Advances in Bioresearch
Adv. Biores., Vol 11 (5) September 2020: 143-149

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.5.143149

Advances in Bioresearch

REVIEW ARTICLE

An extensive view of COVID-19: its diagnostic method and therapeutic potentials

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ABSTRACT
Recently, a new coronvirus (SARS-CoV-2) first reported in December 2019 in Wuhan city of China has expanded rapidly and become global health concern with 4,534,731 confirmed cases in more than 216 countries and regions as per 17th may, 2020. SARS-CoV-2 marked as third highly pathogenic and epidemic coronavirus introduced into human population in 21st century along with middle east respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV). Researchers have identifies SARS-CoV-2 as β-coronavirus with high identical genome to bat coronavirus pointing to bat as natural host along with pangolin as intermediate host. Clinical features of COVID-19 infection include cough, fever, fatigue and gastric disturbance sometime. At present, there is no vaccine or any effective therapeutic has been developed for the treatment of this viral infection although some antiviral drugs (Remdesivir, chloroquine/hydroxychloroquine, lopinavir/ritonavir, nitazoxamide) and convalescent plasma therapy are some potent therapeutic options used by professionals to treat COVID-19. This article summarise current update about its origin, evolution, diagnostic methods and potential therapeutics.
Keywords: *SARS-CoV-2, SARS-CoV, MERS-CoV, COVID-19*

Received 21.04.2020 Revised 01.06.2020 Accepted 21.07.2020

How to cite this article:
T Sharma, V Khandelwal. An extensive view of COVID-19: its diagnostic method and therapeutic potentials. Adv. Biores., Vol 11 (5) September 2020: 143-149

INTRODUCTION

On 12th December 2019, first case of virus induced respiratory syndrome was reported in Wuhan city, Hubei province, China and soon it becomes the centre for the outbreak of pneumonia of unknown cause. Outbreak of this viral induced respiratory distress syndrome makes a pause in the world and become major health concern globally [1]. On 7th January 2020, novel corona virus has been isolated from a patient by Chinese scientists. First it was identified as pneumonia of unknown etiology as its causative agents were not identified. On 30th January 2020, WHO declared COVID-19 to be a Public Health Emergency of International Concern. On 11th February 2020, WHO announced that the disease caused by COVID-19, which is the acronym of "corona virus 2019." Risk assessment at the time was guarded but the outbreak was more like the caused by Middle East Respiratory Syndrome (MERS) corona virus than sever acute respiratory syndrome (SARS) corona virus [2].
As per the report of WHO, there has been 4,534,731 confirmed cases of COVID-19 including 307,537 deaths reported worldwide since December 2019 to may 17, 2020 [3].

ORIGIN, EVOLUTION AND STRUCTURAL ANALYSIS

Belonging to the family *coroniviridea* and sub-family *coronavirinae,* there are four genera of coronaviruses: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Deltacoronavirus, in which alpha and beta infects mammals and gamma and delta infects birds, fishes and also mammals sometimes. Before 2019, only six types of coronaviruses were known to infect humans in whom SARS-CoV and MERS-CoV cause severe respiratory syndrome. Symptoms of new CoV are milder than SARS and MERS. Other members of this family are also responsible for infection in different species including camels, cats, bats, cattle and cause respiratory, enteric, hepatic and neurological diseases, amongst them seven human corona viruses have been identified to infecting humans as given in table 2 [5].

Table 1. Confirmed cases of COVID-19 in different countries till 17th may, 2020 as per the report of World Health Organisation

Country	Confirmed cases	Deaths
United State of America	1,409,452	85,860
Russian Federation	281,752	2,631
The United Kingdom	240,165	34,466
Spain	230,698	27,563
Italy	224,760	31,763
Brazil	218,223	14,817
Germany	174,355	7,914
Turkey	148,067	4,096
France	140,008	27,578
Iran	120,198	6,988
India	90,927	2,872
Peru	84,495	2,392

Table 2. List of human pathogenic coronavirus

Host	Virus	Genus	Symptoms
Human	Human CoV-229E	Alpha	Mild Respiratory tract infection
	Human CoV-NL63	Alpha	Mild Respiratory tract infection
	Human CoV-HKU1	Beta	Pneumonia
	Human CoV-OC43	Beta	Mild Respiratory tract infection
	SARS-CoV	Beta	Sever acute Respiratory syndrome, 10% mortality rate
	MERS-CoV	Beta	Sever acute Respiratory syndrome, 37% mortality rate
	SARS-CoV-2	Beta	Lower Respiratory tract infection, Pneumonia

