How to Cite:
Maryam, S., Fatima, R., Ashfaq, M., Hassan, N., Bibi, A., Syed, A., & Kamran, M. (2023). Epidemiological studies of COVID-19 disease: A story of SARS CoV-2 infection from origin to hospital. *International Journal of Health Sciences*, *7*(S1), 990–1030. https://doi.org/10.53730/ijhs.v7nS1.14303

Epidemiological studies of COVID-19 disease: A story of SARS CoV-2 infection from origin to hospital

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***Abstract*---**The Chinese population having epidemiological link with wholesale market of seafood develop respiratory illness with pneumonia like clinical presentations in Hubei Province, Wuhan City, recently in December 2019. Laboratory results revealed a novel coronavirus that was named severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) and disease it caused given name of coronavirus disease 2019 (COVID-19). COVID-19 turn out to be third outbreak of human pathogenic coronaviruses and first pandemic of 21st century. The previous coronaviruses proved merely as a tip of iceberg after emergence of recently identified SARS-CoV-2 with potential of pandemic status that significantly revealed the concealed

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2023.
Manuscript submitted: 09 Jan 2023, Manuscript revised: 18 March 2023, Accepted for publication: 27 April 2023

capabilities of virulence and contagiousness of betacoronaviruses group. The aim of this review is to discuss the origin of COVID-19, transmission patterns, susceptible hosts, factors that increase disease severity, nucleic acid base diagnosis, mortality and morbidity rates and therapeutic option against COVID-19. The diagnostic methods like microarray and RNA-targeting CRISPR were adopted recently for COVID-19 diagnosis after advancing them further for rapid and accurate detection of COVID-19 and high throughput.

***Keywords*---**COVID-19, pandemic, coronaviruses, origin, epidemiology.

Introduction

At the end of 31st December 2019, Chinese people were diagnosed with respiratory tract infections of an unidentified etiology in the hospital of Wuhan, Hubei Province [1]. Later, a novel coronavirus was identified on 7th January 2020 and named Nov-19 novel coronavirus 2019 by WHO (World Health Organization) [2]. The viral nomenclature, ICTV (International Committee on Taxonomy of Viruses), replaced the Nov-19 with SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) name as it was geneically parallel to the previous SARS coronavirus [3]. The disease caused by SARS-CoV-2 named Coronavirus Disease 2019 (COVID-19) officially by WHO [4]. After its emergence, COVID-19 spread rapidly globally [5], becoming a significant public health concern for researchers worldwide [6-8]. The size of the betacoronavirus genome is 26-32kb approximately while overall virus size is about 60-140nm [9, 10]. The recently identified SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) genome entirely contain of 29844 to 29891 coding nucleotides approximately with lacking the gene of hem agglutinin-esterase1. The genome of virus is roofed in capsid which symmetry is helical and constructed from structural proteins, the genome and capsid is collectively termed as nucleocapsid. Further, nucleocapsid is wrapped by a membrane known as envelope that is assembled by lipids and proteins [11]**.** All these membranous structures prevent the environmental factors influence on virus and ensure its safety in unfavorable conditions [12]. Moreover, spikes are protruded from envelope which is made of glycoproteins and these structures give the virus appearance like crown [13]. Based on this morphology, it has been named as coronavirus by ICTV [14]. Furthermore, those glycoproteins in spikes are the essential part of viral structural proteins (VSP), additionally, it play role in the binding of virus to susceptible host cell and entry of genetic material [15]**.** There are two domains in spikes glycoproteins with different roles, one is associated with viral envelope and other is the part of receptor binding portion [16]. So far there is total 7 different coronavirus have been reported throughout the world, among which 3 are emerged in past 18 years in the 21st century while other were reported in 20th century, which are briefly discussed here.

The Emergence of Coronaviruses and its association with economy loss

Before the emergence SARS coronaviruses and MERS coronaviruses in 21st century [17], the first coronavirus was reported in 1960, which infection was associated with respiratory tract and after that other coronaviruses were also started reporting and a total of 4 coronaviruses were emerged in 20th century. All of them target the respiratory tract of humans, those viruses are 229E, NL63 (alpha coronaviruses) and OC43, HKU11 (beta coronaviruses) [18]. All these coronaviruses were infecting animals and birds, caused various important diseases from agriculture point of view essential disease. Among which avian infectious bronchitis virus (IBV) causes great economy loss in Brazil, about of $3567.40/1000 birds by infecting kidney of the birds and causes diminish eggs production [19]. IBV also reported to cause infection in other animals like cattle; elk, camel and deer by targeting their gastrointestinal tract, which infection led to significant economy loss in the form reduce milk and meat production [20]. Various other agriculturally important diseases has been reported to cause by coronaviruses such as PEDV (porcine epidemic diarrhea virus) that mainly target the pigs, from 2012 to 2014 there have been noticed 3.2% pig production in the North America due to PEDV infection [21]. Other animal viruses include SARS-CoV (swine acute diarrhea syndrome coronavirus) also called TEGEV (transmissible gastroenteritis virus) that causes transmissible gastroenteritis [14]. FCoV (Feline enteric coronavirus) is another highly pathogenic virus infecting animals such as domestic cats which infection is normally symptomatic but sometimes asymptomatic as well and infection patterns are mild. There is another more virulent strain of FCoV was reported that causes persistent infection and the strain named FIPV (Feline Infectious Peritonitis Virus) and the diseases is caused is feline infectious peritonitis (FIP) [22]. Furthermore, coronaviruses also reported to infect the marine animals, the respiratory symptoms and liver failure was observed in deceased whale, the specimens from liver and respiratory tract from whale were evaluated for electron microscopy which did not identify viral pathogen. However, γ-coronavirus was revealed by phylogenetic analysis clearly, although, it was not persuaded the infection of coronavirus pathogen in whale associated with those symptoms nor it was proved experimentally [23]. But there is possibility of damage of marine life as well, in terms of reduction in fish meat production.

Human Pathogenic Coronaviruses in 21st Century

In the 21st century, three highly infectious betacoronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2, had been reported, associated with higher morbidity and mortality rates, it is thought that these viruses emerged due to genetic recombination in animal coronaviruses and have mutated to more virulent strains and transmitted to humans through spill over from animals [17]. SARS-CoV was emerged in in Chinese population in 2002, in Foshan City, Guangdong Province23. However, till July 2003, the infection had been brought under control, during the epidemic period 8,098 were affected from virus and 774 were lost their lives due to this deadly infection [24]. Unexpectedly, another coronavirus was emerged in Kingdom of Saudi Arabia (KSA) in 2012, which was named MERS-CoV (Middle-East respiratory syndrome coronavirus), its infection appeared to be lethal that SARS-CoV which mortality rate was approximately 37% and 2206 people were affected with MERS infection [25].

SARS-CoV-2 (COVID-19)

In recent times, the Chinese population was diagnosed with respiratory illness of unidentified pathogen, with pneumonia like symptoms towards the end of December [26, 27]. Initially, the disease was believed as pneumonia by healthcare officials; however, thorough analysis of patient's throat sample by molecular based diagnostic method and sequencing method detected a novel coronavirus [28]. Hence, on 7th January another coronavirus pathogen of the humans, was discovered [28], provisionally, it was named as novel coronavirus 2019 (nCoV-19) by World Health Organization (WHO) [29]. Because of having genetic similarity with SARS-CoV, it was recognized as SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) by viral nomenclature scheme [30]. The disease caused by SARS-CoV-2 was initially named viral pneumonia but later, WHO given it the name of Coronavirus Disease 2019 (COVID-19) because of some unique disease symptoms of lower respiratory tract like difficulty in breathing [31], other symptoms of COVID-19 are fever, fatigue, coughing, sneezing and sputum production, diarrhea is also reported in severe cases an systemic infection [32-35]. Moreover, the patients also suffered from mental dissatisfaction in severe cases of COVID-19, acute kidney failure and dysfunction of various other organs [36]. The disease symptoms appear after 14 days of incubation [37] and 4-5 days is median [38]. However, incubation period fluctuated based on the patient health status and immunity and it can be shorter in older persons and the persons with weak immune system [39]. COVID-19 transmitted through respiratory droplets [40] very rapidly because of more contagious temperament. After emergence, 42 people were get infection in China in only three days [32]. Across the country, disease spread very rapidly, however, on 13th January, Thailand reported the first outside cases [41]. Similarly, the first death case due to COVID-19 was reported by Philippines, who was the resident of Wuhan and he develops disease symptoms after his arrival in country and after 1 week he died [42]. Comparatively, there is more virulence, pathogenicity and contagiousness observed in newly emergent novel coronavirus disease, which spread very quickly across China, and then China to globe and WHO declared COVID-19 as pandemic on 11th March, 2020 [43]. The infection continued spreading persistently and as of 3rd September, 2020, a total 26,050,603 infection cases reported globally with 863,468 deaths with 3.31% fatality rate [44] and besides toward this, figure is supposed to increases with enduring epidemic. The non- non-availability of vaccines, rapid diagnostic methods and therapeutic options, international travel, highly dense population and frequent contact between human to human and human with surfaces have played significant role the disease spread. Literally, the emergence of COVID-19 revealed hidden potential of coronaviruses and stressed the 21st century [13].

