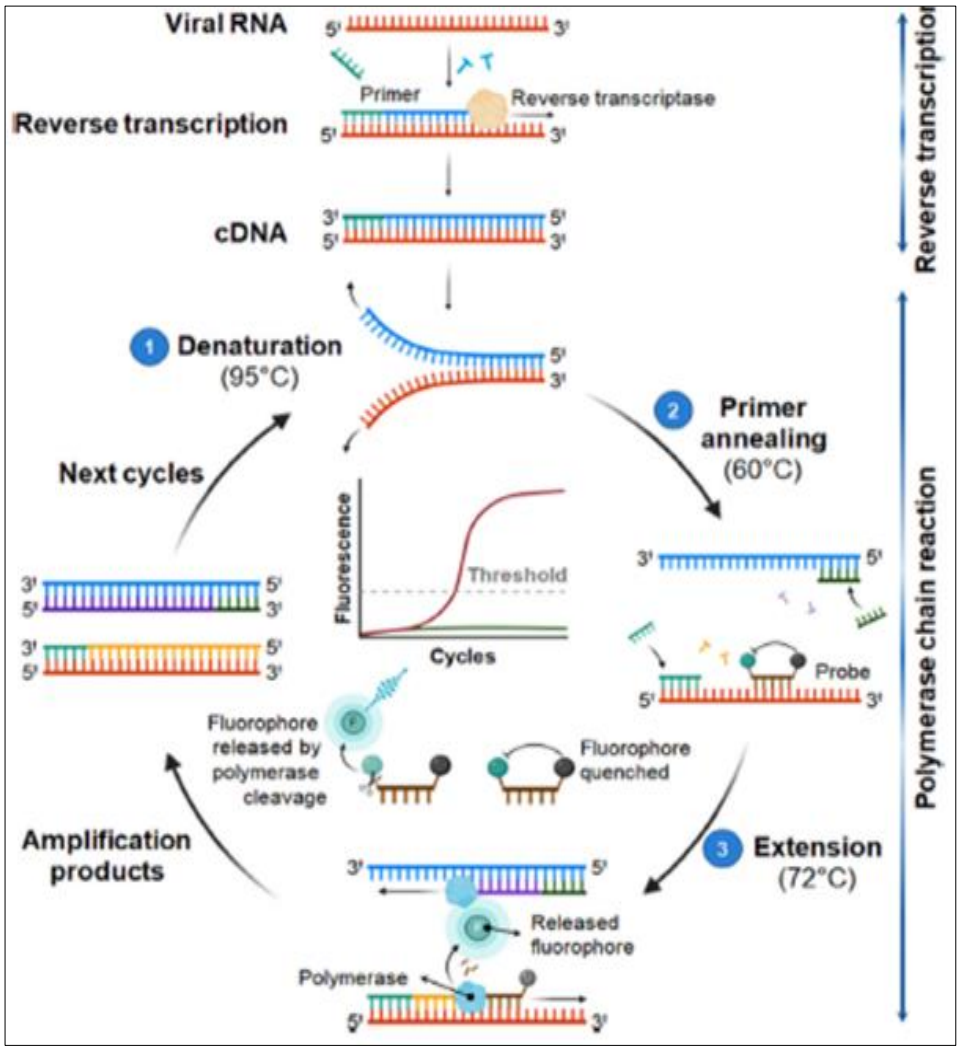


Fig 7: Molecular representation of the real-time RT-PCR principle. Viral RNA is converted to cDNA by reverse transcriptase. Complementary DNA is amplified in PCR in three steps as indicated in the figure (Afzal, 2020) [2]

12.5 Serological diagnosis

Serological tests are rapid and simple alternatives for screening infected individuals. Serological methods do not directly detect the presence of the virus but instead detect evidence of viral infection at some time point in the past. Serologic methods detect and/or measure the amount of antibodies to SARS-CoV-2 or antigen present quantitively or qualitatively (Zhan *et al*., 2020) [71]. Serological surveys can aid in investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. There are various types of serological tests are present such as enzyme-linked immunosorbent assay (ELISA), indirect immune fluorescent test assay (IIFA), lateral flow immune assay, and neutralization tests (Ortiz-prado *et al*., 2020) [52]. A new kit is developed using SARS-CoV-2 IgM with 86.6% and 90.63% sensitivity and specificity respectively as studies indicated (Li *et al*., 2020) [4].

