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Review Article

SARS-COV-2: A STUDY IN LIGHT OF OTHER HUMAN CORONAVIRUSES

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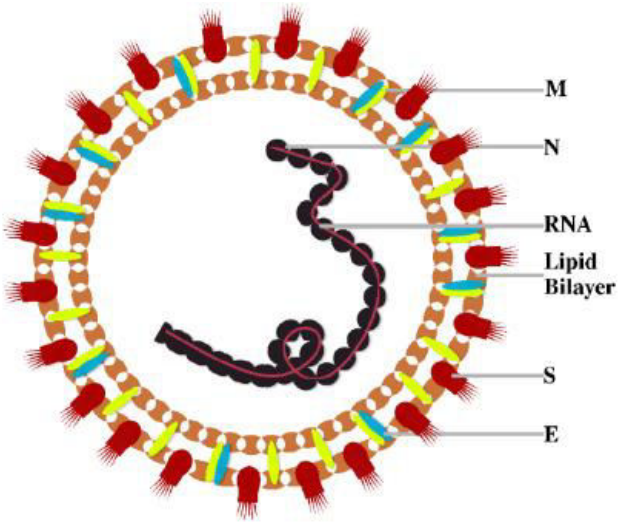
ABSTRACT

Four mild human coronaviruses (HCoVs), viz. HCoV-229E, HCoV-OC43, HCoV-HKU1, and HCoV-NL63 have already been prevailing in the human population causing mild respiratory diseases. Thereafter, the two major human infecting coronaviruses responsible for the production of symptoms that are potentially severe and cause a widespread infection, MERS-CoV and SARS-CoV, came into human notice; and now in December 2019, another species called Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been a cause of major nuisance created all over the world. For a thorough analysis of SARS-CoV-2 and its pathogenicity, it is required to study about the past events of various other human coronaviruses' emergence. A systematic and relative review of Human Coronaviruses based upon their origin, epidemiology, pathogenicity, molecular aspects, lifecycle as well as antiviral strategies considering the current Coronavirus disease 2019 (COVID-19) pandemic would be of great help in a better understanding of SARS-CoV-2.

Keywords: Human Coronavirus; COVID-19; Zoonotic; Epidemiology; Pathogenicity; Molecular Aspects; Vaccines.

1. INTRODUCTION

Coronavirus (CoV) is the common name for the family *Coronaviridae* and is taxonomically placed under the realm *Riboviriae* and order *Nidovirales*. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses ranging from 60 nm to 140 nm in diameter (Fig.1).



M: Membrane glycoprotein; S: Spike glycoprotein; N: Nucleocapsid protein; E: Envelope glycoprotein.

Fig. 1: Diagrammatic representation of the structure of Coronavirus

They have spike-like projections on their surface giving them a crown-like appearance under the electron microscope [1]. They are responsible for causing persistent and long-term health problems like respiratory infections, gastrointestinal tract infections, and central nervous system illnesses in amphibians, birds, and mammals. They have a strong potential to adapt to new environments through mutation and recombination, and to change host domain and tissue tropism [2, 3]. The high rate of mutation in RNA viruses has led to the evolution of various severely pathogenic strains like SARS-CoV and MERS-CoV. Presently, the emergence of SARS-CoV-2 from Wuhan, China responsible for the worldwide pandemic situation has grabbed the attention of scientists towards its unique character of high transmissibility but reduced pathogenicity compared to its relative SARS-CoV.

This is not the first time in history that zoonosis (capability of a disease-causing organism to transfer from animals to humans) is being a cause of global health care problems. Four mild human coronaviruses, *viz.* Human coronavirus 229E (HCoV-229E), Human coronavirus OC43 (HCoV-OC43), Human coronavirus HKU1 (HCoV-HKU1) and Human coronavirus NL63 (HCoV-NL63) have already been prevailing in the human population causing mild respiratory disease or

just mild self-limiting symptoms of their infection like common cold [4]. Thereafter, in beginning of the first decade of 21st century, the two major human coronaviruses, Middle East respiratory syndrome coronavirus (MERS-CoV) and Severe acute respiratory syndrome coronavirus (SARS-CoV), caused severe and widespread infection; and now in December 2019 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been a cause of major nuisance created all over the world.

2. HISTORY, EPIDEMIOLOGY AND PATHOGENICITY

The first human coronavirus 229E species, came to the notice around 1966 when a 45-year-old school teacher in Athens, Greece, arrived at the emergency room of the Hygeia Hospital having clinical symptoms of upper respiratory tract infection and later on, the pathogen was discovered by University of Chicago as Human coronavirus 229E [5, 6]. It has been placed under the genus *α-Coronavirus* falling under the sub-family *Orthocoronavirinae* of the family *Coronaviridae* (Fig.2). Another species of coronavirus, HCoV-OC43, causing common cold was discovered in 1967 and was isolated by McIntosh et al. from nasopharyngeal wash fluid and propagated on human embryonic tracheal organ cultures [7]. It has been placed under the lineage A-*Embecovirus* (sub-genus) of genus *β-Coronavirus* falling under the sub-family *Orthocoronavirinae* of family *Coronaviridae* (Fig.2) [8]. HCoV-229E acts on the human receptor aminopeptidase N [9] present on the cell surface of apical membranes of intestine, lung, kidney, and epithelial cells, macrophages, and synaptic junctions while HCoV-OC43 acts on the receptor 9-O-Acetylated sialic acid. Both the viruses are distributed globally and are at the peak mainly during winter and early spring seasons in temperate climate countries [10, 11] and are responsible for causing respiratory infection of the upper respiratory tract and may also cause severe lower respiratory tract infections (LRTI) in infants and elderlies. HCoV-229E has also been found to be involved in the development of Kawasaki syndrome [12]. These are transmitted through respiratory Droplets and Fomites and have an incubation period of about 2-5 days. The major symptoms include malaise, headache, nasal discharge, sneezing, sore throat, fever, and cough. Killerby et al. have reported that during July 1, 2014 to June 30, 2017, HCoV-229E caused about 7001 cases whereas, OC43 was responsible for about 18,804 cases implying that a total of 0.8% population was tested positive for HCoV-229E whereas 2.2% of the total population for HCoV-OC43 [13].

SARS-CoV, one of the three deadly zoonotic viruses, caused an epidemic in human populations of severe pulmonary disease with a mortality rate of 10% [14]. It was first reported in Guangdong Province, China in late 2002 [15], and then spread rapidly to four continents, infecting 8,096 individuals and claiming 774 victims before it was controlled [16].