The world has become grabbed by the COVID-19 pandemic and alarming situations has been caused throughout the world. A cluster of questions has been posed by this pandemic, which need to inquired simultaneous investigation about COVID-19. This review illustrated the etiology of COVID-19, nature and origin of etiological agent, mode of transmission, host susceptibilities, molecular based diagnostic methods, therapeutic options, vaccines and prevalence throughout the world with the purpose of valid and utmost awareness concerning circumstances and update intercessions. Figure 1 is the schematic diagram of the present review

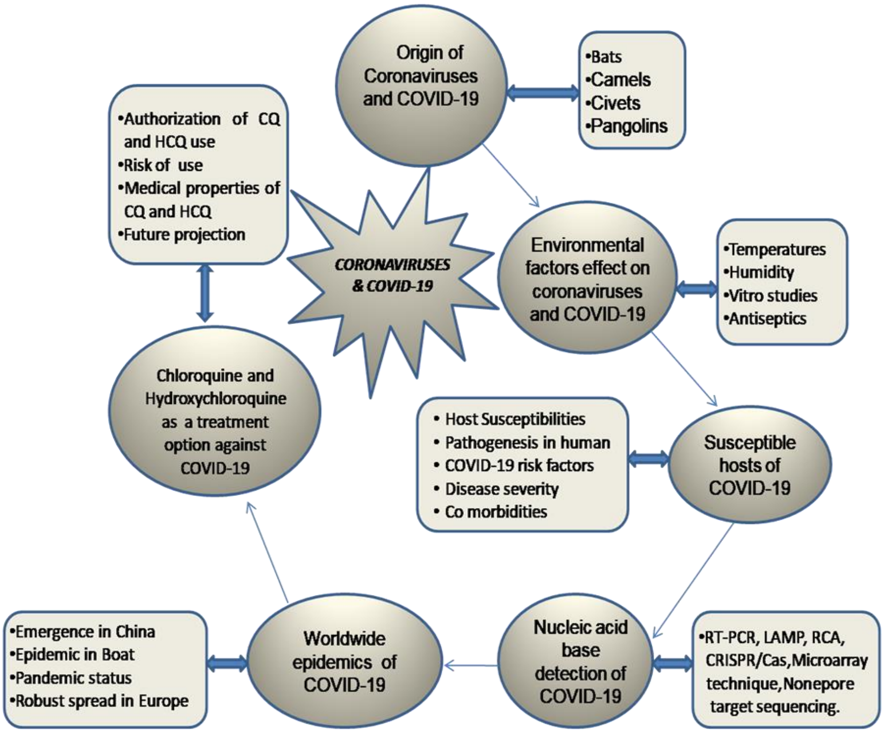

Figure 1. The schematic diagram illustrated the origin of COVID-19, transmission patterns, susceptible hosts, factors that increase disease severity, nucleic acid base diagnosis, mortalities and morbidity rates and therapeutic option against COVID-19.

Origin of SARS CoV-2

Globally, emergence of viral infections has threatened the safety and security of human health and has been a major public health problem [45]. The sudden and unexpected emergence of recently identified novel coronavirus in China [27] poses a cluster of questions related to its origin. In absence of intentional and firm epidemiological studies it was difficult to determinedly indicate the SARS-CoV-2 origin. However, the origin of previous SARS-CoV has broadly discussed while marking the origin of SARS-CoV-2 origin [46]. Unexpectedly, all the hospital enrolled patients affected with COVID-19 were found in link with Wholesale market of seafood and wet animals in Wuhan [47]. Globally, the scientific community has been troubled considerably by newly emergent coronavirus and concern of various researchers around the world has been provoked in the tracing of exact origin. Retrospectively, the previous human coronaviruses were emerged from animals, SARS-CoV from bats and MERS-CoV from camels and also reported in bats [48]. In the outbreak of SARS-CoV, various animals were pointed to mark the exact reservoir of the virus, however, Himalayan palm civets and raccoon dogs was marked as predictable reservoir of SARS CoV [49]. During analysis of bats revealed genetically similar virus to SARS-CoV [50, 51]. In 2002, another coronavirus (MERS- CoV) was emerged in KSA, with similar disease symptoms to SARS-CoV disease. Enthusiastic search for origin of MERS-CoV was initiated due to lack of serological evidence and previous history of exposure [5, 52-54]. Founded on the experience of the SARS coronavirus, MERS coronavirus was believed to be transmitted from bat as well, however eventually a coronavirus similar to the one isolated from infected patient in Saudi Arabia was identified in camels subsequent to concise analysis of supposed animals, hence camels were recognized as origin of MERS coronavirus [55]**.** The coronavirus isolated from camel was 90% genetically similar to the one isolated from infected patients [56]. Bur literally, camels were recognized as potential reservoir of MERS CoV [48].

Role of SARS and MERS-CoV in marking the origin of SARS-CoV-2

The origins of SARS and MERS CoV have played significant role in the marking exact origin of SARS-CoV-2, mammals like bats and pangolins were predicted as potential reservoir of SARS-CoV-2. The pathogens identified from various throat samples of hospital enrolled patients in Wuhan, revealed the 99.9% genetic similarity, which indicated the new host genetic shift [39]. Earlier, the zoonotic origin was already clued by the link of infected patients with seafood and wet animals market [57, 58], which were postulated as potential origin of SARS-CoV-2 and animal origin was recommended [47], but intense pursue was initiated to trace the exact origin [32]. The similarity of SARS-CoV-2 with SARS coronavirus, led the researcher to analyze the bats systematically, but still to detect the exact origin of SARS-CoV-2 was significant task for researchers in the presence of very limited evidence. All the animals epidemiologically linked lo initial outbreak, were determined serologically and SARS-CoV-2 like coronavirus genetically parallel to it was identified from *Rhinolophus affinis* species of bats (RaTG13) [59]. The genetic similarities between both viruses were 96% [45]. Based on genetic similarity, it was claimed that the SARS-CoV-2 is mutated from bats during evolutionary patterns and turn into more pathogenic state [60]. The emergence of viral infections is drive by human influence on natural territories and intermittent amalgamation of different animals to any particular point [4]. It was thought that virus emerged from animal into humans, however, close contact of human and animals increase the chances of zoonotic emergence of viruses under the influence of different environmental factors. Though, emergence of novel viruses also possible in the form of evolution of previously animal limited viruses that needs human receptor to cause infection. Bats were recognized as perpetrator for the COVID-19 outbreak, as similar viral agent was identified from them however, literal origin is still not spotted yet [59]. Similar bat species have been frequently found in other territories of the world included China, Asia, Africa and Europe [61]. These bats harbor the coronaviruses which are present in them naturally; evolutionarily stages led them to be mutated when environmental conditions are altered [62, 63] or accidentally virus transmitted to other animals. Previously, during investigation for virus origin, pangolins were highlighted to be intermediate host or origin of the coronavirus [64]. Other highlighted animals were cats, snakes and camels besides bats and pangolins. Shortly, SARS-CoV-2 like coronavirus genetically parallel to it was identified from *Manis javanica* species of pangolins in the Guangdong Province, China, which were shifted in this province illegaly [65]. Additionally, partial spike gene was identified from both SARS coronaviruses, SARS-CoV-2 and bats derived SARS coronavirus [66-68]. Previously, pangolins were formerly considered to have the probable of intermediate host of SARS-CoV-2 [69]. After pointing out the origin and intermediate host of SARS-CoV-2, various previously posed questions are still need to be answered like the forces involve in the emergence of SARS-CoV-2 from origin into host and evolutionary patterns [70]. On the other hand, very productive information had been granted by the preceding studies of previous human pathogenic coronaviruses such as SARS and MERS, which led the researchers to look for animal origin of SARS-CoV-2 as previous both were emerged from animal origin. Moreover, phylogenetic analysis has contributed greatly, to accurately identify and classify the newly emergent SARS-CoV-2

Environmental factors impact on SARS-CoV-2

The emergence of SARS coronaviruses is highly associated with various factors of environment including temperatures, pressure and humidity, as both SARS coronaviruses, SARS-CoV and SARS-CoV-2 have been emerged in similar weather patterns and month SARS-CoV in China 2002 [71] and SARS-CoV-2 in China, 2019 [72] in December month. In China, winter is at peak during the last two months of year according to the meteorological studies. Severe drought season was observed with 5.5mm precipitation during the month of December [73, 74] while at the time of SARS-CoV-2 emergence, the precipitation was 0mm [75]. Similarly, initial outbreaks of COVID-19 in various countries were observed at peaks in dry and colder temperatures [76] that indicated that SARS-CoV-2 spread efficiently in dry and low environmental temperatures while in humid conditions the disease cannot spread efficiently [77]. The very similar weather patterns were observed in peak epidemic of SARS-CoV in 2002 [78]. The temperatures in ranges of 16-28°C, was optimum environmental temperature for SARS-CoV, the incidence was decline with increase in temperature while the incidence was at peak in optimum temperatures [79]. In vitro study, SARS-CoV-2 appeared to be viable at 22 to 25°C temperature while on rise in temperature its stability reported to decline [80]. Though, discursively, low temperatures favor the viral infection and overall pathogenesis cycle as immunity is weak due to condensed blood circulation, similarly, cilia associated eradicating virus particles mechanism is decline in low moisture, all these conditions enhance the viral pathogenesis cycle that ultimately lead to development of successful infection [81]. According to vitro study, conducted to check the humidity impact on COVID-19 survival, HCV/229E human coronavirus comparable to SARS-CoV-2 was evaluated for study purpose. The study results indicate that half-life of virus is 1 day and 3 hours at 30% humidity and rises of humidity from 30 to 80% significantly reduced the half-life to only 180 minutes [82] while virus propagation was increased on decline in temperature. The half-life of virus is increased significantly in the environment of low temperature and high moisture [82]. Similarly, the previous SARS-CoV was susceptible for high temperature in vitro while remain viable at low temperatures [83], similar consequences of environmental factors was observed in case of MERS-CoV [84]. The survival and viability of *Betacoronaviruses* in environment is varied according to the nature of surfaces contain respiratory secretions, landed from sneezing of infected patient. The SARS and MERS coronaviruses stay viable and remain infectious from 2 hours to 9 days metals, glass and plastic however, the duration of two hours to 9 days can be increased on colder region with low humidity [84, 85,86].

