Natural selection versus creation: a literature review on the origin of SARS-CoV-2

Debmalya Barh[1], Bruno Silva Andrade[2], Sandeep Tiwari[3], Marta Giovanetti[3,4], Aristóteles Góes-Neto[5], Luiz Carlos Junior Alcantara[3,4], Vasco Azevedo[3], Preetam Ghosh[6]

[1]Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakuri, Purba Medinipur, WB, India;
[2]Laboratório de Bioinformática e Química Computacional, Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia (UESB), Jequié, Bahia, Brazil;
[3]Laboratório de Genética Celular e Molecular, Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;
[4]Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil;
[5]Laboratório de Biologia Molecular e Computacional de Fungos, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil;
[6]Department of Computer Science, Virginia Commonwealth University, Richmond, VA, 23284, USA

SUMMARY

SARS-CoV-2 has created a global disaster by infecting millions of people and causing thousands of deaths across hundreds of countries. Currently, the infection is in its exponential phase in several countries and there is no sign of immediate relief from this deadly virus. At the same time, some "conspiracy theories" have arisen on the origin of this virus due to the lack of a "definite origin". To understand if this controversy is also reflected in scientific publications, here, we reviewed the key articles published at initial stages of the COVID-19 pandemic (January 01, 2020 to April 30, 2020) related to the zoonotic origin of SARS-CoV-2 and the articles opposing the "conspiracy theories". We also provide an overview on the current knowledge on SARS-CoV-2 Spike as well as the Coronavirus research domain. Furthermore, a few important points related to the "conspiracy theories" such as "laboratory engineering" or "bioweapon" aspects of SARS-CoV-2 are also reviewed. In this article, we have only considered the peer-reviewed publications that are indexed in PubMed and other official publications, and we have directly quoted the authors' statements from their respective articles to avoid any controversy.

Keywords: SARS-CoV-2 origin, convergent evolution, recombination, conspiracy theories, synthetic virus, COVID-19.

INTRODUCTION

The novel coronavirus of 2019 (SARS-CoV-2) so far has infected 3090445 persons and claimed more than 217769 deaths worldwide (as of April 30, 2020, WHO COVID-19 situation report - 101). The first case of infection was identified between December 7, 2019 to December 12, 2019 from patients linked to a local Huanan seafood wholesale market (wet market) in Wuhan, Hubei province, China; the "person-to-person transmission" characteristic of SARS-CoV-2 was reported by Chan et al. on January 24, 2020, and WHO declared it as the COVID-19 pandemic on March 11, 2020 by the time the virus has already infected 118,319 people with 4,292 deaths across 114 countries [1-8]. By April 30, 2020, it has already spread to almost all the continents infecting 3,090,445 people with 217,769 deaths, and by May 15, 2020, a total of

Corresponding author
Debmalya Barh
E-mail: dr.barh@gmail.com

4,338,658 people are infected with 297,119 deaths [9, 10]. The most severely affected countries, so far, are the USA, Italy, Spain, UK, and France in terms of infection and fatality, and, more recently, Brazil and India are becoming the emerging hotspots [9, 10]. Currently, the infection is at its second or third phases in several countries, except for its origin country, China, where the spread was controlled within the Wuhan city by March, 2020. Currently, the infection is at its second or third phases in several countries, except for its origin country, China, where the spread was controlled within the Wuhan city by March, 2020 and some other countries such as South Korea, Singapore, New Zealand etc. [11].

Coronaviruses (CoVs) are single-stranded RNA viruses that can infect several animals including humans and are classified into four genera (*Alpha-coronavirus, Beta-coronavirus, Gamma-coronavirus*, and *Delta-coronavirus*). The *Alpha* and *Beta*-CoVs can infect humans [12]. Among the *Beta*-CoVs, especially the SARS-CoV, which caused the pandemic in China during 2002-2003 and the MERS-CoV, in Middle-East in 2012-2015, are clinically important [13, 14]. Available genomic sequences and phylogenetic analyses predicted that, both these viruses probably originated in bats and, perhaps, through some intermediate mammalian hosts, were zoonotically transmitted to humans [15, 16]. The SARS-CoV-2, which is responsible for the current COVID-19 pandemic, also belongs to the genus *Beta*-CoV (subgenus *Sarbecovirus*) [2].

The virus was initially designated as 'WH-Human 1' coronavirus by Wu et al. 2020 and later 2019-nCoV by WHO [2, 17]. Nonetheless, the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses on February 11, 2020 designated the virus as SARS-CoV-2 [18]. Later, on March 20, 2020, a group of virologists from China proposed a distinct name for this virus as HCoV-19 which is now also used in several publications [19].

Meanwhile, due to the lack of a "definite origin", since February, 2020, "there are speculations, rumours, and conspiracy theories" that SARS-CoV-2 could be of "laboratory origin" or "artificially", or "intentionally made by humans in the lab" or a "laboratory-engineered CoV" that have "leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported", or "even for the purpose of use as a bioweapon" [20, 21]. Some authors in their article have directly termed the virus as the "Chinese coronavirus" [22].