Corona viruses are enveloped positive sense single stranded RNA virus (+ss RNA virus) which looks like crown under electron microscope due to the presence of spike glycoproteins on the envelop which targets primarily the human respiratory system. Genome-wide phylogenetic analysis indicates SARS-CoV-2 has 79.5%, 50% and 96.3% sequence identity to SARA-CoV, MERS-CoV and a bat coronavirus respectively. Virion of SARS-CoV-2 possesses a neucleocapsid composed of genomic RNA and phosphorylated neucleocapsid protein. These nucleocapsid covered by two different types of spike proteins: the spike glycoprotein trimmer and hemagglutinin-esterase. Location of membrane protein and envelop protein found among the spike protein in the viral envelop. Its genome is composed of 11ORF (Open Reading Frames), with the length of 29,903bp with gene order 5'-ORF1ab-S-E-M-N-3' which encodes for 9860 amino acids and 29 proteins [6]. Four structural proteins: spike surface glycoprotein (S), small envelop protein (E), nucleocapsid protein (N) and matrix protein (M) involved in the formation of SARS-CoV-2 virion in which S genes codes for the receptor bind spike proteins that enable the virus to infect cells and other three proteins are involved in RNA and/or in protein assembly, envelop formation, budding and pathogenesis.

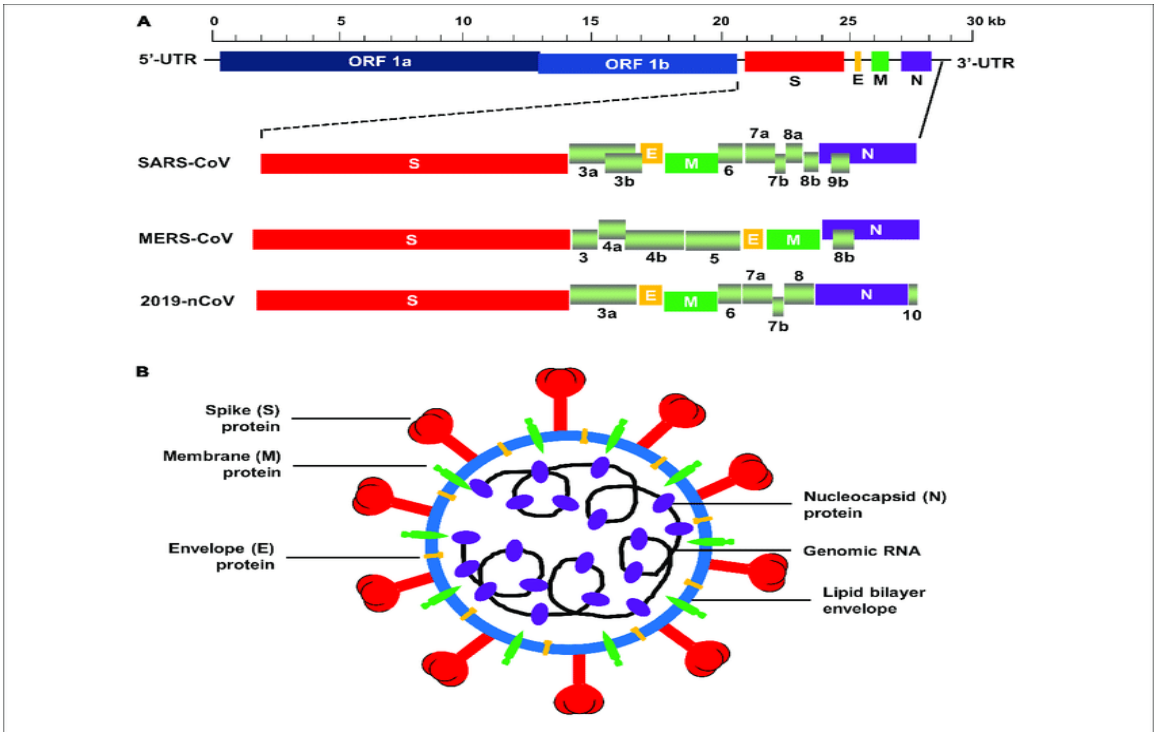

Figure 1- Diagram of SARS-CoV-2 virion with gene sequence of S, E, M, N proteins in SARS-CoV, MERS-CoV, SARS-CoV-2

