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The novel SARS-CoV-2 pandemic: Possible environmental transmission, detection, persistence and fate during wastewater and water treatment

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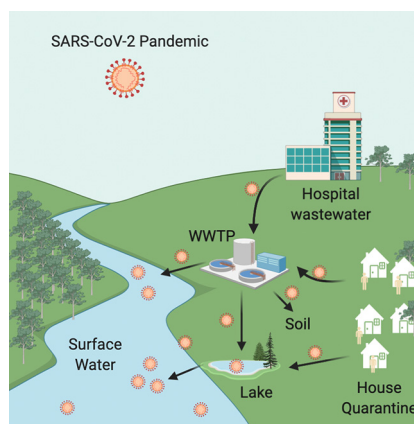
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HIGHLIGHTS

- Humidity and temperature decide survival and viability of SARS-CoV-2 in droplets.
- SARS-CoV-2 contamination of water bodies may be possible through faecal-oral route.
- SARS-CoV-2 RNA was detected in wastewater across the globe.
- Coagulation-flocculation, filtration can remove SARS-CoV-2 RNA.
- Complete inactivation of SARS-CoV-2 is possible through chlorination.

GRAPHICAL ABSTRACT



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ABSTRACT

The contagious SARS-CoV-2 virus, responsible for COVID-19 disease, has infected over 27 million people across the globe within a few months. While literature on SARS-CoV-2 indicates that its transmission may occur predominantly via aerosolization of virus-laden droplets, the possibility of alternate routes of transmission and/or reinfection via the environment requires considerable scientific attention. This review aims to collate information on possible transmission routes of this virus, to ascertain its fate in the environment. Concomitant with the presence of SARS-CoV-2 viral RNA in faeces and saliva of infected patients, studies also indicated its occurrence in raw wastewater, primary sludge and river water. Therefore sewerage system could be a possible route of virus

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outbreak, a possible tool to assess viral community spread and future surveillance technique. Hence, this review looked into detection, occurrence and fate of SARS-CoV-2 during primary, secondary, and tertiary wastewater and water treatment processes based on published literature on SARS-CoV and other enveloped viruses. The review also highlights the need for focused research on occurrence and fate of SARS-CoV-2 in various environmental matrices. Utilization of this information in environmental transmission models developed for other enveloped and enteric viruses can facilitate risk assessment studies. Preliminary research efforts with SARS-CoV-2 and established scientific reports on other coronaviruses indicate that the threat of virus transmission from the aquatic environment may be currently non-existent. However, the presence of viral RNA in wastewater provides an early warning that highlights the need for effective sewage treatment to prevent a future outbreak of SARS-CoV-2.

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1. Introduction

The novel COVID-19 disease, caused by the SARS-CoV-2 virus was declared a pandemic by the World Health Organization (WHO) on 11th March 2020 (Yeo et al., 2020). The first cases of SARS-CoV-2 infection were reported in Wuhan, China, in late 2019, when a cluster of patients was diagnosed with primary atypical pneumonia (Lu et al., 2020). Later genetic analyses revealed this virus to be a member of the family Coronaviridae, which comprises of retroviruses, most of which are pathogenic to animals and some to humans (Lu et al., 2020). At this point, statistics pertaining to this pandemic, including the number of people affected, in a critical state and dead around the globe, are rapidly increasing with each passing minute. Currently, SARS-CoV-2 has infected >27,000,000 people across the globe and claimed >8,91,000 lives by 08-09-2020. Comparatively, the SARS-CoV outbreak of 2002, which also started in China, infected only about 8000 people and claimed <900 lives. Likewise, in 2012, the Middle Eastern respiratory syndrome (MERS), caused by another novel betacoronavirus, the MERS-CoV, had its first outbreak in Saudi Arabia. Its spread was contained rapidly and completely by July 2015 (Lu et al., 2015). Similar to the SARS and MERS outbreak, COVID-19 outbreak has possible zoonotic origins, revealing the capacity of coronaviruses to frequently jump the species barrier (Hanscheid et al., 2020).

The current transmission of COVID-19 appears rampant and out of control. Although the infection by SARS-CoV-2 is characterized as generally mild, its reproduction number (R_0), which is an index of the transmissibility of a virus, is much higher than SARS-CoV or MERS-CoV (Liu et al., 2020a, 2020b). Therefore, it is entirely plausible that the epidemiology, pathogenesis, dormancy, and other physicochemical aspects may also be distinctly different from SARS-CoV. The SARS-CoV-2 virus is an enveloped, enteric virus (Kampf et al., 2020). While most enteric viruses comprise of genetic material within a protein coat, enveloped viruses have an additional envelope predominantly composed of lipids and proteins (Wigginton et al., 2015). Enveloped viruses, such as the novel SARS-CoV-2 are usually not associated with faecal contamination and are rapidly inactivated in the aquatic environment. However, numerous copies of SARS-CoV-2 genetic material were quantified inside the inpatient toilets in a hospital in Wuhan, which was initially attributed to the aerosolization of the virus (Liu et al., 2020a, 2020b). Later, additional investigations pointed towards faecal shedding of the virus by infected patients (Xu et al., 2020a, 2020b), thus making the sewage system a possible route of SARS-CoV-2 release into the environment. A recent scientific brief by the WHO confirmed the presence of non-infectious SARS-CoV-2 viral RNA in wastewater influents and/or sludge from Milan, Italy; Paris, France; Murcia, Spain; Brisbane, Australia; multiple locations in the Netherlands; New Haven, Connecticut and eastern Massachusetts, United States of America; and in poliovirus surveillance sites across Pakistan (WHO, 2020a). Additionally, the presence of SARS-CoV-2 viral RNA has been confirmed in water drawn from Seine River and Ourcq canal, Paris, France (Lesté-Lasserre, 2020) and in rivers in Italy and Ecuador (Rimoldi et al., 2020; Guerrero-Latorre et al., 2020). At present, these evidences are utilized to better understand viral shedding

dynamics, to monitor viral circulation, to facilitate early detection in regions with limited clinical surveillance, and as a possible early warning tool for COVID-19 outbreak (Orive et al., 2020). Surveillance of SARS-CoV-2 RNA in water and wastewater systems can increase preparedness in the event of an outbreak.

Currently, there are no reports suggesting the transmission of SARS-CoV-2 from wastewater, and cell culture model-based evaluation of virus infectivity from environmental samples is lacking till date. Environmental surveillance for SARS-CoV-2 is a rapidly evolving field and the WHO has recommended several additional focused studies to obtain insights on possible persistence of infectious SARS-CoV-2 and SARS-CoV-2 viral RNA fragments in the environment and also in untreated and treated sewage. Although currently there are no studies available to indicate the transmission of SARS-CoV-2 via sewage or wastewater systems, the possibility of such transmission has not been scientifically eliminated as yet. Contrary to the general character of enveloped viruses, published reports indicate that SARS-CoV-2 RNA may persist in wastewater for several days (Ahemed et al., 2020). This aspect was also stressed by the WHO in its latest scientific brief (WHO, 2020a, 2020b). Reports on other CoVs, such as HCoV, suggested that these viruses could survive for ten days in primary sewage and 5 days in secondary sewage at wastewater treatment plants (WWTPs) (Gundy et al., 2009). Aerosolization during different stages of the wastewater collection and treatment and also during sludge treatment and handling has been linked with the risk of rotavirus and norovirus infection (Pasalari et al., 2019; Uhrbrand et al., 2017). Inhalation of aerosol borne viruses might give rise to adverse health effects, including gastrointestinal and respiratory diseases among the workers at WWTPs. The prolonged presence of SARS-CoV and MERS-CoV in the environment was linked with faecal excretion and subsequent environmental contamination (Yeo et al., 2020; Zhou et al., 2017; Goh et al., 2013). This route of transmission is hence, also plausible for the SARS-CoV-2, although there is no evidence for the same till date. Currently, evidences suggest that WWTPs receiving sewage from hospitals and isolation centres treating patients infected with the novel coronavirus have elevated viral load. Although the viral load undergoes considerable dilution by the time it enters wastewater treatment plants, recent reports suggest that approximately 2 copies/100 mL of viral RNA to 3×10^5 copies/100 mL are detected in the WWTP influent depending on the scale of community spread in the area (Foladori et al., 2020). With the growing demand for water reuse and reclamation, the role of WWTPs in the transmission of SARS-CoV-2 and potential health risk needs to be assessed critically.