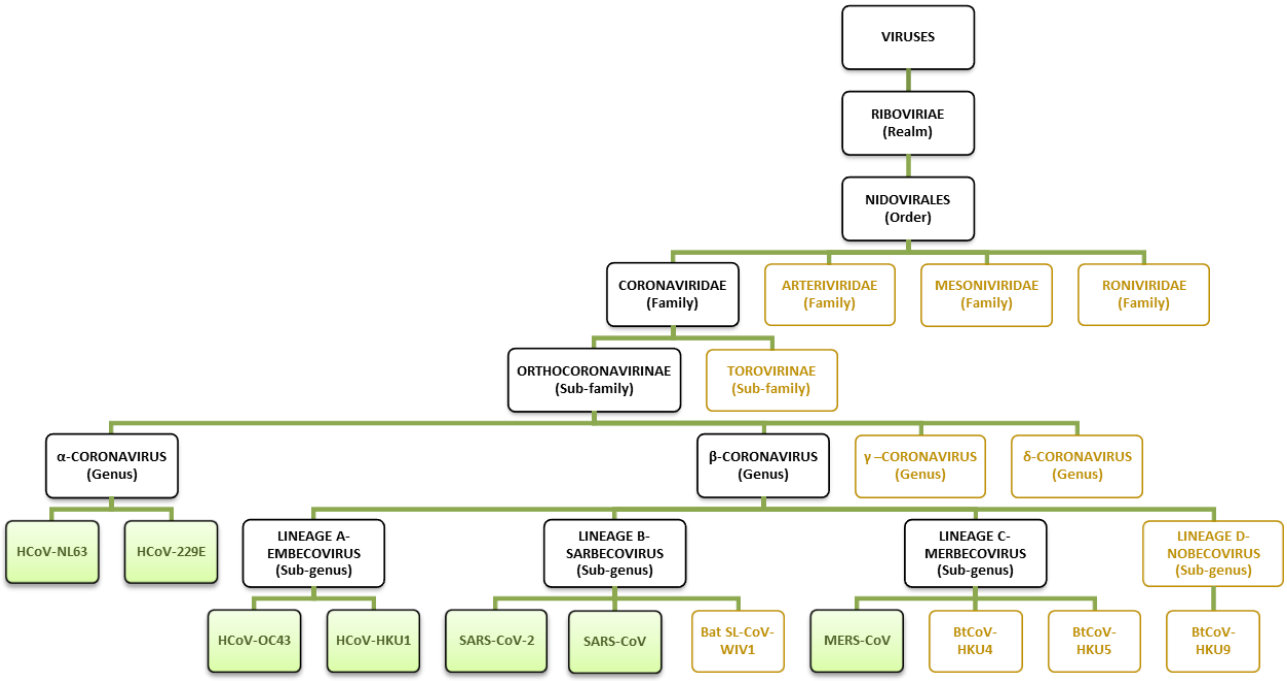


Fig. 2: An overview of the classification of various human coronaviruses (green boxes) [17-19]

Taxonomically it belongs to the lineage B- *Sarbecovirus* (sub-genus), genus β-*Coronavirus,* sub-family *Orthocoronavirinae*, family *Coronaviridae* (Fig. 2). SARS-CoV interacts with the angiotensin-converting enzyme 2 (ACE2) receptor present mainly in the arterial and venous endothelium, arterial smooth muscle, small intestine lining and respiratory tract epithelium in human. It also attacks alveolar monocytes and macrophages resulting in reduced immunity. Its property of spreading at a high rate is credited to its high reproduction number (R_o) of 2-5 [10]. The disease caused by SARS-CoV mainly involves gastrointestinal and respiratory problems. It causes severe acute respiratory syndrome, having an incubation period of 2-11 days. The SARS-CoV is transmitted *via* respiratory droplets, fomites and by faecal-oral routes. The major symptoms of Severe acute respiratory syndrome include fever, myalgia headache, malaise, dry cough, dyspnoea, respiratory distress and diarrhea.

In the year 2004, an α-Coronavirus HCoV-NL63 was isolated from a 7-month-old child suffering from bronchiolitis and conjunctivitis in the Netherlands [20] and then in the year 2005, a novel β-coronavirus HCoV-HKU1 was discovered in Hong Kong responsible for causing respiratory tract infection [21]. HCoV-NL63 is considered as an important cause of (pseudo) croup and bronchiolitis in children, while HCoV-HKU1 causes respiratory infection of the upper respiratory tract. Both viruses are transmitted via respiratory droplets and fomites. HCoV-NL63 has been placed under the genus α-*Coronavirus*, whereas HCoV-HKU1 has been placed under the lineage A- *Embecovirus* (sub-genus) of genus β-*Coronavirus* both falling under the sub-family *Orthocoronavirinae* of family *Coronaviridae* (Fig. 2). Like HCoV-229E and HCoV-OC43, HCoV-HKU1 and HCoV-NL63 are also distributed globally and are at the peak mainly during winter and early spring seasons in temperate climate countries [22]. HCoV-NL63 acts on the angiotensin-converting enzyme 2 (ACE2) receptor in humans which is distributed mainly in the arterial and venous endothelium, arterial smooth muscle, small intestine, respiratory tract epithelium, alveolar monocytes, and macrophages leading to the susceptibility in human cell lines like that of intestinal tract and kidney, whereas the receptor for HCoV-HKU1 is still unknown. According to Killerby, from July 1, 2014 to June 30, 2017, HCoV-HKU1 caused about 5,225 cases whereas, NL63 was responsible for about 8,558 cases, implying that a total of 0.6% population was tested positive for HCoV-HKU1 whereas 1.0% of the total population for HCoV-NL63 [13].

MERS-CoV is the second deadly zoonotic virus witnessed by the human race in the early second decade of the 21st century. It emerged in Saudi Arabia and affected 2,494 people causing 858 deaths [23, 24] with a fatality rate of 34%. MERS is a lethal respiratory disease which had a higher fatality rate than that of SARS[25]. Taxonomically it belongs to the lineage C-*Merbecovirus* (sub-genus), genus β-*coronavirus*, sub-family *Orthocoronavirinae*, family *Coronaviridae* (Fig.2). MERS-CoV interacts with the Dipeptidyl peptidase 4 receptor in humans which is distributed mainly in the respiratory tract epithelium, kidney, small intestine, liver and prostate activated leukocytes leading to the susceptibility in human cell lines like that of the respiratory tract, intestinal tract, genitourinary tract, liver, kidney, neurons, monocyte, T-lymphocyte and histocytic cell lines. It spread from the Middle East in 2012 to South Korea in 2015 with its spreading property attributed to its R_o which is 2-5 [10, 26]. MERS-CoV is responsible for causing Middle East respiratory syndrome (MERS) having an incubation period of 2-13 days. The infection caused by MERS-CoV is highly transmissible *via* respiratory droplets and fomites. The main symptoms of the Middle East respiratory syndrome include fever, cough, chills, sore throat, myalgia, arthralgia, dyspnoea, pneumonia, diarrhea and vomiting and acute renal impairment [23].

