



Review paper

COVID-19 rhapsody: Rage towards advanced diagnostics and therapeutic strategy



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ABSTRACT

The deadly global outbreak of coronavirus disease-2019 (COVID-19) has forged an unrivaled threat to human civilization. Contemplating its profuse impact, initial risk management and therapies are needed, as well as rapid detection strategies alongside treatments with existing drugs or traditional treatments to provide better clinical support for critical patients. Conventional detection techniques have been considered but do not sufficiently meet the current challenges of effective COVID-19 diagnosis. Therefore, several modern techniques including point-of-care diagnosis with a biosensor, clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins that function as nuclease (Cas) technology, next-generation sequencing, serological, digital, and imaging approaches have delivered improved and noteworthy success compared to that using traditional strategies. Conventional drug treatment, plasma therapy, and vaccine development are also ongoing. However, alternative medicines including Ayurveda, herbal drugs, homeopathy, and Unani have also been enlisted as prominent treatment strategies for developing herd immunity and physical defenses against COVID-19. All considered, this review can help develop rapid and simplified diagnostic strategies, as well as advanced evidence-based modern therapeutic approaches that will aid in combating the global pandemic.

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has resulted in a human disease called coronavirus disease-2019 (COVID-19), which has been currently evidenced in more than 200 countries worldwide, with the rapid spread of the disease being reported [1]. From its initial emergence in late 2019 until December 2020, the number of active cases rose to 74.8 million worldwide with 1.7 million casualties [2]. In this situation, accurate detection of suspected cases, proper isolation of infected patients, and active treatment procedures are required to arrest the pandemic.

Conventional nucleic acid-based detection techniques used in virological diagnosis have established themselves as a concrete technology. Detection of viral nucleic acids using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) is a well-known method owing to its high specificity and sensitivity [3]. In contrast

to rRT-PCR, other modified PCR-based methods are also available. However, these diagnostic methods have various disadvantages to them such as a high rate of false-negative results [4,5], longer detection time, and compromises in the accurate detection of COVID-19 owing to the presence of inadequate genetic material in the throat or nasopharyngeal swab (NPS). Therefore, the development of novel detection methods and treatment strategies is imperative to supplement the current diagnosis.

Recent advancements in emerging techniques have led to the development of potential toolkits with advanced molecular biology and digital technology. The developed method includes utilization of clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins, which function as nuclease (Cas) for the detection of COVID-19 [6,7]. In addition, based on the CRISPR-Cas system technology against SARS-CoV-2, a group of Indian scientists have established an efficient, rapid, highly sensitive, and economical FnCas9 Editor Linked Uniform Detection Assay

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(FELUDA) kit for the successful detection of COVID-19, which provides results within 1 h [8]. Apart from the CRISPR-Cas system, aptamer-based technology has also gained attention for its sensitive detection of SARS-CoV-2 [9]. Additionally, isothermal amplification, rolling circle amplification, microarrays, biosensors such as field-effect transistors (FETs), plasmonics-based technologies [10], ultrafast advanced strand exchange amplification (ASEA) [11], immunoassays, next-generation sequencing (NGS) [12], and digital technology have also been used in COVID-19 detection.

In addition to developing diagnostic strategies, it is equally necessary to find treatments for the novel coronavirus (nCoV). Various treatment strategies, such as conventional drug treatment, plasma therapy, and vaccine development, have been proposed for COVID-19 treatment [13,14]. In this context, complementary and alternative medicines, such as Ayurveda, have also gained immense interest because of adequate clinical evidence [15] of their beneficial actions such as generation of herd immunity, anticancer and antimicrobial activities [16,17]. Recent reports have suggested that Ayurvedic treatment, involving an in-depth therapeutic strategy, can efficiently treat COVID-19 [18]. From past history, it has been deciphered that a combination of herbal formulations such as Shadanga Paniya and Ayurvedic medicine Agastya Harityaki is effective in treating respiratory turbulence and, recently, has been prescribed by the Ministry of Ayush, Government of India, for treatment against COVID-19 [19]. The inclusion of homeopathy, Unani, and herbal medicine has also been proven efficient in battling against COVID-19 [20,21].

In this review, we abridged the overview of the current updates on the diagnosis of COVID-19 and therapeutic strategies that are using traditional medicine as an immunity booster to help fight the COVID-19 global pandemic.

2. nCoV disease and clinical manifestations

Coronaviruses (nCoVs) are large positive-sense single-stranded RNA viruses with lengths of approximately 26–32 kb [22,23] and with a spike glycoprotein on the envelope, which gives them the crown-like appearance. nCoVs are beta-coronaviruses of the subgenus *Sarbecovirus* and family *Coronaviridae*. nCoVs mainly originate from the B-lineage of the beta-coronaviruses and are closely related to their earlier strain SARS-CoV [24]. SARS-CoV-2 is a highly contagious pathogen that is transmitted by aerosols, mucous membranes, and respiratory droplets. The broad clinical spectrum of the illness ranges from asymptomatic or mildly symptomatic to severely symptomatic, leading to multi-organ dysfunction syndrome. The most common symptoms include dry cough, fever, shortness of breath, fatigue, tiredness, chest pain, increased risk of blood clotting, and blockage of brain blood vessels [25,26] (Fig. 1).

3. Advance towards detection methods

Recent critical events have reinforced the need for a global sensitive surveillance system that can detect the coronavirus during its early phase of outbreak and prevent its spread and amplification. Conventional methods are most often used for the identification of COVID-19, but several drawbacks to these methods have resulted in failure to effectively control the spread of the virus. Rigorous assessment using advanced high-end techniques during COVID-19 pandemic came into existence and thereby gained attention for their rapid and sensitive detection. Fig. 2 provides a diagrammatic representation of SARS-CoV-2 detection methods.

3.1. Conventional RT-PCR and its pitfalls

The standard reference test for coronavirus detection is reverse

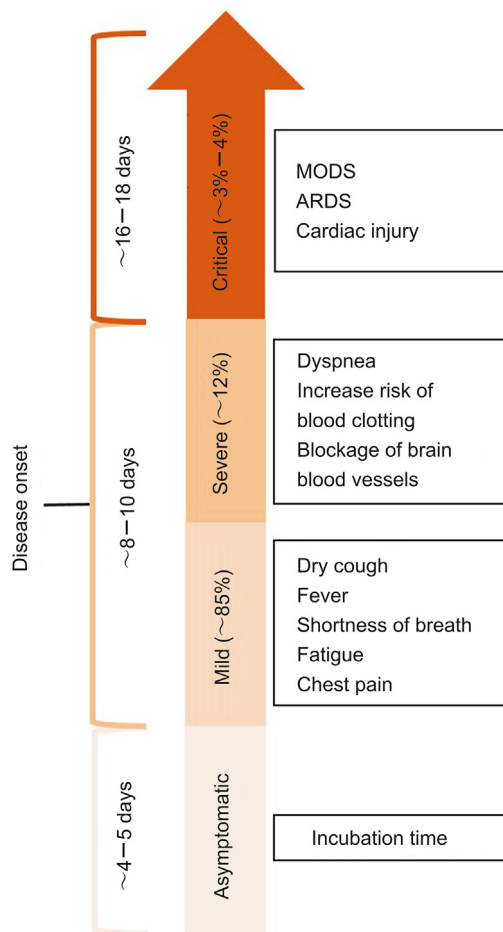


Fig. 1. Different stages of clinical manifestations of COVID-19. Symptoms of COVID-19 mainly include fever, fatigue, dry cough, dyspnea, acute respiratory distress syndrome (ARDS), multi-organ dysfunction syndrome (MODS), and cardiac injury, with varied incubation time and disease onset.