Virus (Disease)	Origin Virus	Intermediate host	Host
SARS-CoV-1 (SARS 2002)	SARS-like Bat-CoV	Civet Cat	Humans
MERS-CoV (MERS 2012)	SARS-like Bat-CoV	Camel	
SARS-CoV-2 (COVID 2019)	BaT-CoV RaTG13	Pangolin (could be origin as well [Pangolin-CoV])	

Figure 2- Transmission of SARS-CoV, MERS-CoV and SARS-CoV-2

A suspect case is identified with cough, sore throat and fever, clinical features may be asymptotic or without fever. Virus may isolated from respiratory samples (throat swab/nansopharyngeal swab/branchoalveolar lavage/sputum) and spread through primarily respiratory tract, droplet, direct contact and respiratory secretions in acute condition whereas in chronic conditions virus has been also isolated from stool and blood, indicating the possibility of multiple route transmission. Studies have been found that elderly and patients underlying diseases like lung infections, cardiovascular, diabetes, hypertension account for a large proportion of COVID-19 [7]. Studies have found that patients underlying lung infection, cardiovascular, diabetes and hypertension account for a large proportion of COVID-19 [8].

DIGNOSTIC METHODS

National Medical Products Administration (NMPA), China, approved 11nucleic acid based methods and eight antibody detection kits for the diagnosis of SARS-CoV-2. Laboratory tests available for the diagnosis of SARS-CoV-2 includes Real-Time Polymerase Chain Reaction (RT-PCR), immunological tests for the detection of antibodies and antigens, viral culture techniques for the isolation of virus, indirect fluorescent antibody techniques, immunofluorescent techniques and rapid immunochromatographic tests assisted by flow cytometry analysis for CD4+ and CD8+ T cell counts, complete blood picture (to demonstrate lymphopenia), chest radiography (pneumonia) and serum biochemistry (serum protein and others) [9].

Nucleic acid testing: RT-PCR involves the reverse transcription of SARA-CoV-2 RNA into complimentary DNA (cDNA) strands followed by amplification of the cDNA. In this process first step involves sequence alignment and primer design followed by assay optimization and testing. Studies have been reported that there are 3 regions of conserved sequences: the RdRP gene (RNA dependent RNA polymerase gene) in the open reading frame ORF1ab region, the N gene (nucleocapsid protein gene) and the E gene (envelop protein gene) in which RdRP and E genes showed highly analytical sensitivity for detection than N genes. RT-PCR predominantly used for the diagnosis of respiratory samples. Currently, nucleic acid tests using isothermal amplifications are developed for the detection of SARS-CoV-2. These techniques involve recombinase polymerase amplification, loop mediate amplification (LAMP) and helicase dependent amplification. Currently, protocol of SHERLOCK, a detection strategy that uses Cas13a ribonuclease for RNA sensing has also been released and used by many laboratories for clinical diagnosis of SARS-CoV-2 [10]. **Protein testing:** protein testing method utilises the viral protein antigens and antibodies created in response to SARS-CoV-2 infection. Serological tests use enzyme-linked immunosorbent assay (ELISA) which detect IgG and IgM from serum of COVID-19 patient [10]. **Point-of-care test:** these tests are used to diagnose the patients without laboratory infrastructures. Lateral flow assay and microfluidic devices are example of piont-of-care tests. In lateral flow assay, sample of patient (blood, urine etc.) deposited on a paper like membrane strip coated with two lines each contain gold nanoparticle-antibody conjugates and captured antibodies. Antigen binds on the gold nanoparticle-antibody conjugate and further immobilised by the captured antibodies leads to red or blue coloured visible lines [10]. There are some emerging diagnostic techniques describes in table 3, which can play an important role in identifying and managing the spread of COVID-19.