In recent COVID-19 pandemic, it was thought that the incidence rate of this disease will decline in summer season, surprisingly, in vitro study there was no negative impact observed on SARS-CoV-2 [87]. Conversely, high temperatures can reduce the survival of SARS-CoV-2 in environment as respiratory secretions landed on surfaces will evaporated in high temperatures. Thus, transmission chain can be interrupted because of high temperatures [88, 89]. To predict the viability of SARS-CoV-2, another human coronavirus (HCoV-229E) was evaluated deliberately to infect human hands and till one hour 45% of viruses remain viable [82]. Moreover, same coronavirus was evaluated to infect human fingers to check the effect of water and hand sanitizers on it, about 70% of viruses inhibited with water while hand sanitizers (alcohol based) reduced the viral concentration up to 99.99% within half minute [90]. Other disinfectants like sodium hypochlorite and ethanol based disinfectants also reported to inactivate the virus within one minute [86]. SARS-CoV reported to inactivated by ultraviolet light though it is tested so far against SARS-CoV-291, but in vitro, ultraviolet light considerably denatured the various microorganisms including viruses, fungi and bacteria [92]. It is effective strategy in the disinfection of contaminated surfaces; the photo dimers of ultraviolet light has the potential to penetrate in the nucleic acid and denature the nucleotides [93] and can be tested against COVID-19 as well. In comparison with other coronaviruses, the human pathogenic coronaviruses such as SARS, MERS and SARS-2 appeared to more susceptible for hand sanitizers, disinfectants and environmental factors like moisture and temperatures [94]. The ongoing COVID-19 pandemic has remain headache around the world especially for developed countries like European and USA regions, they are looking for miracle in this pandemic and waiting for summer and warm season for reduction in incidence of disease. The hot weather played significant role in the controlling of previous SARS-CoV, which was also emerged in December but then outbreak was reported to be decline on rise temperature

Emergence and transmission routes of COVID-19

The COVID-19 infected patients had epidemiologically liked to super market of seafood and wet animals in Wuhan [32]. The researchers started investigating the origin of SARS-CoV-2 among market animals. Shortly, the origin of newly emergent coronavirus was traced that were bats in Wuhan, China, as similar coronaviruses were isolated from bats which have 88% genetic similarity with SARS-CoV-2 [95]. However, by mode SARS-CoV-2 transmitted from bats to human is still not identify yet but bats were recognized as potential origin of the newly emergent human pathogenic coronaviruses and the market was considered as reservoir and soon and to prevent the further transmission market was closed immediately. But still, many other peoples that do not have direct link with Wholesale market were reported to diagnose with COVID-19 and its incidence reported to increased very quickly. The healthcare officials were perplexed with increased incidence as theoretically, only that population was expected to infect who have linked with market, which was closed but still infection cases were reporting [96-98]. The transmission of disease from human to human was not emphasized yet as it was thought that infection would be only limited to initially affected population and for person to person spread it need contagious nature like previous SARS-CoV32, [99]. The previous human pathogenic coronaviruses, SARS and MERS had the potential to transmit from human to human [39] and it was reported in the COVID-19 epidemics as well when a cluster of family members reported to diagnose with infection [96-98], and manifest the infection although none of them had linked with Wholesale market [99]. Further, human to human transmission of COVID-19 was confirmed decisively when infection was diagnosed in various healthcare officials including other staff. It was asserted that the infection came from infected patients that were already enrolled in hospital wards and transmit the infection during close persistent contact. Later, it was come to know that the coughing and sneezing of infected patients have contaminated the entire wards, which encouraged the human to human transmission significantly [59,100]. Subsequently, most of the hospital enrolled patients affected with COVID-19 were didn't have any direct link with Wholesale market. After thorough analysis of hospital admitted patients, it was stated that the COVID-19 spread efficiently from human to human [101], which was largely reported among healthcare officials and hospital staff. The highest human to human transmission cases in hospital and healthcare center were reported by China and Italy till 15th March, 2020, the highest [102,103]. The droplets of infected patients in the form of coughs and sneezes were responsible for transmission of infection to other healthy individuals [104]. The droplets cannot remain suspended in the air because of large size and landed on ground in the near area of affected person in almost 2 meter. Further, the stay of the landed droplets on ground varied with the nature of surfaces and environmental factors such as temperatures and moistures [105]. At this moment, virus from landed droplets can transmitted by means of respiratory route to susceptible hosts by direct and indirect ways [32,61] . The transmission route is same like previous SARS-CoV but transmission rate is high, additionally, previous SARS was reported to transmit by feco-oral route and handling infected animals [106]. The transmission rate of SARS-CoV-2 is comparatively higher than SARS and MERS coronaviruses, because of which it spread very quickly across the china after initial outbreak and then to globe [32]. The considerable role has been played by nasal secretions as infected patients coughs and sneezes frequently and infection can be transmitted to other humans. However, gaining entry into other hosts by nasal route, the viral pathogenesis is started in respiratory system that ultimately leads to infection. After one week of infection the viral load is at peak and steady flow of virus is started in the form of coughing and sneezing from infected individual [101, 107]. The other expected route involve in SARS-CoV-2 transmission is infected person stool as it has been identified from stool samples of infected patient. Yet, there is no official documented case reported that is transmitted by feco-oral route [59,108,109]. Additionally, SARS-CoV-2 also isolated from serum [110] and other body fluids of human such as saliva, urine and rectal fluids [111], but not involved in the infection transmission. Asymptomatic individuals that have lack of disease symptoms also transmitted the infection, peak viral concentrations found in their nasal secretions and still they don't have apparent COVID-19 disease symptoms [112]. So far, throughout pandemic all the cases transmitted by horizontal mode and no vertically transmitted cases is reported by means of sexual intercourse and breastfeeding but the COVID-19 infected lady delivered abnormal infant [113]. Similarly, no disease case reported due to blood transfusion but still National Blood Center of the National Institute of Health (ISS) actively follow preventive procedures during the blood transfusion practices [114]. In relation to Chinese report, the highest transmission cases (41%) are reported by human to human, 29% cases were reported by healthcare officials 12.3% were family cases and only 8.7% infected population were directly exposed to potential source (Huanan fish market)100. However, in the SARS-CoV epidemic, the highest transmission rate was observed among hospital staff [115]. In the ongoing pandemic of COVID-19, there are some countries including Angola, Nepal, Bhutan, Papua New Guinea, Somalia, Sudan, Namibia and Mongolia did not report the local transmission till March 2020 [116], and disease only limited to imported cases. Local transmission of COVID-19 disease is still under inquiry in several countries like, Timor-Leste, Tanzania and Holy [117].

Vulnerabilities of susceptible hosts

The host susceptibility for COVID-19 is determined by the presence of cellular receptor that is present in various animals and humans. The receptor of SARS-CoV-2 is angi-otensin-converting enzyme-2 (ACE-2) to with virus bind and enter its genetic material. The ACE-2 in human is present in the respiratory tract that was found and confirmed by radiological graphs [118]. The similar receptor was using by previous SARS-CoV as well [119], however, comparatively, SARS-CoV-2 binds weakly while SARS-CoV strongly [120]. The expressing of ACE-2 receptor in higher magnitudes significantly increases the susceptibility of the individuals. ACE-2 receptor in Asian people, expressed in high amount while its expression is lower comparatively in Americans, white and Africans people [121]. Thus, it's indicated that ACE-2 receptor is varied based on the race. The human's immune system does not have memory cells or pre-existing immunity for the SARS-CoV-2 as according to the initial report of COVID-19 disease in China revealed that every human being is susceptible for it [122]. Moreover there are certain risk factors that increase the susceptible host vulnerability those risk factors include smoking, elder people, diminished immunity, hypertension and the people with background sicknesses like renal disease, cardiovascular diseases and diabetes [122]. The smokers have higher magnitudes of ACE-2 receptor cells as in their body the expression of ACE-2 gene is high that significantly increase risk for infection [123]. The smokers have unfettered ACE-2 receptors in altered cell types although it is mediate by ingredients of cigarette; smoking period and its cession also significantly influence it [123]. The receptor Dipeptidyl peptidase IV (DPP4) of other human pathogenic coronavirus MERS that was broke out in Kingdom of Saudi Arabia (KSA) also reported to have expression in individuals who smoke [124]. The people with habit of smoking develop COVID-19 disease more severely and particularly they are also observed to have COPD (Chronic obstructive pulmonary diseases) [125].