transcriptase polymerase chain reaction (RT-PCR), which is highly sensitive, specific, time-consuming, simple, and predominantly used for the detection of all types of CoVs [27,28]. Conventional RT-PCR detects viral RNA using fluorescent primers and thermal cycling steps. The process is sensitive, specific, and affordable; however, an error in the amplification process results in an adverse outcome, because of sample mishandling or the rapidly mutating nature of nCoV. A few reports on RT-PCR-based detection include targeting of the envelope (E) and RNA-dependent RNA polymerase (RdRp) genes, exhibiting 100% specificity [27]. Another RT-PCR-based detection by Nalla et al. [29] targeted the E gene and potentially detected samples with 100% specificity and sensitivity. To this end, Konrad et al. [30] also developed an approach that targets the E gene, rendering the present approach more sensitive than the earlier two RdRp gene assays; however, at later stages of the RT-PCR cycle, unspecified signals were obtained. In addition, the commercially available conventional kit for COVID-19 detection targeting the goal gene and direct saliva test is also advantageous over NPS, owing to easy and less contagious methods of detection, and is in the line towards routine diagnostics of COVID-19 [31,32].

3.2. Loop-mediated isothermal amplification (LAMP)

LAMP is a nucleic acid amplification technique that enables effective diagnosis and isolation strategies with high sensitivity,

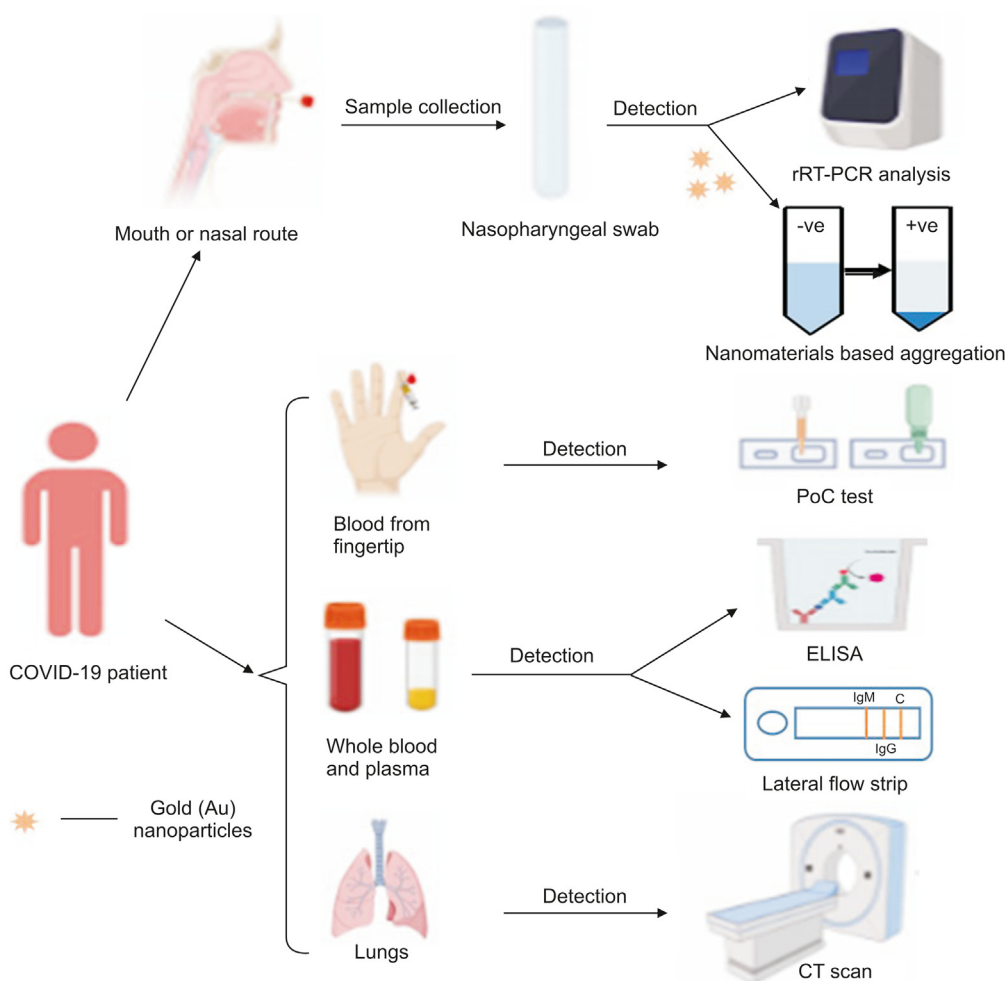


Fig. 2. Schematic of several diagnostic methods for COVID-19 detection. All detection methods have been illustrated schematically. The methods include real-time reverse transcriptase-polymerase chain reaction (rRT-PCR), nanomaterials based aggregation assay, point-of-care (PoC), enzyme-linked immunosorbent assay (ELISA), and computed tomography (CT).

specificity, and efficiency under isothermal conditions at a single point temperature for pathogen detection. It is a significantly rapid, affordable, and highly reliable technique as compared to the conventional RT-PCR, and it establishes itself as a strong contender for direct use in diagnosis [33]. The method utilizes a single tube for DNA and RNA amplification, four primers specific to the experiment, and a DNA polymerase capable of strand displacement enabling the synthesis of 10^9 copies of target DNA in less than an hour at 65°C [34]. Recently, Song's group [35] elucidated the design of a two-stage LAMP strategy (COVID-19 Penn-RAMP) that could be performed in closed tubes by means of colorimetric or fluorescence detection. These assays exhibited 10-fold higher sensitivity than conventional RT-PCR when tested with purified targets. Interestingly, Lamb et al. [36] demonstrated rapid SARS-CoV-2 RNA detection within 30 min. However, both these experimental evidences were executed in simulated patient samples with artificially spiked swabs and blood samples. Moreover, Zhang et al. [37] successfully performed LAMP-based experimentation to identify SARS-CoV-2 RNA from a pool of purified RNA or patient cell lysis by visual, colorimetric-based detection. For further validation, the experimental results were verified using RNA samples obtained from respiratory swabs of patient samples in Wuhan, China. Furthermore, the obtained results may be compared with spiked RNA samples, as well as patient samples. In addition, an innovative LAMP-based COVID-19 diagnostic kit was developed by the Sree

Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India, named Chitra-Gene LAMP-N [38]. This test is regarded as a confirmatory sensitive diagnostic test for the SARS-CoV-2 nucleocapsid (N) gene. The detection time was determined to be 10 min, whereas the sampling to test time was highly remarkable at less than 2 h.

