



The novel coronavirus 2019-nCoV: Its evolution and transmission into humans causing global COVID-19 pandemic

Y. R. Rastogi¹ · A. Sharma¹ · R. Nagraik¹ · A. Aygün² · F. Şen²

Received: 28 April 2020 / Revised: 8 May 2020 / Accepted: 18 May 2020
© Islamic Azad University (IAU) 2020

Abstract

A novel coronavirus strain 2019-nCoV has caused a rapid global pandemic-COVID-19. Scientists have taken onto the task of characterizing this new virus and understanding how this virus has transmitted to humans. All preliminary studies have found some striking similarities between this new virus and the SARS-CoV that caused a similar kind of epidemic in 2002–2003. Through bioinformatics tools, a great deal of information has been gathered about the origin, evolution and zoonosis of this virus. We, in this review, report the symptoms, mode of transmission and available and putative treatments to tackle 2019-nCoV infections. We also comprehensively summarize all the information so far made available regarding the genome, evolution and zoonosis of this virus.

Keywords 2019-nCoV · COVID-19 · Homologous recombination · orf1a/b · Phylogenetic analysis · Receptor binding domain (RBD) · RdRp enzyme · Spike glycoprotein

Introduction

Since December 21, 2019, China has been under the grip of a severe epidemic with cases even being reported at a rapid rate from neighboring countries like Japan, Thailand, South Korea, Nepal and India as well as far flung countries like the USA, the UK, Italy, Germany and France (<https://www.who.int/docs>). The cause of this epidemic is a novel strain of coronavirus earlier temporarily designated by World Health Organization (WHO) as 2019-novel coronavirus (2019-nCoV)¹, and the infection due to 2019-nCoV has been designated as COVID-19 (CO—corona, VI—virus, D—disease and 19 stands for 2019).

The previous related outbreaks like the 2002–03 global severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak that originated in the Guangdong province in China

or the Middle East Respiratory Syndrome coronavirus (MERS CoV) outbreak that originated in Saudi Arabia had a mortality rate of 9.6% and 37% respectively (Drosten et al. 2003; Fouchier et al. 2003; Zhong et al. 2003; Zaki et al. 2012; de Wit et al. 2016). The 2019-nCoV is the seventh coronavirus known to infect humans. Of these, four coronavirus strains HCoV-229E, HCoV-OC43, HCoV-NL6 infect infants, the upper respiratory tract of the immunocompetent and lower respiratory tract of immunocompromised individuals causing mild cold and flu-like symptoms. In contrast, the highly pathogenic SARS CoV, MERS-CoV and 2019-nCoV infect the lower respiratory tract (bronchial epithelial cells) of immunocompetent individuals causing chronic respiratory ailments.

Almost all the index patients of the 2019-nCoV outbreak were either sellers or visitors at the Huanan seafood market in Wuhan where besides aquatic animals even mammals and snakes were sold. It is speculated that the zoonosis of the virus happened from an intermediate host animal being sold at the market. However, no bats were being sold. It is similar to how Himalayan civets

Communicated by M. Abbaspour.

✉ F. Şen
fatihsen1980@gmail.com

¹ Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Himachal Pradesh 173229, India

² Sen Research Group, Biochemistry Department, Faculty of Arts and Science, Dumlupınar University, Evliya Çelebi Campus, 43100 Kütahya, Turkey

¹ The International committee on Virus Taxonomy has officially named the novel coronavirus as Severe Respiratory Coronavirus-2 (SARS-CoV-2) (Gorbalenya et al. 2020). However, many scientists consider the suggested name inappropriate as it causes confusions regarding the disease it causes and leads to a misconception that the new virus is another strain of the previous SARS-CoV (Jiang et al. 2020a). We, therefore, as of now, refer to the novel coronavirus as 2019-nCoV for the purpose of this article.



(*Paguma larvata*) acted as the intermediate host for the zoonosis of SARS CoV in 2002–03 although horseshoe bats (belonging to genus *Rhinolophus*) were primary hosts coronaviruses are positive-sense single-stranded RNA viruses enclosed in a solar corona shaped envelope (hence the name). They belong to the sub-family *Coronavirinae* of the family *Coronaviridae* of the order *Neovirales*. They are further divided into four genera—*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. *Alphacoronavirus* and *Betacoronavirus* infect only mammals, whereas the *Gammacoronavirus* and *Deltacoronavirus* mostly infect birds. The 2019-nCoV along with the SARS and MERS CoV belongs to the genus *Betacoronavirus*. Data from the phylogenetic analysis show that 2019-nCoV is distantly related to SARS CoV (with less than 79% sequence identity at genome level) or MERS CoV (less than 50% sequence identity). This proves that the 2019-nCoV is novel and distinct from the previously reported pathogenic coronaviruses. The 2019-nCoV is further classified under the sub-genera Sarbecoviruses along with two closely related (about 88% identity) bat-derived SARS-related coronaviruses (SARSr-CoV) designated as SL-CoVZC45 and SL-CoVZXC21. This throws light on the fact that 2019-nCoV like all previously reported pathogenic coronaviruses (SARS and MERS CoV) has its ancestry to coronaviruses with bats as the primary host. However, the ancestry of 2019-nCoV is not directly linked to the two bat-derived coronaviruses and homologous recombination events in an intermediate host have played a key role in the evolution of this novel strain.