In this article, we sequentially reviewed the key publications, as per their official peer-reviewed publication dates (during January 01, 2020 to April 30, 2020), on the natural origin of SARS-CoV-2, and the published articles that defend the "conspiracy theories". Subsequently, we discuss the evolution and recombination aspects of the SARS-CoV-2 Spike protein. Since the "conspiracy theories" also talks about potential "bioweapon" and "synthetic virus" aspects of the SARS-CoV-2, additionally, we have reviewed the related knowledge on these two aspects available in peer-reviewed publications from the public domain. Similarly, we have briefly presented the current research scenario on SARS-CoV-2. To provide a historical prospective of the review, for all the key articles, we have also analysed the origin of the articles (country and institutes), date of publication, scientific evidences, and use of authentic citations etc. In order to avoid any controversy, in most of the instances, we have directly used authors' statements with proper citations (*ipsis litteris*).

Selection of articles for review

To retrieve key articles, we searched the PubMed (www.ncbi.nlm.nih.gov/pubmed) literature database using specific combination of key words limiting the search from January 01, 2020 to April 30, 2020. We used search words: "Novel Coronavirus" or "SARS-CoV-2" or "COVID-19" or "nCoV-2" in combination with any of these words- "conspiracy theories", "laboratory-engineered", "man-made", "laboratory origin" "bioweapon", "evolution", "natural selection", and "origin" etc. The retrieved articles are manually curated at the abstract level and the most relevant papers were selected to compile the main review section.

We found a total of 2152 articles related to SARS-CoV-2 that are published and indexed in PubMed during this time frame. However, we found only fourteen articles that can be used for our review. Among these fourteen, eight publications have investigated the zoonotic origin of the virus, but none of these eight articles have used any terminology related to the "conspiracy theories" in their publications. In our search, six articles are found to reject the "conspiracy theories" on SARS-CoV-2 origin. We have considered these fourteen

articles to compile the main sections of the use our review. We have used additional articles to describe the other sections of this review.

SARS-COV-2 has "probably" originated from bat or pangolin

As per our knowledge, Zhu et al., was the first to report about the novel coronavirus of Wuhan on January 24, 2020; however, the first case of SARS-CoV-2 was recorded on December 07, 2019 from Wuhan [1, 3]. The SARS-CoV-2 genome is a single stranded and positive sense RNA of 29903 bp, and on January 05, 2020, researchers from Fudan University (China) submitted the complete genome sequence of the virus to GenBank (GenBank: MN908947, RefSeq: NC_045512). Based on the sequence identity, on January 30, 2020, Lu et al. from the National Institute for Viral Disease Control and Prevention, the Chinese center for disease control and prevention, and ten other Chinese institutes, in their *Lancet* article, first reported that the SARS-CoV-2 genome is overall 88% identical to two bat coronaviruses, bat-SL-CoV-ZC45 (GenBank:MG772933) and bat-SL-CoV-ZXC21 (GenBank:MG772934) but distant from MERS-CoV (~50% identity) and SARS-CoV (~79% identity) [23]. Using similar kind of analysis, Wu et al., from Shanghai Public Health Clinical Center of the Fudan University, in their *Nature* article, published on February 03, 2020, have also indicated that SARS-CoV-2 is closely related to the bat-CoV-SL-CoVZC45 (82.3% amino acid identity), and the nucleotide fragments from 1 to 1,029 and 1,652 to the end of the sequence are most closely related to bat-CoV-SL-CoVZC45 and bat-CoV-SL-CoVZXC21. Nevertheless, the Spike protein's receptor binding domain (RBD) (critical for infection and transmission of the virus) region 1,030 - 1,651 is closely related to SARS-CoV and bat-SARS-like CoV (bat-CoV-RsSHC014, GenBank: KC881005) and these two CoVs can directly transmit to human [2]. Based on their findings, Wu and colleagues suggested that bats are the possible host of SARS-CoV-2 and the Spike-RBD of the SARS-CoV-2 might be generated due to a probable recombination between SARS-CoV-2 and SARS-CoVs or bat SARS-like CoVs (WIV1 and RsSHC014). However, the complete genome of the SARS-CoV-2 did not emerge due to recombination [2]. Similar to the report of Wu et al., Zhou and colleagues from Wuhan Institute of Virology along with four other Chinese institutes, in their February 03, 2020 *Nature* publication, claimed that SARS-CoV-2 originated from bat-CoV-RaTG13 (GenBank: MN996532) and not due to any kind of recombination [2, 17]. The SARS-CoV-2 is highly similar to bat-CoV-RaTG13 (96.2%) throughout the genome and the S gene that encodes the Spike protein displays 93.1% identity [17] (Figure 1A).

