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Latest updates on SARS-CoV-2 genomic characterization, drug, and vaccine development; a comprehensive bioinformatics review

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ABSTRACT

Amid the COVID-19 outbreak, several bioinformatic analyses have been conducted on SARS-CoV-2 virus genome. Numerous studies rushed to fill the gap about this novel virus. Comparison with other related sequences, structural predictions of the produced proteins, determination of variations in amino acid residues and depiction of possible drug and vaccine targets have been the focus of scientific research from the beginning of this year. In addition to discussing the viral taxonomy, clinical features, life cycle, and genome organization, this review will focus on the recent updates in genome and viral proteins characterization and potential therapeutic and vaccine candidates developed so far. Comparative studies with related genomes and proteins provide understanding for the viral molecular mechanisms and suggest targets for therapeutics and vaccinology trials to stop the escalation of this new virus. This pandemic, with its resulting social and economic afflictions, will definitely have significant marks on our lives in the following years.

1. Introduction

The speedy dissemination of COVID-19, which in less than 6 months has shifted from affecting some individuals in Wuhan (Hubei province, China) to greater than 7 million people and causing more than 400,000 deaths in nearly every country in the world [1], has alarmed all public health systems and governments. The outbreak was believed to emerge from Wuhan City, Hubei province, in China at the end of 2019 and was allied to the Huanan Seafood Wholesale Market [2]. Suspected patients were admitted to a selected hospital in Wuhan and by Jan 2, 2020, a total of 41 patients had been recognized as having laboratory-confirmed COVID infection. During the Spring Festival travel season in January 2020, the virus extended to other areas in China, and was subsequently transmitted to other countries through international travelers [3].

Worobey et al. (2020) explained the establishment of the earliest SARS-CoV-2 transmission networks in Europe and North America. Their findings propose that the virus was disallowed to take hold in USA and Germany early due to the swift and early interferences. Introductions of the virus from China to Italy and Washington later initiated the earliest continuous transmission networks [4].

However, a recent study unexpectedly detected SARS-CoV-2 antibodies in the pre-pandemic period in Italy. Beginning from September 2019, SARS-CoV-2 RBD-specific antibodies were detected in 11.6% of people in Italy. This study proves that SARS-CoV-2 circulated between asymptomatic persons in Italy a few months before the first patient was recognized. Detecting SARS-CoV-2 antibodies in asymptomatic people prior to the outbreak in Italy may restructure the pandemic history [5].

Due to a marked increase in the infections in several countries, the virus outbreak was declared to be a Public Health Emergency of International Concern by the World Health Organization (WHO) on January 30, 2020 [6]. On the 11th of February 2020, the International Committee on Taxonomy of Viruses called the virus "severe acute respiratory syndrome coronavirus-2" (SARS-CoV-2) based on the phylogenetic analysis [7]. That day, the WHO also named the illness resulting from this virus "Coronavirus Disease 2019" (COVID-19) [8]. Due to the very quick rise in the total affected cases and countries, the WHO announced SARS-CoV-2 a pandemic on March 11, 2020 [9]. As of December 27th, 2020, over 79.2 million cases and over 1.7 million deaths have been reported [10].

1.1. Virus/disease characterization

1.1.1. Taxonomy and general characters

Coronaviruses form part of the subfamily *Orthocoronavirinae,* family *Coronaviridae*, suborder *Coronavirinae*, order *Nidovirales* [11]. This subfamily is classified into 4 genera – *alpha, beta, gamma* and *delta*

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coronavirus. Until this year, only 6 coronaviruses are proven to evoke infections in humans. Four of them are endemic in humans, and cause only mild upper respiratory symptoms, known as human coronaviruses (HCoV) 229E, NL63 (both from genera *alphacoronavirus*), OC43 and HKU1 (both from genera *betacoronavirus*) [12]. The remaining two, both from genera *betacoronavirus*, are epidemic human coronaviruses and cause severe lower respiratory symptoms: Middle East respiratory syndrome coronavirus (MERS-CoV), which appeared in 2012 in Saudi Arabia [13], and severe acute respiratory syndrome coronavirus (SARS-CoV), which originated in China in 2002–2003 [14]. *Gamma* and *deltacoronavirus*, on the other hand, include several avian coronaviruses [12]. SARS-CoV-2, as revealed by sequence analysis, has an archetypal genome structure of coronaviruses, specifically *Betacoronavirus*, subgenus *Sarbecovirus*. It is regarded as the seventh coronavirus that infects humans till now [15].

Coronaviruses are pervasive pathogens in nature, leading to gastrointestinal or respiratory infections and sometimes affecting vital systems in humans and animals. They are inactivated by UV rays, high temperatures, lipid solvents like ether, 75% ethanol, chlorine-containing disinfectants, and chloroform [16].

1.1.2. Transmission

When COVID-19 first started in China, human to human transmission was doubtful, up till the appearance of patients not connected to the seafood market. As outbreaks emerged all around the world, human-to-human transmission became obvious [17].

SARS-CoV-2 transmission, like other respiratory viruses, occurs via respiratory droplets, indirect or direct contacts [18]. However, its transmission rate is greater than SARS-CoV and MERS-CoV. The infection risk rises the longer and nearer a person is to an infected person, and unventilated enclosed packed places are more hazardous than the open air. Some reports have proposed a relation between the quantity of virus exposure and the harshness of disease, though this relation remains questionable, as the severity of disease is more likely to depend on the person's immunity [19].

Fomite transmission's role is not yet completely known. Accurate information on the environmental stability of SARS-CoV-2 is needed to verify the risks of fomite transmission from contaminated surfaces. SARS-CoV-2 was described to last on numerous surfaces including stainless steel, plastic, glass, and cardboard for a minimum of several hours [20]. In September 2020, studies suggested that a protein-rich medium like airway secretions could protect the virus and may augment its persistence and spread by contaminated fomites. Consequently, virus contaminated fomites may have a substantial role in the indirect transmission of COVID-19. This conclusion verifies surface cleaning as it may have a vital role in stopping SARS-CoV-2 transmission [21]. Moreover, Riddell et al. (2020) determined the existence rates of SARS-CoV-2 at diverse temperatures. Infectious virus was isolated from glass, stainless steel and paper for up to 28 days at 20 °C. On the contrary, it lasted fewer than 24 h at 40 °C on some surfaces [22].

Alternatively, a recent study suggested that environmental contamination leading to SARS-CoV-2 transmission is not likely to happen, provided that standard cleaning precautions are imposed. Hence the chance of transmission through inanimate surfaces is less common than formerly recognized [23].

Airborne transmission might also probably occur in specific settings that generate high concentrations of aerosols in a closed environment, but this still has to be evidenced scientifically [18]. Whole blood and urine of COVID-19 patients were found to contain SARS-CoV-2 RNA, which suggests that transmission through these routes is likely [24]. Moreover, studies proved that stool samples also contained SARS-CoV-2 prior to and after treatment, indicating that the fecal-route transmission of SARS-CoV-2 should not be neglected [25].

Concerning the vertical spread of COVID-19, an early study suggested that no vertical transmission of SARS-CoV-2 occurred throughout 3rd trimester of pregnancy, where no virus was detected in the newborns of 9 infected women [26]. However, the small number of cases analysed in the study makes further studies to prove this essential. Moreover, 2 of 6 neonates of COVID-19 infected women had high IgG and IgM antibodies to SARS-CoV-2 in another study [27]. In July 2020, a meta-analysis of SARS-CoV-2 infection and pregnancy was performed and showed that vertical transmission of the virus is possible, though in a small percentage of cases [28].

1.1.3. Clinical features of COVID-19

The predicted incubation period of SARS-CoV-2 is 2–14 days, with the median incubation period being 4 days [29]. The illness starts with flu-like symptoms that most commonly consist of fever, fatigue and dry cough. Other symptoms include sore throat, shortness of breath, chest tightness and muscle pain. Non respiratory manifestations including palpitation, diarrhea, or headache sometimes precede respiratory symptoms. Patients may have runny nose, nausea and vomiting [30]. SARS-CoV-2 may cause gastrointestinal symptoms due to a direct damage to the intestine [29].

In addition to the gastrointestinal symptoms, several retrospectives studies reported that patients with COVID-19 experienced hypogeusia and hyposmia which may be due to the swelling of the nasal mucosa [31]. Olfactory and gustatory dysfunctions are currently being listed to be from the initial warning signs and for improved case detection.

COVID-19 may result in complications leading to severe infections. Serious cases frequently suffer dyspnea and/or hypoxemia 7 days following the beginning of the illness, which can swiftly progress to Acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, coagulopathy and multiple organ failure. about 80% of patients have leukopenia, and 72.3% have lymphocytopenia and elevated C reactive protein (CRP) levels [32]. Most chest computed tomography (CT) images of lungs show lesions (sometimes dense) in numerous lung lobes. Ground-glass opacity together with consolidation shadows have been reported [33]. However, a wide range of severity patterns is exhibited during the course of the disease. Dyspnea occurs within 8 days after the disease onset in a few patients, whilst it may not happen in other patients [34].

Patients with mild disease can recover after 2 weeks, while severe cases last nearly 3–6 weeks, with death occurring from 2 to 8 weeks [35]. Current cases prove a good prognosis for that the majority of patients, but not the old-aged and those with chronic illnesses or malignancy [16]. Moreover, although mild symptoms have been reported in children, ethnicity and previous comorbidities might be independent risk factors for critical illness [36]. It must be noted that asymptomatic persons may also be infected with SARS-CoV-2 [17,37] which allows a more effective spread of the virus from one person to another.

1.1.4. Structure of SARS-COV-2

Coronaviruses are enveloped, single-stranded RNA viruses ranging from 80 to 220 nm in diameter. The envelop possesses crown-like spikes (20-nm in length) similar to the sun's corona under electron microscopy, thus its name coronavirus (Fig. 1A) [12]. They also bear the largest genome among the presently known RNA viruses, ranging between 27 and 34 kB [38]. Electron micrographs of the novel SARS-CoV-2 particles were mostly spherical with a diameter of 60–140 nm, and spikes about 9–12 nm in length [15].