Ongoing conditions of pandemic occurred due to the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) worldwide on a massive scale. It is a new coronavirus, which was earlier named as 2019 novel coronavirus (2019-nCoV) by the World Health Organization (WHO) on January 7, 2020, and subsequently renamed to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [27]. Lower fatality rate has been observed in SARS-CoV-2 infections than that of SARS-CoV and MERS-CoV infections. However, SARS-CoV-2 infection is spreading like a forest fire leading to more than two hundred thousand new cases per day. As per the clinical observations, the virus appears to be more fatal in elderly patients or patients with comorbidities (additional conditions like smoking or lung disease) [28]. SARS-CoV-2 use ACE2 mammalian receptor, like SARS-CoV, for cellular entry for human-to-human transmission [29]. It has an incubation period of 3-6

days. The infection caused by SARS-CoV-2 is severely transmissible *via* respiratory droplets and fomites. The major symptoms of COVID-19 include fever, dry cough, dyspnoea, myalgia, headache and diarrhea [30]. As per the statistical data till- 12:00 am IST, 7th November 2020, SARS-CoV-2 has caused about 49,388,405 cases, leading to 1,244,717 deaths creating a fatality rate of 3% and recovery rate of 97%. SARS-CoV-2 is the third highly pathogenic coronavirus that crossed the species barrier to cause fatal pneumonia in humans after the HCoV-SARS and MERS-CoV viruses [31]. Taxono-mically, SARS-CoV-2 belongs to the order *Nidovirales,* family *Coronaviridae*, genus β-*coronaviruses*, species *Severe acute respiratory syndrome-related coronavirus*, strain *Severe acute respiratory syndrome coronavirus 2* (Fig. 2) [32, 33].

3. ORIGIN AND TRANSMISSION ROUTES OF HUMAN CORONAVIRUSES

Human coronaviruses (HCoVs) have large and complex RNA genomes and are believed to have a zoonotic origin. With the constantly evolving and mutating genomes of RNA viruses, their adaptability to different hosts also changes. Among the seven known human coronaviruses, five of them namely HCoV-NL63, HCoV-229E, MERS-CoV, SARS-CoV and SARS-CoV-2 are believed to be originated from bats and the remaining two human coronaviruses, HCoV-HKU1 and HCoV-OC43, are thought to have originated from rodents [34]. According to Vijgen, HCoV-OC43 was probably passed on to humans from cattle and might have been circulating in cattle since the 18th century [35].

The possible origin of SARS-CoV from bats was first suggested in 2005 by two independent studies reporting the discovery of SARS-related coronaviruses isolated from Chinese horseshoe bats (*Rhinolophus spp.*) [36, 37] along with a few more strains discovered in the following years [38]. A similar case has been reported for MERS-CoV as well, which was found to be closely related to coronaviruses isolated from bamboo bats (*Tylonycteris spp.*) and pipistrelle bats (*Pipistrellus spp.*), respectively termed Tylonycteris bat coronavirus HKU4 and Pipistrellus batcoronavirus HKU5 [39]. Thus, bats play an essential role in coronavirus ecology and evolution, as they are believed to accommodate an exceptionally wide diversity of coronaviruses. Inter-mediate hosts play a crucial role in the transmission of the HCoVs to other susceptible hosts like humans. The presumed intermediate host for SARS-CoV, that led to the transmission to humans from bats is believed to be palm civet cats (*Paguma larvata*), belonging to the Viverridae family was identified even before the identification of natural bat carriers, when highly similar SARS-CoV strains were found in the civets and in the workers, supposed to handle them from a wet market in China [40]. One of the evidence which suggested that SARS-CoV was probably transmitted to the market civets by other animals was reported during comparing samples from market civets to those in the wild [41].

A similar scenario was reported for MERS-CoV and its intermediate host, the dromedary camel (*Camelus dromedaries*), with the possibility that at some point bats infected camels [26], as MERS-CoV strains obtained from the infected human patients were highly similar in sequence to those isolated from camels [42, 43]. An evolutionary process involving a single cross-species transmission of bovine coronavirus from cattle to humans must have operated in the evolution of human coronavirus OC43. Just like that, owing to rapid change in the genome of RNA viruses, HCoV-229E is believed to be transmitted from bats to Camelids possibly [44]. It has been hypothesized by many researchers that all HCoVs were initially flourishing in bats as CoV-related viruses (SARS related-CoV, MERS related-CoV, and SARS-CoV-2). With time they first evolved and adapted to their intermediate host and finely transmitted to humans.

According to Boni et al. [45], SARS-CoV-2 is not a recombinant of any of the *Sarbecoviruses* (the viral subgenus containing SARS-CoV and SARS-CoV-2) identified so far. They found that the receptor-binding motif of SARS-CoV-2 is not a recently evolved trait, but an ancestral characteristic shared with bat viruses. They used phylogenetic dating methods and found that the lineage giving rise to SARS-CoV-2 has been circulating unnoticed in bats for decades. Based on their genomic sequencing analysis, Andersen and his collaborators proposed that the virus evolved to its current pathogenic state through natural selection in a non-human host and then jumped to humans, suggesting that an intermediate host was likely involved between bats and humans [46]. Later, Lam et al. through metagenomics identified SARS-Cov-2related coronavirus in pangolins (*Manis javanica*) seized in anti-smuggling operations in southern China [47].