Entry and Pathogenesis of SARS-CoV-2

The attachment to receptor is the first step of viral pathogenesis that eventually leads to infection. After binding, virus enters its genetic material in the host cell which is then replicated inside host cell by using the cellular machinery. ACE-2 receptor in humans mainly expressed in respiratory tract on which virus attached to facilitate the entry of its genetic material into cell and initiate pathogenesis [126-128]. The virus reached to respiratory tract of the susceptible host when virus containing aerosols are inhaled, that is mostly occur when a person regular stay near the infected person [129-132]. Subsequent to entering, virus bind with cellular receptor after attachment to epithelial cells in respiratory tract that is further accompanied by entry of genome into cell [133,134]. In vitro study, initially ciliated cells are infected with lower airways [135] and could be diagnose from specimen of nasal secretions but disease symptoms are not appeared. In this stage, concentration of virus is not adequate but virus started proliferated due to short supply of innate immune cells [136]. Due to lacking of memory cells, host immunity can trounce the virus neither anti-viral drug is available to kill the virus and eventually lead to regions of lower respiratory tract. Viral infection becomes apparent in this phase with typical disease symptoms of COVID-19. At this phase COVID-19 infection the, clinical condition of the patient represented fever, cough and fatigue whereas production of sputum, lymphopenia, haemotysis, dyspnoea and diarrhea are included in other symptoms which appeared in severe conditions [32,35]. The viral proliferation is triggered the host innate immunity reactions and its early markers can be detected in sputum and other nasal secretions. However, in diagnosis of previous SARS coronavirus, the important infection marker was CXCL10 gene [137], which is released in the form of cytokines because of immune system reactions during the clinical course of infection [138]. Most of infections didn't progress to this phase of infection and eventually virus is cleared by immune system. However, 20% experience this phase of infection but this value varies with the immune status of patient and age. In this phase, patient needs intensive care because of severe disease symptoms and there is death chances is well [139]. Moreover if patient is not treated in early stages, patient may experience the extreme circumstances and that can lead to expansion of acute respiratory syndrome, septic shock, coagulopathy and metabolic acidosis. It is necessary to diagnose and isolate the patient with supportive treatment with general fever and respiratory symptoms even without the symptoms of severe stages a patient [33].

Co morbidities and risk factors that increase COVID-19 disease severity

During initial outbreak of COVID-19 in China, every age person of both gender appeared to be susceptible for COVID-19 infection, according to demographic data of initial hospital enrolled patients affected with viral infection [140]. The susceptibility of individual is increase with pre-existing various disease including cardiovascular, diabetes mellitus and renal diseases, moreover, the risk factors that increase the infection chances are smoking, old age and diminished immunity [141,142]. According to a study of hospital admitted COVID-19 patients, the 16.37% patients were previous affected with hypertension and 12.11% were cardiovascular diseases [143]. The presence of comorbidities significantly reduces the immune system function, which largely increase the chances of COVID-19 infection [139]. In the initial outbreak in China of ongoing pandemic, almost half of patients were previously affected from other comorbidities like hypertension, cardiovascular diseases and diabetes while almost 75% of patients were older male with 49 years age median [6]. Comparatively, more incidences was found in male population, for which exact and scientific reason was not identified but claimed that it might be associated with smoking or those comorbidities that worsen the infection patterns [144]. The incidence in female is low because of limited outdoor activities and they do not smoke but conversely, female develop more severe symptoms of COVID-19 in comparison with male and they may require intensive care [145]. The infants affected with COVID-19 in age ranges of 1.5 month to 12 months, appeared to be moderately affected with disease symptoms145. Similarly, the incidence of disease in children is low and infections symptoms are mild while the older people particularly those suffering from comorbidities have high incidence rate and severe symptoms with highest fatality rate [146], additionally, they require intensive treatment and care [147]. The reason of low incidence in children is little exposure to disease source as they are mostly limited to their home. Equally, they are not frequently exposed to harmful pollutants thus; their respiratory tract is healthier in comparison with adults. Additionally, children's can infect with COVID-19 disease of mild symptoms as the intensity of cytokines storm is less in them [148]. Moreover, those underlying diseases such as hypertension, diabetes mellitus and cardiovascular diseases, are very infrequent in children; immune system is become weak due to underlying diseases [149]. Furthermore, another reason behind mild symptoms of children in COVID-19 is less prevalence of C-reactive proteins, which significantly reduces the immune damage and has mild immunological responses. However, C-reactive proteins are present in higher frequency among adults that increase the chances of immune damage in adults [150]. According to initial report, 73% of people were 40 plus in age among 169,930 infection cases and no fatality case is reported in children of less than 10 years [151] while older age population reported the highest fatality rate according to the reports of various countries [152]. In the start of COVID-19 pandemic, in China, highest fatality rate was found in age group of 60-69 while Italy reported highest fatality rate was in 70-80 [153]. In the older people, the most frequent trouble is hypertension due to which COVID-19 disease severity is increased with involvement of rennin angiotensin system (RAS) that maintain the fluids balance especially salts [154]. The healing process is slow in the older people and during COVID-19, the epithelium is damaged during infection and mucociliary clearance mechanism is slow. In these circumstances, virus spread efficiently in respiratory tract and reached to gas exchange units of lungs, which increased disease severity significantly [155]. Certainly, older people are greater at risk to develop severe symptoms of disease and even they can die due to weak immune system. Other factors involve in disease severity are bacterial and viral infection such as *Klebsiella* and influenza infection that only increase the COVID-19 disease severity but also delay it diagnosis and can be detect poorly [156]. The other vital organs such as heart is reported to affected with COVID-19 infection as indicated by increase in myocardial enzymes that is prominently observed in older people and in adults and children as well [32]. Additionally, the COVID-19 disease burden is reported to increase with surgeries of infected patients, according to (Wu Z., 2020) study, 34 patients were required surgeries among [138] hospital admitted patients that were diagnosed with COVID-19 however, after surgery disease symptoms reported to accelerated and lead to 7 death cases among 34 [139]. Therefore, the most susceptible population for COVID-19 is post-operative patients and they shifted to intensive care unit immediately due to greater chances of death [157]. Similarly, in another study, a cluster of patients diagnosed positive with COVID-19 right after surgery and those were negative before surgery, and were recently returned from Wuhan [158]. Thus, it was considered that surgeries reduce the incubation period and make the individuals more susceptible for COVID-19 disease and comparatively, the disease manifest with more severe symptoms because of surgical pressure [158]. The recent surgically operated patients have low immunity [159] and in addition to that the response of systemic inflammation is stimulated with surgeries [160]. The immune system is robustly diminished due to any previous viral infections and makes the patients more vulnerable for COVID-19 and patients may face disease severity accordingly, the recent recovered patients from viral infections are highly at risk to get COVID-19 [161]. In viral infections, the immunity is weak and cytokine storm developed further in the form Cytokine release syndrome (acute systemic inflammatory patterns), which lead to dysfunction of many organs [162]. Moreover, in cardiovascular diseases the Pro-inflammatory cytokines are diminished that significantly increase the individual susceptibility towards severe infections [163]. The tumors, chemotherapy and taking of anti-cancerous drugs also reported to increase the individual susceptibility for COVID-19 infection by slow down the immune functions [164]. Interestingly, the people with A blood group are more susceptible for infection in comparison with other blood groups while the individual with blood group O is appeared to be less susceptible [165]. The SARS-CoV-2 receptor ACE-2, also found in other animals such as ferrets and cats, receptor is present on type-2 pneumonocytes of the sub mucosal glands and epithelial cells. Efficient replication has been reported in these animals thus, these animals can evaluated to check the efficacy of anti-viral and vaccine against COVID-19 [166]. The infection of SARS-CoV-2 is also reported Egypt fruit bats but typical COVID-19 disease symptoms didn't observe neither the infection transmitted to other surrounded fellow animals [167]. Overwhelmingly, all the humans of both genders are susceptible for COVID-19 but the consequence of disease are vary on the basis of individual age, immune status, presence of underlying diseases/infection and various risk factors (Table).

Table 1. The Humans susceptibility to COVID-19 and disease severity is increases with the following underlying diseases, infections, risk factors and various conditions.