Coronaviruses have 4 main structural proteins: the nucleocapsid (N), spike (S), envelope (E) and membrane (M) structural proteins. The viral envelope is formed of the latter three structural proteins [39].

The S glycoprotein is a transmembrane protein (molecular weight 150 kDa) projecting from the surface of the virus. S protein monomers assemble into homotrimers and aid in binding of the virus to host cells ACE2 receptor. To further understand the SARS-CoV-2 S protein, its structure was determined by cryo–electron microscopy [40]. This glycoprotein is split into 2 sub units, S1 and S2, by a host cell protease. The S1 subunit, defined by the C-terminal domain (CTD) holding the receptor binding domain (RBD) and the N-terminal domain (NTD),

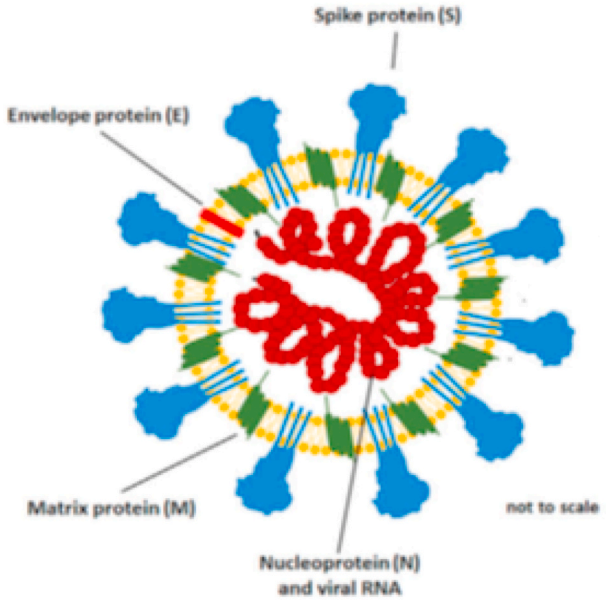

Fig. 1. Severe Acute Respiratory Syndrome Coronavirus 2 structure [12].

determines the host virus range and cellular tropism. S2 subunit, containing an internal membrane fusion peptide (FP), two 7-peptide repeats (HR), a membrane proximal external region (MPER), and a trans-membrane domain (TM), is involved in membrane fusion [41–43].

The most abundant surface structural protein, the M glycoprotein (molecular weight 25 kDa) is concerned in virus assembly [44]. The E protein, the smallest protein in SARS-CoV-2, is concerned with the production and maturation of this virus [45]. The N protein is bound to the nucleic acid of the virus, and consequently, is involved in viral genome and replication cycle related processes [45].

1.1.5. Genome Organization and Characterization

Laboratories around the world have been working on sequencing SARS-CoV-2 genome in an attempt to provide better understanding of the virus structure, mechanisms, and possible drug targets. By the 1st of June, a total of 4988 sequences have been submitted to the NCBI GenBank database including the reference sequence for SARS-CoV-2 genome (NCBI RefSeq NC_045,512). Homology analysis of the submitted sequences were conducted in several studies and revealed that the overall homology among the full-length SARS-CoV-2 genomic sequences was more than 99% [46]. SARS-CoV-2 is a positive-sense single stranded RNA virus. Their genomes typically begin with a 5′-methylguanosine cap (also referred to as 5′UTR) and end with a 3′-poly-A tail (3′UTR). The genome of SARS-CoV-2 is approximately 29.8 Kb in length with G + C content of 38% and is annotated to possess 11 ORFs encoding at least 26 proteins [47,48]. These proteins play a crucial role in viral survival and virulence. More than two thirds of the genome consist of ORF1a and ORF1b containing replication and transcription related genes which encode 16 non-structural proteins (nsps). The rest of the genome encodes the 4 previously mentioned structural proteins in addition to other

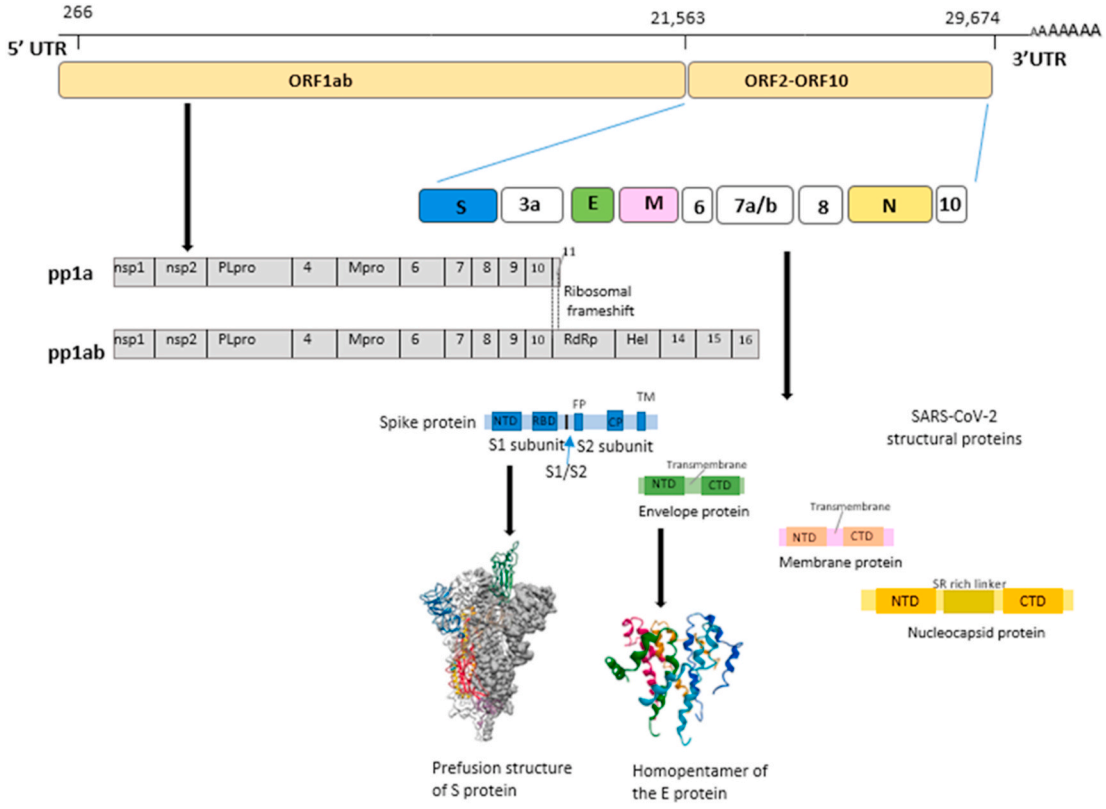

Fig. 2. Schematic representation of SARS-CoV-2 genome organization. The genome consists of a single long open reading frame with two flanking untranslated regions (UTRs). The genome is arranged in the order of 5′ UTR (265 nucleotides)-*orf*1ab (the replicase gene)-orfS- orf3a-orfE-orfM- orf 6- orf 7a-orf 7b- orf8-orf N-orf 10–3′UTR (358 nucleotides). ORF1ab encodes for two large polyproteins, pp1a and pp1ab. Expression of these two polyproteins is achieved by ribosomal frame shifting. These two polyproteins are cleaved into 16 non-structural proteins by the action of two proteases, PLpro encoded by nsp3 and Mpro encoded by nsp5. The rest of the genome encodes for 4 structural proteins; Spike protein (S), Envelope protein (E), Membrane protein (M), nucleocapsid protein (N) in addition to six accessory proteins (coloured white); 3a, 6, 7a, 7b, 8 and 10. The main domains of the four structural proteins are shown. The prefusion structure of S protein is displayed showing the RBD highlighted in green, adopted from Wrapp et al. [40], and the Homopentamer of E pentameric protein which modulates ion channel activity of the E protein is also illustrated, adopted from Gupta et al. [49].

6 accessory proteins encoded by ORF3a, ORF6, ORF7a, ORF7b, ORF8 and ORF10 genes (Fig. 2). Being a positive sense RNA virus, the viral genome is ready to be translated into proteins once entry into the host cell is achieved and the genomic material released. The next part will discuss the different ORFs of the viral genome in order, the produced proteins, their functions, and homology studies with relevant proteins shedding light on the most important outcomes of recent studies on SARS-CoV-2.

1.1.6. ORF1ab

The ORF1ab encodes two large non-structural polyproteins upon translation. Translation takes place by ribosomal frame shifting producing the two large replicase polyproteins pp1ab and pp1a**.** These polyproteins are then processed into sixteen nsps. Cleavage of the polyproteins takes place by the action of two proteases; papain-like protease (PLpro) which is encoded by nsp3 and a serine type Mpro which is the main protease and also referred to as 3CLpro (chymotrypsin-like protease) encoded by nsp5 [50]. These nsps play a vital role, whose functions could be predicted from the studies conducted on SARS-CoV-1 due to the homology between the sequences of SARS-CoV-1 and SARS-CoV-2 (Table 1). Several studies focused on comparing sequences of nsps of SARS-CoV-2 to homologous proteins in other related coronaviruses. A study conducted homology modelling to compare between nsp2 and nsp3 of SARS-CoV-2, SARS-CoV-1 and bat SARS-like CoV. It was observed that the amino acid in position 501 (321 of the nsp2 protein) was a polar amino acid in SARS-CoV-2 conferring a higher stability to the protein whereas the corresponding site in Bat SARS-like CoV has a non-polar amino acid. The same study reported that the position 723 in the SARS-CoV-2 (543 in nsp3) has a serine instead of the glycine residue present in bat SARS-like CoV and SARS-CoV-1. In the amino acid position 1010, the SARS-CoV-2 has a proline residue, the bat SARS-like coronavirus has a histidine residue and the SARS-CoV-1 has an isoleucine residue. The study assumes that the stabilizing mutation in the nsp2 could account for SARS-CoV-2 highly contagious ability, while the destabilizing mutation in nsp3 could suggest a mechanism that differentiates COVID-2019 from SARS [51]. Khailany et al. analysed 95 SARS-CoV-2 complete genome sequences and reported that nsp3 displayed more variants than other nsps [52]. A huge comparative study analysed the genomes of 10,022 SARS-CoV-2 sequences and also noted that nsp3 had the largest number of missense variants among nsps [53]. The same study reported mutations in nsp12 (the RNA-dependant RNA polymerase) and deletions in nsp14 (the 3′-5′ exonuclease). A recent study on molecular characterization of a SARS-CoV-2 in a COVID-19 cluster in France reported a deletion of an amino acid in nsp2 (Asp 268 Del) when compared to the sequence of the reference strain (isolate Wuhan-Hu-1, EPI_ISL_402,125). The study suggested the spread of this deletion in the SARS-CoV-2 clusters in Europe and mentioned that the impact of this deletion on the transmission and pathogenicity of the virus had be evaluated [54].