3.3. Rolling circle amplification-based method

Another time-saving method for the detection of target nucleic acids is rolling circle amplification (RCA), which can undergo signal amplification of 10^9 -fold/circle within 90 min [39]. This method has been used for SARS-CoV detection. The setup was accomplished in solid and liquid, and presented preliminary results with a few clinical respiratory samples [40]. The foremost advantage of this molecular technique is the minimal reagent requirement and low false-positive results compared to those with traditional PCR techniques. However, the method is yet to be deployed for SARS-CoV-2 detection.

3.4. Microarray-based method

Microarray is a rapid, efficient, and high-tech SARS-CoV nucleic acid detection method. The process mainly relies on the generation of cDNA labeled with specific probes using reverse transcription.

Before loading in each well for hybridization with solid-phase oligonucleotides fixed on the microarray, numerous washing steps were carried out to remove free DNA, thereby resulting in the occurrence of a signal due to the presence of specific viral nucleic acids [41]. Later, Shi et al. [42] designed an oligonucleotide microarray of 60-mer based on the TOR2 sequence and used it for SARS-CoV detection of clinical samples. Owing to the rapid mutation phenomenon of SARS-CoV, Guo et al. [43] designed a microarray for the detection of 24 single nucleotide polymorphism (SNP) mutations in the spike (S) gene of SARS-CoV with 100% sample detection accuracy. In addition, immunoglobulin profiling of IgG and IgM in response to SARS-CoV-2, resulting in a SARS-CoV-2 proteome microarray, has been constructed with approximately 18 of the 28 predicted proteins [44]. The results were simultaneously analyzed using a dual color strategy, proving that S1 and N proteins are suitable for COVID-19 diagnostics.

3.5. Immunodiagnostic tests

3.5.1. Lateral flow immunoassay (LFIA)

Recently, LFIA or immunochromatographic tests have drawn special attention in contrast to the available contemporary technologies. LFIA potentially offers economical and rapid diagnostic tests, which can be easily distributed and self-administered or performed by competent healthcare workers to test for SARS-CoV-2 antibodies. The LFIA test can detect the SARS-CoV-2 antibody and efficiently differentiate between IgG and IgM using whole blood, serum, and plasma [45]. The technique mainly follows the principle of the receptor-binding domain of the spike as well as nucleocapsid proteins. According to the study by Ragnosola et al. [46], LFIA often works as a cassette to determine the binding of SARS-CoV-2 antibodies found in convalescent plasma. The developed Clungene® SARS-CoV-2 IgG/IgM rapid test cassettes arbitrate the presence of antibodies in convalescent plasma of donors with a previous history of COVID-19. Bendavid et al. [47] illustrated a test by LFIA in approximately 3330 adults and children, and the results exhibited 82% sensitivity and 99.5% specificity. Another rapid LFIA test introduced by Premier Biotech (Minneapolis, MN, USA) demonstrated a clinical sensitivity of 80.3%, specificity as high as 99.5%, and an assay time as short as between 12 and 20 min [48]. Other LFIA assays with outstanding analytical performance for SARS-CoV-2 detection have been reported by various diagnostic companies such as Shanghai Kinbio Inc. (Shanghai, China), Auto Bio Diagnostics (Zhengzhou, China), and Artron Laboratories (Burnaby, Canada) [49,50]. Additionally, a rapid (10 min) and sensitive lanthanide-doped polystyrene nanoparticle has also been developed to detect anti-SARS-CoV-2 IgG [51]. Similarly, antibody-coated lateral flow strip-based detection has shown excellent specificity for rapid COVID-19 detection, and in most cases, IgG and IgM-class antibodies are primarily used. IgG and IgM expressions together exhibited positive results, while IgG or IgM alone displayed negative results for COVID-19 detection [52].

3.5.2. Enzyme-linked immunosorbent assay (ELISA)

ELISA is a qualitative or quantitative laboratory-based test with high sensitivity and selectivity. Typically, ELISA detects the presence of antibodies such as IgG and IgM in whole blood, plasma, or serum samples from patients [53]. Briefly, the patient samples were incubated on a plate-like platform coated with targeted viral proteins such as S-protein to achieve antibodies from patients and viral protein complexes. Following this, the bound protein-antibody complex is detected with another wash of antibodies, which produces different detection modalities such as colorimetric, electrochemical, and fluorescent readout. In the context of COVID-19, the analytical sensitivity of ELISA was reported to be as low as in the

picomolar (pM) range, and the time of the assay was 2–5 h [54,55]. Interestingly, Krammer's group [56] described a two-staged ELISA protocol for the measurement of human antibody in response to that of the recombinant receptor binding domain or S-protein of nCoV. The first stage of the technique includes cutting-edge sample screening in a single-serum dilution with a receptor-binding domain (RBD). In contrast, the next step consists of samples with positive results obtained from the earlier step, and at the last step a thorough confirmatory ELISA test was performed for total S-protein. Another study by Liu et al. [57], conducted from the sera of 214 COVID-19 patients, involved detecting IgG and IgM using N and S ELISA. The study corroborated that the S ELISA showed higher sensitivity than N ELISA. Moreover, a comparative analysis between ELISA-based and gold-immunochromatographic assay (GICA) has also been performed [58]. The high sensitivity and short testing time suggest that immunoassays are a superior alternative to conventional detection methods. Moreover, Li et al. [59] recently reported a point-of-care diagnostic device with combined IgG–IgM testing platform, and the testing time was as short as 15 min. This over-reliance and effective management of pathogens may necessitate the rigorous development of antibody-based assays to help stop the uncontrollable spread of COVID-19.

3.5.3. Antigen detection test

Antigen detection test mainly detects viral antigens using the immune-capture method. Viral antigens can be detected during the active replication process of the virus, thereby establishing a highly specific test. The potential use of the antigen detection test can act as a triage test for the rapid identification of COVID-19 patients. Interestingly, recent reports have shown that monoclonal antibodies can be used against the N protein of SARS-CoV-2, and numerous simple, non-complex, and rapid test kits are under gradual development [60].