Infection symptoms, host immunological response and transmission

Symptoms exhibited by COVID-19 patients include pneumonia (which includes fever, dyspnoea, malaise, dry cough and respiratory distress), leucopenia and lymphopenia (a low lymphocyte count below a threshold has been suggested as a method for initial clinical diagnosis) (Hui et al. 2020; Chen et al. 2020a; Huang et al. 2020). Less common symptoms include headache, diarrhea, sputum production and hemoptysis (Zhang et al. 2020). Moreover, chest radiographs exhibit multifocal ground glass lung opacities. The incubation period of the virus is relatively long of about 5–14 days (Bouadma et al. 2020; Jiang et al. 2020b). Many patients that go asymptomatic during incubation period become major cause of the spread of this virus.

In a study on nine index patients (out of which eight had visited the Huanan seafood market in Wuhan), there was a sequence similarity of greater than 99.9% among the isolated and cultured 2019-nCoV virus particles. Thus, all the patients were infected from the same animal source in a short span of time. All the patients had almost simultaneously developed the symptoms quite quickly.

Most of the severely ill patients were reported to be in their old age, had some previously underlying conditions like diabetes or cardiovascular or cerebrovascular diseases or were co-infected with infectious bacteria or fungi like *A. baumannii* and *C. albicans*. The number of males infected with 2019-nCoV is higher than the number of females. This trend was observed even at the time of the SARS-CoV outbreak. A reason behind this trend can be that X chromosome and sex hormones positively regulate innate and adaptive immunity enhancing anti-viral immunity in females (Channappanavar et al. 2017). Moreover, the 2019-nCoV like the previous SARS and MERS CoV infections elicits strong cytokine responses in the host causing increased pulmonary inflammation and lung damage. Elevated levels of IgA and IgM in the lungs of an infected patient had been detected. The viral infection leads to the production of IL1B, IFN γ , IP10 and MCP1 which further lead to T-Helper 1 cell production (pro-inflammatory). However, a striking observation is that unlike the previous SARS CoV the 2019-nCoV elicited the production of T-Helper 2 cell-related cytokines like IL-10 and IL-4 as well (which are associated with anti-inflammatory signature). This can be one of the reasons for relatively less severe symptoms in many patients.

The ninth patient from the above-mentioned study had, however, never visited the Huanan seafood market but stayed in a hotel near the market. It is speculated that this patient must have been infected either through an unknown source or through droplet transmission. Moreover, spread of infection in family clusters from these patients establishes the possibility of human to human transmission of infection (Chan et al. 2020b). All the early patients diagnosed with novel coronavirus infection outside China like in Japan, Thailand or India had visited Wuhan just days before the outbreak (Zhao et al. 2020). Given the cases where health workers or other patients and visitors to hospitals across Wuhan have contracted the infection, airborne droplets due to coughing, touching and using personal hygiene items (like towels and cosmetics) of the infected and nosocomial transmission seem the most probable modes of further spread of infection. However, further investigations are required to understand the exact mode of transmission of this virus among humans.

Isolation and characterization of the novel coronavirus

All the earliest patients of the outbreak in Wuhan were diagnosed with pneumonia from an unknown cause. Bronchoalveolar lavage fluid (BALF) was collected from patients for the isolation of nucleic acid samples which was tested for 22 pathogens (18 viruses and 4 bacteria) using PCR. All the tests resulted in negative.

BALF along with throat swab from one patient was then cultured on human airway epithelial cells (HAL) (Zhu et al. 2020). Viral particles from HAL showing cytopathic effects