Diseases/Infections/Risk factors	References
Cardiovascular, Diabetes Mellitus and Renal Diseases	[141,142]
Tumors, Chemotherapy, Anti-cancerous Drugs	[164]
"A" blood group are more susceptible for COVID-19 infection	[165]
Hypertension,	[6]
The magnitudes of ACE-2 receptor, Asian people are more susceptible for COVID-19 due to high expression of ACE-2 receptor	[121]
Pro-inflammatory Cytokines	[163]
High amount of C-reactive proteins	[150]
Disease severity is increased with rennin angiotensin system (RAS)	[154]
Bacterial and viral infection such as *Klebsiella* and *influenza* infection.	[156]
Older age population is more susceptible for infection	[152]
Smoking increase the chances of COVID-19	[144]

However, all the mentioned comorbidities and risk factors significantly increased the chances of COVID-19 and make individual more susceptible for infection, which need to strict implementations of all SOPs released by concerned health authorities such as using of strong disinfectant on nearby surfaces, frequent hand washing with efficient sanitizer, regular using of mask and gloves and avoiding the crowded areas [168]. The health authorities need to strictly implement the standard operating procedures (SOPs) and aware the people about all those risk factors and susceptibilities of COVID-19 [169, 170.] Infected patients can be isolated and treated prior to further transmission of COVID-19. However, to break the transmission chain and prevent further spread of disease, the accurate and rapid diagnosis of COVID-19 is required based on nucleic acid detection.

Diagnosis of COVID-19

Nucleic Acid based detection of COVID-19

At present, public health community around the world has been challenged by the high infections and deaths rate of recently emerged COVID-19. Such scenario required the infection to be diagnosed accurately and rapidly to control the ongoing epidemic of COVID-19, which is only possible by molecular based diagnosis. There are certain other viral diseases having same symptoms like COVID-19 thus accurate detection of infection is recommended. Among molecular based diagnostic methods, the most frequent and reliable technique is based on nucleic acid that can help in infection source calculating and ultimately epidemic control of COVID-19. The highly recommended economical molecular based method for rapid diagnosis of COVID-19 is RT-PCR (Reverse transcription-polymerase chain reaction). Clinically it shows results of false-positive/negative and slow sensitivity as well. However, the disease severity can have increased significantly with False-negative results and facilitate COVID-19 spread through delayed patient isolation from uninfected individuals and treatment can be affected ultimately. Another most commonly applied technique for COVID-19 identification, detection and its evolution monitoring is sequencing. The current situations of pandemic reflect the unsuitability of sequencing technique because of disaster, as it is very lengthy process and expensive. Additionally, the RNA viruses with high mutation rate can affect the regions specific that are targeted for detection and thus reduce the accessibility of targeted regions for detection. Consequently, for accurate detection of COVID-19, some new molecular based methods have been developed recently in the pandemic. Those methods include microarray-based method; loop mediated isothermal amplification (LAMP) method, RNA-targeting CRISPR diagnosis, rolling circle amplification-based method and nanopore target sequencing (NTS) method.

Polymerase chain reaction (PCR)

In the molecular biology, the short and broadly used practice is PCR that make DNA copies from thousands to millions containing the fragment of gene. A very small quantity of DNA (genetic material) cans be amplified in PCR and provide adequate evidence of the DNA or gene segment for comprehensive study. Most of the microbial diseases are diagnosed by PCR because of its broader impact and reliability, which give the importance to PCR to be used routinely in diagnosis of various infections including COVID-19 [171]. The etiological agent of COVID-19 is SARS-CoV-2 that is RNA virus and can be converted to cDNA by process of reverse transcription through the reverse transcriptase enzyme. Next, with using of specific primers the DNA sample is amplified by PCR subsequently further accompanied by the gel visualization and gene sequencing [172]. Even though, for the diagnosis of COVID-19, the most recommended test is PCR. However, there are chances of certain limitations with the use of PCR. Those limitations include false positive results, more time consumption and low specificity and sensitivity.

Real-time reverse transcriptase PCR

In cases of COVID-19 detection the most widely used and preferential method is RT-PCR (Real-time reverse transcriptase-PCR) due to RNA as genetic material and several advantages. Comparatively, Real-time RT-PCR has high specificity and sensitivity than the usual PCR and its procedure is simple and quantitative [173]. Currently, the most favored method around the world used for COVID-19 diagnosis is Real-time RT-PCR that helps significantly in the detection of infection in early phases [174]. In the advancement of real-time RT-PCR method improvement, considerable efforts have been executed in order to overcome its disadvantages that can be expected during the procedure [175]. Those disadvantages are mentioned earlier; however, the lack of positive control is the major disadvantage of this technique that play key role in the detection of disease. Yu et al., 2008 have established real time RT-PCR to address that problem of positive control in coronaviruses detection through armored RNA, which is non-infectious, quantifiable and can be live or inactivated [176]. It was designed by engineering to be used in the COVID-19 diagnosis as positive control. Genetically diverse viruses like coronaviruses have high mutation rate that led the sequences to be changes rapidly and need detection of coronaviruses precisely. The risk of false results has reduced in precise detection of COVID-19. However, the detection sensitivity can be increased by establishment of multiplex real time RT-PCR that significantly helps to identify the SARA-CoV-2. The other most frequent molecular based technique is quantitative RT-PCR (rRT-PCR), which is very effective in many viral diseases including COVID-19. In routine the diagnostic method with better specificity and sensitivity for COVID-19 can be TaqMan chemistry of single step rRT-PCR, which is further improved with the use of dual TaqMan probes [177]. The application range of Dual TaqMan probes is very broad and can be performed very easily because of easy procedure. The reaction components are almost same as other viruses with exception of slight modification while additionally it requires pre-designed probe and specific primers. There was another method such as RT-PCR SYBER green dye based assay, was also examined but appeared to be less effective and have lower specificity than the TaqMan probe based assay [178]. The methods of rRT-PCR are using commonly in the ongoing COVID-19 outbreak as diagnostic tool and now it can be used in both domains, diagnostic and research domains. Conversely, the false negative results are may be disposed with the using of rRT-PCR. According to a study, recently, PCR reports reported the negative result of COVID-19 of 5 patients. Though, all the patients found positive with COVID-19, based on the analysis of CT scan of chest examination. The samples were collected again and analyzed through rRT-PCR, which results the COVID-19 infection in all 5 patients [179]. The sensitivity of rRT-PCR is depend on sampling, storage of samples such as duration, location and location temperature, however, sensitivity normally ranged from 50-70% [180,181]. The major limitation of the rRT-PCR is biosafety problems such as biosafety problems in sampling, laboratory handling, and non-availability of biosafety cabinets [182].

Loop mediated isothermal amplification (LAMP) method

Among molecular based diagnostic methods, that detect COVID, the most prominent method in the recent outbreak of COVID-19 is Loop mediated isothermal amplification (LAMP) method. It has higher sensitivity and specificity rates and amplifies the nucleic acids (DNA/RNA) very rapidly. The method procedure involves the DNA polymerase and specific primers that synthesize the targeted DNA. The uniform temperature for this experiment is 60-65°C that is change than ordinary process, as it did not denature the strands only displace it [183]. At the end point of detection through LAMP method, amplified products are further analyzed by gel electrophoresis. Additionally, because of exponential amplification feature of LAMP, 4 different primers that can spot 6 different targeted sequences at the same time [184]. Within very short time or in an hour maximum up to more than 109 copies of targeted sequence of loop form DNA amplified by LAMP assay, and final product is in the form of many inverted repeats. The clinical diagnosis of COVID-19 through LAMP application is appeared to be very useful, reliable and cost effective as it does not require expensive instruments or reagents/chemicals. In the case of COVID-19 diagnosis through LAMP, the ORF1b region of SARS-CoV-2 is targeted and amplified by 6 primers to establish the effective viability. The sensitivity and detection rate of LAMP and conventional PCR based techniques appeared to be similar when the amplified products are analyzed by gel electrophoresis [185]. In the avoiding of limitations of COVID-19 diagnosis during endpoint and for more efficient diagnosis, the LAMP technique is recommended by many studies based on the analysis of amplified products through gel electrophoresis. Retrospectively, the previous human coronaviruses (NL63), have been detected with better sensitivity and specificity when gel electrophoresis was used to analyze the amplified product extracted from cell culture and clinical samples. The LAMP based methods are developed by Shirato et al. 2014 with new modifications, for the epidemiological studies and diagnosis of human coronaviruses the technique called RT-LAMP [185] was developed which have no cross reaction with respiratory viral pathogens and high specificity [186]. The method of non-specific signal transduction is involving in the sequence specific LAMP-base methods. Pyrophosphates releases by double stranded DNA amplicons intercalated with fluorescence dyes and polymerization that causes the turbidity of solution [187]. Moreover, to improve the performance the of RT-LAMP technology, a researcher start using QProbe(quenching probe) in signal monitoring [186] that increase the RT-LAMP technology proficiency. This development has been high applicable for the diagnosis of human pathogenic viruses. Furthermore, there was another technique developed called RT-LAMP-VF, in which naked eyes was able to visualize the nucleic acid by vertical flow visualization strip [188] (Fig. 2).