1.1.7. ORF 2-10

This part is made of 9 sub-genomic mRNA sequences which code for the structural proteins (Spike, Envelope, Membrane and Nucleocapsid) in addition to 6 accessory proteins (3a, 6, 7a, 7b, 8 and 10). Some of these proteins undergo glycosylation in the Golgi apparatus forming glycoproteins.

ORF S: has a length of 3822 nucleotides [46], and codes for the spike surface glycoprotein which is formed of S1 and S2 subunits as mentioned previously. Accordingly, several studies conducted sequence alignments of this protein with those of related coronaviruses to detect amino acid conservation and variation. A study by Chan et al. observed very high homology (99%) between the S2 subunit of SARS-CoV-2, the two bat SARS-like CoVs and human SARS-CoV-1. This suggests that antiviral peptides which are active against S2 could be used against SARS-CoV-2. Subunit S1 was found to share 70% identity with bat SARs-like CoVs and human SARS CoV-1 [47]. However, although the core domain of RBD in

Table 1
List of non-structural proteins (nsps) of SARS-CoV-2, their amino acid lengths, and functions.

Non-structural protein nsp	Amino acid length and position	Function	References
nsp1	180 aa (residues 1–180)	Suppresses host innate immune functions. It supresses type I IFN expression in infected cells. This antagonism takes place by degradation of host mRNA and inactivation of host translational machinery.	[47,55–57]
nsp2	638 aa (residues 181–818)	Interacts with host factors prohibitin 1 and 2 which are involved in many cellular processes.	[47,58]
nsp3	1945 aa (residues A819-G2763)	It is the largest protein in the coronavirus genome. It is a large transmembrane protein, a papain-like protease (PLpro) responsible for the processing of viral polyproteins encoded from the genomic RNA to individual protein components. It was also shown to antagonize host innate immunity.	[47,59,60]
nsp4	500 aa (residues K2764 – Q3263)	A multipass membrane protein responsible for membrane rearrangement. It interacts with nsp3 and 6 to form double membrane vesicles (DMV). This interaction is crucial for viral replication complex formation.	[47,59,61]
nsp5	306 aa (residues S3264 – Q3569)	Mpro, chymotrypsin-like protease (3CLpro), the viral main protease considered the key enzyme which digests the viral polyprotein at no less than 11 conserved sites including its autolytic cleavage from polyproteins pp1a and pp1ab.	[47,59,62,63]
nsp6	290 aa (residues S3570-Q3859	A transmembrane scaffold protein that forms a complex with nsp3 and nsp4 (DMV formation)	[43,47,59]
nsp7	83 aa (residues S3860 – Q3942)	A cofactor that binds to nsp8 forming hexadecameric complex and acts as processivity clamp for RNA polymerase and primase	[47,62,64]
nsp8	198 aa (residues A3943 – Q4140)	A cofactor that binds to nsp7 forming hexadecameric complex and acts as processivity clamp for RNA polymerase and primase	[47,62,64]
nsp9	113 aa (residues N4141 – Q4253)	A ss-RNA binding protein. Putatively stabilizes nascent nucleic acid during replication or transcription protecting it from nucleases.	[47,60,62,65]
nsp 10	139 aa (residues A4254 – Q4392)	A small protein believed to act as a multifunctional cofactor. In replication it interacts with nsp14 involved in replication fidelity and also interacts with nsp16. It interacts with nsp14 and 16 stimulating exonuclease and O-methyl transferase activity.	[43,47,62]
nsp 11	11-23 aa (residues S4393 – V4405)	It is a short peptide at the end of ORF1a and represents the frameshift boundary. It is a pp1a cleavage product of an unknown function.	[43,47,62]
nsp12	932 aa (residues S4393 – Q5324)	It is the RNA-dependant RNA polymerase (RdRp) responsible for both replication and transcription of the viral	[43,47,62,66]

(*continued on next page*)

Table 1 (*continued*)

Non-structural protein nsp	Amino acid length and position	Function	References
		genome. Formation of new viral RNA mediated by RdRp is believed to be the key step in viral life cycle.	
nsp 13	601 aa (residues A5325 – Q5925)	A helicase composed of an N-terminal metal binding domain and a helicase conserved domain. It plays an important role in viral replication by having the ability to unwind duplex RNA and DNA.	[47,62,67]
nsp14	527 aa (residues A5926 – Q6452	It has 3′-5′ exoribonuclease activity which is important in proof reading during viral RNA replication and has N-7-Guanine methyl transferase activity which is involved in mRNA capping.	[47,56,62]
nsp15	346 aa (residues S6453 – Q6798)	It is a poly(U)-specific endoribonuclease enzyme which cleaves RNA at the 3′ end of uridylates. Loss of nsp15 was found to affect viral replication and virulence.	[47,56,62]
nsp16	298 aa (residues S6799 – N7096	It has 2-O methyl transferase activity which is essential for capping of viral mRNA to escape host detection.	[47,56,62]

S1 was found to be highly conserved, the external subdomain which is responsible for the interaction with the host receptor was found to share only 40% amino acid identity with other SARS-related CoVs [47]. Another study assessing the variants in S protein revealed 427 non-synonymous variants many of which are located within RBD and B-cell epitopes [53]. Sequence alignment revealed difference in five critical amino acids in RBD of S1 subunit between SARS-CoV-2 and SARS-CoV and 3D structural analysis showed that the spike protein of SARS-CoV-2 has a higher affinity to the receptor ACE2 than S protein of SARS-CoV-1 [40,68].

In November 2020, the WHO published an alert reporting a mink-associated variant strain of SARS-CoV-2 in Denmark (referred to as the cluster 5 variant) [69]. This variant strain displayed several amino acid changes in the spike protein [70]. A more recent alert was published reporting a variant strain in UK which displayed a range of 14–17 mutations, the most significant of which is an N501Y mutation in the spike protein resulting in alteration in the key residues in the RBD [71].

In a research assessing the effect of D614G spike mutation on the pathogenicity and transmissibility of SARS-CoV-2, where glycine replaces aspartic acid at position 614 of spike protein, 614G was reported to have become more prevalent than 614D though initially 614D was the more prevailing variant strain suggesting a selective advantage for 614G. The variant 614G was found to be associated with higher viral loads in younger patients. However, no increase in the severity or mortality of COVID-19 patients was observed [72].

ORF 3a: is 828 nucleotides in length and codes for 3a accessory protein [46]. It is also worth mentioning that some of the early studies on SARS-CoV-2 reported the presence of accessory protein 3b [47,48]. One study described it as a short novel putative protein with four helices [47].

ORF E: formed of 228 nucleotides in length [46]. It codes for the envelope protein which is conserved across β-coronaviruses [73]. Envelope protein is an integral membrane protein that consists of N-terminal, transmembrane and C-terminal domains [45]. Previous studies showed that E proteins oligomerize to form ion channels which is important for pathogenesis. Despite being a minor component of the virus membrane, it plays a key role in viral pathogenesis and induction of overexpression of inflammatory cytokines. It also participates in viral assembly and release of new viral particles [45,74]. It is also worth mentioning that a study by Gupta et al. reported that α-helix and loops present in the E protein of SARS-CoV-2 experience random movement. This happens under optimal conditions modulating the ion channel activity of E protein which is associated with pathogenesis [49].

Comparison between the SARS-CoV-2 E protein with homologous proteins from closely related viruses showed E protein to be highly conserved with only 3 variants found across 797 studied genomes. A distinguishing feature of SARS-CoV-2 E protein was observed which is the presence of Arg at position 69 instead of Glu, Gln, Asp in other homologous SARS-CoV E proteins followed by a deletion in position 70 instead of Gly or Cys in the other proteins. The study with this finding hypothesized these changes may have a significant effect on protein-protein interaction [73].

ORF M: consists of 669 nucleotides [46]. It codes for the membrane protein (M) which is the most abundant structural protein and constitutes the majority of the viral membrane. It is thought to play a central role in viral assembly and budding through interaction with other structural proteins, mainly E and N proteins [75]. Like the E protein, M protein consists of three domains, the N-terminal domain, trans-membrane helices and the C-domain. A study on SARS-CoV-2 E and M proteins revealed that M protein shared 98% identity with sequences from Bat and Pangolin isolates. Among 797 studied genomes, M protein displayed 7 variants. The study reported a unique feature in M protein of the current SARS-CoV-2, a serine residue at position 4 present in all the M variants that the study concluded. In homologous proteins, the corresponding position to this Ser was either a deletion or an Asp. The study assumed that since this mutation from relevant Coronaviruses lies in the N-terminus region which lies outside of the virus particle, it may play an important role in virus-host interaction [73].

ORF 6: formed of 186 nucleotides and codes for Orf6 accessory protein which is formed of 61 amino acids [46].

ORF 7a and ORF 7b: formed of 366 and 132 nucleotides, respectively [46]. Produced protein 7a is believed to play an important part in viral pathogenesis where it functions to induce apoptosis of the infected cell and arrest the cell cycle in addition to promoting the production of pro-inflammatory cytokines [45]. Amino acid identity between 7a and 7b proteins of SARS-CoV-2 revealed more sequence identity with Bat SARS-like CoV than human SARS-CoV-1 [48].