Later, Zhang and colleagues from Yunnan University (China), in their *Current Biology* article published on March 19, 2020, pointed out that Pangolin-CoV is 91.02% and 90.55% identical to SARS-CoV-2 and bat-CoV-RaTG13, respectively, at the whole-genome level [24]. The mutations in the Spike-RBD of Pangolin-CoV (MP789) (GenBank: MT084071) are more closely related to SARS-CoV-2 than to those of the bat-CoV-RaTG13, but do not have the unique Furin recognition motif (-PRRA-) at S1/S2 cleavage site found in SARS-CoV-2. The authors finally suggested that, although Pangolin species are a natural reservoir of SARS-CoV-like Coronaviruses, it is still unclear whether pangolin is the origin of SARS-CoV-2 [24]. On March 26, 2020, in the same line with Pangolin origin, Lam et al. from Shantou University, Guangdong (China), in their *Nature* publication, showed that the Guangdong Pangolin-CoVs (sub-lineages GDP1L and GDP2S: GISAID: EPI_ISL_410544) genomes have 85.5% to 92.4% sequence similarity to SARS-CoV-2, respectively, and thus, are very closely related to SARS-CoV-2 [25]. Regarding the Spike-RBD, the SARS-CoV-2- RBD is 97.4% identical to Guangdong Pangolin-CoVs at amino acid level and shows identical amino acids at five critical residues, whereas bat-CoV-RaTG13 shares only one amino acid with SARS-CoV-2 (residue 442 of human SARS-CoV-2) (Figure 2A). These Pangolin-CoVs also lack the polybasic insertion (-PRRA-) at S1/S2 cleavage site that is unique to SARS-CoV-2 [25] (Figure 2B). Based on these findings, the authors finally concluded that, the SARS-CoV-2-RBD is originated from Guangdong Pangolin-CoVs "due to selectively-mediated convergent evolution rather than recombination" and pangolins may have acquired SARS-CoV-2 related viruses independently from bats or another animal host [25]. Nevertheless, such sequence similarity cannot be sufficient to either confirm or rule out any role of the pangolins as an intermedi-

ate host for SARS-CoV-2 [26]. Based on sequence analysis, Lau et al. from The University of Hong Kong, on April 21, 2020, published an article in *Emerging Infectious Diseases* showing that the SARS-CoV-2 is probably a novel recombinant virus having close relationship with bat-CoV-RaTG13 and its RBD is the closest to that of the Pangolin-CoV-RBD but none of these "represents its immediate ancestor", and its origin and direct ancestral viruses are yet to be identified [27] (Figure 1A, B).

On May 11, 2020, Zhou and colleagues from six Chinese institutes, in their article published in *Current Biology*, have shown that the SARS-CoV-2 is most closely related to bat-CoV-RmYN02 (GISAID: EPI_ISL_412977) with 93.3% nucleotide identity at complete genome and 97.2% identity with 1ab gene of SARS-CoV-2 [28]. However, bat-

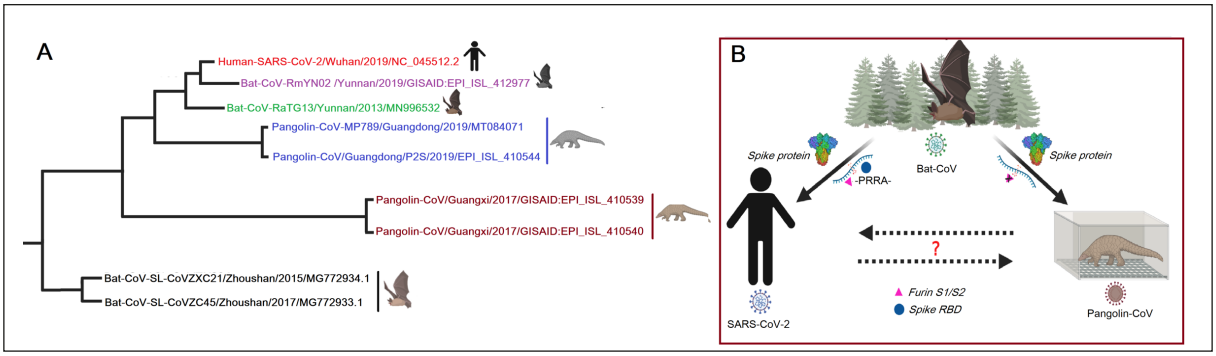

Figure 1 - A) Diagrammatic representation of phylogenetic relationship of SARS-CoV-2 with its nearest relatives shows that bat-CoV-RmYN02 is most closely related to this virus. B) The diagrammatic representation of Spike protein of SARS-CoV-2 and its probable origin and transmission. The figures are developed based on the publications presented in this review.