Table 3. Some emerging diagnostic methods developed for COVID-19

Technique	Clinical sample used	Biomarker	How it works
RPA	Serum	Nucleic acid	PCR, perform CRISPR/Ca9-mediate lateral flow nucleic acid (CASLFA)
RT-RPA	Nasopharyngeal swab		RPA, SHERLOCK multiplexed single detection via fluorescence
LAMP	Throat swabs		Isothermal DNA synthesis using self-reoccurring strand displacement reactions; positive detection leads to increased sample turbidity
RPA	Fecal and nasal swabs		forward and reverse primers blind to DNA and amplify strands at 37 °C
Real-time NASBA	Nasal swabs		transcription-based amplification for RNA targets
Rolling circle amplification	Serum		DNA polymerase used to extend a circular primer and repeatedly replicate the sequence
LAMP	Nasopharyngeal aspirates		reverse transcriptase LAMP reaction for RNA target
Magnetic biosensor	Serum	Protein	Magnetic separation of protein targets
ELISA	Serum		enzymatic reaction to produce colored product in presence of target
Digital ELISA	Serum		digital readout of colored product by enzymatic reaction in presence of target
Lateral flow	Serum		gold-coated antibodies produce colorimetric signal on paper in presence of target

PATHOGENESIS

Clinical manifestations of COVID-19 include fever, non-productive cough, myalgia, fatigue, normal or decreased leukocyte counts and radiographic evidence of pneumonia. Symptoms are very similar to SARS-CoV and MERS-CoV infections.

Virus entry and spread: predominantly SARS-CoV-2 transmitted through respiratory droplet contact and potential in fecal-oral. Primary viral replication occurs in mucosal epithelium of upper respiratory tract, with further multiplication in lower respiratory tract and gastrointestinal mucosa. Some patients have also demonstrated non respiratory symptoms such as diarrhoea, acute liver and heart injury, kidney failure, implying multiple organ involvement. **Pathological findings:** in first report of pathological finding of COVID-19, right lung showed acute respiratory syndrome due to desquamation of pneumocytes and hyaline membrane formation whether left lung tissue demonstrate pulmonary edema with hyaline membrane formation which suggest early phase acute respiratory distress syndrome. In death cases with COVID-19, both lungs had been found with massive mucus secretions. **Acute respiratory distress syndrome (ADRS):** ADRH accounts for mortality of most respiratory disorder; this condition prevents enough oxygen from getting to the lungs and into circulation. In fatal cases individual require mechanical ventilation. **Cytokine storm:** rapid viral replication predominantly causes massive endothelial and epithelial cell death and vascular leakage, eliciting the production of exuberant pro-inflammatory cytokines and chemokines. Recently, a well known virological phenomenon, antibody dependent enhancement (ADE) has also been confirmed in multiple viral infections. **Immune dysfunction:** lymphopenia found to be a common feature of COVID-19 in many cases. Patients with severe infection showed reduction and hyperactivation of CD4 and CD8 T cells. Such conditions are accounting for severity and mortality [11].

Therapeutic potential

There is no vaccine or antiviral drug has been developed for the potential treatment of COVID-19, therefore some supportive treatment including oxygen therapy, antiviral drugs, plasma transfusions, vaccines etc. are currently in use based on the previous therapeutic experience of SARS and MERS. World Health Organisation (WHO) activated R and D Blueprint to accelerate diagnosis, vaccines production and therapeutics for this pandemic. Aim of this blueprint is to accelerate research and development process and provide coordination between scientists and global health professionals [12].

Table 4. Potential antiviral drugs against SARS-CoV-2

Drug	Antiviral mechanism	Therapeutic effect
Lopinavir/Ritonavir	Viral protease inhibitors	Didn't decease the viral load and have gastrointestinal side effect, No benefit was observed in hospitalized adult patients with severe Covid-19
Disulfiram		Has inhibitory activity against SARS and MERS
Favipiravir	Viral nucleoside analogs	Could suppress SARS-COV-2 infection at a high concentration
Remdesivir		Could block SARS-CoV-2 enter Vero E6 cells, Reveled the possible inhibition mechanism of SARS-COV-2 RNA polymerase by Redesivir effector molecules, effective in treating severe COVID-19
Ribavirin and Galidesivir		No evidence, Have therapeutic effect on patients with SARS and MERS but lacking the evidence for SARS-COV-2
Chloroquine	Viral host fusion inhibitors	Suppression of COVID-19 pneumonia exacerbation, reduction of symptom duration,
Hydroxychloroquine		significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin
Arbidol		can effectively inhibit SARS-CoV-2 infection at a concentration of 10-30 μM
Nitazoxanide		Have inhibition activity of the SARS-COV-2
IDX-184		Have effective against SARS-CoV-2 strain.
Sofosbuvir		Tightly combine with SARS-CoV-2 RdRp for viral clearance
Corticosteroids		Low-dose corticosteroids may be beneficial for the survival of severe COVID-19 patients,
Baricitinib	JAK signaling pathway inhibitor	Baricitinib could also abate SARS-COV-2 virus infectivity