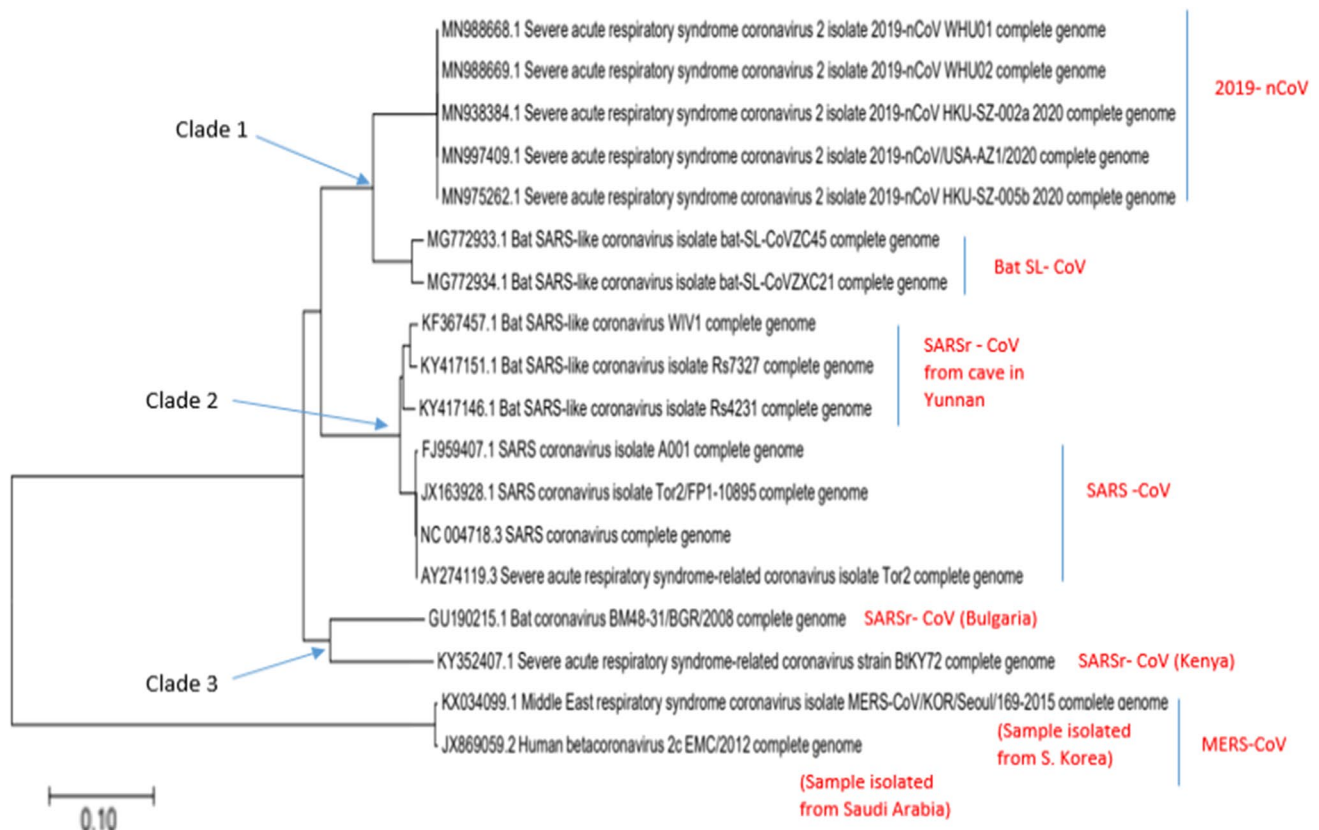


Fig. 1 Phylogenetic analyses of full-length genomes of 2019-nCoV and other closely related reference genomes of the genus *Betacoronavirus*

were collected for RNA isolation. The isolated RNA samples were then cloned as cDNA and sequenced.

A BLASTn of the sequenced cDNAs (query sequences) was performed for which no matching sequence was found in the GenBank database. However, the query sequences had high similarity with the *Betacoronavirus* sequences pertaining to lineage B (which include SARS CoV and SARSr-CoV). The highly similar sequences were then used as references for analysis and gene annotation of the query sequences. Further electron microscopic analysis of the viral particles in the inclusion bodies in the cytoplasm of the cytopathic HAL revealed spiked envelope with a solar corona-like shape, confirming that the viral particles belonged to the family Coronaviridae. Phylogenetic analysis of the novel query sequences along with other *Betacoronavirus* genome sequences revealed that the closest relatives of the 2019-nCoV are bat-derived SL-CoVZC45 and SL-CoVZXC21 (forming one clade), whereas the SARS-CoV is distantly related forming a separate clade (Fig. 1). However, the 2019-nCoV formed a distinct monophyletic cluster within the clade with a long branch separating away the two bat-derived SARSr-CoV (Fig. 2).

The RNA-dependent RNA polymerase (RdRp) gene in the ORF1a/b region of the *Betacoronavirus* genomes is highly conserved across the genus (Lu et al. 2020). The RdRp gene thus formed the basis for real-time-PCR (RT-PCR)-based

laboratory diagnosis of the 2019-nCoV infection in early index patients. The cycle threshold or ct values of the patient-derived samples ranged from 22.85 to 32.41. These low ct values may be indicative of a high viral nucleic acid abundance in patient samples due to a very high rate of virus replication resulting in enhanced severity of the infection. The S gene in the 2019-nCoV genome that codes for the spike glycoprotein has also formed the basis for RT-PCR-based diagnosis of infection. The spike protein on the outer surface of coronaviruses is responsible for host cell receptor binding and invasion.