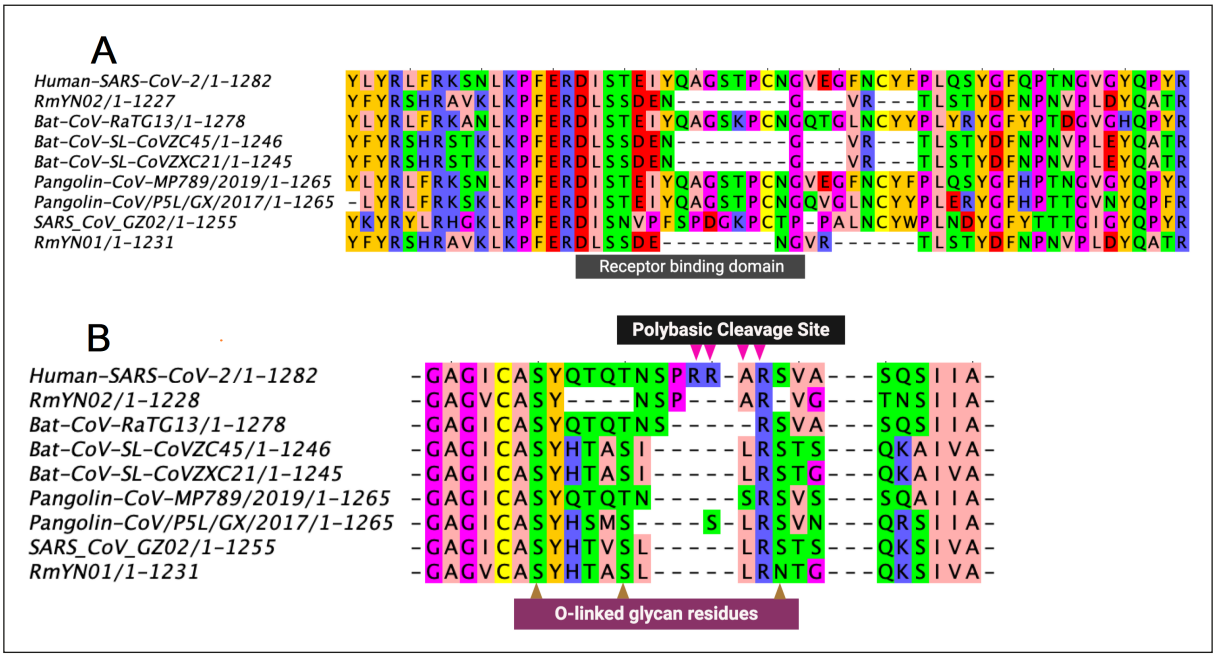

Figure 2 - A) Sequence alignment of the Spike-RBD of SARS-CoV-2 with its nearest relatives. B) Sequence alignment of the S1/S2 Furin cleavage site of SARS-CoV-2 with its nearest relatives. The figures are developed based on the publications presented in this review.

CoV-RmYN02 has only 61.3% sequence identity to the Spike-RBD of SARS-CoV-2 and has two loop deletions (Figure 2A) that shorten the RBD and, therefore, the RBD of bat-CoV-RmYN02 may not bind to human Angiotensin I Converting Enzyme 2 (hACE2). Similar to the bat-CoV-RaTG13, bat-CoV-RmYN02 also has only one amino acid similar to the critical six amino acids of SARS-CoV-2-RBD responsible for effective binding to hACE2. Therefore, the amino acid changes in the SARS-CoV-2 are probably due to a combination of complex recombination and natural selection events. The most interesting feature is that the bat-CoV-RmYN02 has a –P-AA- insertion at the S1/S2 Furine cleavage site that is not present in any other known bat-CoVs or Pangolin-CoVs closely related to SARS-CoV-2 (Figure 2B), indicating that such insertion is an independent natural event probably due to a recombination. Finally, based on their findings, Zhou et al. concluded that SARS-CoV-2 has a natural zoonotic origin probably from the bat [28].

It is worthwhile to indicate that most of these researches were funded by Chinese agencies and all of these genomes: bat-CoV-SL-CoVZC45, bat-CoV-SL-CoVZXC21, bat-CoV-RsSHC014, bat-CoV-RaTG13, and bat-CoV-RmYN02 were isolated from various provinces of China and submitted to GenBank and/or GISAID by various Chinese Institutes, including Institutes of Military Medicine Nanjing Command and Wuhan Institute of Virology, China during the years 2013-2020 (Supplement Table-S1). Similar to the Bat-CoVs, Pangolin-CoVs (MP789, GD/P2S) were also collected from China (Guangdong province) and submitted to GISAID by Chinese Academy of Fishery Sciences, Guangdong in 2020 (Supplement Table-S1).

Are convergent evolution and recombination associated with hACE2 specific Spike RBD?