The SARS-CoV-2 virus has not been detected either in water treatment plants (WTPs) or in drinking water till date. However, possible contamination of water sources via non-point discharges cannot be ruled out (Kumar et al., 2020a, 2020b). For instance, the practice of open defecation or use of pit toilets, which are common in low-income countries and are used by approximately 900 million people around the world, has now been cited as a possible source of viral contamination of soil and groundwater (Foladori et al., 2020). Similarly, leaky sewer lines may contaminate the water distribution networks

during negative pressure events when wastewater from the surrounding environment enters the water distribution network. Specifically, countries with intermittent water supply and those that do not maintain the required residual chlorine levels would be more susceptible (Mohapatra et al., 2014). Therefore, inadequate and/or faulty sewer systems leading to improper sanitation may pose a concern for viral contamination in the aquatic environment.

This review emphasizes the role of environmental factors that may affect the spread and fate of the virus. Attenuation of the virus in WWTPs is expected due to the temperature, pH, effect of UV in sunlight, and co-existence of other pollutants (Foladori et al., 2020). However, since in-depth studies on the infectivity and transmission of SARS-CoV-2 are lacking, it is important to consider the possibility of environmental contamination and spread through wastewater. In WWTPs, the current need is to understand the fate of SARS-CoV-2 and its capacity to retain infectivity. Treated wastewater may be utilized as reclaimed water. In developing countries, poor sanitation and inadequate treatment can lead to non-point contamination of surface waters with SARS-CoV-2. Bioaerosols generated by forced aeration, mixing, and pumping may further aggravate the spread of the virus. A schematic illustrating these aspects and the possible fate of the virus in the environment is shown in Fig. 1. However, retention of infectivity of the virus under these conditions is not completely understood.

2. A brief history and architecture of the coronaviruses

When viewed under an electron microscope, a ring of small bulbous structures can be observed around the viral envelope, giving the characteristic appearance of a crown and hence the name “Coronavirus”

(Chafekar et al., 2013). They are enveloped viruses containing a single strand of positive-sense RNA (Lu et al., 2020) of 26–32 kbp length (Yeo et al., 2020). A list of important pathogenic coronaviruses is provided in Table A1 in the electronic supplementary material (ESI). The tendency to cause pathogenicity in humans is seen only in alpha and beta coronaviruses. The *Betacoronavirus*, SARS-CoV-2 clusters with its sister virus SARS-CoV in the same group and shows maximum sequence homology-based on phylogeny, taxonomy and established practice (Fig. A1; ESI) (Gorbalenya et al., 2020). The virus particles are spherical in shape and are about 50–200 nm in diameter, and their volume and mass are 10^6 nm^3 and 10^3 MDa , respectively (Chen et al., 2020).

Coronavirus envelope comprises of three major proteins, namely, M, E, and S (Alsaadi and Jones, 2019). The M protein is a transmembrane protein with a net positive charge and basic nature with an isoelectric point of 9.6 (Hu et al., 2003). The E protein comprises of a short hydrophilic domain and a long trans-membrane hydrophobic domain with an overall net zero charge (Schoeman and Fielding, 2019). The S protein is a class-I fusion protein (Bosch et al., 2003), which is responsible for virus attachment and entry into the host cell. The overall charge on the envelope of SARS-CoV-2, hence becomes amphoteric due to positive charge of M protein and the negative charge of sugar residues on the S protein. The fusion complex of SARS-CoV-2 is stabilized over a wide range of pH, indicating that pH does not have much effect on the entry of the virus into the host (Aydin et al., 2014). The envelope surrounds the nucleocapsid, which protects the genetic material of the virus. The nucleocapsid is formed of N protein, which is a heavily phosphorylated RNA binding protein encoded by the virus. N protein is also an important component of the RNA polymerase complex, which is responsible for the replication of the virus (Fehr and Perlman, 2015).

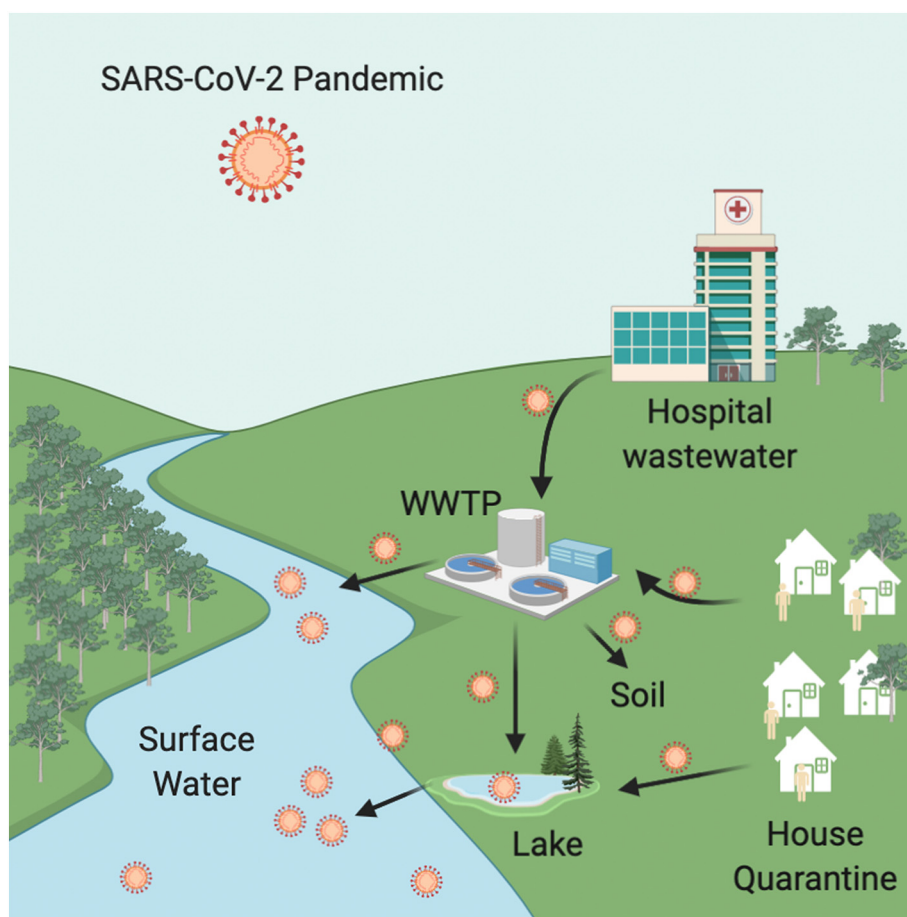


Fig. 1. Schematic representation of the possible transport processes and fate of SARS-CoV-2 in the environment.

SARS-CoV-2 utilizes its densely glycosylated spike (S) protein to enter a host cell (Fig. 2). The S protein is cleaved by host proteases or fusion enzymes at the polybasic cleavage site (RRAR). Upon invading the host cell, the S protein complex undergoes a substantial conformational rearrangement to fuse with the host cell membrane. The spike subunit, S1 binds to the host cell receptor, angiotensin-converting enzyme 2 (ACE2 protein) to mediate the fusion event. The distal part of S1 subunit harbors the receptor-binding domain (RBD) and contributes to the stabilization of the prefusion complex. Overall the binding mechanism to ACE2 is quite similar for SARS-CoV-2 and SARS-CoV, and the ACE2 domain is conserved across different mammalian species, thus allowing infection in a wide range of hosts (Andersen et al., 2020; Gorbalenya et al., 2020; Walls et al., 2020).