There is no cross-protection between SARS-CoV and COVID-19. The Challenge of serological test/diagnosis is Cross-reactivity of COVID 19 to other coronaviruses. The N protein antibodies of SARS-CoV cross-react with COVID-19 but not provide cross-immunity (Kannan *et al*., 2020) [37]. The sensitivity of antibody detection is generally lower than the molecular method of diagnosis.

13. Treatments and prevention mechanisms of sars-cov-2

To date, there are no specific antiviral drugs or vaccines available against COVID-19 infection for potential therapy and prevention of humans infection (Adhikari *et al*.; Gennaro *et al*., 2020) [1]. General treatment of COVID-19 infected patients needs complete bed rest and supportive treatment, ensuring adequate calorie and water intake to reduce the risk of dehydration. Water electrolyte balance and homeostasis need to maintain along with monitoring of vital signs and oxygen saturation; keeping the respiratory tract unobstructed and inhaling oxygen in more severe cases (Hafeez *et al*., 2020) [29]. The effective option of antiviral therapy and vaccination is currently under evaluation and development (Lai *et al*., 2020) [42].

Infected patients will receive supportive care including oxygen therapy, fluid therapy, and antibiotics for treating secondary bacterial infections according to WHO guidelines management strategies (Dhama *et al*., 2020) [19]. In the case of hypoxia nasal catheter and mask oxygen should be immediately provided to the patients.

The CDC recommends multiple steps to prevent the transmission and risk of SARS-CoV-2 (Ramphul and Mejias, 2020) [56]. Frequent hand washing lasting at least 20 seconds by using soap and water, use of hand sanitizers with at least 60% alcohol, refrain from touching eyes, nose, and mouth with unwashed hands, and wear a face mask is advised (Unhale *et al*., 2020) [65]. Maintain social distancing and avoid close contact with people who are coughing or sneezing (Hafeez *et al*., 2020) [29].

Early detection, diagnosis, treatment, quarantine, border closure, and screening at the airport are classical effective prevention and control measures to block human to human transmission as well as secondary infections among close contact and health care worker. The major challenges for the prevention and treatment of SARS-CoV-2 viral infection are due to the presence of many potential natural hosts, intermediate hosts, and final hosts. And also asymptomatically infected persons and patients in the incubation period or recovered from COVID-19 may pose serious challenges for disease prevention and control (Duan, 2020b) [21]. In general, epidemiologic investigations, risk assessment, surveillance, rapid diagnostic tests, and vigilance are required by all countries to combat the potential outbreak of SARS-CoV-2 (Dagur and Dhakar, 2020) [15].

14. Conclusion and Recommendation

COVID-19 is a newly emerged epidemic pathogens of humans caused by SARS-CoV-2, which was detected first in China. The virus now spread and became global issues. The newly emerged virus mainly causes respiratory and intestinal tract infections in humans. World health organization announced that COVID-19 is a public health emergency of international concern and pandemic. The virus has the potential for rapid and extensive spread among people and countries. It has high transmissibility and a low mortality rate compared to SARS-CoV.

SARS-CoV-2 is an enveloped, non-segmented, single-stranded, and positive-sense RNA virus that belongs to beta coronavirus based on genomic structure and phylogenic analysis. The genome of the novel coronavirus is composed of four main structural proteins. The immediate ancestor of SARS-CoV-2 is most likely originated in a bat species based on the genomic and phylogenic analysis. COVID-19 infects both wild and domestic animals either naturally or experimentally. Coronavirus can skip species boundaries and adapt to new hosts. Currently, there is no effective therapeutic agent and vaccine for the emerged viruses. Therefore; epidemiologic investigations, risk assessment, surveillance, investigation of the possible intermediate host, rapid diagnostic tests, and attention are required to combat the potential outbreak of SARS-CoV-2.

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