The Spike glycoprotein (S-protein) of *Beta*-CoVs is very important since it binds to the hACE2 receptor for viral attachment and subsequent membrane fusion [29]. The receptor binding ability of the Spike is crucial for the transmission capability of the CoVs [12]. The S1 domain of the Spike protein (318–510 amino acid position *i.e.* C-terminal domain of S1 of SARS-CoV) contains the RBD for hACE2 and the S2 domain is involved in membrane fusion [30]. However, the Spike protein of SARS-CoV-2 is quite different from other coronaviruses and is highly specific to hACE2. Compared to SARS-CoV, in SARS-CoV-2, several key residues (Asn439, Asn501, Gln493, Gly485 and Phe486) in Spike-RBD responsible for hACE2 binding are altered, making SARS-CoV-2 much more aggressive in binding to hACE2 [23, 31]. Furthermore, a unique four additional amino acid residue insertions (-PRRA-) is found in the Furin cleavage site between S1 and S2 subunits (S1/S2, residues 682 and 685) of the SARS-CoV-2, which increases the transmissibility of this virus [32]. Furin protease is ubiquitously expressed in almost all the human vital tissues including lung, liver, pancreas, gastrointestinal (GI) tract, brain, and reproductive organs. The Spike protein is cleaved by Furin at the S1/S2 site, and this cleavage is essential for S-protein-mediated cell-cell fusion and viral entry into human cells. The -PRRA- insertion optimizes the S1/S2 cleavage and increased the cell-cell, organs, and systemic infection of the SARS-CoV-2 in the entire human body [32, 33]. Therefore, the transmission and systemic infection potential of the SARS-CoV-2 is much higher as compared to other CoVs due to these specific mutations in the C-terminal domain of S1 and the -PRRA- insertion at S1/S2 Furin cleavage site of its Spike protein.

Nonetheless, it is also reported that the SARS-CoV-2 did not originate due to any recombination event, and that the alterations in the Spike-RBD and -PRRA- insertion at the S1/S2 Furine cleavage site is probably due to a combination of complex recombination and natural selection [2, 17, 28]. Lam et al. suggested that the origin of SARS-CoV-2 Spike-RBD is not due to recombination but due to "selectively-mediated convergent evolution" [25]. Recently, on February 18, 2020, it was reported in a BioRxiv post by Patiño-Galindo et al. that Spike-RBDs of beta-CoV species, including the SARS-CoV and MERS-CoV, generally carry recombination hotspots [34]. This group of American researchers further hypothesized that bat-CoV-RaTG13 acquired the SARS-like-RBD before 2009 through recombination and, later, accumulated additional specific nucleotide substitutions to give rise to the SARS-CoV-2-RBD [34].

As previously reported, the SARS-CoV-2 is predicted to be most closely related and probably originated from bat-CoV-RaTG13 or bat-CoV-RmYN02 or Pangolin-CoV [17, 24, 28]. But only

the bat-CoV-RaTG13 Spike-RBD can use hACE2 as its receptor and may infect humans. However, there are three amino acid changes (L486F, Y493Q and D501N) in SARS-CoV-2 as compared to the bat-CoV-RaTG13 Spike-RBD, which have increased the hACE2 binding and transmission efficacy of SARS-CoV-2 [31].

On March 02, 2020, Wu and colleagues from various American and Chinese Institutes in their phylogenetic findings posted in BioRxiv pointed out that considering the overall genome sequence, the SARS-CoV-2 is closely related to bat-CoV-RaTG13; however, the Guangdong pangolin-CoV shares near identical amino acid sequence in the RBD (aa 315-550) than the bat-CoV-RaTG13, indicating a possible recombination between bat-CoV-RaTG13 and Guangdong pangolin-CoV in the development of SARS-CoV-2. Moreover, the authors also noted that divergences of most proteins between bat-SARS-like CoV and human SARS-CoV occurring during 1990-2002 and during 2005- 2012 happened between bat-CoV-RaTG13 and SARS-CoV-2 [35].

Taken together, it is presumed that the SARS-CoV-2 is closely related to bat-CoV-RaTG13, bat-CoV-RmYN02, and Pangolin-CoV-GD/P2S. The unique Spike protein of SARS-CoV-2 that is essential for its aggressive transmission and systemic infection is also very important to understand its origin. While the SARS-CoV-2 shows genome-wide identity with bat-CoV-RaTG13, bat-CoV-RmYN02, and Pangolin-CoV-GD/P2S, its Spike-RBD is very much identical to Pangolin-CoV-GD/P2S and polybase insertion at the S1/S2 Furine cleavage site is more similar to bat-CoV-RmYN02 when compared to the bat-CoV-RaTG13 and Pangolin-CoV-GD/P2S. Therefore, the SARS-CoV-2 has a "probable" zoonotic origin and the Spike protein may have originated from recombination or convergent evolution among Bat-CoVs and Pangolin-CoVs (Figure 2A, B). However, so far available genome sequence based similarity is not sufficient to either confirm or reject recombination and/or convergent evolution of the origin of SARS-CoV-2 Spike protein alteration for its human specific virulence.