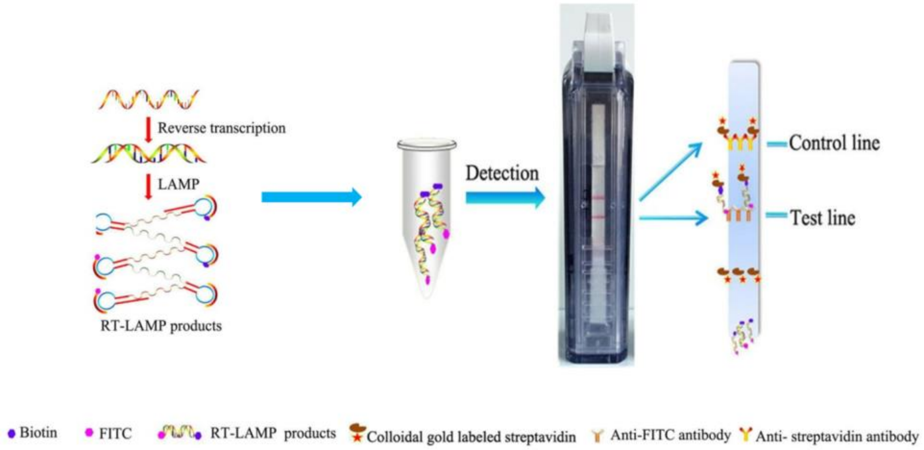

Figure 2. Schematic illustration of the RT-LAMP-VF assay. Figure was adopted from Huang et al., 2018 [188]

The fluorescein isothiocyanate (FITC) and biotin play the role of primers labeling, during isothermal amplification. A complex is formed between colloidal gold labeled by streptavidin and amplicons labeled by biotic, the strip coated with anti-FITC antibody captured the complex and a visible colored line is formed apparently. In the development of LAMP technology, there was another method designed, comprises of strand exchange signal transductions with thermo stable invertase which involve transductions of glucose signals from template of MERS-COV, the signals can be detected by glucometer with 20-100 copies/µL [189]. Similar mechanism is involved in another diagnostic process, the signals of hCG (human chorionic gonadotropin) are determined considerately in commercially available pregnancy kits [190]. Same procedure was applied for detection of COVID-19 through LAMP based method, involves incorporation of engineered proteins in hCG reported on commercial pregnancy tests to transducer signal to hCG from LAMP. The human blood and saliva can be used in this diagnostic technique and at least 20 copies of pathogens can be detected. All these developments reported in LAMP based method have the potential to detect COVID-19 disease rapidly and accurately. The applications of LAMP based detection are broadly applying in the diagnosis of COVID-19 [185, 186,191]. Beside all these developments in LAMP based method, it is still associated with various limitations including reactions repetition due to internal PCR inhibition control during the experiment. Other limitations include the designing of target specific primers that is crucial step in the detection of COVID-19. The fragment obtained from LAMP assay is usually large and cannot be evaluated for additional required applications e.g. cloning. Though, LAMP is still highly recommended method for nucleic acid amplification, and can identify nucleic acids in very minute quantity almost within 60 minutes. This capability of LAMP made it suitable detection method for reliable diagnosis of COVID-19 with better sensitivity. The burden of COVID-19 patients on hospital has been increased significantly and unfortunately, many of them remain untested due to lengthy process and largely depended on preventive and control strategies to control the disease. However, in such scenario LAMP based method for COVID-19 detection is suitable and and has been largely recommended for rapid detection of COVID-19. The features of LAMP methods are compared with PCR method in detection of viral diseases.

Rolling circle amplification-based methods (RCA)

Rolling circle amplification-based methods has been used largely to detect the sequences of nucleic acids, which sensitivity is comparatively better than PCR, however, this method is also known as padlock probes [192.193]. The Rolling-circle amplification (RCA) technique hybridizes the targeted sequence of nucleic acid (DNA/RNA) by probe which both ends are found with DNA ligase. The template for the reaction of RCA is formed by the catalysis of circular DNA by DNA polymerase enzyme. The ligation process is major advantage of RCA over other amplification methods that reduce the chances of non-specific binding of hybridize probe (with base pairing at both sides) with complementary strands. Additionally, in PCR, RCA also avoid the non-specific amplification and also permit the single-nucleotide polymorphism. RCA doesn't need the reverse transcription it can recognize the template of both nucleic acids (DNA/RNA), this capability of RCA made it uniform and reliable methods in the detection DNA and RNA both [194]. There is no requirement of thermocycler, as primers under thermal conditions easily bound with the single-stranded DNA isolated by DNA polymerase enzyme. The sensitivity of RT-PCR based technique which is preliminary widely used diagnostic tool, varies but depends on various factors sample type, duration of collection etc but estimated sensitivity to be as low down as 30% [193, 195]. Besides, the magnitude of RT-PCR amplification is excellent but the reagents used in the experiment are expensive and procedure is relatively lengthy, which required time and additionally there are chances of false-positive results due to cross contamination. On the contrary, RCA is operational with several advantages over RT-PCR amplifications as it is depended on signal amplification rather than on amplification. Retrospectively, in 2005, the most promising detection technique that was using in routine in the diagnosis of previous SARS-CoV was RCA, with broader impact in identification of coronavirus having greater sensitivity and specificity [194]. In molecular modeling, the RCA has the potential to use artificial DNA templates to sense the single template level. Conclusively, RCA appeared to be one of the most reliable detection methods with exceptional features in the diagnosis of various microorganisms included SARS-CoV, and clinically can be used in the ongoing pandemic of COVID-19. Although there may need of additional work to improve the RCA method and make suitable for COVID-19 diagnosis.

Nucleic acid hybridization through microarray technique

A high amount of coronaviruses nucleic acid can be obtained by nucleic acid hybridization through microarray technique in very short time. It requires reverse transcription to convert coronavirus RNA into cDNA, which is then marked with specific probes, wells of microarray trays are filled with cDNA probes. The surfaces of microarray plate surrounded the adhered oligonucleotides. After washing several times, the hybridized probe remains bounded on plate, although it will eliminate the unbound DNA, and the existence of virus specific nucleic acids can be detected. The microarray method was widely used in the detection of various viruses included SARS CoV [196]. Later, microarray technique was further improved to overcome the high mutation rate in the genome of RNA viruses, with this improvement the single nucleotide polymorphism (SNP) can be detected from sample of SARS-CoV with excellent accuracy [197]. However, there is still improvement needed in the microarrays method to detect multiple strains of coronaviruses as it has the potential to emerge suddenly. In past 18 years 3 different coronaviruses has been emerged throughout the world. In 2007, oligonucleotide array with low density and lacking of florescence was planned that can sense the whole genus of coronavirus [198]. The sensitivity of microarrays method and real time RT-PCR are same. Further developments were intended in the convenient diagnostic platform that relies on technology of microarray chip and originated as effective detection method for virus [199].

RNA-targeting CRISPR diagnosis

The family of DNA sequence found in prokaryotes, bacteria and archae both can be signified by clustered regularly interspaced short palindromic repeats (CRISPR). CRISPR comprises set of various enzymes that cut the DNA at specific sites after recognition. These enzymes include Cas9, Cas12, and Cas13. However similar mechanism was programmed for identification and cutting of targeted sequence of viral RNA by Cas12 and Cas13 enzyme families [200] Cas13 was modified further to sense the nucleic acids sequences rapidly [201]. By using Cas13 enzymes, a method was developed named SHERLOCK, specific high-sensitivity enzymatic reporter unlocking, with potential of cutting down the reporter RNA sequences. Recently, in the COVID-19 pandemic, this method was activated for SARS-CoV-2 detection [202]. This procedure involves reported RNA cleavage which is detected by cleavage enzyme, subsequently targeted viral RNA sequences are amplified by isothermal amplification, which is then visualize by fluorophore [203]. The CRISPR based methods can be performed on paper strips in the detection of COVID-19, and there so any requirement of specific or complex equipment's but still it have better sensitivity and specificity. The advantage of these techniques is being cheap in prices and can be performed within 45-55 minutes. The trials of CRISPR diagnosis in the targeting of RNA sequences have potential for the diagnosis of viral diseases [200]. The RNA viruses such as dengue and Zika viruses can be detected by CRISPR based technology and their mutations as well. The agreement for CRISPR diagnostics as a detection tool for COVID-19 has been discussed. Various specific points of this technique like multi-nucleic platform, rapid and portable, are available for researchers who are intended to improve this method further.

Nanopore target sequencing (NTS)

Nanopore target sequencing (NTS) can sense the long fragments of nucleic acids on the nanopore platform; it can also amplify the fragments of virulence genes and analyze the data same time. The major advantage of this technique is the confirmation of viral infection rapidly by forming sequence map on the genome of virus. Moreover, the various respiratory infections are detected by NTS within very short time from six to ten hours. This property of NTS makes it suitable detection method for the COVID-19 diagnosis; additionally it may require additional changes in the framework [204]. Single MinION sequencer chip is used to perform NTS and it applies on testing samples and the sequence data is analyzing via bioinformatics channels at systemic intervals. The determination of high grade readings takes place to increase the plasmid concentration through mapping out the genome. Positive samples of infection cannot be detected by NTS by using one or two sites like standard conventional PCR [204, 205].