Genomic structure and replication cycle of the 2019-nCoV

Gene annotation studies have successfully deciphered the genome of the novel coronavirus in great detail (Chan et al. 2020a). The single-stranded RNA of the 2019-nCoV consists of 29,881 nucleotides coding for 9860 amino acids. The either ends of the RNA genome are flanked by 5' and 3' untranslated region (UTR) which is similar to that of other *Betacoronavirus*. Starting from the 5' end, the genome consists of ORF1 a/b that codes for 16 non-structural proteins (nsp) including RdRp followed by other ORFs coding for





Fig. 2 A simplified phylogenetic analysis of spike protein of 2019-nCoV with different reference genomes from the genus *Betacoronavirus*

accessory and structural protein genes—spike (S), envelope (E), membrane (M) and nucleocapsid (N). There is no major difference between the functions of various nsp of 2019-nCoV and SARS-CoV.

Coronaviruses produce a set of nested sub-genomic (SG) mRNAs of varying sizes which are then translated to produce various structural and accessory proteins (Sztuba-Solińska et al. 2011). These SG mRNAs are synthesized by a ‘discontinuous template synthesis’ method in which a 5′ leader sequence is joined to an ORF(s) via the transcription regulatory sequence (TRS). TRS is a conserved 5–7 nucleotide sequences upstream of ORFs in coronavirus genomes. Bioinformatics studies have revealed that in 2019-nCoV around 12 ORFs produce a set of 9 nested SG mRNAs. Inside a host cell when the virus makes its copies, the *orf1a/b* is directly translated to produce two polypeptides pp1a and pp1ab which are cleaved to form 16 nsp including RdRp. These nsp together form a replication transcription complex (RTC). RTC then replicates the virus genome and produces various structural and accessory protein SG mRNAs inside a double membrane vesicle.

Receptor binding and animal to human transmission of the 2019-nCoV

The 2002–03 SARS-CoV binds with the angiotensin-converting enzyme 2 (ACE 2) receptor present on bronchial epithelial cells and type II pneumocytes in the lower respiratory tract via the receptor binding domain (RBD) of the spike protein.

The spike protein is divided into two functional subunits—S1 domain and S2 domain. The S1 domain is responsible for receptor binding, whereas the S2 domain is responsible for fusion of virus with the host cell membrane. The SARS-CoV forms a clover-shaped trimer with three S1 heads and a trimeric S2 stalk (Cui et al. 2019). The S1 domain is further sub-divided into an amino terminal domain (S1-NTD) and a cysteine-rich carboxy terminal domain (S1-CTD). The S1-CTD at the tip of S1 domain acts as the RDB. The RBD is further sub-divided into a core region and a receptor binding motif (RBM). The compatibility of spike protein with the host cell receptor is largely dependent on the amino acid residues in the RBM. Any changes in the RBM or even core region amino acid residues can decrease or increase the affinity of spike–receptor binding. Changes in amino acid residues in RBM can even alter the virus’ tropism (Song et al. 2005).

Residues 442, 472, 479, 480 and 487 in the SARS-CoV RBM contribute to host receptor specificity and in determining host range (Wu et al. 2012). The residues at each of these positions form a salt bridge with specific ACE-2 receptor residues. Asparagine or arginine at residue 479 of RBM increases binding affinity with human and civet (intermediate host of SARS-CoV) ACE-2, whereas lysine at the same position decreases this binding affinity. Serine at residue 487 is required for RBM binding with civet ACE-2, but it fails to bind efficiently with human ACE-2. On the other hand, mutations in SARS-CoV leading to threonine at residue 487 lead to very efficient RBM and human ACE-2 binding. The early phase SARS-CoV strain-hcGd03 (Ser at residue 487) that infected humans from civets had low infectivity and failed to transmit between humans. However,



middle- and late-phase SARS-CoV strains especially hTor02 had a high infectivity and rapidly transmitted between humans as it had Thr at residue 487. Therefore, it can be inferred that RBD of spike protein in *Betacoronavirus* is the cornerstone of infectivity and animal to human transmissions.

The overall phylogenetic analysis of spike protein of 2019-nCoV with various reference genomes of *Betacoronavirus* is more or less similar to the full-length genome phylogenetic analysis. In fact, the S2 domain of 2019-nCoV has a high degree of similarity with its two bat-derived ancestors—SL-CoVZC45 and SL-CoVZXC21, but interestingly, on the other hand, the S1 domain shares very little similarity. Rather the S1 domain of 2019-nCoV is very similar to that of SARS-CoV with around 50 conserved amino acid residues despite the fact the two falls in different clades. Consistent with this, the homology modeling of RBD of 2019-nCoV with SARS-CoV RBD as template revealed that the RBM of the 2019-nCoV is very similar in structure to that of SARS-CoV. These results led to the conclusion that 2019-nCoV may also bind to the human ACE-2 receptor. Another study using HeLa cells expressing ACE-2 also reached the same conclusion that 2019-nCoV may bind with the ACE-2 receptors (Zhou et al. 2020). However, many residues that are crucial for binding of SARS-CoV RBD with ACE-2 vary in 2019-nCoV. The significance of these variations on ACE-2 binding needs to be further investigated.