The "conspiracy theories" and their rebuttals by scientific community

Until February 03, 2020, at least three peer-reviewed articles were officially published in *Nature* and *NEJM* suggesting a "probable" bat origin of the SARS-CoV-2 [2, 3, 17]. On February 14, 2020 Xiao et al. from five American Institutes along with one Chinese University published a comment opposing the "man-made" "genetic manipulation", and "bioweapon" conspiracy theories of SARS-CoV-2 [21]. The authors wrote the article in response to an article posted in BioRxiv on February 02, 2020 that perhaps claims that the SARS-CoV-2 Spike carries four "unique inserts" (TNGT-KR, HKNNKS, RSYLTPGDSSSG, and QTNSPR-RA). These "inserts" are very similar to the V1, V4, and V5 regions of Envelope or Gag protein of some HIV-1 strains found in certain countries, and these insertion sequences have increased the host range and host cell receptor binding ability of the SARS-CoV-2 Spike [21, 36]. Using genome sequences and bioinformatics analysis, Xiao et al., rejected the claim of HIV-1 sequence "inserts" as these "insert" sequences have poor identities and are very rare in HIV-1 genome sequences [21]. Moreover, these specific insertions are present in other bat originated *beta*-CoVs, and many CoVs have similar insertions but a different sequence is present at the first position. Furthermore, three inserts naturally exist in three bat-CoVs, and these inserts do not have any positional or sequence advantages in enhancing the receptor binding of the virus. The authors emphasized that virus gains any additional sequence from other organisms through recombination and, in order for this to occur; the virus needs direct interaction with that host organism. Therefore, to gain the four HIV-1 inserts, the bat-CoV and HIV-1 should co-infect the same cell, but this possibility is negligible as the hosts of bat CoVs and HIV-1 are different. Since these sequences are widely present in various mammals, it is much more probable that bat-CoV gained these sequences from their hosts through recombination [21]. Finally, Xiao et al suggested that the SARS-CoV-2 may have originated from bat-CoV-RaTG13-like coronavirus as reported by Zhou et al. [17, 21].

On February 26, 2020, Liu et al. from three American Universities claimed that there are "No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2" [20]. In their commentary they wrote "there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin" and that "some people have alleged that the human SARS-CoV-2 was

leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported". However, authors have not cited any authenticated source or literature that has claimed the "laboratory engineering". In this commentary, Liu et al. have also considered a "claim in Chinese social media" (without revealing the source) that a construction of a chimeric CoV (SL-SHC014-MA15 virus) with Spike gene from bat-CoV-SL-SHC014, present in the backbone of a SARS-CoV and capable of infecting human cells is the origin of SARSCoV-2 [20]. The information on the chimeric SL-SHC014-MA15 virus was published in 2015 by Scientists from seven American Institutes, Wuhan Institute of Virology, and the Swiss Bellinzona Institute of Microbiology [37]. The authors indicated that SARS-CoV-2 has >6,000 nucleotides difference from this chimeric SL-SHC014-MA15 virus and, thus, they completely rejected that SARS-CoV-2 has originated from it as an engineered virus [20]. The authors further emphasised that "synthetic constructs" are typically generated using a known backbone where sequences are inserted logically, and it is unlikely that randomly occurring mutations that are present in natural isolates such as bat-CoV-RaTG13 will be kept. Therefore, Liu et al. finally concluded that, currently, they do not have any credible evidence to support SARS-CoV-2 is a "laboratory-engineered CoV" [20].

Later, in order to defend the natural origin of SARS-CoV-2 against the "most likely constructed via laboratory recombination" hypothesis, Hao et al. from three Chinese Institutes, published a letter on March 08, 2020 in response to a blog/website of James Lyons-Weiler [38]. The authors show that the 1378 bp Spike sequence of SARS-CoV-2 (as claimed by James Lyons-Weiler) is not "unique" and is found in naturally occurring other CoVs and, thus, the SARS-CoV-2 is not generated in the laboratory. Besides, the "unique" sequence of Shuttle-SN vector that James Lyons-Weiler claimed to have been used in developing SARS-CoV-2 is not true since the Shuttle-SN having a fragment of the spike gene from SARS-CoV, is a common expression vector for laboratory use. All these three comment/commentary/letter were published in *Emerging Microbes & Infections* [20, 21, 38].

Meanwhile, a group of 27 public health scientists from 25 Institutes across nine countries issued a "spontaneous" "statement of support" for medical and public health professionals and scientists of China who are combatting the COVID-19. The correspondence was published in *Lancet* on February 18, 2020 stating that "We stand together to strongly condemn conspiracy theories suggesting that COVID-19 does not have a natural origin". The authors cited the publications supporting the zoonotic origin of the virus; however, did not cite any authenticated source of these "conspiracy theories" [39].