3.5.4. Other immunoassays

Other rapid immunoassay-based diagnostic methods include a multiplexed single-molecule array (SIMOA) established by the renowned company Quanterix (MA, USA). It targets the responses of three important immunoglobulins, IgG, IgM, and IgA, to four viral proteins (spike protein, S1 subunit, receptor binding domain, and N protein). The method is executed in only one sample, aiming towards 12 antibodies for isotype-viral protein quantification. An ultrasensitive immunological response SIMOA with high specificity and sensitivity was further hypothesized compared to that with a single interaction in ELISA for COVID-19 detection. During the first week of infection, the method showed sensitivity and specificity of 86% and 100%, respectively, and after the first week classified as later stage, 100% sensitivity and specificity were observed [61].

The proteomic screening tool, a protein microarray method (PMM), simultaneously analyzes a mixture of proteins. Moreover, the PEPperCHIP® SARS-CoV-2 Proteome Microarray (PEPperPRINT) was developed based on the genomic construction of SARS-CoV-2 isolated from the virus Wuhan-Hu-1 (GenBank ID: MN908947.3), which has been used for serological screening of at least 5000 individual peptides present throughout the viral proteome. Since 2002, PMM has successfully diagnosed Chinese subjects as SARS-positive due to its adequate sensitivity to ELISA tests. Therefore, the method is considered to be more reliable than conventional immunoassays, owing to its determination of more protein antigens of the virus [62].

3.6. Next-generation sequencing (NGS)

NGS plays a decisive role in virus detection. NGS platform was recruited for the famous Human Genome Project and has been

implemented to detect SARS-CoV-2. “Billion To One (BTO)”, professional cancer molecular diagnostic company, has designed a technique called the qSanger-COVID-19 test capable of a detection 30 times faster than that with conventional qPCR methods [63]. Numerous NGS-based COVID-19 toolkits have been used for the detection of viral mutations. Additionally, a UK-based company, Youseq, recently developed a highly sensitive COVID-19 NGS Library Prep Kit, which functions effectively in the presence of minimal viral titers [64]. Following this, BGI Biotechnology, a Chinese company, developed a metagenomic-based sequence detection kit, NGS COVID-19 Kit, for the rapid detection of SARS-CoV-2 [12]. The kit can also detect viral sequences and has been approved by the National Medical Products Administration, China. Additionally, in this alarming situation, the rapid spread of the variants of SARS-CoV-2, such as the UK B.1.1.7 strain, defined by multiple spike proteins such as deletion 69–70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H, and the Brazilian B.1.1.28 strain with the first detected S protein mutation E484K in the sequence of a patient from Rio de Janeiro, Brazil [65], is gaining increased concern. This 484 K.V2 variant has also spread to several other countries, such as England, Singapore, and Canada. Therefore, the gradual emergence of these new strains and the increase in reinfection critically urge the sequencing of more SARS-CoV-2 samples. Public health agencies leverage sequencing technology to provide critical data for viral surveillance of SARS-CoV-2 variants. Moreover, single-cell RNA sequencing can also firmly address SARS-CoV-2 infection along with the strong guidance of previous literature study [66].

3.7. Point-of-care (PoC)-based detection for COVID-19

The PoC device can be a foremost option for rapid, highly sensitive detection of COVID-19, which can be used at home. PoC device can be an alternative and a much superior solution for widespread detection of SARS-CoV-2 among various populations.

PoC platforms, receiving emergency use authorization (EUA) from the US Food and Drug Administration (USFDA), are manufactured by renowned groups such as Abbott (IL, USA) and Cepheid (CA, USA) [67]. Abbott ID NOW COVID-19 test kits are also on the frontline by the fabrication of 6.6 lb, a small toaster-like lightweight box known as Abbott's ID NOW™. The Cepheid Xpert® Xpress SARS-CoV-2 system works in a random manner to achieve the result within 30 min. It is also noted that the Abbott technology missed one-third of the samples detected as positive by Cepheid Xpert Xpress [68]. Another reputed non-government company in India, the Fast Sense Diagnostics, has proposed a technology called CovE-Sens by developing two modules for early detection of COVID-19, within a few minutes [69]. ePlex® SARS-CoV-2 is a qualitative automated nucleic acid multiplex platform capable of automated extraction, amplification, and detection using a single-use cartridge. This entire setup is a very simple visualized readout similar to that of the pregnancy test kit. The developed PoC system is based on a specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) technology and is called SHERLOCK testing in one pot (STOP COVID) [70]. The system utilizes the AapCas12b protein, which is efficiently integrated with the guided RNA containing spacer sequence for reliable, specific, and sensitive detection of the nCoV N gene. This efficient technique may be further used at home using samples such as saliva. However, most of the aforementioned EUA-approved technologies need rapid testing to achieve their sensitivity, specificity, and reliability.

3.8. Biosensors

Due to the lack of clinical evaluation of PoC devices, other

emerging techniques such as biosensors have been developed for rapid, ultrasensitive detection of COVID-19. The technology is successful in detecting the earliest point of infection from NPS within minutes, owing to smart exposure and a rationalized protocol for virus diagnosis.

Currently, a study has reported a photonics-technology that manipulates light; the ultrasensitive demonstrator could detect “day 1” infections in patients with low viral load, representing a breakthrough in tackling the coronavirus pandemic [71]. A plasmonic biosensor with dual attitude, such as the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction, has been demonstrated as a phenomenal approach towards the medical diagnosis of SARS-CoV-2 [10]. The detection procedure was initiated through nucleic acid hybridization using gold nano-islands with a two-dimensional architecture and was functionalized using complementary DNA receptors. Moreover, the fabricated biosensor precisely detected a distinct target from a multigene mixture with a detection limit as low as 0.22 pM. Another biosensor for sensitive S1 protein detection underwent steady and thorough monitoring of asymptomatic patients. Moreover, the bioelectric response change in membrane-engineered cell lines constituted with human chimeric spike S1 antibody was measured using the fabricated biosensor following the combined bioelectric recognition assay principle and the membrane engineering-based molecular identification technology [72,73]. The viral antigen was detected at a lower level of pg/ng under approximately 3 min using this fabricated biosensor, and no sample pre-processing was required [74]. In addition, immunological field-effect transistor (FET)-based sensing technology is a promising tool for the sensitive detection of viral antigens from clinical samples [75,76]. Recently, a graphene-based FET sensor was fabricated for the rapid detection of SARS-CoV-2 antigen in clinical samples using a specific antibody against the SARS-CoV-2 spike protein [77]. However, all these technologies need to be extensively explored for tackling the COVID-19 pandemic outbreak.