ORF8: This gene is 366 nucleotides in length [46] and codes for a 121 amino acid protein. Orf8 of SARS-CoV-2 is different from that of other related coronaviruses. It lacks a known useful aggregation motif (VLVVL) which is found in SARS-CoV-1 orf8b and was reported to trigger intracellular stress pathways and to activate inflammasomes [76]. Instead, secondary structure prediction of this novel orf8 short protein revealed it is a protein with an alpha-helix and a beta-sheet that contains six strands [47]. Multiple sequence alignment showed the protein sequence of SARS-CoV-2 Orf8 shared very low similarity with that of SARS-CoV-1 [48,68] It belongs to the group that includes the closest genome sequences of bat SARS like CoVs ZXC21 and ZC45 whereas it is distant from the orf8 of human SARS-CoV-1 [47] Several studies believe examining the biological function of this particular protein (orf8) will be critical in understanding the pathogenesis of SARS-CoV-2 [52].

ORF N: it is 1260 nucleotides in length. It codes for the N protein which is a highly phosphorylated protein, 419 amino acids in length and constitutes the only protein in the nucleocapsid [46]. It is formed of two main structural domains that are independently folded, an N-terminal domain (NTD) and a C-terminal domain (CTD). The two domains are capable of binding to RNA but each uses a different mechanism and the contribution of both domains is required for optimal RNA binding [77,78]. In the central region there is a third domain, the Ser/Arg (SR rich) linker, which acts as a third RNA binding domain [79,80].

Sequencing of N protein of SARS-CoV-2 and comparing it to homologous proteins is a crucial point in understanding structural and

mechanistic basis of this virus and identifying the critical residues for RNA binding and virus infectivity. Sequencing and phylogenetic analysis of different studies came as follows; N protein was closest to that of bats according to a study based on the first three genomes of novel SARS-CoV-2. Another study reported N protein of SARS-CoV-2 to share 94% identity with both bat SARS-like CoVs and human SARS-CoV-1 [47]. Sequencing of N protein encoding regions along genome datasets revealed high conservation indicating that SARS-CoV-2 shares identical characteristics with other CoVs. Similarity with other N proteins of SARS-CoV-1, MERS-CoV and HCoV-OC43 was found to be 89.74%, 48.59% and, 35.62%, respectively, as observed from Clustal Omega multiple sequence alignment [80]. However, crystal structure of the N protein revealed a unique nucleotide binding pocket in NTD which can be a potential drug target. The SARS-CoV-2 N-NTD crystal showed orthorhombic crystal with 4 monomers which was different from SARS-CoV-1 N-NTD [80]. Being abundantly expressed and highly immunogenic, N protein of SARS-CoV-2 represents a good target for drug design.

ORF 10: formed of 117 nucleotides and codes for a 38 amino acid short protein [46]. It was depicted that this protein has no comparative proteins in the NCBI GenBank Database [53]. A study by Tang et al. also reported that ORFs from SARS-CoV-2 were found to be conserved in other related viruses with the exception for ORF8 and ORF 10 [68]. It is therefore thought that this protein is opt be used to distinguish SARS-CoV-2 infection more rapidly than PCR based strategies [52].

It is worth mentioning that SARS-CoV-2 lacks the gene encoding hemagglutinin-esterase (HE) which is a structural protein characteristically present in a subset of beta coronaviruses and thought to be involved in cell entry mechanisms [43,47]. As for the 5′- and 3′-UTR sequences, they are similar to those of other betacoronaviruses sharing nucleotide identities of more than 83% [47].

1.2. Molecular phylogeny and divergence between SARS-CoV-2 and related coronaviruses

The importance of phylogenetic studies and multiple sequence alignments lies in their ability to identify the mutations which enabled SARS-CoV-2 to acquire its characteristic pathogenic traits and cross species. Scientific literature has examples that show how even single point mutations in virus proteins can alter their pathogenesis and biology significantly [61,73].

1.2.1. Viral evolution

Several studies showed that the genome of SARS-CoV-2 shares high nucleotide similarity with bat SARS-like CoVs more than with human SARS-CoV-1 [47,81]. All studies point to bats as the natural host of SARS-CoV-1, MERS-CoV and SARS-CoV-2. Two bat coronaviruses (BatCoV RaTG13 and BatCoV RmYN02) were found to share approximately 95% sequence identity with SARS-CoV-2 [81,82]. However, the intermediate host which caused SARS-CoV-2 to evolve and infect human has not been fully confirmed yet. Pangolins were the first -and so far the most-suggested intermediate hosts. Several studies reported that the critical functional sites of the S protein are almost identical in pangolin CoV and SARS-CoV-2 assuming that in one scenario, pangolins have provided a partial spike gene to the SARS-CoV-2 [68,83–85]. SARS-CoV-2 E and M proteins were also found to share high structural similarity to their counterparts from bat and pangolin CoVs [73]. A second scenario assumed that although the amino acid similarity between the RBD of SARS-CoV-2 and that of pangolin (97.4%) is higher than the similarity with that of bat CoVs (89.2%), SARS-CoV-2 and pangolin CoVs may have originated independently where cross-species transmission from bats took place [86]. Another opinion about pangolins role in SARS-CoV-2 is that pangolins are natural hosts for *Betacoronaviruses* but does not support that SARS-CoV-2 emerged directly from pangolin CoV [87]. As depicted by some studies, other possible intermediate hosts that may have transmitted SARS-CoV-2 to human include snakes and turtles [88]. Yet, a study by Zhang et al. refuted snakes being the intermediate host for SARS-CoV-2 [89]. Several molecular phylogeny studies still need to be conducted to understand how SARS-CoV-2 evolved and determine its intermediate host, if any.

Studies comparing SARS-CoV-2 to other human CoVs showed that SARS-CoV-2 genome shared 79–82% nucleotide identity with human SARS-CoV-1 [47,90] and was found to be less related to and distant from MERS-CoV [90]. Although at the whole genome level SARS-CoV-2 was closer to bat CoVs, the receptor binding domain of SARS-CoV-2 was reported to be closer to that of SARS-CoV-1 [90]. Studies of the amino acid substitutions in the proteins of SARS-CoV-2 are thus important to explain how these proteins differ structurally and functionally from those of SARS-CoV-1. The major distinction between SARS-CoV-2 and SARS-CoV-1 was found to be in orf 3, Spike and orf8 as mentioned earlier [40,47,52,68]. Specifically, the S1 subunit of the spike protein of SARS-CoV-2 displayed a higher binding affinity to human angiotensin converting enzyme II (ACE-2) than that of SARS-CoV-1. This was attributed to difference in 5 amino acid residues in the RBD of S1 which affected the 3D structure of the spike protein [40,52].

1.2.2. Mutations in SARS-CoV-2 genome

Phylogenetic analysis of SARS-CoV-2 genome has been paralleled by studying mutations in its genome to determine the different variants and most common mutation sites. This should provide better understanding for the continuous viral evolution which might affect its spread and pathogenicity. In addition to this, it helps in accurate design for primers and probes. A study by Koyama et al. identified 5775 genome variants among 10,022 studied genomes, 1905 of which were found in ORF1ab. Mutations in the RdRp (nsp12) were detected as well as deletion in the 3′-5'exonuclease [53]. In another study, a number of 116 mutations were found in 95 analysed genomes, the most common were found in ORF1ab, ORF8 and the N gene [52]. A study by Tang et al. identified 149 mutation sites across 103 sequences and categorized SARS-CoV-2 viruses into two major lineages, L and S lineages with L being the major lineage (~70%). This was based on the observation mentioned in the study where 101 of 103 studied SARS-CoV-2 sequences displayed complete linkage with one of two SNPs (single nucleotide polymorphism); L lineage where T28,144 is in the codon of Leucine and S lineage where C28,144 is in the codon of Serine [68]. Homology analysis also reported that although sequence variation among SARS-CoV-2 genomes was low, mutation hotspots in ORFs 1a, S, 8, and N were detected and suggests this might sometimes be the reason behind the false negative RT-PCR result which is not uncommon in SARS-CoV-2 diagnosis [46]. A genotyping analysis also showed that the genes encoding RNA polymerase, RNA primase, S and N proteins undergo frequent mutations [91]. It is therefore clear that studying mutations in SARS-CoV-2 is critical for understanding viral pathogenesis and vaccine development.

1.2.3. Virus life cycle

The inhaled SARS-CoV-2 enters the human cell through three main steps which include receptor binding, proteolysis, and activation of membrane fusion within endosomes [92]. The Receptor binding of S glycoprotein with ACE2 receptor involves conformational changes of both moieties. Proteolysis occurs by different proteases for example furin, trypsin, cathepsins, transmembrane protease serine protease-2 (TMPRSS-2), TMPRSS-4, or human airway trypsin-like protease (HAT) [93]. In case of SARS-CoV-2, TMPRSS2 and lysosomes proteases hydrolyze S protein into two parts: S1 and S2. This process is highly facilitated by the presence of multibasic amino acids at the cleavage point [94,95] The S1 part contains the receptor binding domain (RBD) which attaches to the cell receptors and S2 part is responsible for fusion with cell membrane [96]. At the boundary between S1/S2, a proprotein convertase motif was found which is known to be the hallmark of pathogenicity of avian influenza. However, Wall et al. (2020) found that this motif did not improve the entry of SARS-CoV-2 inside the cells. One

interesting finding was reported by Ou et al. who speculated SARS-CoV-2 S protein can react with the receptor by protease-independent entry which may explain the rapid progress of this disease. After attachment is completed, the virus transmembrane undergoes fusion through an endocytic pathway where viral RNA is freed into the cytoplasm [97]. The endosomal pathway guarantees the integrity of plasma membrane during passage of any extracellular substance. The interior of the endosomes is characterized by acidic pH due to the presence of a proton pump inside it [98]. Moreover, cysteine proteases, likely cathepsin B or L, enabled the pH-dependent release of the virus from the cytoplasm [95].

The ACE2 receptor is a zinc binding carboxypeptidase found in different body cells like type II pneumocytes, myocardial cells, cholangiocytes, enterocytes, and oral mucosal epithelium [99]. The interacting region in this receptor composes of fifteen residues ordered into a beta-sheet conformation surrounded by two capping loops. This region is highly conserved between SARS-CoV-1 and SARS-CoV-2 and share 76% similarity [100]. However, some modification in the amino acid residues L455, F486, Q493, and N501 improved the interaction in SARS-CoV2. Ortega et al. reported the presence of two loops around the RBD of S protein which strengthen the receptor binding due to involvement of more molecules in the binding [101]. Moreover, phenylalanine in the loop (F486) can bind to hydrophobic pocket of ACE2 and helps in receptor recognition [102]. Many studies concluded that ACE2 receptor mutation does not affect its binding to S protein or promotion of S protein syncytia formation [103,104].