The most cited article for its critical observation on the origin of SARS-CoV-2 was published in *Nature Medicine* on March 17, 2020. In this correspondence, Andersen et al. from six American institutes with one British and an Australian university claimed that they "do not believe any type of laboratory-based scenario is plausible" in the origin of SARS-CoV-2 with its optimized Spike-RBD and insertion of polybase at S1/S2 cleavage site [40]. The authors, taking the references of published articles on zoonotic origins of SARS-CoV-2 and based on their own analysis, suggested that the SARS-CoV-2 could have originated either through "natural selection in an animal host before zoonotic transfer" or "natural selection in humans following zoonotic transfer". They rejected the possibility of "laboratory release" or the "SARS-CoV-2 acquired RBD mutations during adaptation to passage in cell culture" [40]. Nevertheless, it was claimed that the mutations in RBD are possible during adaptation to passage in cell culture [41]. However, Andersen et al. suggested that, nearly identical Spike-RBD of Pangolin-CoV with the SARS-CoV-2 supports a recombination or mutation event in the development of SARS-CoV-2 Spike-RBD probably from Pangolin-CoV [40]. It was previously reported that, insertions and deletions near the S1/S2 of Coronavirus Spike can occur due to natural evolutionary process (or prolonged passage or sub-culturing [42-44]. However, in order to generate such virus through passage, a "progenitor virus with very high genetic similarity" needs "prior isolation" [40]. Introduction of a polybasic cleavage site specific to hACE2 requires repeated sub-culturing of this virus in cell culture or animals with hACE2. But neither such progenitor virus nor sub-culturing based polybasic cleavage to hACE2 has "previously been described". Hence, Andersen and colleagues concluded that SARS-CoV-2 is not generated or released/escaped from laboratory [40]. Thus, according to these au-

thors, without prior knowledge in public domain, we may not precisely identify the origin of SARS-CoV-2. The authors finally concluded that "although the evidence shows that SARS-CoV-2 is not a purposefully manipulated virus, it is currently impossible to prove or disprove the other theories of its origin described here" (Figure 1B). The authors have acknowledged the funding supports from Wellcome Trust NIH, and Australian funding agency [40].

Is SARS-CoV-2 a potential "bioweapon"?

Since some "conspiracy theory" doubted the SARS-CoV-2 is a "bioweapon" as mentioned by Xiao et al. [21], we tried to investigate if any knowledge in the public domain is available that has suggested SARS-CoV-2 to be a potential "bioweapon".

According to biological warfare experts, natural or genetically engineered virus with novel characteristics, higher transmissibility, adaptability, communicability, ethnic specificity, higher morbidity and mortality rate, difficult to detect or diagnose and treat, with no known vaccine would be an ideal bioweapon. "Tailoring" classical pathogenic virus could achieve all these goals [45, 46]. The history of virus based bioweapon is quite old and dates back to the age of World War-I. While Anthrax, Alphaviruses, Smallpox have already been used in biological welfare, Ebola, Marburg, and SARS coronavirus are the new candidates [47-49].

The SARS pandemic of 2002 caused by SARS-CoV was spread to 29 countries with 8422 cases with ~10% fatalities within 7 months of its outbreak [50]. Compared to SARS-CoV, the SARS-CoV-2, which is responsible for the current COVID-19 pandemic, was first reported on December 07, 2019 from Wuhan, China and until April 25, 2020, the pandemic spread across 185 countries infecting 2,812,557 people with 197,217 deaths [1]. The overall mortality rate from SARS-COV-2 is around 7.01% [51]. The SARS-CoV and SARS-CoV-2 are both predicted to have originated in bats and transmitted to human through some intermediate hosts [2, 17, 24, 25, 52]. Both the SARS-CoV and SARS-CoV-2 belong to the same *beta*-CoV genus and show ~79% genome sequence identity minimal difference in replication proteins, no difference in Nsp13 (helicase protein), 87% identity with Spike protein, and have an additional Orf10 [23, 53]. Compared to SARS-CoV, the SARS-CoV-2 has significantly developed the required changes in its Spike protein for high transmission and infection efficacy in human as previously discussed, and till date no effective drug or vaccine is available to combat SARS-CoV-2 [54, 55].

Casadevall and Pirofski have described a calculation for weapon potential of any new microbial agent, and based on that estimate, they showed that "SARS coronavirus has a weapon potential that is intermediate between *Variola* and *Bacillus anthracis*" [48]. We have not found any such calculation available so far (till April 30, 2020) in any peer-reviewed publication that has estimated the weapon potential of SARS-CoV-2.

Engineered and synthetic virus in public knowledge

Similar to the "bioweapon" conspiracy theory, "synthetic construct" or "laboratory engineering" theories are also floated that are rejected by Liu et al. [20]. Therefore, we also reviewed literatures on synthetic viruses and likewise tried to find if any peer-reviewed publicly available article has designated SARS-CoV-2 as a "synthetic virus".