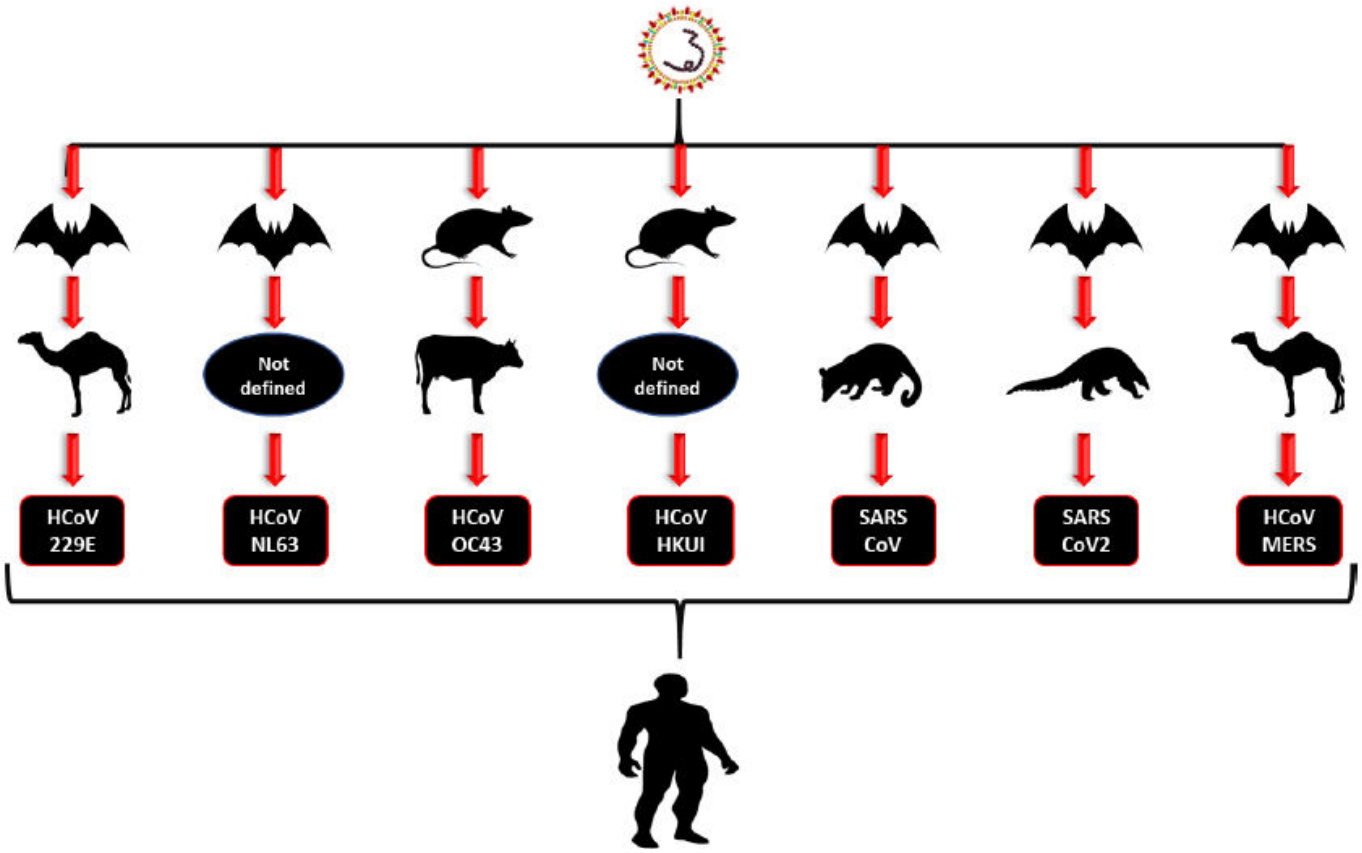


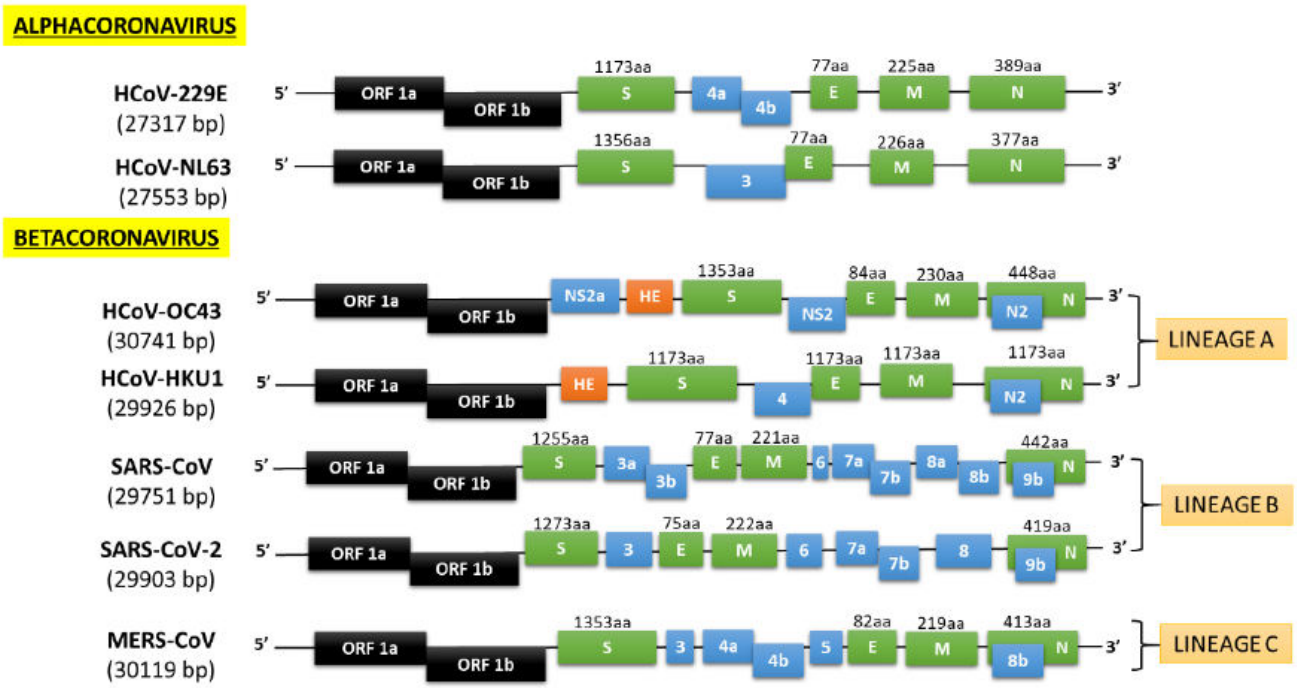
Fig. 3: Flowchart depicting the possible transmission routes of Coronaviruses from their natural reservoir