3. Transmission of the virus

Coronaviruses are characterized by their frequent species shifting events, from animal to human (zoonosis), human to animal (reverse zoonosis) and animal to animal (Graham and Baric, 2010). The novel SARS-CoV-2 virus is also believed to be of zoonotic origin. However, the reservoir species and intermediate host identities remain unclear and currently they are topics of controversy. However, the sheer magnitude of the pandemic indicates very high human to human transmission potential. Initial studies from the epicenter of the outbreak of the disease, Wuhan, indicated that the primary mode of transmission of this virus was via aerosols or droplets (Liu et al., 2020b) originating from both symptomatic and asymptomatic carriers (Li et al., 2020). Another well-established mode of transmission of SARS-CoV-2 is through fomites, i.e., inanimate objects (Kampf et al., 2020). Fomite transmission is deemed extremely dangerous as the virus can survive longer on metal and plastic surfaces than in air or in droplets (Kampf et al., 2020), causing severe threats of nosocomial infections and spread of infection among healthcare workers (Wang et al., 2020a, 2020b). Viral RNA was detected in saliva of patients, thereby indicating the possibility of salivary gland infection and subsequent transmission (Gu et al., 2020). Preliminary evidence suggests the potential of the virus to replicate in the small intestinal epithelium (Gao et al., 2020). The stool and urine samples of patients and asymptomatic carriers also contained the virus (Zhang et al., 2020) and faecal shedding was found to continue up to 21 days or more after the symptoms subsided. While currently there are no evidences establishing the infectivity of SARS-CoV-2 in wastewater, viral RNA has been quantified in both raw sewage and in surface water bodies worldwide (Wu et al., 2020; Ahmed et al., 2020a, 2020b). Thus, Faecal-oral transmission of the virus is also a possibility (Foladori et al., 2020; Heller et al., 2020). The established modes of transmission together with other possible modes of transmission of the virus are shown in Fig. 3 and the transmission modes are discussed further in the following sub-sections.

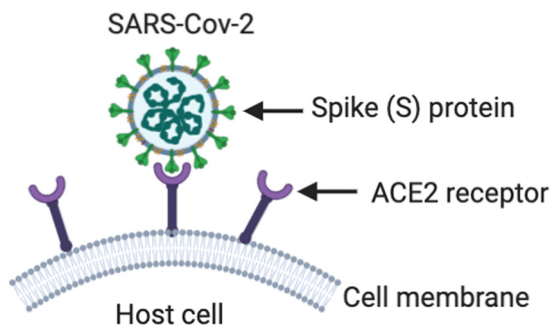


Fig. 2. Interaction of SARS-CoV-2 with the host cell membrane at ACE2 membrane receptor.

3.1. Droplet transmission

Droplet transmission is recognized as the primary mode of disease transmission in SARS-CoV-2. Generation of aerosols or droplets laden with infectious viral particles during bodily functions, such as coughing, sneezing, talking and exhaled breath, during toilet flushes, and sewage treatment has been well documented for other viruses (Chattopadhyay and Taft, 2018). Such functions can produce droplets ranging in size from <1 to $2000 \mu\text{m}$ diameter, which later evaporate, releasing the viral particles into the atmosphere, where they can remain in suspension (Chattopadhyay and Taft, 2018). Similar studies with SARS-CoV-2 resulted in detection of the viral particles from aerosol droplets of size ranging from 0.25 to $2.5 \mu\text{m}$ diameter (Liu et al., 2020b). A number of environmental conditions affect the size and viability of these aerosols in air, including temperature, relative humidity, and ultraviolet light intensity (Gunthe et al., 2020; Sooryanarain and Elankumaran, 2015). Evaporation of these carrier droplets vary according to the size of the aerosols and the environmental conditions. Upon evaporation, the viral particles remain in air and can be transmitted via air currents to nearby locations (Christian et al., 2004; McKimmey et al., 2006). The size of aerosols generated can vary between various sources, such as from a sick person, toilet flushes, or between various processes in a WWTP (Chattopadhyay and Taft, 2018). In general, smaller particles are generated during toilet flushes and from sick persons, while much larger particles are released from treatment plants (Fig. 4). Evaporation of the initial carrier droplets generated from toilet flushes leads to the formation of smaller droplet nuclei containing the viral particles (Chattopadhyay and Taft, 2018). Such droplet nuclei are typically $<10 \mu\text{m}$ in diameter and have been identified to travel through the air causing SARS-CoV infection (Chan et al., 2011). Researchers quantified viral RNA in aerosol samples collected from an intensive care unit (ICU) of a hospital in Wuhan (deposition rate of $113 \text{ copies m}^{-2} \text{ h}^{-1}$), even when the viral load in the air, as well as in aerosol samples collected from other locations in the hospital, was negligible (Yuan et al., 2020). The sampling was conducted for a period of 3 h.

The larger aerosol particles generated in treatment plants are normally more sluggish and can only travel over short distances. However, some of the aeration processes employed during the biological treatment of sewage have been reported to create aerosols of smaller size, which may travel longer before being evaporated (Chattopadhyay and Taft, 2018). Nevertheless, workers at treatment plants may be exposed to a high risk of infection from aerosols generated at WWTPs (Wigginton et al., 2015). Particles generated via sneezing, coughing, or exhalation follow different fate and are comprised of the smallest droplets. These actions generate muco-salivary droplets which follow short-range semi ballistic emission trajectories. The peak exhalation speed is $10\text{--}30 \text{ m/s}$ ($33\text{--}100 \text{ ft/s}$). Such a multiphase 'puff' may remain suspended in ambient air and can travel up to $7\text{--}8 \text{ m}$ (23 to 27 ft) (Bourouiba, 2020). Van Doremalen et al. (2020) compared the stability of SARS-CoV-2 and SARS-CoV aerosols, in which the decay rate of the virus was evaluated using a Bayesian model. It was observed that in both cases, the virus particles retained their infectivity up to 3 h. This study highlights the high risk of transmission of the viruses through functions, such as exhalation, sneezing, and coughing (Van Doremalen et al., 2020). Through this route, the viral transmission may occur over longer distances within a short time period. Therefore, the current social distancing norms set by the WHO (2 m distance between people) may not be sufficient to curb viral transmission.

The droplet transmission mechanism can be summarized in three steps, as shown in Fig. 4. The first step is the release of airborne particles containing the virus (Step 1), followed by the evaporation of the aerosol leading to the virus remaining suspended in air (Step 2). However, some of the drops containing the virus precipitate on the ground. Transmission of either the virus in suspension or the aerosols via air currents



Fig. 3. Known and possible modes of human-to-human transmission of SARS-CoV-2.

The red arrows indicate those transmission modes that are hitherto scientifically and medically validated and constitute a known threat of transmission. The black arrows indicate possible transmission mechanisms that need more scientific evidence for confirmation as threats. 1. The virus is shed via small droplets or aerosols generated by sneezing, coughing or exhalation by a sick person. 2. Evaporation of the droplets lead to viruses remaining suspended in the air, infecting healthy individuals. 3. Further, from air, the virus can settle on inanimate surfaces, including wood, plastic or metal surfaces, where they survive for long periods. 4. Shedding of SARS-CoV-2 virus is observed in faeces, urine and saliva of infected individuals, thus leading to entry of the virus into the sewer systems and sewage treatment plants. 5. Aerosolization of water containing the virus during toilet flushes or during wastewater collection and aeration can again lead to transmission of COVID19 disease via droplets. 6. Viral particles in the saliva and stools of patients can lead to nosocomial infections as the viral load increases with increase in number of infected patients. 7. While there are no evidences of infection hitherto available, viral contamination can occur via sewage treatment plant effluents, which enter surface waters and/or non-potable water sources. This pathway can also indirectly affect healthy individuals who are engaged in recreational activities, such as, swimming or gardening utilizing contaminated water resources.