Silicon-based integrated Point-of-Need (PoN) transducer

Nucleic acids sequencing can be performed by chemically enhancer method as well, like Silicon-based-Point-of-Need (PoN) transducers (TriSilix). Even though, advances in nucleic acid based detection methods, the rapid detection of infections with high sensitivity and low cost is still needed at the Point-of-Need (PoN). It is more necessary in a case in which disease symptoms is similar to other diseases and having no treatment option like COVID-19. In a high standard typical laboratory, TriSilix can be created unlike other silicon-based technologies. A series of chemical-based methods for metal (water), electroplating, thermal bonding and laser cutting to enable low-density fabric formation that does not need advanced semiconductor detection [206]. Toward the making of economical device, the planned construction develops the exceptional silicon possessions and joins three modes of maneuver on a single chip, that is, electric heater (Joule), temperature sensor with negative confliction that can straight endow with the temperature of the sample solution through reaction, and an electrochemical sensor to perceive target nucleic acid. Hence, TriSilix can stand with the interruption in supply chain throughput the world as manufacturing of device can take place any place in the world [206]. There are several disadvantages reported in the TriSilix technology like foe sample operation it requires purified DNA. It required high grade temperature of 110 °C for the attachment of sample reservoir and polymer films. Relatively, high temperature is required like 110 °C for the amplification of nucleic acid. There may requirement of modified thermoplastics in the form fabrication process, in cases of high grade temperatures, which cannot be melt at higher temperatures [206]. Various diagnostic methods have been described along with advantages and disadvantages. Usually, the viral infections are detected by RT-PCR, but it has various limitations like sensitivity and specificity. LAMP is another molecular based diagnostic toll with high sensitivity and specificity, and it amplified the genome very quickly. Correspondingly, in coronavirus nucleic acid detection the Rolling-circle amplification (RCA) technique and Microarray exhibited high sensitivity with high throughput rapidly. Moreover, CRISPR related technologies are appeared to be very significant in the diagnosis of COVID-19. Furthermore, a new nucleic acid based detection method can be developed by advancing the already existing methods mentioned earlier to detect the COVID-19 infection rapidly with higher specificity and sensitivity at low cost.

Outbreaks of SARS Coronaviruses

SARS Coronaviruses (2002-03)

The first coronavirus was broke out in Chinese population 2002, Guangdong Province, Foshan [25]. Globally, SARS-CoV affected 8422 people with 916 fatalities from emergence till the end of epidemics [207], among 8422, mainland China reported 5327 and mortality rate was 7%, highest incidence was reported in Beijing [208]. The virus was emerged from zoonotic origin; the highest transmission rate was reported among hospital staff and healthcare officials [116]. Beside China, the coronavirus affected other 26 countries globally included Hong Kong, Taiwan, Toronto and Singapore.

Hong Kong reported the first case of SARS-CoV on 4th March, 2003, the infected patient was Southern Chinese nephrologist, who landed in Hong Kong on 21st February, 2020 and shifted hotel. Later, it was diagnosed positive with SARS-CoV infection [209]. In the same hotel, other 16 people diagnosed with SARS-CoV who were living in the same floor with nephrologist room and contacted directly or indirectly. The SARS-CoV infection was confirmed by thorough analysis of nasopharyngeal aspirate by reverse transcriptase-polymerase chain reaction (RT-PCR) additionally, rise in antibody titer against SARS CoV was also increased [210]. In a while, various other visitors came to Hong Kong from other countries were start developing disease symptoms after entrance due to long period of incubation period. There were 138 more individuals including healthcare workers diagnose positive with SARS-CoV infection in the general medical unit where nephrologist was admitted [211]. In the Hong Kong, there is another outbreak of SARS-CoV is reported, in which 329 people were affected at the end of March, 2003. The origin for this outbreak was male patient who was recently shifted from southern China and admitted in hospital on 15th March due to kidney problem [212]. Later, he developed the symptoms of fever, rigor and chills and diagnosed with Influenza A virus. After taking different antibiotics like levofloxacin, oseltamivir, defervescence and cefotaxime he recovered after 2 days and discharged from the hospital and shifted in brother home at the Amoy Gardens. Meanwhile his stay, he develop symptoms of severe respiratory distress and identified with SARS-CoV infection in the hospital. Consequently, a cluster of patients reported positive with SARS-CoV infection from Amoy Gardens community. SARS-CoV was identified from throat samples of community patients [213]. In Vietnam, Dr Carlo Urbani, identified first case of SARS-CoV infection on February 28, 2003, later he too died from SARS-CoV infection [214]. Till June, 2003, sporadic cases of infection reported in the various regions of Hong Kong however, after 23 June, no single infection case reported in Hong Kong, hence it was recognized as SARS-CoV free country.

The SARS-CoV infection outbreak in Vietnam was associated with Hong Kong hotel where nephrologist stayed, coincidently; there were three guests from Singapore as well. After coming to hometown Singapore, they diagnose positive with infection and admitted in hospital where infection spread to healthcare officials then to community. Later two taxi drivers and his fellows also diagnosed with infection [215]. Shortly, in February, 2003, infection number reached to 97 among healthcare workers while overall 238 cases reported. However, sporadic cases were continually reporting till May 5, 2003, but after that no infection case was reported in Singapore because of implementation of vital control strategies of health authorities.

On 25th February 2003, an individual develop the symptoms of SARS-CoV infection in Taiwan, but did not consulted doctor neither went for laboratory diagnosis till to weeks, later, infection transmitted to his son and wife but disease consequences were severe in them and additionally, they necessitated constant ventilation in hospital. In the hospital, the infection spread to healthcare official as well [216]. Retrospectively, the infected patient had travel history of Guangdong province, China and returned to Taiwan on 21st February, from side to side Hong Kong. However, till the end of March the infection was limited to healthcare official and 3 other cases were reported. Genetic analysis of pathogen isolated from throat sample of patient revealed the similar strain of SARS-CoV that was reported in Taiwan [217]. Eventually, the epidemics of SARS-CoV infection were brought under control till July 31, 2003 after nine months of emergence. Globally, infection affected 8098 people with 774 deaths; majorly affected countries were China, Hong Kong and Taiwan (Table-1)218.

Table 2. The overall global incidences of SARS-CoV infection [218].

Countries	Infections	Mortality rate
China	5327	6.55
Hong Kong	1755	17
Taiwan	346	37
Canada	251	10.69
Singapore	238	33
Vietnam	63	13.86
America	29	00
Philippines	14	14.28
Thailand	09	22.22
Australia	06	00
Malaysia	05	40
United Kingdom	04	00
Total	8098	774

SARS-CoV-2, COVID-19 (2019-20)

The recent pandemic of COVID-19 revealed the hidden potential of coronaviruses and previous coronaviruses proved merely as a tip of iceberg, following the appearance of novel coronavirus with prospective of pandemic standing, which appreciably exposed the out of sight abilities of virulence and contagiousness. The COVID-19 was broke out among a cluster of patients in December 2019 in Wuhan, Hubei Province, China [219]. During initial outbreak, one patient was died due to COVID-19 till 29th December [32]. On the second day of January 2020, 41 more patients diagnosed positive with COVID-19 in Wuhan [220]. Brusquely, rapid transmission of COVID-19 comes into sight in China, as people were unaware about transmission routes and disease symptoms additionally, asymptomatic individuals drastically force the rapid spread of the infection across the country. The most significant role in the infection spread was played by movements of people who were gathered in Wuhan from all over the China to celebrate Chinese New Year (Chunyun). Due to emergence of COVID-19 the Chunyun was cancelled and all the people were directed to their home cities from Wuhan, however, on their departure many of them were incubation period and asymptomatic carriers who spread the infection silently to their destinations, which is then transmitted to community by person to person contact [221]. Later, 278 more infection cases were reported in China, 258 from Wuhan and 14 from Guangdong Province while 4 cases reported outside China, Thailand 2 and Korea and Japan 1, 1, respectively [222]. On January 19, the first cases of COIVD-19 were reported in United States America [223]. Brusquely, the infection rate noticed with unexpected increase and cases reached to 571 as affecting 25 districts in China as of January 22, 2020 [224]. After two days 17 cases reported outside China while in China infection cases reached to 878. Outside China cases reported from different countries included Thailand, Singapore, Vietnam, Japan, South Korea, United States and Taiwan and these cases were epidemiologically associated with China, Wuhan [225]. Because of high transmission rates, infection distributed very quickly across population like outbreaks of SARS-CoV [226]. Nishiura *et al,* reported 5502 infection cases till January [227]. Next day, on 25th January the total infection cases reached to 1975 very quickly along with 56 death reports, in accordance with report of Chinese National Health Commission [34]. However, in various regions of USA, person to person transmission of COVID-19 infection start reporting as of January 30, 2020 [228], 443 suspected patients from 41 states were tested for COVID-19 and 15 were positive with infection whereas the rest cases were still in pending [228]. There were almost 30000 passengers screened by CDC on airports prior to entry in USA. At the end of January, 2020, the COVID-19 infection cases in China reached to 7734 and 90 cases reported from different countries around the world included USA, Thailand, Canada, Taiwan, Singapore, Vietnam, Germany, Republic of Korea, Sri Lanka, Philippines, Finland, India, Nepal, Malaysia, France, Japan, Cambodia, Australia and United Arab Emirates [229].