The binding between the S protein and ACE2 receptor itself was puzzling and affected the infectivity of SARS-CoV2. The RBD has two positions, standing up and lying down to enable receptor binding and immune evasion, respectively. It was found that SARS-CoV-1 RBD is always in the standing position while SARS-CoV-2 is in the lying down state. Thus, SARS-CoV-2 has higher binding to ACE2 receptor and less accessibility to the immune system. This hidden RBD also contributed to the difficulty of treatment and vaccination.

The activation of ACE2 receptors lead to alteration in the renin-angiotensin-aldosterone system (RAAS) which is the reason behind the inflammatory lung disease accompanied with SARS-CoV-2 infection. Moreover, the proinflammatory mediators such as interleukin-8/cytokine-induced neutrophil chemoattractant-3 and interleukin-6 expression lead to vasoconstriction and pulmonary edema [105].

After the virus entry inside the cell, synthesis of RNA starts using two different processes: genome replication and transcription of subgenomic RNA [106]. The replication is initiated by the translation of gene 1, which encodes the replicase enzyme. This enzyme is processed by two viral proteases, a papain-like protease (PLpro) and a 3C-like protease (Mpro) into 16 non-structural proteins [107]. The cleavage of N-terminus of the replicase poly-protein is carried out by PLpro into nsp1, nsp2 and nsp3. Moreover, PLpro is believed to antagonize the host's innate immunity by interfering with type I interferon signaling pathway [108]. Mpro, on the other hand, is important in maturation of non-structural proteins. These nsps combine into the replicase–transcriptase complex (RTC) for RNA replication and transcription of the sub-genomic RNAs. Afterwards, the RNA-dependent RNA polymerase (RdRp), which is encoded by the RNA virus begins the continuous process of RNA synthesis from the RNA templates. The replication involves the formation of negative strand RNA to serve as a template for genomic RNA. It is worth mentioning that RdRp sequence is homologues in both of SARS-CoV-1 and SARS-CoV-2 [109].

Subgenomic RNAs acts as mRNAs for the structural and accessory genes which are downstream of the replicase polyproteins. The transcription process to synthesize subgenomic RNA is highly complicated among positive strand RNA viruses. It requires initiation and premature termination. Moreover, the subgenomic RNA contains at 5' end, a leader sequence which is unique in coronaviruses. This 5′ leader sequence may protect SARS-CoV-1 mRNAs from nsp1-induced endonucleolytic cleavage of capped mRNAs, which explains the effective buildup of viral mRNAs and proteins during the infection [106]. The transcription process is regulated by transcription-regulating sequences (TRSs) located at the 3′ end of the leader sequence (TRS-L) and preceding each viral gene (TRS-B). The transcription process is discontinuous because RdRp ceases the transcription at each TRS sequence. Finally, the subgenomic RNA is translated into structural (N, M, E and S), non-structural and accessory proteins (AP), assembling new virions.

After replication, transcription, and translation, the assembly of virion particles takes place by cooperation between endoplasmic reticulum and Golgi apparatus [99]. Meanwhile, the assembly of SARS-CoV-2 virus is not yet studied. Therefore, information in the following paragraphs is based on the study of SARS-CoV-1 in general. The release of the virions from coronavirus-infected cells consists of two processes, assembly of the helical nucleocapsids and of the viral envelopes.

First, the assembly of helical nucleocapsids occurs in the cytoplasm [110]. The nucleocapsid composes of N protein and RNA. Sometimes the M protein could also be found [111]. N protein is a multifunctional protein. It binds to leader RNA to maintain the RNA conformation suitable for replication and transcription. It binds to viral RNA genome and packs it into a helical nucleocapsid structure referred to as ribonucleoprotein (RNP) complex [79]. The packaging of RNA into virus like particles (VLP) was found to be nucleocapsid dependant [112]. It is also involved in host pathogen interactions and viral pathogenicity; it blocks the S phase in mammalian cells [113], in addition to being highly immunogenic as its CTD acts as an interferon antagonist [79,80]. As depicted from studies on N protein of related coronaviruses, NTD binds to the transcriptional regulatory sequence (TRS) and melts TRS-cTRS RNA duplex complex, CTD is responsible for oligomerization which is crucial for viral ribonucleoprotein packaging and SR rich linker is responsible for primary phosphorylation [80]. N protein also binds to nsp3 and M proteins. These interactions are key for the formation of replicase-transcriptase complex (RTC) and packaging of the genome into VLP [78,112].

Second the viral envelope assembly occurs in the intracellular membranes. Normally, enveloped viruses obtain their membranes through budding of the viral nucleocapsid through cellular membranes. SARS-CoVs assemble at the intracellular membranes in the Endoplasmic reticulum-Golgi intermediate compartment (or ERGIC) after infection. From there, they bud within the lumen and are consequently carried outside the cell through 'exocytosis' within the cargo vesicles [49]. The viral membrane proteins play a clear role in formation of the viral envelope. As studied in SARS-CoV-1, M and E proteins are required for virion assembly and release but E protein is found in trace amounts. Thus, the assembly depends mainly on interactions among M proteins [110]. In addition, M protein provides the matrix for nucleocapsid attachment and thus budding takes place. The other proteins such as S and E, are integrated into the lattice through interactions with M, whereas N and viral RNA interact with M C-terminal domain, which is exposed to the cytosol [114]. Other studies showed that protein E is very important in pathogenesis of SARS-CoV-1 and proved that the absence of E protein lead to virus attenuation [49,115].

2. Treatment and drug discovery

2.1. *In silico and in vitro drug targeting for COVID-19*

Finding a treatment for COVID-19 has become the focal point of research in the past few months. Several previously known drugs have been tested for their activity against SARS-CoV-2. In this review, we will try to summarize the potential drug candidates for SARS-CoV-2 treatment that were deduced from *in silico* and *in vitro* studies.

There are three main strategies for developing an anti-SARS-CoV-2 drug, the first is to test already existing antivirals and repurposing of therapeutic drugs that were reported to have antiviral activity, the second depends on using *in silico* molecular docking to screen molecular

databases for compounds with a therapeutic effect on SARS-CoV-2. The third strategy depends on using the genomic information of the virus to develop novel targeted drugs from scratch which might take very long time.

Based on the first strategy, different antiviral drugs were tested. According to some studies, it was found that the common antiviral drug Ribavirin showed no effect against SARS-CoV-2 while the new antiviral drug Remdesivir effectively inhibited viral replication [116]. It is worth mentioning that an *in silico* docking study to analyse the binding sites of potential protease (PLpro) inhibitors showed multiple active site residues in the protease structure for Remdesivir while for the other tested ligands (nelfinavir, ritonavir, lopinavir and aketoamide), there was only one active site residue [117]. Testing different combinations of drugs (Lopinavir, Oseltamivir and Ritonavir) using molecular docking and dynamics were also investigated [118]. Another *in silico* study explored the molecular mechanism of binding between Mpro and its suggested inhibitors. The study referred to particular regions of the Mpro pocket as the "anchor" site and reported a newly discovered mechanism in which the binding of the ligand improved when part of it occupied this anchor site inside Mpro pocket. The study also suggested nelfinavir as a potent drug against SARS-CoV-2 [119].

As an application to drug repurposing strategy, chloroquine and Hydroxychloroquine were tested as they were previously reported to effectively inhibit SARS-CoV-1 viral replication *in vitro*, Hydroxychloroquine being more effective [120]. Combining hydroxychloroquine and azithromycin showed synergistic effect on SARS-CoV-2 *in vitro* [121]. Determining the therapeutic efficiency and cardiovascular side effects of hydroxychloroquine has been a focal point in clinical studies recently [122].

According to the second strategy, molecular databases were screened for compounds with a therapeutic effect on SARS-CoV-2. Homology modelling was used to build all possible SARS-CoV-2 protein structures for screening of potential inhibitor molecules from marketed drugs. Important protein targets in SARS-CoV-2 include PLpro, Mpro, RdRp, helicase in addition to structural proteins. A series of antivirus drugs, antibacterials, muscle relaxants, anti-tussive drugs and a spectrum of natural products were reported to bind with high affinity to PLpro, the papain-like protease of SARS-CoV-2. The well-fitting of the anti-asthmatic montelukast drug into the active pocket of Mpro, the chymotrypsin-like protease of SARS-CoV-2, was also reported. Potential inhibitors of RNA-dependant RNA polymerase (RdRp), helicase and structural proteins like S protein were also depicted in this study [108].

Zhou et al. used network proximity analyses of different drug targets and SARS–host interactions in the human interactome where they reported three possible combinations (e.g., sirolimus plus dactinomycin, mercaptopurine plus melatonin, and toremifene plus emodin) to be efficient against SARS-CoV-2 [123].

In silico docking studies using medicinal plant libraries have also been conducted for the identification of potential anti-viral phytochemicals. One study screened 32,297 compounds and suggested anti-SARS-CoV-2 molecules that need further investigations [124]. Three phytochemicals were also found to reduce the random movement of SARS-CoV-2 E protein thus inhibiting its function [49]. Another study showed that luteolin, the main flavonoid in a traditional Chinese medicinal formula, binds strongly to the active sites of the main protease (PLpro) of SARS-CoV-2 indicating a possible antiviral activity [116]. A study carried out by Abdelli et al. tested the binding affinity for ACE2 receptor by several natural products. This study showed that Isothymol showed the highest binding score and the most stable interaction with ACE2 receptor [125]. Joshi et al. studied about 318 phytochemicals from 11 plants and then screened their activity against main protease (Mpro) and ACE2 [126]. Moreover, they reported that the typical pharmacophore consists of hydrophobic, aromatic, negative, and positive functional groups in addition to hydrogen bond acceptor and hydrogen bond donor groups.