4. MOLECULAR ASPECTS OF CORONA VIRUSES

Coronaviruses possess a positive sense, large (26.4 to 31.7 kbp) RNA which is linear and unimolecular in nature, making them a group of the largest RNA-genome viruses known so far (Fig. 4). This huge genome provides extra plasticity in accommodating and modifying genes with higher chances of mutations. Also, the G+C content of coronavirus genomes is high and varies from 32% to 43% [17]. In coronaviruses, proteolytic processing produces 15 or 16 mature products, commonly denoted to as non-structural proteins (nsps) and numbered according to their position from N- to C-terminus in the viral polyproteins. Many nsps are unique enzymes involved in one or more essential step(s) in viral replication. Along with nsps the genome of coronaviruses also codes for certain structural proteins that are common to all coronaviruses. The viral envelope consists of a lipid bilayer, in which the membrane (M), envelope (E), and spike (S) structural proteins are fastened. The ratio of E:S:M in the lipid bilayer is approximately 1:20:300 [48]. On average a coronavirus particle has 74 surface spikes [49]. A subset of coronaviruses (specifically the members of β-*coronavirus* subgroup A) also has a shorter spike-like surface protein called hemagglutinin esterase (HE). Nucleocapsid protein (N) is the only protein present in the core of the viral particle, forming the nucleocapsid (Fig. 1). The RNA viruses have mutation rates as high as one million-fold than their host [50]. Being an RNA virus, SARS-CoV-2 also has high mutation rates. Spike glycoprotein, ORF1ab, ORF8, and NSP-1 are among the highly mutated regions of SARS-CoV-2 [51]. These overwhelming rates of mutations are the major cause of concern in the process of development of therapeutics for the treatment of COVID-19 disease. A high mutation rate in corona-viruses provides the possibility for this newly introduced zoonotic viral pathogen to adapt to become more efficiently transmitted from person to person and possibly become more virulent [24].

Nucleocapsid Protein (N) is a 349 to 470 aa long conserved phosphoprotein, encoded by the N gene, and constitutes the only protein present in the

nucleocapsid. Multiple copies of the nucleocapsid (N) protein bound to the positive-sense single-stranded RNA genome in a continuous beads-on-a-string type conformation [52, 53]. It is heavily phosphorylated and basic in nature. It is suggested that phosphorylation triggers a structural change leading to the enhancement of the affinity for viral RNA than that of the non-viral RNA. It is a multifunctional RNA-binding protein that is responsible for the assembly of viral genomic RNA into a ribonucleoprotein complex [54]. It is also considered to be involved in RNA synthesis and translation [55, 56]. It exhibits RNA chaperone activity and acts as an antagonist of type I interferon [57].



Corona viruses possess a capped, polyadenylated and structurally polycistronic infectious genome having a general genome organization represented as, 5′-leader-UTR-replicase-S (Spike)-E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′ UTR-poly (A) tail with the genome functioning as mRNA for the replicase gene. The replicase gene (black in color) encoding the non-structural proteins (nsps) occupies two-thirds of the genome. The 5' end has a leader sequence and untranslated region (UTR) which comprises multiple stem-loop structures required for RNA replication and transcription. Variable numbers of small accessory genes (Blue) are interspersed within the structural genes (green in colour) at the 3′ end in different coronavirus lineages. Transcriptional regulatory sequences (TRSs) are present at the beginning of each structural or accessory gene for the expression of each of these genes. 3′ UTR also contains RNA structures required for the replication and synthesis of viral RNA. The gene encoding haemagglutinin-esterase (HE) in lineage A of β-coronaviruses is in orange.

Fig. 4: Genome organization of seven known human coronaviruses (not in scale)

Membrane Protein (M) is a 218 to 263 aa long integral membrane protein involved in virus particle assembly [58]. According to Neuman [49], it creates a work-station on the cellular membrane for virus and host factors to make new virus particles. It also plays an important role in determining the particle morphology.

Envelope Protein (E) is a 74-109 aa long pentameric integral membrane protein exhibiting ion channel and/or membrane permeabilizing (viroporin) activities. It is a minor structural component of viral envelope coded by E gene, with around 20 copies per particle. It is involved in virion morphogenesis and is also identified as a virulence factor for SARS-CoV. The E and M proteins are associated with the envelope of all coronaviruses and are very essential for the formation of the viral envelope and maintaining its structure and shape [53].

Spike Glycoprotein (S) is a large (1128-1472 aa long), homo-trimeric type I membrane glycoprotein responsible for receptor-binding and membrane fusion during viral infection (de Groot et al., 2011). The S proteins are responsible for the "spikes" present on the surface of coronaviruses giving the characteristic crown-like appearance to this virus family. It is a protruding

protein outside of the virus envelope, these protrusions are specific to only certain receptors on the host cell and are essential for the host specificity and viral infectivity, with a short transmembrane domain at the C terminus, followed by a short cytoplasmic tail rich in cysteine residues [8]. It is made up of two subunits namely, (N-terminal) S1 and (C-terminal) S2, which are distinguishable as soon as the S protein is cleaved by host proteases [59]. The S1 subunit stabilizes the entire S protein prior to the infection and prevents the S2 protein from premature conformational changes. The S1 subunit forms the head of the spike and possesses a receptor-binding domain (RBD) that can recognize human receptor on target cell membranes. It initiates infection by interacting with the host receptor and cause the virion particle to bind to the host cell membrane, which causes conformational changes in the S glycoprotein. The S2 subunit acts as an anchor and helps in the fusion of the viral and cellular membranes after protease activation. The C termini of the extracellular parts of the S proteins has coiled-coil regions of two heptad repeats (HR1 and HR2) [17]. During the fusion of S protein with the cell membrane, these heptad repeats obtain a trimer-of-hairpins structure, and bring the fusion peptide closer to the C-terminal region of the ectodomain, leading to the fusion of viral and target cell membranes. Due to its specificity and indispensable role during viral infection, the S protein is a significant target for development of vaccines, inhibitors, neutralizing antibodies, etc. for COVID-19.

SARS-CoV-2 when compared with other coronaviruses, it has been found that the percentage of similarity between nucleotide sequence of SARS-CoV-2 and SARS-CoV is subsequently high, yet SARS-CoV-2 shows some explicit characters, specifically in accessory proteins present at the 3′-terminus of the genome like the absence of the 8a protein, a longer 8b protein consisting of 121 amino acids when compared with 84 amino acids in SARS-CoV, and a shorter 3b protein of only 22 amino acids. The new SARS-CoV-2 also differs from the other coronaviruses by encoding an additional hemagglutinin (HE) glycoprotein that possesses acetyl-activity, which might enhance the cell entry and pathogenesis of the virus [60].