In our PubMed literature search, we found that genetic engineering for manipulation of a virus is an old practice. Adeno-associated virus is frequently genetically manipulated for gene delivery in gene therapy, and highly pathogenic viruses are genetically modified for vaccine development [56, 57]. Similarly, there are published articles describing synthetic viruses. Artificial poliovirus was chemically synthesized in 2002 by Cello et al. from State University of New York, USA without using any natural template [58]. Synthetic SARS-CoV was created in 2007 by University of North Carolina, USA, and, in 2008, synthetic recombinant bat SARS-like Coronavirus was developed by Vanderbilt University, USA [59, 60].

Moreover, Dutch scientists reported manipulation of Coronavirus genome with interspecies chimeric Coronaviruses in 2008 [61]. Scientists from China, in 2011, had successfully created synthetic Torque teno virus and in 2017, in collaboration with scientists from Canada and Netherlands, had reported synthetic baculovirus [62, 63]. Importantly, the SARS-CoV-2 genome that was first published on January 05, 2020 is reconstructed using a rapid synthetic genomics platform by a team of European scientists, and it was reported on May 04, 2020 [64]. However, there is no knowledge in the public domain as a peer-reviewed

publication supporting that the SARS-CoV-2 responsible for COVID-19 is a "synthetic" or "engineered" virus as of April 30, 2020.

Coronavirus research scenario

As it is predicted that the SARS-CoV-2 has probably originated form Bat-CoV or Pangolin-CoV, we aimed to provide a glimpse on who are the leaders in Coronavirus research as well as the status on the clinical research on COVID-19 in this review.

The major pathogenic CoVs of human (SARS-CoV, MERS-CoV, and SARS-CoV-2) are likely originated from bats, and bats are the major natural reservoirs of various CoVs [42]. SARS-like CoVs are found in bats from China, Europe, Africa and Southeast Asian countries. However, the Yunnan province in China is a "diversity hotspot" for bat-SARS-like CoVs [42]. The close relatives of the SARS-CoV or MERS-CoV are all bat-CoVs that were isolated from bats in China [65]. Similar to the SARS-CoV and MERS-CoV, the SARS-CoV-2 also shows high sequence similarity with bat-CoVs and Pangolin-CoVs from China [2, 17, 23-25]. Because of the availability of a large pool of the natural bat-CoVs, scientists form China have achieved some important milestones in bat-CoVs research. They had isolated and sequenced bat-SARS-CoV genomes in 2006 and in 2008, they successfully mapped hACE2 receptor binding domain of SARS-CoV and bat- SARS-CoV and constructed SARS coronavirus replicon [66-68]. Ge et al., in 2013, first reported that Chinese (Yunnan) horseshoe bats are natural reservoirs of SARS-CoV; they use hACE2 for cell entry, and can directly infect humans. Based on serological surveillance, Wang et al., in 2018, found that Bat-CoVs can directly infect humans [69], and the infections may be subclinical [70]. However, with time, China conducted bat-CoV research in collaboration with scientists/ Institutes from USA, Switzerland, Australia, Singapore, and Pakistan, among other countries [37, 70-75]. Importantly, within one month of the first reported case of COVID-19, they published the SARS-CoV-2 genome [2]. Furthermore, the bat-CoVs (bat-CoV-SL-CoVZC45, bat-CoV-SL-CoVZXC21, bat-CoV-RsSHC014, bat-CoV-RaTG13, and bat-CoV-RmYN02) and Pangolin-CoVs that showed to have high similarity with the SARS-CoV-2 genome are all isolated from their various provinces and sequenced by various Chinese institutes and submitted to GenBank or GISAID during 2013-2020 (Supplement Table S1). Nevertheless, as of May 04, 2020, top five countries involved in COVID-19 clinical research as per ClinicalTrials.gov (https:// clinicaltrials.gov/) are USA (209 studies), France (174 studies), China (74 studies), Italy (59 studies), and Spain (52 studies).

CONCLUSION

The information and knowledge currently available in the public domain as peer-reviewed publications support a probable bat or pangolin origin of SARS-CoV-2. However, the sequence of the evolutionary events in the origin of this virus is still unclear, as to whether it is due to a "natural selection in an animal host before zoonotic transfer" or "natural selection in humans following zoonotic transfer". Furthermore, some genomes are yet to be sequenced or to be available in the public domain that can shed light on its definite origin. Until April 30, 2020, no peer-reviewed article was found to be published supporting any "conspiracy theory" on the origin of the SARS-CoV-2. Finally, although China is leading the various aspects of SARS-CoV-2 research, the USA is currently leading the Clinical trials on COVID-19.

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Authors' contributions

All authors have equally contributed.

Conflicts of interest

Authors declare no conflict of interest.

Supplementary Table is available at:

Harvard Dataverse: Supplement Table-S1: CoV isolates closely related to the SARS-CoV-2. https://doi.org/10.7910/DVN/YJZGZ5

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