The third strategy depends on using homology modelling of viral proteins to discover possible ligand binding sites and predict binding mechanisms. This is crucial for designing new drugs. An example to this is the study by Shankar et al. which suggested two ligands that can possibly be developed into antivirals against SARS-CoV-2, the first (an aza-peptide epoxide) can be used to irreversibly inhibit viral protease and the second as a viral replication inhibitor [60]. Another study used computer-aided drug design to identify a mechanism-based inhibitor of SARS-CoV-2 Mpro and screened more than 10,000 compounds for their ability to inhibit this protease [63]. A study on the crystal structure of nucleocapsid protein of SARS-CoV-2 also revealed unique drug targeting sites that can be used to design new antiviral agents [80]. An *in silico* study by Liu et al. also identified potential compounds possibly targeting Mpro, the main protease of SARS-CoV-2 [127]. In their study, Han and Kral designed inhibitors from the protease domain (PD) of ACE2 and by using the Molecular dynamics simulations, the α-helical peptides were the most stable and highly specific to SARS-CoV-2 [128].

2.2. On-going clinical trials

A lot of clinical trials have proliferated since the beginning of COVID-19 pandemic to slow its spread. All the on-going clinical trials are registered either in ClinicalTrials.gov or the International Clinical Trials Registry Platform (ICTRP) of the WHO. Most of registered clinical trials are grouped either for treatment or prevention of COVID-19. A lot of antivirals drugs previously used for Influenza, Ebola, HIV or Malaria were repurposed for COVID-19 disease. For example, the combination of Lopinavir and Ritonavir was tested in 34 investigational clinical trials. They are both protease inhibitor used originally in treatment of HIV infection [129]. However, Cao et al. mentioned that this combination showed no benefit beyond the standard care. The standard care includes providing supplemental oxygen, antibiotic agents, noninvasive and invasive ventilation, vasopressor support, and extracorporeal membrane oxygenation (ECMO) [130]. Moreover, some drugs were directed against the ACE2 receptor such as Losartan, an angiotensin receptor antagonist [131]. Randomized double-blinded placebo-controlled phase I clinical trials are testing safety of Losartan especially in COVID-19 respiratory failure patients [132]. Other clinical trials targeted the entry of SARS-CoV-2 like phase 2 clinical trial on Camostat mesilate (CM), an inhibitor of TMPRSS2 [133]. In addition, amiodarone, calcium channel blocker, was used against Ebola virus to block its entry by blocking the ion channels [134]. A phase 3 clinical trial now is directed towards using Verapamil and Amiodarone against COVID-19 [135]. Furthermore, the use of monoclonal antibodies against COVID-19 was investigated in different studies. The most important study was on using Tocilizumab, an anti-IL6 drug which reached Phase 3 [129]. Moreover, some monoclonal antibodies were developed against the S protein as a part of the ACTIV-3 master protocol sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) [136]. These antibodies, VIR-7831 [137], BRII-196 and BRII-198 [138] are used for hospitalized patients and reached Phase 3 clinical trials.

As of June 25, the RECOVERY trial (Randomized Evaluation of COVID-19 Therapy) which is a multicenter, open-label trial sponsored by the National Health Service in the United Kingdom announced that dexamethasone with standard of care therapy decreased the mortality rate in confirmed COVID-19 cases by one third. Results of this clinical trial are still not published in a peer review journal but its results were announced in Nature news and NIH site (https://www.recoverytrial.net/news/low-cost-dexamethasone-reduces-death-by-up-to-one-third-in-hospitalised-patients-with-severe-respiratory-complications-of-covid-19 and https://www.covid19treatmentguidelines.nih.gov/dexamethasone/).

Some clinical trials depended on previous computational testing of drugs. For example, Famotidine effectiveness was predicted from *in silico* screening of several FDA licensed compound against the SARS-CoV 2 protease Plpro catalytic site and it showed a high computationally score and favorable pharmacokinetic and safety profile. Now, Famotidine is

tested in a multisite adaptive trial phase 3 for COVID-19 in hospitals [139]. Moreover, Umifenovir is a Russian antiviral drug whose activity was investigated *in Silico* by Huynh et al. [119] against Mpro and it is in now in phase 4 clinical trial [140]. Being highly effective against SARS-CoV-2 protease Mpro [141], Remedesivir was used in a randomized, double-blinded, placebo-controlled trial in China for COVID-19. Interestingly, it was reported that Remdesivir helped the patients to have a clinical improvement in shorter time than those receiving placebo [142]. However, in November 2020, WHO conditionally recommended against the use of Remedesivir in hospitalized patients due to lack of evidence on better outcomes for its usage in comparison to placebo [143]. Other drugs that are being clinically used include Chloroquine and hydroxychloroquine which are believed to impair virus replication at early stages as well as interference with post-translational modifications of the viral proteins. Several clinical studies have so far been conducted to determine the therapeutic and prophylactic activity as well as the safety of hydroxychloroquine [122,144]. A study that used molecular modelling suggested that chloroquine binds with high affinity to sialic acid and gangliosides inhibiting the binding of N-terminal domain of the S protein of SARS-CoV-2 to gangliosides [116].

3. Prevention: COVID-19 vaccine development

The immediate production of a potent vaccine is obviously mandatory to reduce not only the tragic numbers of infections and fatalities caused by the COVID-19 pandemic, but also its disastrous impact on world economies. Even though vaccine development is lagging behind the pandemic spread, it is still essential and critical, since the infection is still growing all over the world. Moreover, COVID-19 infection might be staying for a long time, and become a seasonal disease, just like influenza [145]. Pharmaceutical companies and academic institutions all around the world are working on evolving a SARS-CoV-2 vaccine [146]. It is well believed now that our world will not come back to normal up until successful vaccines become accessible and a global vaccination program is efficaciously applied.

3.1. Why is it taking so long?

It usually takes years to develop vaccines for human use, particularly if new technologies, untested for safety or not produced on a large scale, are used. Typically, the development of new vaccines takes around 10–20 years, with a success of lower than 10%, even for a vaccine that reaches clinical evaluation [146].

Chinese researchers swiftly identified SARS-CoV-2 and quickly revealed its genomic sequence [15,147]. The virus was isolated from naso and oropharyngeal swabs, and its sequence, replication properties, and cell culture tropism was determined. SARS-CoV-2 grew in 2 Vero cell types: VeroE6 and Vero CCL81, the viral titers being a little higher in the former type [148]. The MERS-CoV and SARS-CoV-1 vaccines developed during the past years provided scientists with crucial knowledge to help develop SARS-CoV-2 vaccines, owing to their genetic resemblance with SARS-CoV- 2. The challenge is still great though, since all CoV vaccine candidates proved unsuccessful in primary clinical evaluation, with none being forwarded to licensing [149].

We know from these previous studies that the S protein, which binds with the ACE2 host receptor, is an excellent target for a vaccine. Antibodies can neutralize the virus by targeting the spike and interfering with this binding. Scientists determined SARS-CoV-2 S protein structure by cryo–electron microscopy in record time [40]. Hence, we already have a perfect antigen to be used for advanced vaccine platforms. Basically, two essential steps are required before putting a vaccine into clinical trials.

To begin with, to confirm that a vaccine is protective, it should undergo preclinical evaluation using suitable animal models. Only a few animal models are presently available for the novel SARS-CoV −2 [146]. Since wild-type mice do not support the growth of the virus, studies using the human angiotensin-converting enzyme 2 (hACE2) transgenic mice infected with SARS-CoV-2 have been carried out to study its pathogenicity. However, the virus only brought about moderate disease in these transgenic mice [150]. In addition, of the non-human primates, rhesus macaques were reported to be more susceptible to SARS-CoV-2 than cynomolgus macaques and marmosets. Recent studies also reported that SARS-CoV-2 may cause moderate to serious pulmonary infections in ferrets and hamsters [151].

Next, we need to confirm the safety of these developed SARS-CoV-2 vaccines. Safety is the most critical aspect to consider during vaccine development, and it is important that we ought not to fast track COVID-19 vaccines without satisfactory secure guarantees. Some SARS-CoV-1 vaccines that were previously evaluated in animal models resulted in complications. For example, earlier reports have implicated that full-length S protein can induce serious liver damage and enhanced infection, known as antibody-dependent enhancement (ADE), due to S protein specific antibodies [152]. ADE results when non-neutralizing antibodies against virus proteins enhance virus infectivity through augmenting viral entry into host cells [153]. ADE has been reported for different coronaviruses. This phenomenon occurs due to a reduction in the binding strength of neutralizing antibodies to the virus, which switches these antibodies into suboptimal—non neutralizing ones. This decrease in affinity may be mainly due to a change in the conformation of the viral S-protein. Nevertheless, other factors contributing to antigen drift and antigenic determinant changes may also have a role [154]. In addition, an inactivated whole virus vaccine in non-human primates and a VLP vaccine in mice were found to protect from infection but showed an immunopathologic-type lung disease in challenged animals [155]. Immunization with vaccines expressing SARS-CoV-1 S protein was reported to cause enhanced hepatitis in ferrets as well [156].

After that, to ensure constant quality, human vaccines should be manufactured using procedures that adhere to current Good Manufacturing Practice (cGMP), which demands committed facilities and qualified personnel. Such processes are not available yet for most vaccine candidates and hence need to be generated from scratch.

As soon as reliable preclinical data and preliminary cGMP quality vaccine batches are available, clinical trials may start. These begin with phase I trials to assess the vaccine safety in a small number of individuals. Phase 2 trials are next, where preparation and doses are determined to verify vaccine effectiveness, and lastly phase 3 trials, where vaccine effectiveness and safety are required to be validated in a larger group of people. However, in emergencies like this pandemic, this pathway may be pressed, and a quick plan might be developed to gain a license by regulatory agencies [157]. However, this should be carried out carefully to avoid catastrophic outcomes.

Another important aspect is that we will need to build large scale production capacities to manufacture satisfactory quantities of cGMP-quality vaccines. Current infrastructure may be utilized for conventional vaccines, however, for vaccines using new technologies, e.g., mRNA, it will be challenging and will obviously take some time [157].