The rate of 2019-nCoV spread and resultant deaths have dramatically risen with time in Wuhan despite proper quarantine measures in place. As more strains from the later phases (after the onset) of the 2019-nCoV outbreak are isolated and sequenced, mutations in the S gene leading to the accelerated transmission and enhanced infectivity will be elucidated. These further investigations will also throw light on the recombination events that led to animal–human transmission of this virus.

Recombination events leading to zoonotic 2019-nCoV from bat-derived SL-CoVZC45 and SL-CoVZXC21 must have taken place inside bats or in some unknown intermediate host being sold at Huanan seafood market. A study based on comparative relative synonymous codon usage bias (RSCU) analysis claimed that two species of snakes—*Bungarus multicinctus* and *Naja atra*, acted as intermediate host for 2019-nCoV in which the recombination events happened about 2 years ago (Ji et al. 2020). The principal behind RSCU analysis is that viruses tend to evolve codon usage bias similar to that of their hosts. The study compared the synonymous codon usage bias in 2019-nCoV with that of all possible intermediate hosts—birds, marmots, hedgehog, bats, snakes and humans, and the results were expressed as squared Euclidean distance. The two species of snakes mentioned above had the least squared Euclidean distance value compared to other animals. The RSCU results were further supported by claims that the two species of snakes are found in the central Hubei province and were even being sold at Huanan sea food market. The study has, however, been discredited by a large number of scientists on

the grounds that it is highly unlikely that any coronavirus strain would ever infect a reptile which are so distant from mammals.

According to recent reports (Cyranoski 2020), researchers at the South China Agricultural University in Guangzhou have claimed that the endangered pangolins are most likely the intermediate host of 2019-nCoV before it spread to humans. This claim is based on the fact that genome sequences of viruses isolated from pangolins are 99% similar to the genome sequence of 2019-nCoV isolated from patients and that coronavirus use receptors with similar molecular structure in pangolins as in humans for infection. Further supporting this claim is a study that reported coronavirus infection is one of the causes of deaths in pangolins (Liu et al. 2019). This claim has not been published so far and requires further investigations to precisely ascertain how this zoonosis from pangolins to humans took place.

Molecular evolution of 2019-nCoV from bat-derived ancestors

SARS-CoV originated from SARSr-CoV strains like WIV1 and RsSHC014 isolated from bats in a cave in Yunnan province in China. However, neither of these were direct ancestors of early phase SARS-CoV strains. Recombination events in both bats and civets especially in the S, ORF3b and ORF8 regions of the genome led to the evolution of SARS-CoV strains capable of infecting humans (He et al. 2004). The importance of mutations in S gene in this regard has already been discussed. Experimental analysis of protein coded by ORF3b region of SARS-CoV shows that the protein is required for increased replication rate of the virus inside host cells leading to cell necrosis (Yount et al. 2005; Khan et al. 2006). The protein also acts as interferon antagonist by blocking IFN- β synthesis and signaling (Kopecky-Bromberg et al. 2007). Likewise, ORF8-coded protein activates inflammasomes like NLRP3 inside host cells (Shi et al. 2019).

Similarly, the two bat-derived SARSr-CoV (SL-CoVZC45 and SL-CoVZXC21) isolated from Zhoushan and Nanjing in China are not direct ancestors of the 2019-nCoV despite the fact there is high identity at genome sequence level. Recombination events in bats and an unknown intermediate host especially in the ORF1b region and S gene have led to the evolution of current virulent 2019-nCoV strains (Wu et al. 2020).

The ORF1b, ORF3b and ORF8 regions of the 2019-nCoV have a very low amino acid sequence identity when compared to that of either SARS-CoV or SL-CoVZC45 and SL-CoVZXC21. On the other hand, the 2019-nCoV S1-CTD crucial for determining virus tropism shares not only a high degree of conserved amino acid residues but also a very similar structure of RBM with SARS-CoV instead of SL-CoVZC45 and SL-CoVZXC21. This observation leads to the inference that positive selection pressures and simultaneous evolutionary mechanisms that caused the viral transmission to humans are very similar



in both 2019-nCoV and SARS-CoV. A broader understanding of the molecular events causing mutations favorable for these viruses to switch to human hosts is necessary to prevent future outbreaks and in designing better preventive measures.

Coronaviruses have an average evolutionary rate of approximately 10^{-4} nucleotide substitution per site per year (Homwong et al. 2016). There are three major molecular mechanisms by which coronaviruses evolve—(i) polymerase error rate, (ii) homologous recombination and (iii) viral persistence (Graham and Baric 2010).