Single-stranded DNA or RNA oligonucleotides selected randomly from nucleic acid libraries called aptamers have formerly paved the way for the successful detection of biomolecules, such as proteins and neurotransmitters [78]. Moreover, detection of SARS-CoV N protein using nanoarray RNA aptamer chip at a concentration as low as 2 pg/mL deserves mention [9]. COVID-19 aptamer-linked immobilized sorbent assay (ALISA)-based detection of SARS-CoV-2 with 90% sensitivity and 99% specificity was first developed by an eminent Indian company THSTI [79]. With the gradual progression in the aptameric detection of SARS-CoV-2, another group of researchers from Pinpoint Science's Laboratory in Berkeley, California, USA, developed an efficient handheld device that could potentially detect the targeted protein of SARS-CoV-2 within 30 s [80]. However, the reliability and sensitivity of this technology require further trials with a large sample size.

3.9. Chest computed tomography (CT)

A few recent reports have demonstrated the disadvantages of the conventional RT-PCR technique. An instance was evidenced where two patients infected with nCoV were tested negative with RT-PCR, which led the clinicians to prescribe chest CT scans as an important criterion for clinical diagnosis of nCoV [81]. Recurring issues with a similar rate of false-negative RT-PCR testing during disease progression and treatment have been separately reported [82]. Such observations have prompted practitioners to recommend CT technique as an effective parameter to be considered along with RT-PCR to make decisions on patient discharge and evaluation of treatment protocols. Indeed, a retrospective study has drawn a conclusion in favor of this technique, ascribing 97.2%

sensitivity to CT compared to the 83.3% with RT-PCR [83]. At this juncture, chest CT scans could be a potential diagnostic tool to formulate a standard paradigm indicative of a nCoV infection.

3.10. Digital approach towards SARS-CoV-2 detection

A digital approach has been conceived for the detection of asymptomatic COVID-19 patients through the phenomenon of recording the forced-cough by using cell phones and thereby steadily combined with artificial intelligence (AI) [84]. A varied survey with 5,320 people was conducted by a popular MIT website with cough recordings of COVID-19 patients. A framework of AI speech processing with acoustic biomarker extractors has been developed, and the recordings were converted into a convolutional neural network (CNN) for early screening of patients. Furthermore, a saliency map was created for the real-time monitoring of patients, a non-invasive detection method with no cost. This advanced CNN-based method has a training subset of 4,256 samples and was validated on 1,064 samples. Apart from the CNN architecture, Quatieri et al. [85] from MIT proposed modeling of speech as well as signal-processing framework to be the estimated parameters for the detection of different states of COVID-19. The outcome of the framework was a dynamicity analysis of human behavior in real surroundings to potentially locate COVID-19 patients. Another remarkable study on asymptomatic COVID-19 patients based on speech recordings has elucidated vocal biomarkers as potent indicators for disease prediction. Biomarkers are developed as a consequence of infection due to respiratory, laryngeal, and articulatory muscular movements [86].

3.11. Looming techniques towards nCoV detection

3.11.1. CRISPR-Cas technology and reactive polymers

CRISPR mainly deals with repeated nucleotide sequences along with aced spacer sequences, and Cas is correlated with CRISPR-associated proteins depicting the nuclease enzymatic function. An RNA targeting CRISPR enzyme Cas 13 was recently developed for rapid and portable sensing of nucleic acids [87]. Zhang's group [88] reported that Cas 13 is generally programmed to target, thereby destroying the genomes of diverse mammalian single-stranded RNA viruses. Moreover, they developed a platform known as SHERLOCK that combined isothermal pre-amplification with Cas 13 for the detection of single molecules such as DNA or RNA. It could detect single-stranded RNA of the Zika virus or dengue and the presence of mutations in liquid biopsy samples of patients. Recently, the same group has developed a protocol for COVID-19 detection using CRISPR technology [89]. The protocol mainly includes the use of RNA fragments of synthetic SARS-CoV-2, followed by continuous detection of SARS-CoV-2 target sequences in a range between 20 and 200 aM, that is, 10–100 copies/ μL of input by a dipstick within 60 min, without requiring detailed instrumentation. In addition to CRISPR-Cas 13, CRISPR-Cas 12 system DNA endonuclease-targeted CRISPR trans reporter (DETECTR) also plays an important role in the potential detection of SARS-CoV-2 targeting the E and N2 genes [6]. The all-in-one dual CRISPR-Cas 12a system (AIOD-CRISPR) also exhibited versatile roles in the ultra-sensitive detection of COVID-19 to achieve a detection limit of up to 5–11 copies of viral RNA/microliter within 90 min [90]. CRISPR-Cas 9 gene editing protocol pends establishment of impressive, rapid, highly sensitive, and economical FELUDA kit developed by a group of Indian scientists [8]. The kit can detect SARS-CoV-2 genetic material within 60 min.

Apart from CRISPR-Cas technology, another non-conventional method that may serve as a potential platform for SARS-CoV-2 detection, is the use of reactive polymer-grafter devices that may

utilize antibodies to detect dsRNA [91]. In this device, a polymer-coated surface-reactive poly (pentafluorophenyl acetate) was applied to immobilize the J2 antibodies on the platform surface. The main advantage of this type of graft device is that antibody binding is sequence-independent, and therefore, the platform may serve as a universal virus detection unit.

3.11.2. Exhaled breath condensate (EBC)

This study aimed to establish a potential diagnostic method for SARS-CoV-2 detection. nCoV is mainly spread via droplets, and in the case of bronchoalveolar lavage (BAL), the diagnosis sensitivity and specificity indexes are the highest. Recently, scientists have proposed that EBC could be used as an efficient diagnostic tool for SARS-CoV-2 detection. Moreover, the use of EBC for examination purposes is a novel approach for the assessment of inflammation within the respiratory tract. The collection of samples is non-invasive, easy, and can be repeated several times, and the process may be executed in severe COVID-19 patients and also in small children covered with special facial masks. During the examination process, patients were asked to breathe out normally for approximately 10–15 min into an apparatus supported by a cold system to accumulate the condensate. The probable utilization of this method could be justified in accordance with the perspective of public healthcare; but the number of false-positive results contributes to the continuous spread of COVID-19 worldwide [92,93].

4. Treatment strategies towards SARS-CoV-2

Initially, the generalized treatment of COVID-19 patients included complete bed rest along with an adequate diet and competent medications. In severe cases, a special care or organ support system is also needed to treat or prevent respiration-related complications, along with adequate therapeutic measurements. In this section, we focus on the various established and developed treatment paradigms recommended for proper treatment of the COVID-19 pandemic.

4.1. Respiratory therapy

Hypoxemia patients with COVID-19 with less than 90% oxygen saturation generally require mild to moderate work of breathing resulting from other causes, and the maintenance of greater than 90% oxygen saturation using high-flow oxygen is a much-needed support. In addition, patients with acute respiratory distress syndrome (ARDS), multi-organ dysfunction syndrome (MODS), hemodynamic instability, severe hypoxia, or multiple high-risk conditions such as hypertension, diabetes mellitus, cancer, cardiovascular disease, or chronic respiratory disorder should be immediately treated with mechanical ventilation and endotracheal intubation [94]. Furthermore, a robust circulatory system with improved microcirculation, vasoactive drugs, and regular monitoring of hemodynamics are also needed for active treatment support.