3.2. Categories of SARS-CoV-2 Vaccines under development

So far, 10 separate technology platforms are being researched to generate a successful SARS-CoV-2 vaccine, however, 4 main types are currently in Phase 3 clinical evaluation (Table 2). These technologies are implementing novel approaches for concentrating on the COVID-19 infection mechanisms, accelerating development and addressing COVID-19 mechanisms that target precise population categories, like the old-aged, expecting women, and immunocompromised individuals [158,159].

According to the WHO, as of January 3, 2021, 172 SARS-CoV-2 vaccine candidates are in pre-clinical development while 60 candidates are in clinical evaluation: 11 in Phase 3, 4 in Phase 2/3, 3 in Phase 2, 22 in Phase 1/2, and 20 in Phase 1 trials [159].

The Food and Drug Administration (FDA) issued an Emergency Use

Table 2
Candidate COVID-19 Vaccines in Phase 3 clinical evaluation according to the WHO as of January 3, 2021 [159].

ID	Vaccine platform acronymn	Vaccine platform description	Type of candidate vaccine	Route of administration	Developers	Phase
1	IV	Inactivated virus	SARS-CoV-2 vaccine (inactivated)	IM	Sinovac Research and Development Co., Ltd	Phase 3
2	IV	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	IM	Sinopharm + Wuhan Institute of Biological Products	Phase 3
3	IV	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	IM	Sinopharm + Beijing Institute of Biological Products	Phase 3
4	VVnr	Viral vector (Non-replicating)	ChAdOx1-S - (AZD1222) (Covishield)	IM	AstraZeneca + University of Oxford	Phase 3
5	VVnr	Viral vector (Non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector)	IM	CanSino Biological Inc./Beijing Institute of Biotechnology	Phase 3
6	VVnr	Viral vector (Non-replicating)	Gam-COVID-Vac Adeno-based (rAd26-S + rAd5-S)	IM	Gamaleya Research Institute; Health Ministry of the Russian Federation	Phase 3
7	VVnr	Viral vector (Non-replicating)	Ad26.COV2·S	IM	Janssen Pharmaceutical	Phase 3
8	PS	Protein subunit	SARS-CoV-2 rS/Matrix M1-Adjuvant (Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M)	IM	Novavax	Phase 3
9	RNA	RNA based vaccine	mRNA -1273	IM	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	Phase 3
10	RNA	RNA based vaccine	BNT162 (3 LNP-mRNAs)	IM	BioNTech + Fosun Pharma; Jiangsu Provincial Center for Disease Prevention and Control + Pfizer	Phase 2/3
11	PS	Protein subunit	Recombinant SARS-CoV-2 vaccine (CHO Cell)	IM	Anhui Zhifei Longcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences	Phase 3
12	RNA	RNA based vaccine	CVnCoV Vaccine	IM	CureVac AG	Phase 2/3
13	DNA	DNA based vaccine	INO-4800+electroporation	ID	Inovio Pharmaceuticals + International Vaccine Institute	Phase 2/3
14	IV	Inactivated virus	Whole-Virion Inactivated SARS-CoV-2 Vaccine (BBV152)	IM	Bharat Biotech International Limited	Phase 3
15	VLP	Virus like particle	Coronavirus-Like Particle COVID-19 (CoVLP)	IM	Medicago Inc.	Phase 2/3

Authorization (EUA) for the Pfizer-BioNTech COVID-19 (BNT162b2) vaccine and the Moderna COVID-19 (mRNA-1273) vaccine on 11 and December 18, 2020, respectively [160].

On the other hand, in July 2020, China hurled an emergency use program for essential workers and high risk individuals and has administered more than 4.5 million doses of Sinopharm's two vaccines and Sinovac's CoronaVac. Later, on December 31, 2020, chinese authorities conditionally approved the general public use of Sinopharm vaccine, right after the firm announced its leading vaccine had a 79% efficacy rate in phase 3 trials. Brazil, Indonesia, Turkey, Chile and Singapore signed up for Sinovac's CoronaVac. The United Arab Emirates announced that the Sinopharm vaccine was 86% effective in a phase 3 trials, and was the first other country to approve it to the public, followed by other countries like Pakistan and Egypt. https://www.bbc.com/news/world-asia-china-55498197.

One of Sinopharm and Sinovac's key advantages is that it can be kept in a standard refrigerator at 2–8 °C, whereas Moderna's vaccine needs to be stored at −20 °C and Pfizer's vaccine at −70 °C. Hence, both Sinovac and Sinopharm vaccines are far more suitable for developing countries which can not store huge vaccine amounts at these low temperatures. https://www.bbc.com/news/world-asia-china-55212787.

Herein, we will summarize recent development efforts, mainly focusing on those that reached Phase 3 clinical evaluation (Table 2). All of the COVID-19 vaccines that are presently in Phase 3 trials require 2 doses, except one candidate, Recombinant novel coronavirus vaccine (Adenovirus type 5 vector) produced by CanSino Biological Inc./Beijing Institute of Biotechnology, which requires only one dose [159].

3.2.1. *Inactivated and live attenuated vaccines*

Killed vaccines are conventional vaccines with established preparatory technology, which explains why many of the vaccine candidates that already entered clinical evaluation are from this group. These vaccines use cultured SARS-CoV-2 inactivated by physical methods (eg. UV light) or chemical methods (eg. formaldehyde) [161]. However, to be effective, these vaccines usually necessitate the presence of an adjuvant and repeated administration. In addition, using the adjuvant alum renders them inappropriate for mucosal delivery and they are poor inducers of cytotoxic $CD8^+$ T cells, which are probably needed for an effective COVID-19 vaccine [162].

CoronaVac is an alum-adjuvant candidate vaccine inactivated using formalin and produced by the Chinese firm Sinovac Biotech. Preclinical studies show incomplete or full protection in macaques exposed to SARS-CoV-2 challenge without detectable ADE [163]. Sinovac's phase 2 trials also showed that the vaccine formed neutralizing antibodies without severe adverse reactions [164]. In July 2020, phase 3 clinical trials were started to measure the efficacy and safety of Sinovac's vaccine in Brazil, Indonesia and Turkey [159,165].

Sinopharm (Chinese company) has also carried out Phase 1/2 trials on two inactivated vaccine viruses [166]. The vaccine is prepared by inoculating Vero cells with SARS CoV 2 strain, culturing, harvesting, inactivating, clarifying, concentrating, purifying and adding aluminum hydroxide adjuvant. In July, Phase 3 Trial to Assess the Safety and Protective Efficacy of these vaccines in healthy population was started [167,168]. Recently, the firm announced that its leading vaccine had a 79% efficacy rate in phase 3 trials. https://www.bbc.com/news/world-asia-china-55498197.

Moreover, some companies are developing live viral vaccines that use cultured SARS-CoV-2 attenuated by passaging. Although strong B and T cell responses may be induced by single administration with no adjuvants, reversion to pathogenicity and disease-causing potential limits these attenuated vaccines [169]. Codagenix, Inc. together with the Serum Institute of India, Ltd. are developing a live-attenuated intranasal vaccine against SARS-CoV-2 named COVI-VAC. However, this candidate is still in phase 1 trials to assess its safety and immune response in healthy adults [159].

3.2.2. Subunit vaccines

Subunit vaccines consist of single or numerous strongly immunogenic antigens able to effectively stimulate the immune system. Although this kind of vaccine is harmless and simpler to produce, it demands combining adjuvants to evoke a powerful protective immune response [146]. They also require repeated administration, are usually not suitable for respiratory mucosal vaccination and are poor activators of $CD8^+$ T cell responses [162]. Antigens such as E, M, N and S structural proteins are being investigated for subunit vaccine development [159].

Several institutions have started developing SARS-CoV-2 subunit vaccine, mainly using the S protein as antigens. For example, Novavax produced a stable nanoparticle vaccine in March 2020. In May, it started a 2-part Phase 1/2 trial of SARS-CoV-2 Recombinant Spike Protein nanoparticle vaccine with or without Matrix-M adjuvant in healthy individuals [170]. A phase 3 trial was started in November 2020 to evaluate the effectiveness, immune response, and safety of SARS-CoV-2 rS vaccine with Matrix-M1 adjuvant in the United States, Mexico and UK [171].

Virus-like particles (VLPs) are a type of subunit vaccine based on several virus-derived proteins that are collected to imitate the organization of true native viruses but with no viral genome, which makes them safer than attenuated or inactivated preparations [162]. These viral proteins are in charge of cell penetration, and therefore efficient cell entry is gained, making them a suitable option when producing a vaccine. VLP vaccines combine sound safety profiles with robust immunogenicity and so are safe alternatives to inactivated viruses. VLPs have outstanding adjuvant properties and are able to stimulate both innate and adaptive immune responses. Experience with formerly developed VLP vaccines showed that they can activate efficient immune responses. These properties make VLPs a superior platform for making vaccines. VLPs are designed using various expression systems, like cells from bacteria, yeast, plants or insects [172]. In contrast to subunit vaccines, the array of S protein on the VLP surface crosslinks the B cell receptor and unswervingly stimulates B cells, but, similar to subunit and inactivated vaccines, they necessitate an adjuvant and frequent administration [162].

Coronavirus-Like Particle COVID-19 (CoVLP) is a VLP vaccine produced by Medicago, a Canadian company, from genetically engineered plants. Its unpublished results suggest efficacy in inducing neutralizing antibodies in mice [173]. It entered phase 3 trials in November to compare the efficacy and safety of the CoVLP formulation to placebo [174].

3.2.3. mRNA Vaccines

In this type of vaccine, a synthetic mRNA is inserted into the human body, which acts as instructions for the cells to synthesize that virus' proteins. Our immune system spots these viral proteins and stimulates an immune response against the virus [19].

mRNA vaccines are a propitious substitute to traditional vaccines due to their short and low-cost manufacturing cycles, high potency and safety. While no mRNA vaccine has been previously approved for human use, their potential is reinforced by earlier studies of influenza, rabies and Zika virus infections in animals [162,175]. However, mRNA alone is not very stable to be used as a prophylactic vaccine and are not easily taken up by the cells. Hence it needs lipid nano particle (LNP) carriers which are very effective to stabilize and pack the mRNA into an injectable form [176]. They also require cool temperatures to be stored [19].