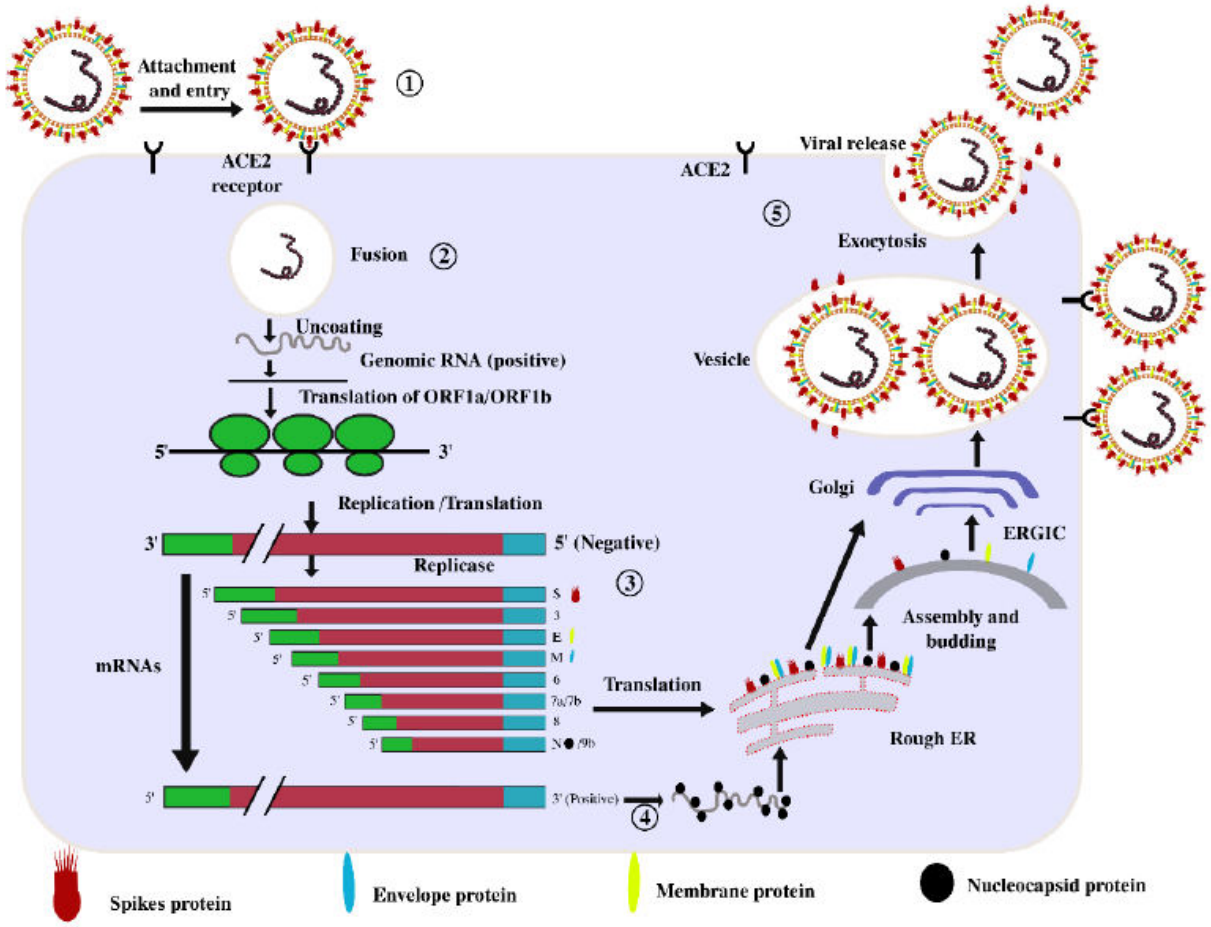
SARS-CoV-2 is closer to SARS-CoV with 77.2% amino acid similarity. However, SARS-CoV-2 possesses a longer spike protein in comparison to SARS-CoV [61]. The spike proteins of both are homologous with 80% identity. SARS-CoV and SARS-CoV-2 present similar receptor-binding domain structures [62] with 79.5% homology [63]. Moreover, the main protease is highly preserved between SARS-CoV-2 and SARS-CoV with a 96% overall similarity, however the SARS-CoV-2 RBD (receptor binding domain) has a significantly higher ACE2-binding affinity than SARS-CoV RBD, based on biochemical data [64]. SARS-CoV-2 and SARS-CoV S2 subunits share high sequence similarities, with 92.6% and 100% overall identities in HR1 and HR2 domains, respectively. In contrast, the resemblance rate between the new coronavirus and MERS-CoV was only 50% [26]. Such atomic-level structural information provides important insights into the molecular basis for coronavirus recognition and infection, and could facilitate ongoing vaccine design and inhibitor screening efforts.

It is important to note that the whole genome sequence as well as the encoded proteins of pp1ab, pp1a, envelope, matrix, accessory protein 7a, and nucleocapsid genes of SARS-CoV-2 shows higher homology with SARS-like bat CoV than that of the human coronavirus SARS-CoV [3, 65]. These data confirm the zoonotic origin of SARS-CoV-2 and support the recent theory that the transmission chain started from bats to humans, with pangolins as intermediate host.

5. THE LIFE CYCLE OF CORONAVIRUSES

The replication cycle of coronaviruses can be divided into five steps: attachment to host cells, viral entry and uncoating, expression of the viral replicase and formation of the replication-transcription complex, viral RNA synthesis, and virion assembly and release (Fig. 5). At first the viral particles bind to the cellular receptors present on the surface of the host cells with the help of spike glycoprotein. S glycoprotein has two functional domains S1 and S2. The S1 domain possesses Receptor-binding domain (RBD) which binds to the cognate host cell receptor. The S2 domain mediates the fusion between the viral membrane and host cell membrane, required for entry of CoVs into host cells. RBD, located at the C-terminal domain in the case of HCoVs, recognizes the cognate cellular receptor which is either proteins or carbohydrates and is present on the plasma membrane of the host cell. HCoV-OC43 and HCoV-HKU1 target glycan-based receptors carrying 9-O-acetylated sialic acid. Whereas, other HCoVs employs surface peptidase such as amino-peptidase N (APN) for HCoV-229E, dipeptidyl peptidase 4 (DPP4) for MERS-CoV and angiotensin-

converting enzyme 2 (ACE2) for HCoV-NL63, SARS-CoV and SARS-CoV-2. Specific binding between S1 and the cellular receptor results in a conformational change in the S2 subunit and large-scale reorganizations of the S protein. The host cell proteases cleave trimeric S protein and expose the fusion peptide of the S2 domain, leading to the fusion of viral and cellular membranes. The cleavage of S protein occurs between the S1 and S2 domains (S1/S2 site) and within the S2 domain proximal to the fusion peptide, at different sites of different coronaviruses. In the case of HCoV-229E, surface proteases like type II transmembrane protease serine 2 are involved in the activation of S protein for host cell entry, whereas, SARS-CoV uses endosomal cysteine protease cathepsin L and another trypsin-like serine protease to activate the S protein.