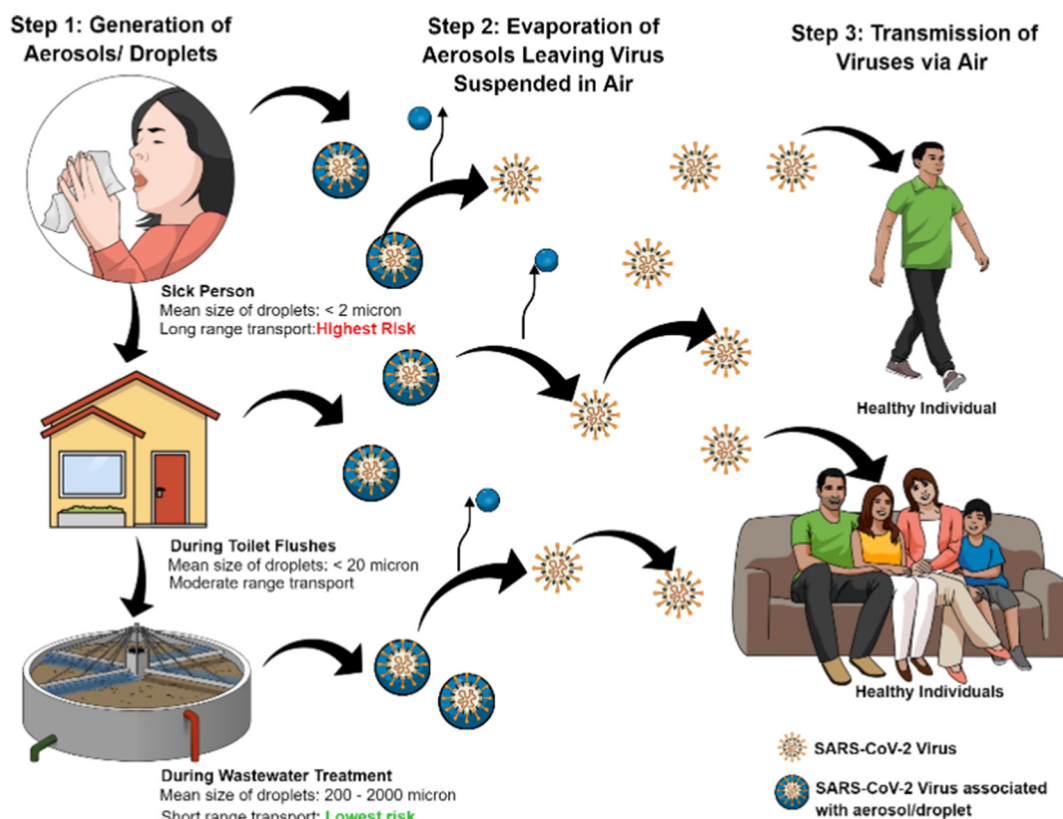


Fig. 4. Steps involved in droplet mode of transmission of SARS-CoV-2 virus.

The vertical arrows are used to pictorially illustrate the evaporation of droplets, leading to suspension of viruses in air. The term 'risk' in the figure denotes the risk of transmission from one point to another and not the risk of infection. The mean droplet size values were obtained from [Chattopadhyay and Taft, \(2018\)](#) and [Liu et al. \(2020b\)](#).

can infect healthy individuals, transmitting the disease (Step 3). The second and third steps are instrumental in rapid transmission of the disease.

Additionally, aerosol or droplet mode of transmission is also known to have the potential to concentrate viral particles ([Chattopadhyay and Taft, 2018](#)). Concentration in the individual droplets is size-dependent; smaller the drops, higher the concentration of virus, as smaller drops are derived from a higher relative amount of water ([Chattopadhyay and Taft, 2018](#)). Another important factor is the cell surface hydrophobicity of the virus. The more hydrophobic the virus, greater is the probability of particles being expelled in a small amount of medium ([Chattopadhyay and Taft, 2018](#)). Initial crystallographic evidences of the SARS-CoV-2 spike and envelope proteins indicated that they are amphiphilic and hydrophobic, respectively ([Lon et al., 2020](#)). The hydrophobic nature of the envelope may explain the rapid expulsion of the virus via smaller droplets. Also, smaller aerosol particles have a greater tendency to be inhaled. Subsequently, they settle in the tracheobronchial and alveolar regions in humans ([Sooryanarain and Elankumaran, 2015](#)).

3.1.1. Effect of relative humidity, temperature and sunlight

The two most important factors that can dictate the infectivity of the virus in droplets are relative humidity and temperature. In general, enveloped viruses are known to retain infectivity longer at a lower relative humidity (RH $\leq 50\%$) ([Chattopadhyay and Taft, 2018](#)). At low RH, the aerosols evaporate rapidly, the droplet size reduces, and the viral particles in the droplets can travel longer distances ([Yang et al., 2012](#)). Although salinity increases due to evaporation can cause virus inactivation, the hydrophobic moieties, and proteins surrounding the enveloped viruses lower the probability of inactivation ([Chattopadhyay and Taft, 2018](#); [Yang and Marr, 2012](#)). A recent study suggested that under high humidity, enveloped viruses, such as SARS-CoV-2, may be

rapidly inactivated due to the interaction of water with the lipid envelope ([Kumar et al., 2020c](#)). Moreover, at low RH, the nasal airway in humans dry up more frequently, such that the mucosal layer is less effective in trapping pathogens ([Sun et al., 2020](#)). The SARS-CoV-2 pandemic started in China when it was experiencing a drought in December 2019 ([Sun et al., 2020](#)). Climate change-induced increased evapotranspiration, leading to a decrease in soil and air moisture throughout the northern hemisphere, may have played a significant role in expediting the disease transmission ([Lian et al., 2020](#)). A recent study conducted in Wuhan reported that SARS-CoV-2 transmission was faster under warm (13–24 °C) and dry weather conditions (RH $\sim 50\%$, and precipitation < 30 mm/month) ([Bu et al., 2020](#)). In the context of aerosol transmission, it is important to determine the minimum infectious dose of the virus that should be present in the air for causing severe infection. However, for data compiled across 80 locations around the world, [Gunthe et al. \(2020\)](#) reported no significant relationship between RH and the number of COVID-19 infected persons.

The prevalence of COVID-19 disease has been remarkably lower in countries along the equator ([Gunthe et al., 2020](#)), indicating a role of temperature and UV index on viral viability and transmission. The findings of [Bannister-Tyrrell et al. \(2020\)](#) and [Shi et al. \(2020\)](#) indicated a possible reduction in SARS-CoV-2 spread with the onset of summer in the northern hemisphere. While early studies over 1–2 months across various countries in the North Hemisphere did not show a decline in transmission of the virus with a rise in temperature ([Luo et al., 2020](#)), a later study found that both temperature and UV index affected aerosol mode transmission ([Gunthe et al., 2020](#)). The temperature range 5–15 °C showed the highest infection rate. These results were in consensus with results reported by [Liu et al. \(2020a\)](#) and [Bu et al. \(2020\)](#). The UV index is also an important factor determining the spread of the disease. [Gunthe et al. \(2020\)](#) reported that the cumulative number of infected cases increased with UV index from 1, peaked at 2.5, and

subsequently decreased. For the areas with a UV index >5 , the number of confirmed COVID-19 cases decreased further. In general, the inactivation of microbes under prolonged exposure to UV irradiation in sunlight is well documented.