Polymerase error rate

Coronaviruses have an unusually large RNA genome (26–30 kb) which makes them prone to errors during replication. The average error rate of RdRp is 10^{-3} – 10^{-5} mutations per nucleotide per replication cycle. However, a set of RNA proofreading and editing enzymes like Exonuclease N synthesized by coronaviruses enhance the RdRp fidelity by around tenfold. It has been observed that during ecological stresses and positive selection pressures to switch between hosts the RdRp fidelity decreases presumably due to decrease in Exonuclease N activity. This in turn makes the coronavirus prone to higher rates of mutations and may lead to altered tropism. Studies have in fact shown when the selection pressures are over and the virus has successfully switched to new host, RdRp fidelity is enhanced again.

Homologous recombination

It is considered to be the most probable mechanism by which coronaviruses evolve. Homologous recombination between coronavirus genomes may take place during co-infection inside a host cell. Coronaviruses take advantage of TRS to carry on homologous recombination using a model similar to copy-choice recombination model. The RdRp while attached to the nascent nucleic acid molecule may switch to an acceptor template from a donor template where the acceptor and donor templates are from different closely related coronaviruses. The 2019-nCoV genome may have undergone a similar kind of homologous recombination with a closely related donor coronavirus during co-infection in an intermediate host.

Viral persistence

The ability of viruses to establish and sustain infections in host cells is known as viral persistence. In case of down-regulation of the expression of host receptor through which the coronavirus binds, the virus may evolve to either be able to bind to a different receptor or may bind to the original receptor with even greater affinity. As mentioned above, the SARS-CoV strains isolated from middle- and late-phase patients of the outbreak were more virulent and had a higher affinity for ACE-2 receptors compared to that of early phase. It is speculated that this happened due to accumulation of

mutations in the S gene of middle- and late-phase strains resulting in greater affinity of spike protein for ACE-2. The steep rise in number of cases as well as deaths in a short span of time due to 2019-nCoV infection can be attributed to viral persistence inside host cell and during human–human transmission.

Medical interventions to combat 2019-nCoV

A very limited dosage of methylprednisolone (1–2 mg/kg/day) has been suggested to alleviate the symptoms like acute respiratory distress and lung inflammation (Huang et al. 2020). Although corticosteroids were found to reduce inflammation in lungs due to SARS-CoV studies had also reported delay in viral clearance due to corticosteroids. Recombinant IFN along with Ribavirin is also being administered to 2019-CoV patients, but the treatment is not very effective (Chen et al. 2020b).

Hydroxychloroquine (an anti-malarial drug) administration has emerged as a popular and effective strategy to treat COVID-19 patients worldwide. The drug alleviates inflammation and resultant respiratory distress by neutralizing the pro-inflammatory cytokine (such as IL-6) and macrophage response (Brufsky 2020). The drug may also act as anti-viral by inhibiting virus replication inside host cells and by decreasing ACE2 affinity for spike glycoprotein (Sinha and Balayla 2020).

Combined lopinavir and ritonavir is an effective regime in controlling HIV infection. The administration of this combination was even found effective for alleviation of clinical symptoms due to SARS-CoV infection (Huang et al. 2020). This combination has also been successfully tried on 2019-nCoV patients admitted in hospitals and can be used as treatment till more effective drugs are found. Transfer of plasma and antibodies from convalescent patients to severely affected patients is also being proposed.

The S2 domain is highly conserved between 2019-nCoV, its two bat-derived ancestors and SARS-CoV. Development of anti-viral peptides targeting the S2 domain has been also been proposed to combat a wide range of SARS-related infections (Du et al. 2009). A study has found that dimerization of nsp9 and its subsequent binding with nucleic acid is essential for nucleic acid replication in coronaviruses (Zeng et al. 2018). The dimerization of nsp9 is brought about by N-finger motif in nsp9. Drugs targeting nsp9 dimerization can also be an effective treatment in curbing 2019-nCoV infection.

As of now, most of the patients have to rely on their immune system to fight 2019-nCoV infection as no vaccine or effective drug is available. It may take several months if not years before one such vaccine is even developed and commercialized. Possible vaccine may include attenuated



2019-nCoV strains, substructures like membrane or envelope proteins, etc.

A recent study has found that coronaviruses can persist on commonly touched inanimate objects like door knobs for about 9 days and for up to a month under low temperatures (about 4–5 °C). Steps as simple as regular disinfection procedures using 70% ethanol or 0.1% sodium hypochlorite are highly effective in eradicating coronaviruses (Kampf et al. 2020). Therefore, use of these simple disinfection procedures in houses, hospitals and various public places will significantly reduce 2019-nCoV spread.

Conclusion

Bats are known to be the reservoirs of many deadly viruses like the Nipa virus and Ebola beside coronaviruses. A reason for this is the inability of bats' innate immunity to sense viral DNAs in the cytosol. Bats have a very high rate of metabolism and increased rates of oxidative phosphorylation cause self-DNA damage leading to its escape from the nucleus and mitochondria into the cytosol. Bats have with time evolved to ignore this self-DNA in the cytosol by dampening of STING mechanism of DNA sensing and downregulating the production of appropriate IFN response. This in turn leads to even viral DNA in bat cytosol to go undetected leading them to host a wide range of viruses (Xie et al. 2018).