1. Attachment to host cells, 2. Viral entry and uncoating, 3. Expression of the viral replicase and formation of the replication-transcription complex, 4. Viral RNA synthesis, and 5. Virion assembly and release

Fig. 5: Diagrammatic representation of the infection process of SARS-CoV-2

After the fusion of the viral membrane and the host cell membrane, the viral genome is released into the cytoplasm. Further, acidification of the endosomal microenvironment is the key requirement for the release of the viral genome into the host cell. After entering the cell, the two open reading frames (ORFs), ORF1a and ORF1b, of the genomic RNA of HCoV are translated to produce two overlapping polyproteins, pp1a and pp1ab, respectively. Subsequently, pp1a and pp1ab are processed into 16 non-structural proteins (nsps) autoproteolytically. These nsps form RTC (replication-transcription complex) for viral RNA synthesis and alter the cellular membranes to form double-membrane vesicles or spherules, onto which the HCoV RTC is assembled and anchored. RTC uses genomic RNA as a template to produce the full-length negative-sense genomic RNA and 5'-nested set of negative sense sub-genomic RNAs (sgRNAs). Full-length negative-sense genomic RNA is used as a template for the production of new copies of the viral

genome and 5'-nested set of negative sense sgRNAs. These negative sense sgRNAs are used as templates to synthesize a 3'-nested set of positive sense sgRNAs, which are translated to structural and accessory proteins. The building units congregate at the assembling site of the ERGIC. After assembly, progeny virions are transported in smooth-wall vesicles. Through the secretory pathway they reach the plasma membrane and are released by exocytosis.

6. ANTIVIRAL STRATEGIES AND VACCINES

At present, no specific treatment is available for HCoV infections. We are relying on supportive therapies only. Though, many efforts were made in this direction since the outbreak of the first coronavirus. Polyclonal antibodies directed for the complete virus or the receptor binding site of spike protein can inhibit the viral infection and monoclonal antibodies directed to the receptor of HCoV-229E *i.e.,* aminopeptidase N can inhibit viral entry into the host cells, but only in *in vitro* conditions [66]. Though not much effective, drugs containing ammonium chloride, chloroquine, or bafilomycin A reduce the infection of both HCoV-229E and HCoV-OC43 virus in the host by increasing the pH of the endosomal pathway, as it disturbs the fusion of the virus with the host cell [67]. The HCoV-OC43 virus has been studied very less as it causes mild infections in humans. There are no clinical trials for this virus, however antiviral strategies at the preclinical stage have been developed against this virus. For example, *Griffithsin*, a carbohydrate-binding agent, specifically binds to oligosaccharides on S glycoprotein, and block virus-host cell binding. *Chloroquine*, an antimalarial drug, is also effective against this virus which acts by endosomal acidification.

In the case of SARS-CoV, the *in vitro* studies shows that the mannose-specific plant lectins derived from *Galanthus nivalis* (Common Snowdrop), *Hippeastrum hybrid* (Amaryllis), and *Allium porrum* (leek) bind to highly glycosylated S protein of coronaviruses and block the S-receptor interaction, thus inhibiting replication of the virus [68]. The CR304 human monoclonal antibody (mAb) and 80R mAb immunoglobulin IgG1 bind to SARS-CoV RBS and block the interaction of S protein and ACE2 receptors [69, 70]. During *in vitro* studies on HCoV-NL63, synthetic HR2 peptide has been found to act as an inhibitor of HR2, which is involved in the membrane fusion, located in the S2 domain. So, blocking this region can be an effective approach for viral treatment. Due to the high mortality rate of MERS-CoV, some of the antiviral drugs were developed for human trials. *Lopinavir-ritonavir* and interferon alfa-2a is a combination of antiviral and immunomodulating agents which can decrease the number of viruses in the blood [71]. Ribavirin, a Guanosine analog that targets the enzyme RNA dependent RNA polymerase, can also act as a potent inhibitor of viral RNA synthesis and mRNA capping. It has shown beneficial effects in some patients with high doses, however with some side effects. Monoclonal antibodies like m336, m337, m338, REGN3051, and REGN3048 are some of the agents which block the virus-cell interaction and therefore reduce the viral infection. Many other strategies ended up at the preclinical stage and did not show any positive effect against this virus [72].

At present, there is no effective medicine for the treatment of COVID-19. However, some clinical trials are going on with pre-designed antiviral therapies to repurpose them for COVID-19. These treatments are characterized into two groups. The first group targets the virus directly and the second group focusses to boost the immune response towards the virus. These therapies consider different modes of action for reducing the viral activities and its ill effects in the human body. Many antivirals inhibit the RNA-dependent RNA polymerase activity. Under this therapy, drugs are designed in such a way that they can inhibit the process of viral replication in the host cell by inhibiting the RNA polymerase reaction which in turn halts the process of viral genome replication. Both *Remdesivir* and *Favipiravir* are antiviral drugs of this category. *Remdesivir* resembles adenosine and *Favipiravir* resembles structurally to guanosine. They get incorporated in the nascent viral DNA, inhibiting RNA dependent RNA polymerase leading to premature termination of the viral RNA.

Some antivirals block the activity of vital viral enzymes. Obstructing the activity of proteases block the breaking of the large polypeptide into small functional proteins, finally, crashing the viral replication machinery. *Ivermectin* is one such drug that has also been proven to exert antiviral activities toward both human immunodeficiency virus (HIV) and dengue virus [73]. *Lopinavir/Ritonavir* is another class of protease inhibitors used for human immunodeficiency virus. Evidence suggests that they can also inhibit the protease found in coronavirus. However, they produce little or no reduction in the mortality of hospitalized COVID-19 patients [74].

Blocking virus cell membrane fusion could be another effective approach to the development of anti-COVID-

19 medicine. Recombinant Human Angiotensin-converting Enzyme 2 (APN01) mimics human ACE2. Viruses instead of binding to the cellular ACE2 receptor, bind to the soluble *ACE2/APN01* and restrict the entry of SARS-CoV-2 by blocking the S protein from interacting with the cellular ACE2. APN01 was originally developed by Apeiron Biologics and is under phase II clinical trial for COVID-19. *Hydroxychloroquine* also serves the same function by increasing the endosomal pH required for the membrane fusion of virus and host cell. It glycosylates the ACE2 receptor and inhibits viral replication. But several placebo-controlled studies suggest that the drug is ineffective to either treat or prevent the disease.