4.2. Conventional drug-based treatment

Drug-based treatment with well-known anti-malarial agents such as chloroquine and its derivative hydroxychloroquine deserves special mention, because it has elicited profound antiviral effects against several viruses such as hepatitis B, HCoV-229E, and human immunodeficiency virus (HIV) type 1. These potent anti-malarial agents block viral entry into cells by glycosylation inhibition of the host receptors and by elevating the endosomal pH, thereby suppressing the production as well as the release of factors that mediate the inflammatory complications of viral diseases [95]. These antiviral effector functions emphasize the rationale for using

chloroquine to treat SARS-CoV-2 infection. In light of the advantages of chloroquine use, several drawbacks also have been gaining attention in recent times. Chloroquine causes tissue damage and several diseases such as retinopathy, neuropathy, cardiomyopathy, and myopathy [96]. A recent study of COVID-19 patients using chloroquine diphosphate in Brazil could not conclude any discrete conclusion and ended early, as most of the patients died due to severe arrhythmias. Moreover, chloroquine simultaneously inhibits proliferation and T-cell activation, inducing apoptosis, which may adversely affect antiviral immune surveillance [97]. It also inhibits antigen-antibody reactions, along with the inhibition of proteolysis, chemotaxis, and phagocytosis. In addition, chloroquine acts as an autophagic inhibitor, thereby preventing fusion between autophagosomes and lysosomes [98]. Thus, autophagy inhibition by chloroquine may lead to cellular death and secondary infection.

As early as in February 2020, hydroxychloroquine was identified as a potential inhibitor of SARS-CoV-2 in vitro. The in vitro activity of hydroxychloroquine in comparison to chloroquine after 24 h of growth resulted in a lower half-maximal effective concentration (EC₅₀) for SARS-CoV-2 [99]. Recently, reports have shown that chloroquine and its analogue hydroxychloroquine have been successfully used to treat a series of COVID-19 cases, as observed by improved radiologic findings, enhanced viral clearance, and reduced disease progression [100]. Experimental evidence from putative randomized clinical trials (RCTs) and prospective open-label studies have claimed that hydroxychloroquine and azithromycin are potentially effective drugs against COVID-19 [101]. Another non-randomized, non-blinded, open-label trial study recruiting 80 patients was conducted. The patients were administered 600 mg of hydroxychloroquine for 10 days, followed by 250 mg of azithromycin to alleviate COVID-19 symptoms [102]. However, the aforementioned claims were offset by three studies, none of which conclude any significant benefit from this treatment [103–105]. However, these two drug candidates—hydroxychloroquine and chloroquine, other antiviral agents, corticosteroids, and inflammation inhibitor agents were found to affect few secondary endpoints, and future clinical trials may verify the current data to suggest a broad spectrum role against COVID-19. The details of various antiviral agents, corticosteroids, and inflammation inhibitors are shown in Table 1 [106–120] and Fig. 3.

4.3. Passive antibody therapy

Passive antibody therapy generally involves the administration of antibodies against a given agent to a susceptible individual to

provide immediate immunity to prevent or treat infectious diseases. Experiences from previous CoV outbreaks such as the SARS-CoV-1 have shown that convalescent sera contain neutralizing antibodies to the relevant virus provide immediate immunity [121]. In recent studies, five critical COVID-19 patients were treated with convalescent plasma for 10 to 22 days. Of these, three patients were discharged from the hospital after a little more than 50 days, and the remaining two were found to be in a stable condition after 37 days of transfusion [122]. In addition, an RCT was designed to properly investigate whether plasma can be used to prevent infectious COVID-19 [123]. With antibody therapy, anticoagulant therapy by enoxaparin reduces intensive care unit (ICU) mortality [124]. Moreover, according to recent reports, early convalescent plasma may slow down the progression of illness in older adults with COVID-19 [125,126]. Early infusion of this simple and inexpensive technique may help high-risk patients.

4.4. Vaccine development

The current SARS-CoV-2 outbreak demands an immense effort to develop vaccines which will help control and reduce disease transmission by creating herd immunity. Vaccines against COVID-19 are currently in progress involving numerous vaccine candidates. In the process of COVID-19 vaccine development, previously noteworthy bacille Calmette-Guerin (BCG) immunization is found to be beneficial against COVID-19 due to its non-specific immune response [127]. However, few clinical trials are underway to evaluate its efficacy among healthcare workers who are in direct contact with COVID-19 patients [128]. There are also other reported vaccines in the clinical stages against COVID-19 (Table 2) [129–138].

4.5. Complementary and alternative medicines

The diverse field of medical practices encompassed by the National Center for Complementary and Integrative Health (NCCIH) of the National Institute of Health (NIH), USA, includes Ayurveda, homeopathy, Unani, and naturopathy, which can aid in accurate diagnosis of different diseases. This globally recognized medical system utilizes the potential of traditional medicines that have been used since primitive times for the treatment of diverse diseases and have been implemented in the treatment of influenza, Japanese encephalitis, and HIV [139]. To suppress the present scenario, the Department of Ayush, Government of India,

Table 1
Drug-based treatment for COVID-19.

Drug types	Drug	Drug target	Trial outcome	Refs.
Antiviral agents (protease/spike glycoprotein inhibitor)	Lopinavir/ritonavir	Inhibiting 3CL ^{pro}	Showing no benefit compared to standard care	[106,107]
	Nelfinavir	Inhibiting 3CL ^{pro}	Potential treatment option against SARS-CoV-2	[108]
	Griffithsin	Inhibiting the spike mediated fusion with the membrane	Preventing >80% infection	[109]
	Nafamostat	Inhibiting the spike mediated fusion with the membrane	Preventing SARS-CoV-2 infection in human lung cells	[110,111]
Antiviral agents (nucleoside analogues)	Remdesivir (GS-5734)	Inhibitor of RNA polymerase, binding to viral RdRp and acting as an RNA chain terminator	Under clinical trial; showing promising results.	[112,113]
	Favipiravir	Inhibitor of RNA polymerase	Showing efficacy against SARS-CoV-2	[114,115]
	Galidesivir (BCX4430)	Inhibitor of RNA polymerase and premature termination of RNA transcription	Broad spectrum antiviral activity	[116]
Corticosteroids	Methylprednisolone	Decreasing host inflammatory response	Reducing death by one-third of critical COVID-19 patients	[117]
Inflammation inhibitors	Tocilizumab	IgG1 monoclonal antibody with IL-6 receptor	Reduction in mortality	[118,119]
	Anakinra	Monoclonal antibody with IL-1 receptor	Clinical improvement in 72% in patients	[119]
	Acalabrutinib	Tyrosine-kinase inhibitor	Improving oxygenation, effect on C reactive protein and IL-6	[119]
Interferons	α- interferon	Producing interferon response	Effective in clinical trial	[120]

IL: Interleukin.