But bats are not solely to be blamed for spread of viral infections to humans. Rather breach of animal–human boundaries due to unchecked urbanization and expansion of agriculture are responsible for zoonosis of viruses from bats and other animals to humans. We need to find solutions to stop this encroachment on habitats of other animals and develop a more vigilant and holistic outlook towards environment. We also need to become more conscious of the source, biology and habitat of the animals that become part of our diet.

Many scientists had predicted the possibility of re-emergence of a 2002–03 SARS-CoV like coronavirus outbreak in future. We still fell short of the requisite preparedness to combat the current outbreak. We need to learn many lessons from this current novel coronavirus outbreak and other epidemics in the past from different geographical regions under the 'One Human–Animal–Environment' approach. If an effective treatment to eradicate novel coronavirus infections is not found quickly the pandemic may turn into a recurring endemic in certain regions which will be even harder to tackle. Biomedical engineers, scientists, chemists and doctors need to come together to find innovative solution. A lot is known about coronaviruses, but still a lot needs to be known about their evolution, transmission and functions of different regions of their genome. Research focusing on development of broad range drugs

and vaccines against coronaviruses based on conserved protein structures needs attention to stay ready for similar outbreaks in future. Newer technologies like artificial intelligence for prediction of disease epidemiology and development of more sensitive and affordable biosensors must also be given attention in this context.

In this time of grave crisis, all the countries need to stand in solidarity with each other. We must also value the sacrifices and unflinching dedication of all the health-workers throughout the world in combating this pandemic.

Acknowledgements The authors are highly grateful to Shoolini University of Biotechnology and Management Sciences, Solan, H.P., India, for their support in helping us accomplish this mini-review. We would also like to thanks Dr. Prasenjit Dhar, CSK HPKV Palampur, Department of Veterinary Microbiology, Palampur, H.P., India, for guiding us in writing this review. We would also like to express our acknowledgement to Department of Science and Technology (DST), Government of India and Indian Council of Medical Research (ICMR), New Delhi, for funding various microbial diagnostic studies through biosensors in our laboratory.

Author contributions Y.R.R., A.S., R.N., A.A. and F.S. participated in the study design and coordination and prepared the manuscript. All authors have read and approved the last article.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Bouadma L, Lescure FX, Lucet JC et al (2020) Severe SARS-CoV-2 infections: practical considerations and management strategy for intensivists. *Intensive Care Med* 46:579–582. <https://doi.org/10.1007/s00134-020-05967-x>
- Brufsky A (2020) Hyperglycemia, hydroxychloroquine, and the COVID-19 epidemic. *J Med Virol*. <https://doi.org/10.1002/jmv.25887>
- Chan JFW, Kok KH, Zhu Z et al (2020a) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect* 9:221–236. <https://doi.org/10.1080/22221751.2020.1719902>
- Chan JFW, Yuan S, Kok KH et al (2020b) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395:514–523. [https://doi.org/10.1016/S0140-6736\(20\)30154-9](https://doi.org/10.1016/S0140-6736(20)30154-9)
- Channappanavar R, Fett C, Mack M et al (2017) Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J Immunol* 198:4046–4053. <https://doi.org/10.4049/jimmunol.1601896>
- Chen N, Zhou M, Dong X et al (2020a) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395:507–513. [https://doi.org/10.1016/S0140-6736\(20\)30211-7](https://doi.org/10.1016/S0140-6736(20)30211-7)
- Chen Y, Liu Q, Guo D (2020b) Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* 92:418–423. <https://doi.org/10.1002/jmv.25681>