The degree of infection caused by a virus is determined by the virulence of the virus and the host's immune response. It has been noticed that elderly COVID-19 patients show severe symptoms and high death rates as compared to infective young ones. The explanation for this observation can be the reduced immunity level of elderly people. Therefore, enhancing host immunity can be one of the strategies to reduce the severity of the viral infection. This can be done by various medicines and therapies like *Natural Killer cells* and *Recombinant interferons*. They both contribute to enhancing the immune responses of the host against the coronavirus.

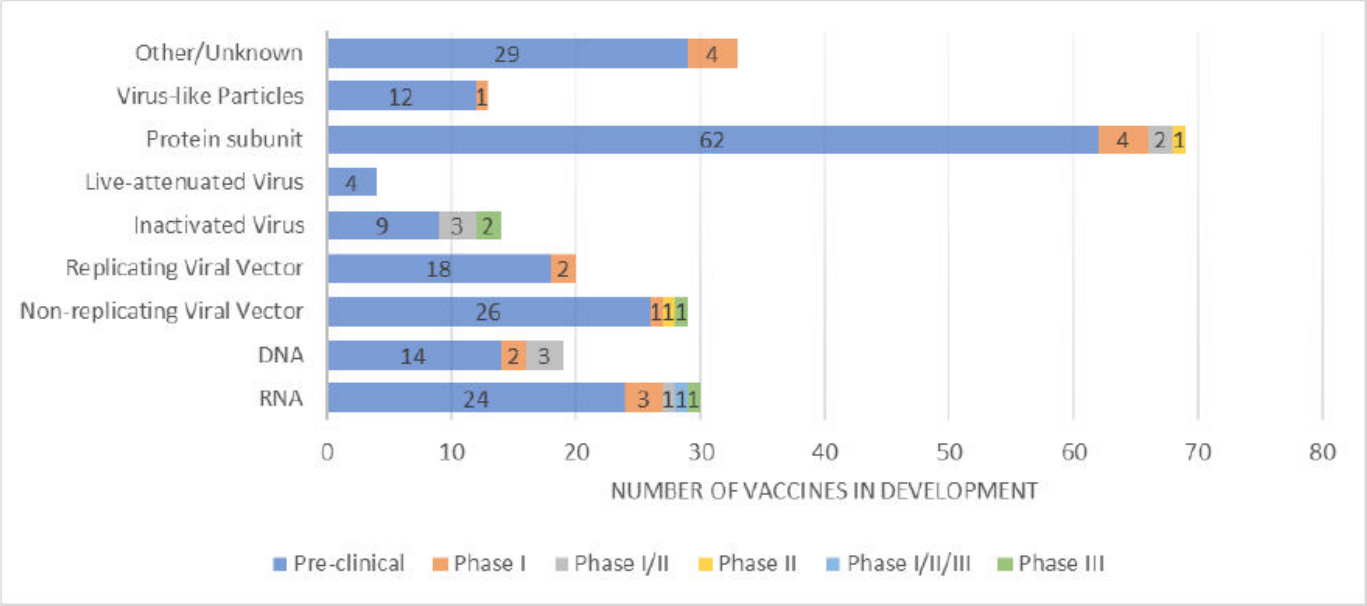


Fig. 6: Status of different types of vaccines under clinical development

A vaccine is usually made up of weakened or killed forms of the microbe, its toxins, or one of its surface proteins, which imitates disease-causing microorganisms and when injected in the host elicits an immune response against it. This memory of the immune response helps the host to produce an elevated immune response on the encounter of that pathogen. More than 200 candidate vaccines are currently being developed against COVID-19 with roughly two dozen at the stage of clinical trials with human volunteers. The strategies used for vaccine development include live-attenuated or inactivated virus; replicating or non-replicating viral vector; DNA vaccine or RNA vaccine and protein subunit or virus-like particles. WHO has developed a "DRAFT landscape of COVID-19 candidate vaccines (https://www.who.int/ publications/m/item/draft-landscape-of-covid-19-candidate-vaccines)" for information purposes only concerning 2019-2020 worldwide of SARS-CoV-2. As of August 8, 2020, the dashboard of the London School of Hygiene and Tropical Medicine (https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/) has described a total of 231 vaccine candidates. Out of these, 33 have already entered clinical trials. Out of 4 candidates at phase-III trial, two candidates are using inactivated virus strategy (ClinicalTrials.gov Identifier: NCT04456595 and ChiCTR2000034780) and rest two are based on the RNA vaccine (ClinicalTrials.gov Identifier: NCT04470427) and Non-replicating Viral Vector (ClinicalTrials.gov Identifier: ISRCTN89951424) approach (Fig. 6). The World

Health Organization has been tracking various COVID-19 candidates and has made no mention of the vaccine clearing all three phases of human trials so far.

According to epidemiologists, to induce herd immunity, at least 70% of the population has to develop antibodies against SARS- CoV-2. However, a very small fraction of the population has been found to develop immunity against the disease. Therefore, the vaccine is the only ray of hope to bring the epidemic to an end.

7. CONCLUSION

Extensive and critical research on HCoVs has been in operation soon after the emergence of novel coronavirus SARS-CoV-2 in Wuhan, China. Due to its high transmissibility yet low pathogenicity, it has become mandatory to control the spread of COVID-19. A comparative analysis of HCoVs that made an appearance in previous years, has made us more informed in deducing the specific zoonotic origin of SARS-CoV-2 and its routes of transmission via intermediary hosts to finally infect humans. A further scientific study of the cross-species transfers of an animal virus to humans (zoonosis) and the adaptations acquired by such zoonotic viruses will surely be helpful in the prevention of future zoonotic events. It has been now a fact that bats harbor a wide variety of CoVs which are potentially pathogenic like SARS-CoV, MERS-CoV, and SARS-CoV-2; and are transmitted to humans via intermediate hosts. Therefore, there are innumerable chances of recombination of various coronavirus species occurring inside their natural reservoirs, which may give rise to new and even more pathogenic HCoV species, threatening human survival. Even though the chances of direct transmission of CoVs from bat to humans are rare but with a constant intervention of human activity in bats habitat and the culture of eating wild animals in some places of China can be a major reason for coronavirus related diseases outbreaks in the future. Presently, the most effective way to prevent such viral zoonosis leading to a global pandemic in the future is to prevent interfering within the ecological niches of the natural reservoirs (bats) of the zoonotic viruses. Most importantly, there is a dire need of enhancing our understanding of coronaviruses and designing vaccines and antiviral drugs targeting human coronaviruses so that we can fight with the current as well as future epidemics.

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Declaration of Competing Interest

The authors declare they have no competing interests.

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