Antiviral drugs

Antiviral drugs can be classified in three categories: viral protease inhibitor, viral nucleoside and agent that disrupt the interaction mechanism between the virus and the host. **Favilavior:** favilavior released by China, which was initially used to treat influenza (in Japan) and nose and throat infections, found to be effective on more than 70 patients with minimal side effects in a clinical trial. **Remdesivir:** remdesivir is an adenosine analogue which can block viral RNA synthesis by targeting RNA dependent RNA polymerase. Originally designed to target Ebola is now used as promising antiviral drug against a wide array of RNA viruses including SARS, MERS and SARS-CoV-2. **Chloroquine and Hydroxychloroquine:** both are used to treat malaria, can reduce viral load and shorten the duration of symptoms. In a study these drugs are found to be more reliable to the exacerbation of pneumonia, improving lung imaging findings, shortening the disease course and promoting a virus negative conversion but there are also some side effects like nausea, worsening vision, digestive disorders and even heart failure in severe conditions. **lopinavir/ritonavir:** antiretroviral drug, designed to treat HIV (AIDS) showed some activity against SARS-CoV in 2003. WHO suggested that it could be beneficial when used with some others drugs like interferon-β, oseltamivir or rivavirin [13, 14, 16]. Some other drugs like Tocilizumab (used to treat sever to moderate rheumatoid arthritis), darunavir (anti-retroviral HIV-1 protease inhibitor), ivermectin (anti-parasite) may also have probable efficiency to treat SARS-CoV-2 however clinical trials need to be completed and to confirm the expediency of the drug in humans.

Convalescent plasma therapy

Convalescent plasma or immunoglobulin has a great therapeutic history against Influenza (1918), measles (1930), H1N1 (2009), Ebola virus disease (2014), SARS (2003) and MERS (2015) without any adverse effect. Plasma utilizes the blood plasma with high neutralizing antibody titer from the recovered patient of COVID-19. This therapy is more effective before the virus cause serious damage in patient's lungs [7]. CR3022, human monoclonal antibody of SARS-CoV can bind with receptor binding domain of SARS-CoV-2 and has the potential to be developed as potential therapeutic of SARS-CoV-2 infection. Some other monoclonal antibodies like m396, CR3014 could also act as alternative for the treatment of COVID-19 [15].

Vaccine

After watching severity and uncontrolled outbreak of this pandemic, development of effective SARS-CoV-2 became first priority of scientists worldwide. There are several vaccination strategies including live-attenuated virus, inactivated virus, subunit vaccines, viral vectors, recombinant DNA, and proteins vaccines tested in animals but it requires additional manufacturing steps and some formal toxicological testing therefore it expect 12-18 months to develop a vaccine against SARS-CoV-2 according to researchers [17].

Table 5. Major vaccine development programs

Consortium	Candidate vaccine	Status
University of Melbourne and Murdoch Children's Research Institute (Australia); Radboud University Medical Center (The Netherlands); Faustman Lab at Massachusetts General Hospital (MGH) (United States)	Bacillus Calmette-Guerin (BCG) live-attenuated vaccine for COVID-19	Phase 3
Kaiser Permanente Washington Health Research Institute	m-RNA 1273	Phase 2
Beijing Institute of Biological Products/Wuhan Institute of Biological Products; the China National Pharmaceutical Group (Sinopharm)	No name announced	
Tongji Hospital; Wuhan, China	Ad5-nCoV	Phase 1
The University of Oxford	ChAdOx1	
Inovio Pharmaceuticals	INO-4800	
Pfizer and BioNTech	BNT162	
Sinovac	PiCoVacc	

Data summarized in table 5 modified from https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker

CONCLUSION

In conclusion occurrence, development and severity of SARS-CoV-2 infection depends upon the interaction between virus and host's Immune system. Age, gender, nutritional status, genetics, neuroendocrine-immune regulation and physical status may contribute to the infection, severity and reinfection of virus. In the former situation, we will urgently require effective therapeutic or vaccine for the cessation of this pandemic, however challenge remains as we saw that SARS-CoV-2 is third coronavirus epidemic found in last two decades includes SARS-CoV in 2000s and MERS CoV in 2010s.

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