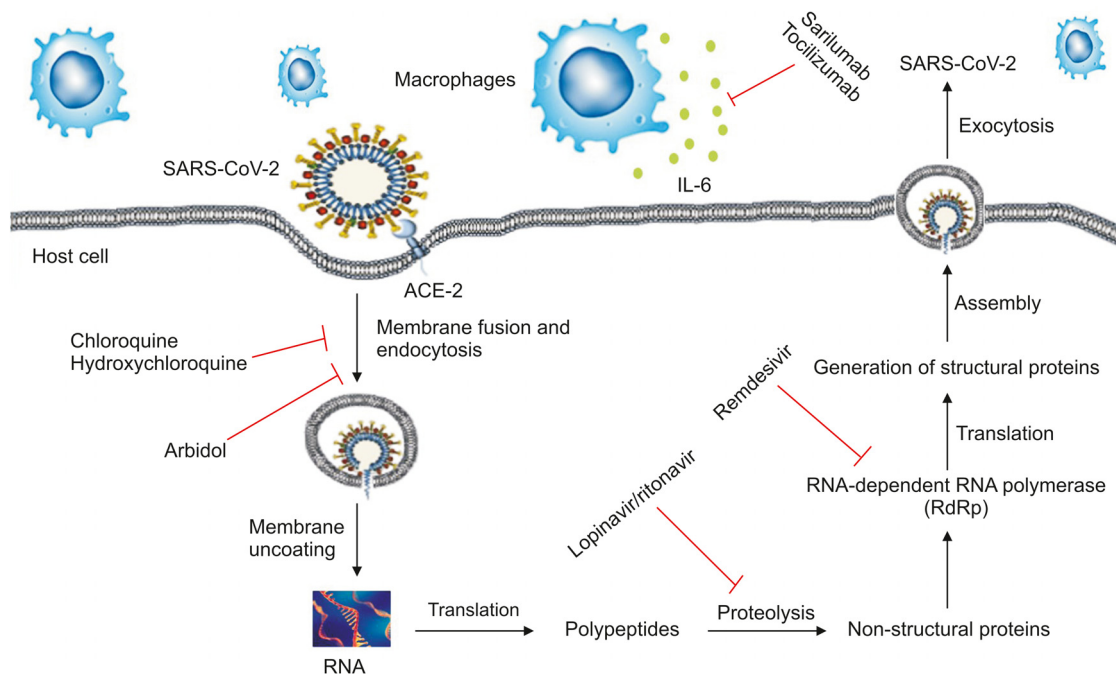


Fig. 3. Simplified representation of SARS-CoV-2 life cycle along potential drug targets. Arbidol, chloroquine, and hydroxychloroquine block membrane fusion or endocytosis. Lopinavir/ritonavir inhibits the proteolysis of translated peptide, whereas remdesivir targets RNA dependent RNA polymerase (RdRp) to inhibit SARS-CoV-2 protein generation. Immunomodulatory agents, sarilumab, and tocilizumab, target IL-6 receptor limit cytokine release. ACE2: angiotensin-converting enzyme-2; IL-6: interleukin-6.

recommended Indian herbal medicines as potential candidates to combat COVID-19 [15].

4.5.1. Ayurvedic treatment

Ancient Ayurvedic treatments have been applied in cases of various infectious diseases, and successful results have been obtained since ancient period. In the modern era of 2020, Ayurvedic treatment has been carried out in 43 patients to diagnose its efficacy against COVID-19 [18]. The treatments included systematic medicines, diet, and sleeping patterns. During the initial phase of 1–13 days of fever, patients were allowed to consume a diet of rice porridge, Yusha, Bhakta [140], Sudarsana Churna tablets [141], Dhanwantara Gutika tablets [142], and Talisadi Churna with honey [143], and patients were prevented from sleeping during the day as well as at night. After the fever subsided, the second phase of treatment, from days 14 to 30, included diets such as milk, ghee [143], and medicine Vidrayadi Ghrutam [144], and the sleeping pattern remained the same as that for the initial phase. Further deterioration from the disease was prevented, and the patients did not reach critical condition [18]. Likewise, traditional Chinese medicine (TCM) has attracted great attention during the COVID-19 pandemic, and more than 60,000 cases have been successfully treated with TCM [145]. Additionally, Shadanga Paniyaan herbal formulation and Ayurvedic medicine Agastya Harityaki are being widely used to treat high fever, headache, shivering, and respiratory problems. Indeed, the Ministry of Ayush, Government of India, recommends a combination of these herbal drugs against SARS-CoV-2. Some of the recommended Ayurvedic formulations such as Pippali rasayana, Laghu Vasant Malati, Sanjeevani Vati, Tribhuvan Keerti rasa, Brihata Vata Chintamni rasa, Mrityunjaya rasa, and Siddha Makardhvaja rasa were also assumed to be effective against moderate to severe SARS-CoV-2 infections [146,147]. Other recommended medicinal formulations by the Department of Ayush include Tirakatu and Tulasi to curb the coronavirus infections in India [20].

4.5.2. Homeopathic treatment

After the emergence of SARS-CoV-2 in India, the Central Council for Research in Homeopathy (CCRH), Government of India, recommended consumption of *Arsenicum album-30* for three consecutive days on an empty stomach, and repeated one month later if the infection prevailed [20]. However, reports regarding the success of the above treatment are still not available to form a concrete conclusion. Fifty patients in Italy with probable, suspected, and confirmed cases were treated with homeopathic medicines under isolated conditions, and no adverse conditions were recorded. Some ancient homeopathic medications that were prescribed against COVID-19 include *Bryonia alba*, *Arsenicum album*, *Phosphorus flavus*, *Atropa belladonna*, and *Natrum muriaticum*. Moreover, homeopathic treatment is considered as an individualized therapy for definite and specific pathological outcomes. Therefore, homeopathic ultra-high diluted solution products, such as *Aconitum napellus*, *Arsenicum album*, *Eupatorium perfoliatum*, *Gelsemium*, or *Ipecacuana*, could help treat early pathogenesis, while *Antimonium tartaricum*, *Baptisia*, or *Camphor officinalis* would be beneficial in later stages for both respiratory and systematic treatment [148–152].