- Cui J, Li F, Shi ZL (2019) Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 17:181–192
- Cyranoski D (2020) Did pangolins spread the China coronavirus to people? *Nature*. <https://doi.org/10.1038/d41586-020-00364-2>
- de Wit E, van Doremalen N, Falzarano D, Munster VJ (2016) SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 14:523–534. <https://doi.org/10.1038/nrmicro.2016.81>
- Drosten C, Günther S, Preiser W et al (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 348:1967–1976. <https://doi.org/10.1056/NEJMoa030747>
- Du L, He Y, Zhou Y et al (2009) The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat Rev Microbiol* 7:226–236
- Fouchier RAM, Kuiken T, Schutten M et al (2003) Koch's postulates fulfilled for SARS virus. *Nature* 423:240–240. <https://doi.org/10.1038/423240a>
- Gorbalenya AE, Baker SC, Baric RS, et al (2020) Severe acute respiratory syndrome-related coronavirus: The species and its viruses—a statement of the Coronavirus Study Group. <https://doi.org/10.1101/2020.02.07.937862>
- Graham RL, Baric RS (2010) Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *J Virol* 84:3134–3146. <https://doi.org/10.1128/jvi.01394-09>
- He JF, Peng GW, Min J et al (2004) Molecular evolution of the sars coronavirus, during the course of the sars epidemic in China. *Science* 303:1666–1669. <https://doi.org/10.1126/science.1092002>
- Homwong N, Jarvis MC, Lam HC et al (2016) Characterization and evolution of porcine deltacoronavirus in the United States. *Prev Vet Med* 123:168–174. <https://doi.org/10.1016/j.prevetmed.2015.11.001>
- Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395:497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)
- Hui DS, Azhar EI, Madani TA et al (2020) The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—the latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis* 91:264–26
- Ji W, Wang W, Zhao X et al (2020) Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol* 92:433–440. <https://doi.org/10.1002/jmv.25682>
- Jiang S, Shi Z, Shu Y et al (2020a) A distinct name is needed for the new coronavirus. *Lancet* 395:949
- Jiang X, Rayner S, Luo MH (2020b) Does SARS-CoV-2 has a longer incubation period than SARS and MERS? *J Med Virol* 92:476–478
- Kampf G, Todt D, Pfaender S, Steinmann E (2020) Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect* 104:246–251
- Khan S, Fielding BC, Tan THP et al (2006) Over-expression of severe acute respiratory syndrome coronavirus 3b protein induces both apoptosis and necrosis in Vero E6 cells. *Virus Res* 122:20–27. <https://doi.org/10.1016/j.virusres.2006.06.005>
- Kopecky-Bromberg SA, Martinez-Sobrido L, Frieman M et al (2007) Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J Virol* 81:548–557. <https://doi.org/10.1128/jvi.01782-06>
- Liu P, Chen W, Chen J-P (2019) Viral metagenomics revealed sendai virus and coronavirus infection of malayan pangolins (*Manis javanica*). *Viruses* 11:979. <https://doi.org/10.3390/v11110979>
- Lu R, Zhao X, Li J et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395:565–574. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8)
- Shi CS, Nabar NR, Huang NN, Kehrl JH (2019) SARS-coronavirus open reading frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discov* 5:1–12. <https://doi.org/10.1038/s41420-019-0181-7>
- Sinha N, Balayla G (2020) Hydroxychloroquine and covid-19. *Postgrad Med J*. <https://doi.org/10.1136/postgradmedj-2020-137785>
- Song HD, Tu CC, Zhang GW et al (2005) Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proc Natl Acad Sci USA* 102:2430–2435. <https://doi.org/10.1073/pnas.0409608102>
- Sztuba-Solińska J, Stollar V, Bujarski JJ (2011) Subgenomic messenger RNAs: mastering regulation of (+)-strand RNA virus life cycle. *Virology* 412:245–255
- Wu A, Peng Y, Huang B et al (2020) Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe* 27:325–328. <https://doi.org/10.1016/j.chom.2020.02.001>
- Wu K, Peng G, Wilken M et al (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *J Biol Chem* 287:8904–8911. <https://doi.org/10.1074/jbc.M111.325803>
- Xie J, Li Y, Shen X et al (2018) Dampened STING-dependent interferon activation in bats. *Cell Host Microbe* 23:297–301. <https://doi.org/10.1016/j.chom.2018.01.006>
- Yount B, Roberts RS, Sims AC et al (2005) Severe acute respiratory syndrome coronavirus group-specific open reading frames encode nonessential functions for replication in cell cultures and mice. *J Virol* 79:14909–14922. <https://doi.org/10.1128/jvi.79.23.14909-14922.2005>
- Zaki AM, Van Boheemen S, Bestebroer TM et al (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 367:1814–1820. <https://doi.org/10.1056/NEJMoa1211721>
- Zeng Z, Deng F, Shi K et al (2018) Dimerization of coronavirus nsp9 with diverse modes enhances its nucleic acid binding affinity. *J Virol*. <https://doi.org/10.1128/jvi.00692-18>
- Zhang J-J, Dong X, Cao Y et al (2020) Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy Eur J Allergy Clin Immunol*. <https://doi.org/10.1111/all.14238>
- Zhao S, Zhuang Z, Ran J et al (2020) The association between domestic train transportation and novel coronavirus (2019-nCoV) outbreak in China from 2019 to 2020: a data-driven correlational report. *Travel Med Infect Dis*. 33:101568
- Zhong NS, Zheng BJ, Li YM et al (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362:1353–1358. [https://doi.org/10.1016/S0140-6736\(03\)14630-2](https://doi.org/10.1016/S0140-6736(03)14630-2)
- Zhou P, Yang X-L, Wang X-G et al (2020) (2020) Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *Nature*. <https://doi.org/10.1101/2020.01.22.914952>
- Zhu N, Zhang D, Wang W et al (2020) A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 382:727–733. <https://doi.org/10.1056/NEJMoa2001017>