COVID-19 in February, 2020

A tragic incident was reported in the start of February, 2020, as COVID-19 outbreak was reported in cruise ship (Diamond Princess) that disembarked at Yokohama port on 3rd February, 2020. The diamond princess had 2666 passengers from different 56 countries, 1281 were from Japan [230, 231]. The diamond princess was departed from Japan, since January 20, 2020, and travelled different countries till 3rd February, 2020, included Vietnam and Taiwan [232]. One of the passengers diagnosed positive with COVID-19 infection on 31st January in Hong Kong, who disembarked from ship on 25th February [233]. With the news of Hong Kong infection case, the boat was quarantined immediately at yokohoma port on 4th February for two weeks after arrival in Japan. Next day on 5th February, 10 more passengers diagnosed with COVID-19 and gradually, after 5th February, many passengers appeared to be infected with COVID-19. However, till February 23, 691 passengers diagnosed with COVID-19 infection and 3 patients died [234], among which 5 were officers and healthcare workers. Besides, total 7 deaths were reported and infection cases reached to 696 [235].

Global Incidences of COVID-19, initial cases in Iran and Italy

In the first week of February 2020, China reported overall 31,161 cases with 631 death [236]. In the second week of February, the brusque increase in the COVID-19 infection observed in China and a total of 70548 cases reported till February 16, 2020 [237] whereas, global cases reached to 683 outside China [238]. During the 3rd week of February, Iran reported the two cases of COVID-19 in Qom Province [239]; unfortunately, both were died next day as said by Health Ministry and Medical Education report [240]. In a minute, 139 more cases were reported in Iran as of February 26 [241]. Various government officers were affected with COVID-19 included ranks included Deputy Health minister, Iraj Harirchi [242], Vice President for Women and Family Affairs; Masoumeh Ebtekar, and Chairman of Parliament's of Foreign Affairs Committee and National Security; Mojtaba Zolnour [243]. At the end of 3rd week of February 2020, overall cases of COVID-19 in China were reached to 75769 and 2239 fatalities while worldwide cases reached to 1200 with death reports [244]. On February 21, a cluster of patients diagnosed with COVID-19 in the regions of Lombardy [245], however, cases spread continually in Italy and within 4 days cases reached to 283. At the end of February, a total of 1827 infection cases reported in Italy, 1520 from Lombardy and 307 from Veneto [246]. Although, worldwide, COVID-19 reached to 66 countries and infection cases were reached to 89.068 with 3,046 deaths. Among 66 countries, the majority of cases came from China, 80,134; Korea 4,212, Italy 1689 and Iran reported 978 cases [247].

COVID-19 in March, 2020, and turning to Pandemic

The brisk increase in the COVID-19 infection was observed in the start of March in several countries throughout the world. However, various countries first time report the initial cases of infection in March. The March month appeared to be very fateful for world because of sudden increase in infections cases, as result various countries implemented lockdown and basic precautionary measures including the closure of government/private educational institutes and bans on social gatherings [248-250] by CDC. Conversely, the infection rate in China was decline comparatively but increases very rapidly around the world especially in Iran and in major countries of European regions including Spain, France and Germany. Very soon, every country of the European continents affected with COVID-19. Because of incubation period and asymptomatic carriers the transmission chain did not noticed and COVID-19 spread to a considerable population in various countries especially in Spain, France and Germany [148]. Till 7th March, 87,317 infection cases and 2977 death cases reported globally [251] in different 60 countries. The highest incidence of COVID-19 was reported in Italy, Iran and South Korea after China [252]. Till March 10, the COVID-19 affected every continent of the world [253]. As of March 11, 2020, the epidemics of third human pathogenic coronavirus (COVID-19) declared as pandemic by WHO [43]. Till pandemic, majorly affected countries affected with COVID-19 are mentioned in Table 3.

Table 3. The countries with highest incidences and mortality rates of COVID-19 disease till March 11, 2020 [252].

Countries	Total Cases	Recovered	Deaths	Mortality rate
China	80,796	62,801	31,169	3.92%
Italy	12,462	1,045	827	6.63%
Iran	9000	29,59	354	3.93%
South Korea	7,869	33	66	0.83%
France	2,281	12	48	2.10%

Spain	2,277	183	55	2.41%
Germany	1,966	25	3	0.15%
United States	1,322	15	38	2.87%
Switzerland	652	4	4	0.61%
Japan	639	118	15	2.34%

The COVID-19 affected 117 countries around the world from every continent [43, 126, 277] individual infected with COVID-19 majorly from China, Italy, Iran and South Korea [254]. By the mid of March, Italy reported highest incidence of COVID-19 after China, Italy reported 24,747 cases, Iran 13,938 [160]. Till the third week of March, worldwide cases reached to 270,069 very brusquely with 11,271 deaths, Italy reported 4,032 fatalities, China, 3,248 cases, Iran 1,433 and Spain reported 1,044 deaths [255].

The increase in the European Countries epidemics

After declaring of COVID-19 as first pandemic of 21st century, coincidently, infections acceleration increases in the European continent with distressing fatalities predominantly in Italy32. As of March 22, global cases of COVID-19 were reached to 292,142 with 12,784 throughout 189 countries [32]. The incidence was high in older and aged people [256]. In robust spread of COVID-19 in European continent was mainly associated with asymptomatic carriers, which play considerable role in the infection distribution. As asymptomatic patients remain unnoticed and transmission series extended due to belated diagnosis of asymptomatic patients [257]. In a while as of March 25, Italy become leading country in terms of infection cases where 74, 386 affected with COVID-19 and 7, 503 lost their lives [258]. Robust spread of COVID-19 continued till 31st March, overall global cases were reached to 693,224 with 33,106 deaths. The 392,757 cases reported in European Region with 29,962 fatalities, after European Region, highest cases 142,081 reported in Americans region with 2457 fatalities, followed by 103,775 in Western Pacific Region with 3649 fatalities, Eastern Mediterranean reported 46,329 with 2813 fatalities, South East Asia regions 40, 84 with 158 deaths while the African regions reported 3486 cases with 60 fatalities [259].

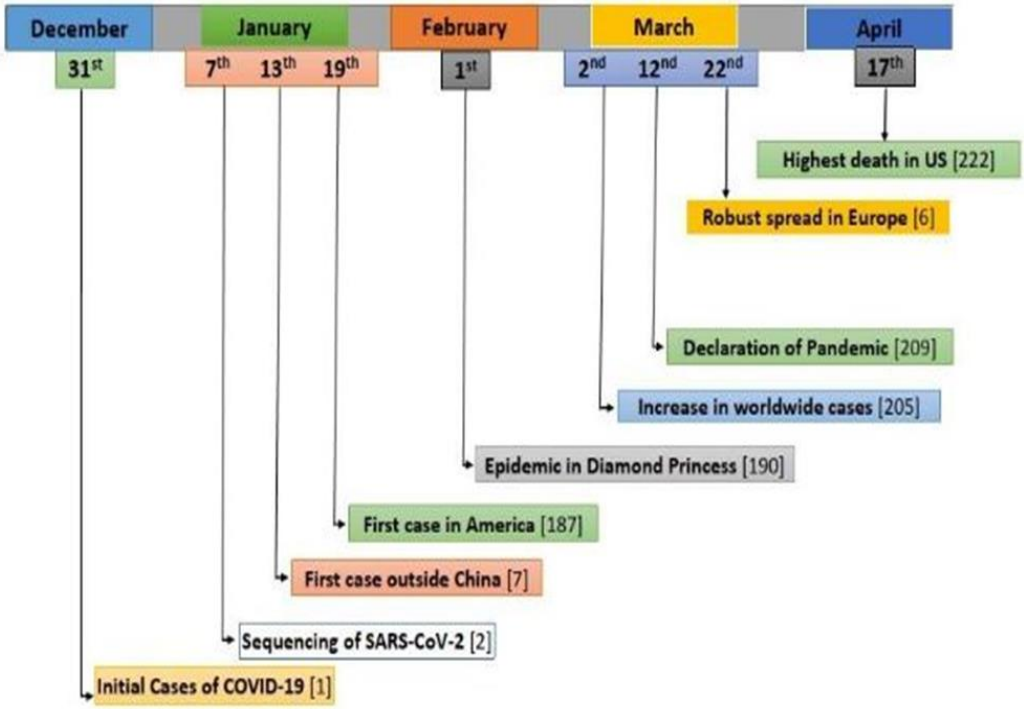

Figure 3. The important events of COVID-19 disease

Conclusion

All the human population appeared to be susceptible for it however, susceptibility and disease severity increases with presence of background diseases like cardiovascular diseases, diabetes and hypertension. The environmental temperatures have no any significant role on the transmission of COVID-19. Initially, disease grabbed the China and then quickly spread throughout the world and declared pandemic in the second week of March. In the March the Chinese control the COVID-19 but outside China infection acceleration increases especially in Europe and then Americas regions. The previous coronaviruses proved merely as a tip of iceberg after emergence of recently identified SARS-CoV-2 with potential of pandemic status that significantly revealed the concealed capabilities of virulence and contagiousness of betacoronaviruses group. Moreover, CRISPR related technologies are appeared to be very significant in the diagnosis of COVID-19. Furthermore, a new nucleic acid based detection method can be developed by advancing the already existing methods mentioned earlier to detect the COVID-19 infection rapidly with higher specificity and sensitivity at low cost.

Conflict of interest

The authors have declared no conflict of Interest.

Acknowledgments

The authors wish to thank and acknowledge their respective universities and institutes

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