4.5.3. Unani treatment

Historically, Unani treatment has been used to combat numerous diseases. According to the eminent Persian scholar Najeebuddin Samarqandi, Unani medicine has a beneficial role against Humma-e-Wabaiya (epidemic fever) and Nazla-e-Wabaiya (epidemic influenza). The symptoms of COVID-19 resemble those diseases mentioned, which enhance anti-COVID possibilities of Unani medicine [153]. Unani medicinal treatment has also been recommended by the Government of India against the virus. Patients were advised to consume Sharbat Unnab (10–20 mL), Sharbat Nazla (10 mL), Tiryaaq Arba (3–5 g), Ikseer Bukhar (2 pills), and Qurs e Suaal (2 tablets) twice daily along with lukewarm water. The Unani drugs *Lactuca sativa* (Tukhm and Kahu Mukashar) and *Rosa*

Table 2
List of vaccines undergoing clinical and preclinical trials.

Candidate	Characteristics of vaccine	Company	Stage/approval along with dates	Efficiency rate (%)	Refs.
Sputnik V	Non-replicating viral vector	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Approved in Russia (12 August 2020)	91.6	[129]
BNT 162 mRNA-1273	mRNA vaccine	Pfizer, BioNTech, Fosun Pharma	Approved in UK, Canada (9 November 2020)	95	[130]
ChAdOx1	Attenuated adenoviral construct expressing SARS-CoV-2 protein	Moderna/NIAID	Approved in USA (23 December 2020)	94.1	[131]
CoronaVac Inactivated	Inactivated	Oxford University/Astra Zeneca	Approved in UK (30 December 2020)	63	[132]
BBIBP-CorV	Inactivated	Sinovac Biotech	Limited use in China (July 2020)		[132]
Covaxin Ad5-nCoV	Inactivated Adenovirus type 5 vector that expresses SARS-CoV-2 spike protein	Sinopharm/Wuhan Institute of Biological Products	Limited use in China, UAE (September 2020)		[132]
NVX-CoV2373	Nanoparticle vaccine	Sinopharm/Beijing Institute of Biological Products	Approved in UAE (9 December 2020), China (31 December 2020)	79.34	[132]
Adeno-based (rAd26-S+rAd5-S)	Non-replicating viral vector	Bharat Biotech	Approved in India (3 January 2021)		[132]
Bacillus Calmette-Guerin GX-19	Repurposed vaccine DNA vaccine	CanSino Biologicals	Phase 3	65.7	[133]
ARCoV	mRNA vaccine	Novavax	Phase 3		[133]
ZyCoV-D Inactivated	DNA vaccine	Gamaleya Research Institute	Phase 3		[133]
—	mRNA vaccine	Murdoch Children's Research Institute	Phase 2/3		[133]
LNP-nCoVsaRNA	RNA vaccine	Genexine	Phase 1/2		[133]
Live attenuated virus	Codon deoptimized live attenuated vaccines	Academy of Military Medical Sciences, Suzhou Abogen Biosciences and Walvax Biotechnology	Phase 1		[133]
GRAd-COV2 QazCovid	Adenovirus type Inactivated	ZyklusCadila	Phase 2		[134]
INO-4800	DNA vaccine	Institute of Medical Biology at the Chinese Academy of Medical Sciences	Phase 2		[134]
'Molecular clamp' vaccine platform	Fast-response technology	CureVac	Phase 2		[134]
MVA-VLP	Modified vaccine Ankara-Virus like particles vaccine platform	Imperial College London	Phase 1/2		[134]
Anhui Zhifei Longcom's vaccine (NCT04466085)	Protein subunit vaccine	Codagenix/Serum Institute of India	Preclinical		[134]
		ReiThera	Phase 1		[134]
		Research Institute for Biological Safety Problems	Phase 1		[134]
		Inovio Pharmaceuticals	Phase 2/3		[135]
		University of Queensland	Phase 1		[136]
		GeoVax-BravoVax	Preclinical		[137]
		Institute of Microbiology and Anhui Zhifei Longcom Biopharmaceutical	Phase 2		[138]

damascene (Gule Surkh) are used to treat sore throat and can possibly curb infections [20]. Moreover, in terms of sanitation, air purification, and immune modulation, Unani medicine could also be effective in reducing SARS-CoV-2 infections [154].

4.5.4. Herbal treatment

Immunity status plays a crucial role in COVID-19 patients, and herbal medicine accounts for immunomodulatory activities exhibiting anti-COVID possibilities [155,156]. The herbal extracts of *Anthemis hyaline*, *Nigella sativa*, and *Citrus sinensis* have been reported to inhibit corona virus replication in vitro [157]. An extract from *Eupatorium fortunei*, a well-known herbal medicinal plant, can inhibit RNA viruses, including human coronavirus [158]. These probable herbal drugs can be tested against SARS-CoV-2 and may become an essential therapeutic agent against it. Additionally, the medicinal plant *Artemisia annua*, recommended by the World Health Organization, may act as a possible treatment against COVID-19 [159]. Interestingly, the extracts of 13 herbs, including *Forsythia suspense*, *Ephedra sinica*, *Lonicera japonica*, and *Isatis indigotica*, were tested in SARS-CoV-2 infected Vero E6 cells [21]. They were found to inhibit viral replication, affect the morphology of viral cells, and reduce the production of pro-inflammatory cytokines. Moreover, many countries have used curcumin for its effective antioxidant, anti-inflammatory, anti-mutagenic,

anticancer, anti-Alzheimer's disease, and antibacterial activities, and owing to its broad range of activities, curcumin could also be used as an efficient anti-COVID therapy [160–162].

5. Conclusion and future perspectives

In the current scenario, steps such as countrywide lockdown, self-quarantine, social distancing, and control of infection sources have been initiated to ensure the safety of the people. These restrictions may have beneficial effects in the short-term, but partially fail in the long-term owing to economic issues. Finding ways to live with this virus is the best option using preventive care, which can reduce the financial crisis. However, the exact mechanism of this viral infection is still unclear and needs to be validated by adequate clinical trials, advanced diagnosis, and therapies to combat this devastating situation.

The optimization of diagnostics and treatment strategies is a dynamically developing field during this pandemic outbreak, supported by contemporary medicine and compatible healthcare protocols. Scientists and clinicians have focused on implementing the most sensitive, specific, rapid, and reliable diagnostic toolkit; however, the COVID-19 lacks sufficient data to determine the standards for interpretation by PoC devices. Advocation of a treatment strategy using a conventional drug, respiratory therapy,

plasma therapy, vaccine development, complementary and alternative medicine may pave the way towards a successful treatment paradigm. Nevertheless, efforts of thorough treatment development might be a gamble because of safety issues. In this context, commercialized vaccines promise the hope to delay or stop disease progression, especially in older adults with a higher risk of being affected by COVID-19.

In conclusion, this review helps collate knowledge about the current diagnosis and therapeutic scenario, including rapid molecular, serological, digital-based detection, traditional drug treatments, molecular therapies, complementary and alternative drug treatments against SARS-CoV-2; these techniques may help stabilize the clinical crisis faced by the world.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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