3.1.2. Effect of air quality

Long-term exposure to polluted air may increase the risk of infection and fatality, as observed for the earlier SARS-CoV infection (Cui et al., 2003). A possible reason could be the deterioration of respiratory tract epithelial lining due to prolonged exposure to various air contaminants, resulting in compromised lung functioning. Similarly, air conditioning leading to air re-circulation also increases the chances of infection (Cheung et al., 2003). In many office spaces, air conditioning causes nasal airways to dry up (Wolkoff, 2018), leading to slow mucociliary clearance (Lowen and Steel, 2014). This makes phagocytosis by the innate immune system difficult in the upper airways, thereby increasing the risk of infections (Conticini et al., 2020). Furthermore, cold and dry air, such as those that are prevalent in air-conditioned rooms, are known to alter the natural rheology of mucous, preventing the natural pathogen trapping function of mucous (Lowen and Steel, 2014). The virus particles also associate with dust and other particulates. The hydrophobic SARS-CoV-2 may retain its infectivity by associating with such particles.

A few studies have highlighted the effect of air pollution on SARS-CoV-2 lethality and morbidity. Stagnant air in indoor spaces and closed structures are reported to cause rapid transmission of the virus (Qian et al., 2020). This study reported that 254 out of 318 confirmed cases of the disease occurred within the houses of identified infected individuals, and a majority of the infections appeared to have been transmitted in an indoor setting or in a transportation system. Good ventilation and proper filtration of re-circulated air can significantly decrease the risk (Faridi et al., 2020). Such systems can flush out contaminated air rapidly, reducing the risk of aerosols remaining suspended in the air. No traces of viral RNA were detected in the air in 5 intensive care units (ICUs) housing COVID-19 patients in a hospital in Iran equipped with efficient air filtration systems (Faridi et al., 2020).

Conticini et al. (2020) showed that air pollution not only led to rapid propagation of SARS-CoV-2 via aerosols but also led to increased lethality. The study area chosen for their research included the Lombardy region, in Northern Italy, which recorded the highest number of infected persons and the highest mortality rates in Italy and the world. Coincidentally, Lombardy was also reported as the most polluted region in Italy and Europe, based on the data from the Ozone Monitoring Instrument on NASA's Aura satellite (Conticini et al., 2020). Increased industrialization led to higher incidences of smog-related air pollution in Northern Italy, which in turn, led to the stagnation of suspended pollutants in the air. These conditions were more conducive for the propagation of SARS-CoV-2 via aerosols. However, another study conducted in Lombardy and its proximity could not establish a clear consensus on the transport of SARS-CoV-2 through the air using PM₁₀ as a carrier (Bontempi, 2020). Piedmont cities in the neighborhood of Lombardy with a higher concentration of PM₁₀ experienced lower infection cases compared to Lombardy cities having lower levels of PM₁₀ and a higher number of infections.

Prolonged exposure to polluted air may permanently alter immune systems, weakening the ciliary defenses in the upper airways, even in young adults. This factor, coupled with a higher proportion of the aged population with other prevailing health conditions, could explain the high lethality in Italy. Higher mortality rates due to COVID-19 were also correlated to increased CO, NO₂, and particulate matter (PM 2.5 and PM 10) in China (Pansini and Fornacca, 2020; Fattorini and Regoli, 2020) and London (Sasidharan et al., 2020; Travaglio et al., 2020). Further studies also indicated that the short term changes in air quality (such as those induced by industry shut down due to government-mandated lockdowns) did not have much impact since irreversible damage to the immune system was caused by prolonged exposure to

polluted air (Bauer et al., 2012). Such irreversible lung damage is also reported in smokers. Recent studies indicated an upregulation of the ACE2 receptor expression in the lung tissue of smokers. Since the entry of SARS-CoV-2 into the host cells is initiated by binding to the ACE2 receptors, smokers are more susceptible to COVID-19 disease (Brake et al., 2020). However, the effects of inhalation of cigarette smoke in healthy individuals and susceptibility to SARS-CoV-2 infection and upregulation of ACE2 expression in the lungs need to be further analyzed.

3.2. Fomites or inanimate surfaces

On inanimate surfaces, SARS-CoV-2 can retain infectivity for several hours. Infectivity retention was maximum on plastic and stainless steel, where the half-life was 6.8 h and 5.6 h, respectively (Holbrook et al., 2020). Kampf et al. (2020) reported that CoVs could remain infective on plastic for 2–9 days at room temperature, depending on the strain (Kampf et al., 2020). In the cruise ship, Diamond Princess SARS-CoV-2 viral RNA remained on surfaces for up to 17 days after the inhabitants had left. Transmission of SARS-CoV-2 in the cruise ship was attributed, not to the air conditioning mediated air re-circulation, but to contamination through fomites (Xu et al., 2020a, 2020b).

Prolonged virulence and infectivity of the virus retained on inanimate surfaces have implications on the spread of the disease among health care workers, hospital staff, scientists, and laboratory workers. Such cases were reported during the SARS and MERS outbreak (Hofmann and Pöhlmann, 2004). Often health care professionals refrain from wearing proper personal safety equipment, such as masks and gloves, while tending to patients undiagnosed with potential viral diseases (Barratt et al., 2019; Hofmann and Pöhlmann, 2004). This not only poses a risk for health care professionals, but it also poses a significant risk for other vulnerable patients. Similar instances were also reported during the MERS-CoV outbreak, mainly due to a scarcity in safety gears. These studies highlighted the importance of ensuring the availability and use of such safety gear by healthcare professionals. Appropriate disposal of used safety gear is also an important consideration (Khan et al., 2016). Disinfection of surfaces and equipment is also necessary for preventing the transmission of viral diseases (Khan et al., 2016). Due to the transmissibility of SARS-CoV-2 through inanimate surfaces, guidelines for proper handling of samples containing SARS-CoV-2 have been released by the WHO (WHO, 2020a, 2020b, 2020c). Disinfectants containing 62%–71% ethanol, 0.5% hydrogen peroxide, and 0.1% sodium hypochlorite can effectively reduce CoV infectivity in less than a minute. Routine sanitization and disinfection of hospital isolation wards, their anterooms, and bathrooms in Singapore and China indicated a remarkable decrease in viral load in the air and on surfaces (Ong et al., 2020; Wang et al., 2020a, 2020b). Hence, these disinfectants should be widely used in hospital wards and laboratories where patient and viral samples are routinely handled (Kampf et al., 2020).

3.3. Faecal-oral route

A possible route of viral contamination in the water bodies is through faecal matter of patients (Yeo et al., 2020). Faecal shedding of viruses can indicate gastrointestinal complications in addition to respiratory infection. Contaminated faecal matter may enter natural water bodies through leaky sewers or due to compromised WWTPs, especially in developing countries (Majumdar et al., 2019). Aerosolization of infectious viruses during toilet flushes has been attributed to disease transmission to household members in the case of SARS-CoV (McKimmey et al., 2006) and SARS-CoV-2 (Mcdermott et al., 2020). Poor hygiene and sanitation can also lead to contamination of the patients' hands, leading to disease transmission during routine activities, such as handshakes and food intake. Such practices can also lead to contamination of fomites and inanimate surfaces.