An LNP-encapsulated mRNA-based vaccine, mRNA-1273, which encodes the SARS-CoV-2 S protein was the first to start phase I clinical trials in the United States, conducted by Moderna and the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID) to evaluate its safety and immunogenicity [177]. mRNA-1273 was developed built on earlier studies on SARS-CoV-1 and MERS-CoV. MERS-CoV. Recently published phase 1 results showed that small doses of two parenteral injections are safe and stimulate a primarily $CD4^+$ T cell response and strong specific antibody responses in tested participants [162,178]. Moderna started phase 2 trials for its vaccine candidate in May 2020, and in July, a Phase 3 clinical study was started to determine the efficacy, safety, and immunogenicity of mRNA-1273 to prevent infection for up to 2 years after the second dose of mRNA-1273 [179]. Clinical trials showed that the Moderna vaccine was 94.1% effective in averting COVID-19 infection in healthy candidates https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/Moderna.html.

In addition, the German company BioNTech together with Pfizer and the Chinese drug maker Fosun Pharma also developed an mRNA vaccine, BNT162b2, which is a lipid nanoparticle-formulated vaccine encoding a SARS-CoV-2 S protein. BNT162b2 proved to be 95% effective against Covid-19 and serious adverse events were minimal [180].

3.2.4. DNA Vaccines

These are plasmid DNA molecules encoding antigens. DNA vaccine are simple, stable, safe, can be easily and rapidly produced and have been proven to activate both T and B cell responses [175]. Moreover, they are superior in delivery efficiency when compared to mRNA vaccines and are heat stable, but they require entry into the nucleus which introduces the threat of vector integration and mutations in the genome of the host [181].

Early DNA vaccines had low immunogenicity in humans. However, many strategies have been tried to solve this problem. One such strategy was the use of adaptive electroporation. Transfection efficiency may be improved by up to one thousand fold by membrane electrochemical permeabilization and electric field created by applied voltages which can drastically increase uptake of DNA plasmids into the cells. Further developments have been described in the latest intradermal electroporation (ID-EP) DNA delivery technology. When compared to intramuscular electroporation (IM-EP) DNA delivery, ID-EP was found to be well-tolerated and dose-sparing, particularly in the induction of antibody responses [182].

INO-4800, produced by Inovio Pharmaceuticals, is a synthetic DNA vaccine expressing the viral S protein *in vitro*. After intradermal injection (and electroporation (EP) by CELLECTRA® 2000 device) of mice and guinea pigs, specific T cell responses and efficient antibodies which counteract the Covid-19 infection were observed. Moreover, blockage of S protein binding to its receptor and biodistribution of SARS-CoV-2 targeting antibodies to the lungs was reported [183]. In June, Phase 1/2 trial was started to assess the vaccine on healthy adult volunteers and in November, Phase 2/3 trial to assess the safety, immunogenicity and efficacy of INO-4800 injected intradermally followed by electroporation in high risk participants was initiated [184].

3.2.5. Live vector vaccines

Live viruses may be genetically engineered to serve as vectors and hold pathogenic genes, coding for foreign proteins that evoke immune responses [185]. Viral vectors are auspicious tools for gene therapy and vaccines. Live vector vaccines are distinguished by possessing the safety of subunit vaccines and the powerful efficacy of live weakened vaccines [146]. They have many advantages including: (a) highly specific delivery of genes to target cells (b) highly efficient gene transduction and (c) stimulation of strong immune responses, and enhanced cellular immunity. Unlike subunit vaccines, which induce a humoral response,

recombinant viral vectors allow expression of intracellular antigens and encourage a strong cytotoxic T lymphocyte response [186].

The use of viral vectors necessitates a high biological safety level to obtain public approval. Consequently, poorly pathogenic viruses are frequently chosen. Viruses are genetically engineered in most cases to decrease or abolish pathogenicity. Furthermore, most of these vectors are replication-defective [186].

Though viral vector platform has been extensively studied and has a well-established track record for infectious diseases, only a few of these vaccines have been accepted for human use. Some viral vectors require one dose for protection and have natural tropism for the respiratory mucosa, making suitable for mucosal vaccination. The expertise for their large-scale clinical grade manufacture and storage already exists, accordingly, they are the second most common platform for COVID-19 vaccine development [162].

Delivery systems based on Adenoviruses have many advantages including their simple administration by oral or nasal route and their lack of pathogenicity in humans, especially for non-replicating mutants [175]. The Oxford Vaccine Group (which formerly developed a MERS vaccine), and AstraZeneca designed a chimpanzee adenovirus vaccine vector called AZD1222 (formerly ChAdOx1), encoding the S protein. The vaccine proved to be protective in mice, inducing a sturdy humoral and cell-mediated response. It demonstrated a considerably lowered viral load in rhesus macaques after a single vaccination dose, with no evidence of ADE after challenge [187].

The AZD1222 vaccine was tested in around 1000 healthy volunteers in April 2020. On 22 May, Oxford researchers declared that they had initiated recruitment for a Phase 2/3 trial of nearly 10,000 healthy adult volunteers to evaluate if individuals of diverse ages could be protected from COVID-19 efficiently [188]. In the beginning of June, AstraZeneca announced it had initiated mass production of its potential vaccine and aimed to reach 2 billion doses by September (https://www.astrazeneca.com/media-centre/press-releases/2020/astrazeneca-to-supply-europe-with-up-to-400-million-doses-of-oxford-universitys-vaccine-at-no-profit.html). A phase 3 study was started in August [159]. However, On 8 September, AstraZeneca stopped clinical trials because of an adverse reaction in one of the tested persons. After a thorough study, the trials recommenced in the U.K., Brazil, South Africa, and India but are still paused in the U.S. as of 23 September [19].

In addition, the Chinese company CanSino Biologics together with the Institute of Biotechnology, Academy of Military Medical Sciences is designing a recombinant adenovirus type-5 (Ad5) vectored COVID-19 vaccine expressing the viral S protein. In March, the safety, tolerability, and efficacy of the vaccine was tested in Phase I trials and proved to be tolerable and protective. Humoral responses culminated at day 28 after vaccination, while quick specific T-cell responses were reported from day 14 post-vaccination in healthy individuals [189]. It must be mentioned that these are the first results from Phase I trials of any SARS-CoV-2 vaccine to appear in a scientific journal. Phase 2 clinical trials for the vaccine started last April [159]. Results indicated the candidate has a sound safety profile, with no serious adverse events. Single-dose vaccination stimulated quick immune responses within 14 days and substantial humoral and cellular immune responses within 28 days in most cases [190]. A global phase 3 trial was initiated in August to assess the efficacy, safety and immunogenicity of the vaccine in individuals. The Chinese government has permitted the vaccine only for military use https://clinicaltrials.gov/ct2/show/NCT04526990?term=vaccine&cond=covid-19&draw=6.

The Chinese government has approved the vaccine for military use only [19].

As shown in the table, most of the current COVID-19 vaccine are administered parenterally. However, a respiratory mucosal vaccine strategy able to stimulate strong responses straight in the respiratory mucosa will be most efficient in the primary control of the virus. This route is also superior in being needle-free and demanding a much lower dose than the parenteral route. Yet, the need for inhalational devices may be a restrictive factor for extensive application. DelNS1-2019-nCoV-RBD-OPT1 (Intranasal flu-based-RBD) is an intranasal vaccine candidate based on Influenza viral vectors and produced by Jiangsu Provincial Center for Disease Prevention and Control. In November 2020, a phase 2 trial to assess the immunogenicity and safety of this vaccine was started [191].

It is interesting to note that some SARSCoV-2 vaccine candidates based on viral vectors are being developed to be administered by the oral route. For example, VXA-CoV2-1 Ad5 adjuvanted Oral Vaccine is an oral pill produced by Vaxart (US) and is based on different SARS-CoV-2 antigens [192]. However, it is still in Phase 1 trials [159].

3.2.6. *Nonspecific vaccines*

Vaccines already protective against other infections may also be used against COVID-19. The Murdoch Children's Research Institute in Australia is running a Phase 3 trial on the BCG vaccine (developed in the early 1900s against tuberculosis), and numerous other tests are in progress to check if this vaccine confers any protection against SARS-CoV-2 [193]. Recently, BCG vaccine has been suggested to prevent SARS-CoV-2 infection, due to its potential to lift up innate immunity and preliminary epidemiological studies which detected decreased COVID-19 severity in nations with universal BCG vaccination guidelines. However, these studies were not systematically corrected for confounding variables. In October 2020, Hansel et al. reported that, after correction for confounding variables, their epidemiological results do not provide evidence to link overall BCG vaccination policy with the spread and mortality of covid-19 [194].

4. Concluding remarks

The COVID-19 outbreak, due to its rapid spread, has affected our daily lives in every way. When we will fully mend from these effects and whether normal life will ever return is still unknown. During the past months, a lot has been learned about this disease and its causative virus. Therefore, here, we reviewed the updated advances about diverse aspects including clinical features, source of infection, transmission dynamics, genomic characterization and uncovering of novel approaches for disease control. Yet, there are still many knowledge gaps around many aspects including the differences in susceptibility, progression, and outcome of the infection by SARS-CoV-2 which, undoubtedly relies on a multifaceted interaction of human body, the virus and environmental influences. Also, there is no definite and highly effective treatment yet. All these topics need to be further researched quickly to reach successful outcomes and happy endings.

Coronaviruses are genetically dissimilar and have a great predisposition to recurrent genetic mutations. These mutations have clearly been central for the virus evolutionary history, and unfortunately, may well result in other analogous epidemic outbreaks in the coming years. Accordingly, in addition to restricting this epidemic, we should work hard to develop complete measures to avert upcoming outbursts of zoonotic basis.

Considering the strong influence of this pandemic on economies worldwide, funding a vaccine producing framework that would permit a quick reaction to upcoming infections is indeed a huge investment. This will generate satisfactory and ready vaccine candidates that can be used swiftly and effectively to stop an emerging infection immediately. Smart emergency strategies that help us to check, manufacture and spread vaccines within months is also urgently needed. Hopefully, many lessons will be gained from this pandemic which will render us ready in the future.

Declaration of competing interest

The authors declare no conflict of interest.

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