During the outbreak of SARS and thereafter, that of MERS, a much higher number of patients suffered from diarrhea compared to that

during bird flu (Yeo et al., 2020). Some in-vitro studies indicate that the pulmonary implications of MERS-CoV infection may be secondary to gastroenteric implications (Zhou et al., 2017). The initial indication of a possible faecal route of transmission came from the detection of SARS-CoV-2 viral RNA from stool and anal swabs of patients at the People's Hospital of Wuhan University, Wuhan, China (Gao et al., 2020). The SARS-CoV-2 infection is known to develop diarrhea in 2–10% of the patients (Yeo et al., 2020). In a cohort of 1099 patients tested positive for SARS-CoV-2 infection from 552 hospitals in China, 5.0% exhibited symptoms, such as nausea or vomiting, and 3.8% presented symptoms of diarrhea (Hindson, 2020). Approximately 49% of the patients who tested positive for the disease came to the hospitals because of digestive symptoms and not respiratory symptoms. It was also noted that the number of discharges and disease alleviation was higher in patients who did not show any digestive symptoms (Hindson, 2020). Furthermore, electron microscopy of four SARS-CoV-2 positive faecal specimens detected viable virus in stool samples from two patients who did not have diarrhea (Hindson, 2020). A significantly high concentration of SARS-CoV-2 was quantified in the air inside the inpatient toilets in a hospital in Wuhan, which was attributed to aerosolization from faeces and urine (Liu et al., 2020a, 2020b). Similarly, eight out of ten children who tested positive for SARS-CoV-2 infection was reported to persistently shed viral particles even when there was no trace of nasopharyngeal viral shedding (Xu et al., 2020a, 2020b). Recently, published data from China reported presumed transmission of SARS-CoV-2 from one patient with gastrointestinal symptoms to 10 healthcare workers and four other patients (Mcdermott et al., 2020). Moreover, since these viruses remain active at temperatures as low as 4 °C and under highly alkaline conditions because of their lipid envelope, they might retain infectivity in hospital wastewater and domestic wastewater for a considerable time, as shown for SARS-CoV (Wang et al., 2005b). Thus, with an increasing number of diarrhea cases, the role of water systems in the transmission of these viruses needs to be accessed critically. These observations, coupled with the high survival rates of coronaviruses, such as SARS-CoV and MERS-CoV, in stool samples (Yeo et al., 2020), implies that a faecal transmission route for SARS-CoV-2 may be possible. Some recent reviews have highlighted frameworks for possible SARS-CoV-2 faecal-oral transmission routes (Foladori et al., 2020; Heller et al., 2020). The studies indicating the presence of SARS-CoV-2 RNA in gastrointestinal tract samples are summarized in Table 1.

Many researchers have linked the presence of infectious viruses and viral RNA in faecal samples to the expression of ACE2 receptors in the gastrointestinal tract. Additionally, structural analyses indicated that the binding affinity of SARS-CoV-2 virus to the ACE2 receptors is more potent than that of SARS-CoV (Yeo et al., 2020). ACE2 is known to be abundant in the epithelia of the lungs and intestine in humans, suggesting a probability of faecal-oral transmission of the disease. Specifically, some studies have suggested that ACE2 is primarily located on the luminal surface of differentiated small intestinal epithelial cells, while the expression of ACE2 receptors was lower in the colon (Gao et al., 2020). Furthermore, a larger number of ACE2 viral host receptors were demonstrated in the intestinal epithelial cells as opposed to the respiratory epithelium in several patients (Hindson, 2020).

The fate of a large variety of enteric viruses has been explored in the urban water cycle (Guerrero-Latorre et al., 2018). The presence of these viruses in faeces and subsequently in the sewer systems were thus predicted effectively (Fongaro et al., 2015), and their concentration was reported to vary from 10^3 to 10^{11} copies/g of faeces (Gerba et al., 2017). These studies have primarily focused on non-enveloped enteric viruses as they can better survive the harsher environments than their enveloped counterparts (Fong and Lipp, 2005). The hypothesis that enveloped viruses are generally absent in wastewater systems, and lakes infiltrated with sewage was proven wrong when the human influenza virus was found to be persistent in urban water systems (Pinon and Vialette, 2019). However, even during a major influenza outbreak,

the concentration of influenza viruses in the water bodies was not found to be high enough to pose a risk (WHO, 2007). Nevertheless, the likelihood of a mutant form of human influenza virus to get shed into these water bodies to achieve higher concentrations and be infectious for a prolonged duration cannot be eliminated (Wang et al., 2005a, 2005b; Wigginton et al., 2015). No studies have conclusively shown the transmission of infective SARS-CoV-2 from WWTPs, although numerous studies have demonstrated viral RNA in sewage in the WWTPs.

3.4. Transmission modeling and quantitative microbial risk assessment (QMRA)

The impact of most zoonotic events is often underestimated due to limited surveillance and paucity of disease burden data, especially in developing countries. Mathematical models have been used to predict how super spreaders transmit SARS-CoV-2. The risk of disease introduction and the strategies and decisions for mitigation of the onset and spread of such outbreaks can be predicted by mathematical models. With a shift towards a data-driven approach, simple mathematical models can be used to provide an estimation of possible impacts in a timely fashion, while complex models can be used to simulate real-world scenarios by incorporating several complex parameters in the model (Wiratsudakul et al., 2018). Modeling results of 3200 sub-populations from 200 different countries and regions indicated that sustained 90% travel restrictions to and from Mainland China would only modestly affect the epidemic trajectory unless combined with a 50% or higher reduction of transmission in the community (Chinazzi et al., 2020). By using time-dependent contact and diagnosis rates, Tang et al. (2020) proposed a risk transmission model for the novel coronavirus (SARS-CoV-2). They highlighted the incidence of the peak of this pandemic. Since human mobility plays a significant role in the spreading of SARS-CoV-2, a large-scale agent-based transport simulation (MATSim) model may be used to simulate the seasonal variation of the outbreak. This model considers the real-world behavior of an individual's daily path in an urban setting. A similar analysis was done for the influenza outbreak (Hackl and Dubernet, 2019). In addition to human mobility, human behavior integrated hierarchical (HiHi) model based on the SIR (Susceptible, Infectious, and Recovered) model, and the Wells-Riley equation may be utilized for ranking potential risk locations, such as home, offices, and schools. Such models were successfully used to predict the spread of smallpox (Zhang et al., 2018). Bayesian phylogeographic studies may further be implemented to uncover regional routes of transmission and factors responsible for the rate of viral diffusion within a particular country (Lu et al., 2017a). This approach incorporates the role of economic, agricultural, environmental, and regional climatic factors on viral diffusion in a community and hence can be used for region-specific predictions.

Considering the evidence of faecal shedding for both SARS-CoV and MERS-CoV, and their ability to remain viable in conditions that could facilitate faecal-oral transmission, it is possible that SARS-CoV-2 may also be transmitted via this route (Yeo et al., 2020). Studies already show that patients with SARS-CoV-2 can potentially contaminate the environment through respiratory droplets and faecal shedding (Ong et al., 2020). In order to assess the health risk of workers and residents associated with exposure to this virus from WWTPs, a combination of Gaussian plume dispersion model and quantitative microbial risk assessment (QMRA) may be useful, as has been reported for Rotavirus (RoV) and Norovirus (NoV) bioaerosols (Pasalari et al., 2019). Knowledge of several factors, including aerosol size profile, deposition dose (Lim et al., 2015), and water to air transfer coefficient (Hamilton and Haas, 2016), is essential for QMRA modeling of bioaerosols. It is vital to consider two critical mechanisms, i.e., aerosol dispersion followed by the transfer of the virus from water to air. Although data on SARS-CoV-2 in the aerosols generated from WWTPs are not available to date, previous studies have reported the distribution of other viruses, such as somatic

Table 1
Studies indicating the presence of SARS-CoV-2 virus in gastrointestinal samples.

Sample	Country	No. of patients tested	No. of patients positive for oral-faecal shedding	Type of virus detected	Concentration of detected in the study	Total number of days of faecal-oral shedding ^a	Reference
RT-PCR of rectal swabs	China	10	8 (including 1 asymptomatic case)	Viral RNA	-	27	Xu et al., 2020a, 2020b
RT-PCR of stool	USA	1	1	Viral RNA	~2.2 × 10 ³ copies/mL	-	Holshue et al., 2020
RT-PCR of urine and stool	China	73	39 (+ in faeces), 0 (+ in urine)	Viral RNA and nucleocapsids	-	1-12 days during disease period, up to 17 days after symptoms subsided	Xiao et al., 2020
ACE2 and viral nucleocapsid staining from gastric, duodenal, and rectal tissues							
RT-PCR of urine and stools	China	153	44 (+ in faeces), 0 (+ in urine)	Viral RNA and live, infectious viruses (using imaging)	<2.6 × 10 ⁴ copies/mL	47 days (cumulative)	Wang et al., 2020a, 2020b
Stool cultures followed by electron microscope imaging ^b							
RT-PCR of oral (saliva) and anal swabs	China	39 (15 after medical treatment)	8 (+ in saliva), 4 (+ in anal swabs), 2 (+ in both)	Viral RNA	~4.7 × 10 ³ copies/mL (in saliva) ~1.3 × 10 ³ copies/mL (in anal swabs)	-	Zhang et al., 2020
ELISA based detection of anti-SARS-CoV IgG and IgM							
RT-PCR of stool samples	China	9	6 (5 were readmitted after discharge due to prolonged faecal shedding)	Viral RNA	-	-	Su et al., 2020
RT-PCR of stool samples	China	14	5	Viral RNA	-	-	Zhang et al., 2020
RT-PCR of stool samples	China	74	41	Viral RNA	-	Mean of 27.9 days after first symptom onset	Wu et al., 2020
RT-PCR of stool samples	Hong Kong	59	9	Viral RNA	5.1 × 10 ⁵ copies/mL in patients with diarrhea 7.9 × 10 ³ copies/mL in patients without diarrhea	-	Cheung et al. (2020)
RT-PCR of stool and urine samples	China	42	28 (+ for faeces), 0 (+ for urine)	Viral RNA	-	6-10 days after nasal samples were negative	Chen et al., 2020
RT-PCR of stool	China	3	3	Viral RNA	~7.9 × 10 ³ copies/mL in 1 patient on 8-20 days after throat swab	8-20 days after throat swab	Xing et al., 2020a
RT-PCR of urine, faecal or anal swab and gastric fluid samples	China	16	1 (+ in urine), 6 (+ in gastric fluid), 11 (+ in faeces), 4 (+ in anal swabs) ^c	Viral RNA	-	20th day after negative throat swab	Huang et al., 2020
RT-PCR of anal swabs	China	69 discharged patients	4	Viral RNA and nucleocapsid protein RNA	255 copies/mL of viral RNA, 1.3 × 10 ² copies/mL of nucleocapsid protein RNA	9-25 days after discharge	Liu et al., 2020a, 2020b

^a From date of admission or initial testing.

^b The studies were conducted in 4 patient samples that had a high concentration of viral RNA.

^c Some patients tested positive in more than one sample drawn but were reported as independent cases in the study.

coliphages, F-specific coliphages, adenoviruses, norovirus, and enteroviruses at WWTPs (Kitajima et al., 2020). Based on a study conducted on adenovirus exposure, the WWTP workers were found to be at a higher risk when they were exposed to bioaerosols that emerged from the influent and from the biological oxidation ponds (Carducci et al., 2018). Recently, Zaneti et al. (2020) estimated health risks to WWTP workers by applying QMRA in WWTPs for extreme, aggressive, and moderate scenarios. Although this paper was published as a preprint (open-source platform), it elucidated a step by step approach for estimation of viable SARS-CoV-2 at WWTPs, their dose-response curve, and finally, the risk of infection. For the aggressive and extreme scenarios, the risk of infection to WWTP workers was found to be 6.5×10^{-3} and 3.1×10^{-2} , respectively. For the extreme scenario, the tolerable risk was higher than the benchmark set by WHO, i.e., 10^{-3} (Zaneti et al., 2020). Franklin and Bevins (2020) proposed a conceptual model on SARS-CoV-2 spillover from WWTPs to the natural water bodies that can further infect wild animals and, subsequently, human beings. Although the model was proposed with several assumptions, the basic hypothesis behind this model was that infective SARS-CoV-2 could be transmitted via sewage into the natural water bodies (Franklin and Bevins, 2020). Thus, SARS-CoV-2 may infect workers at WWTPs. Modeling studies performed should take into account the persistence, infectivity, and aerosolization of SARS-CoV-2 under various WWTP operating conditions. Moreover, the integration of environmental factors might provide more accurate risk estimation, especially in places where WWTPs are located in densely populated areas.

4. Detection of coronaviruses

4.1. Trends in virus detection

Detection of SARS-CoV-2 in various environmental matrices is a major bottleneck at present, as there is a lack of robust protocols depicting adequate sensitivity, specificity, and reproducibility. While most of the detection techniques are optimized for non-enveloped enteric viruses due to their high infectivity and stability in the environment, the same methods may not be suitable for enveloped viruses. Enveloped viruses differ significantly from non-enveloped viruses in terms of structural and genetic characteristics. Owing to difficulties in culturing human pathogens, detection of viral pathogens in the environment is tedious and cost-intensive. In general, the overall detection techniques broadly fall under qualitative and quantitative molecular approaches or in-vitro counts through plaque-forming units (PFU). In addition to RT-PCR (Kittigul et al., 2019; Ouardani et al., 2015; Wang et al., 2005a), other methods known for enteric virus detection include enzyme-linked immunosorbent assay (ELISA) (Atabakhsh et al., 2019; Pimenta et al., 2016), filtration techniques (Ikner et al., 2011) and bio-sensor technology (Altintas et al., 2015). Irrespective of the method used, it is important to achieve the lowest possible detection limit, which is usually achieved by preconcentrating a large volume of water or wastewater. Similarly, isolation of the infective virus, which can be analyzed through PFU, requires the preconcentration of a large number of viruses from the sample. Preconcentration of environmental samples is challenging due to the extremely low viral loads in environmental samples. During the preconcentration of viruses from water/wastewater, other constituents of the matrix may also accumulate and may interfere with subsequent analysis. After preconcentration, the high concentration of organic matter and suspended solids in WWTP influent samples were reported to interfere with virus analysis (Prado et al., 2019). The problem may be more severe while analyzing sludge samples characterized by a high concentration of organic matter (Matsui et al., 2003).

Various studies have focused on preconcentration of enteric viruses or their genetic materials from tap water (Ahmed et al., 2015), surface water (Ahmed et al., 2015), wastewater matrices (Fumian et al., 2019), and hospital effluent (Wang et al., 2005a) through membrane

filtration (Ahmed et al., 2015), electropositive filter media (Miao et al., 2019), electronegative filters (Cashdollar and Wymer, 2013), ultrafiltration (Ikner et al., 2011), hydroextraction and flocculation (Calgua et al., 2013), and quaternary amine (QA) methacrylate monolith columns (Rački et al., 2015). Among other chemicals, chloroform is commonly used to disperse virus aggregates (Gerba and Betancourt, 2017) and extract and concentrate the viral nucleic acid (Bae and Shin, 2016; Young et al., 2019) from various types of environmental samples. Studies with non-enveloped viruses have shown variable recovery after the preconcentration of different viruses (Shi et al., 2017). Based on a study with two bacteriophages and rotavirus, Pisharody et al. (2021) highlighted the need for optimization of the eluent for ensuring improved recovery of the virus of interest in adsorption elution based preconcentration protocols (Pisharody et al., 2021). Similarly, optimization of eluent would also be required for ensuring adequate recovery of enveloped viruses, since they show greater susceptibility to organic solvents (chloroform), pH variation, and temperature variation. Moreover, different assays may produce conflicting results when the concentration in wastewater is low. As coronaviruses tend to sorb onto organic matter and are protected by the suspended solids (Gundy et al., 2009), the true concentration of viruses cannot be determined in the absence of a robust method that can take into account both the liquid phase and solid phase concentration of viruses. Thus, these methods require further optimization before concentrating SARS-CoV-2 or its genetic materials, which are present at low concentrations (Table 2). Several recent reviews have focussed on preconcentration of viruses from environmental matrices (Corpuz et al., 2020; Kitajima et al., 2020; Michael-Kordatou et al., 2020).

Among several techniques, RT-PCR has increased the sensitivity, precision, and accuracy with which viruses can be detected (Fumian et al., 2019). Metagenomic analyses have recently made headway in the detection of human pathogenic viral genotypes in the environment. Shotgun metagenomic tools have been utilized simultaneously and independently to identify DNA and RNA of viral genotypes from different environmental matrices, including wastewater effluents, sewage sludge, and tap water (Bibby and Peccia, 2013; Jumat et al., 2017; Wang et al., 2018). The probability of identification of viruses depends both on the abundance and size of the viral genome (Bibby et al., 2011). For the first time, a metagenomic analysis was performed using MetaVir2 as a basic tool to evaluate the diversity of DNA viruses in wastewater effluent in the United States and France (O'Brien et al., 2017). Similar attempts were also made to study the diversity of RNA viruses in environmental matrices (Adriaenssens et al., 2018; Kittigul et al., 2019). However, based on a study conducted in Hong Kong, <60% of the metagenomic viral contigs recovered could be identified by the current NCBI and IMG/VR viral databases (Wang et al., 2018).

4.2. Trends in SARS-CoV-2 analysis in the environmental matrices

Recently, several attempts were made to detect SARS-CoV-2 genetic material in wastewater matrices (Ahmed et al., 2020a, 2020b, 2020c; Medema et al., 2020; Nemudryi et al., 2020; Wu et al., 2020a; Wurtzer et al., 2020), river (Rimoldi et al., 2020) and sludge samples (Peccia et al., 2020). A schematic representation of the sample preparation steps is shown in Fig. 5. The wastewater sample is subjected to a preconcentration/pretreatment step based on centrifugation (Medema et al., 2020), membrane filtration (Nemudryi et al., 2020), or pH adjustment (Ahmed et al., 2020a, 2020b). The sample volumes handled during preconcentration/pretreatment was found to range from 11 to 500 mL. Some studies suggested the use of a small sample volume to overcome the matrix effect. However, for samples with low viral load, detection of the viral RNA may not be possible when small sample volumes are used. Thus, a higher sample volume of 500 mL was preconcentrated to 150 to 200 μ L by some researchers to achieve a concentration factor of the order 10^3 (Nemudryi et al., 2020).

Table 2
Details of sample collection, analysis and concentration of SARS-CoV-2 and other enveloped viruses.

Country	Sewage or hospital wastewater	Sampling (grab or composite)	Sample pre-processing steps	Concentration method	Amount of sample (mL)	Quantitative technique used	Recovery	Concentration (copies/L)	Limit of quantification (copies/L)	Target virus	References
Australia	Sewage	Composite	a) pH adjustment, b) Centrifugation	a) Electronegative membrane filter b) Ultrafiltration	100–200	RT-qPCR	-	0–120	-	SARS Cov-2	Ahmed et al., 2020a
USA	Sewage	Composite and grab	Centrifugation	Ultrafiltration/electronegative filter	1000	RT PCR	54–56%	0–10 ³	10 ²	SARS-CoV-2	Sherchan et al., 2020
USA	Sewage	Grab and composite	-	NanoCeram	18,000	RT PCR	-	10 ⁴ –10 ⁵	-	SARS-CoV-2	Miyani et al., 2020
USA	Sewage	Grab and composite	Membrane filtration	Ultrafiltration	500	RT PCR	-	10–10 ⁴	-	SARS Cov-2	Nemudryi et al., 2020
USA	Sewage	Composite	Membrane filtration	Polyethylene glycol (PEG) precipitation	40	RT PCR	-	0–10 ⁵	-	SARS Cov-2	Wu et al., 2020 (preprint)
Brazil	Sewage	Composite	-	Ultracentrifugation	40	RT PCR	-	Qualitative	-	SARS-CoV-2	Prado et al., 2020 (preprint)
Spain	Sewage	Composite	-	Aluminum hydroxide adsorption-precipitation	200	RT PCR	-	0–5.5	4.45	SARS Cov-2	Randazzo et al., 2020
France	Sewage	Composite	Centrifugation & filtration	50 kDa MWCO filter membrane	50	RT PCR	-	0–10 ³	-	SARS-CoV-2	Trottier et al., 2020
France	Sewage	Composite	Not available	Ultracentrifugation	11	RT PCR	-	>10 ⁶	10 ³	SARS Cov-2	Wurtzer et al., 2020
Italy	Sewage	Composite	Filtration	PEG/dextran	250	Nested PCR	-	Qualitative	-	SARS-CoV-2	Rosa et al., 2020 (preprint)
Germany	Sewage	Composite	Centrifugation	Ultrafiltration	45	RT PCR	-	2.7×10 ³ to 3.7×10 ⁴	-	SARS-CoV-2	Westhaus et al., 2020
Switzerland	Sewage	Composite	Membrane filtration	a) Electronegative membrane filter b) Polyethylene glycol (PEG) precipitation	500	RT PCR	12–71%	0–10 ⁶	10 ⁴	Hepatitis E virus, adenovirus and norovirus	Masclaux et al., 2013
Netherlands	Sewage	Composite	Centrifugation	Ultrafiltration	250	RT PCR	70 ± 50%	Qualitative	-	SARS Cov-2	Medema et al., 2020
India	Sewage	Composite	Centrifugation & filtration	PEG precipitation	50	RT PCR	-	0–8.05 × 10 ²	-	SARS Cov-2	(preprint) Kumar et al., 2020d

(continued on next page)

Table 2 (continued)

Country	Sewage or hospital wastewater	Sampling (grab or composite)	Sample pre-processing steps	Concentration method	Amount of sample (mL)	Quantitative technique used	Recovery	Concentration (copies/L)	Limit of quantification (copies/L)	Target virus	References
Turkey	Sewage	Composite	Centrifugation	Ultrafiltration and PEG precipitation	250	RT PCR	-	0-9.33 × 10 ⁴	-	SARS Cov-2	Kocamemi et al., 2020a
Israel	Sewage	Composite	Filtration	PEG/alum precipitation	250-1000	RT PCR	-	Qualitative	-	SARS Cov-2, Adenovirus, MS2	(preprint) Bar Or et al., 2020
Australia (air-cruise)	Sewage	Grab	-	Ultrafiltration/electronegative filter	1000	RT PCR	-	0-8.8 × 10 ³	-	SARS-CoV-2	Ahmed et al., 2020c
Japan	Sewage and river water	Grab	-	Electronegative membrane-vortex (EMV) and membrane adsorption	200-5000	qPCR and nested PCR	-	0-10 ⁴	-	SARS-CoV-2	Haarimoto et al., 2020
Italy	Sewage, river	Composite and grab	-	-	500	RT PCR	-	Qualitative	-	SARS Cov-2	Kimoldi et al., 2020
Ecuador	River water	Grab	Preconditioning with 1 N HCl	Skimmed milk flocculation	2000	RT PCR	-	10 ⁵ -10 ⁶	-	SARS-CoV-2 and Adenovirus	(preprint) Guerrero-Latorre et al., 2020
Turkey	Sludge	Grab	Centrifugation & filtration	PEG 8000 adsorption	250	RT PCR	-	1.17×10 ⁴ to 4.02×10 ⁴	-	SARS-CoV-2	Kocamemi et al., 2020
USA	Primary sludge	-	-	-	2.5	RT PCR	-	1.7 × 10 ⁶ -4.6 × 10 ⁸	-	SARS-CoV-2	(preprint) Peccia et al., 2020
USA	Sewage, sludge, soil and pond water	Composite	-	Aluminum Flocculation	5000-20,000	PCR	-	Qualitative	-	HIV	(preprint) Ansari et al., 1992
Saudi Arabia	Surface water samples	Composite	-	Glass wool (VIRADEL)	10,000	RT PCR	4.5%-5.1%	10 ¹ -10 ⁴	-	Hepatitis A virus and coronavirus	Blanco et al., 2019
South Africa	Sewage	Composite	Filtration	HA filter	1000	qPCR	-	0-10 ⁵	10 ⁴	Hepatitis A virus, adenovirus norovirus, and coliforms	Adefisoye et al., 2016
China	Hospital effluent	Composite	-	Electropositive filter media	100	RT PCR	-	Qualitative	10 ³	SARS Coronavirus	Wang et al., 2005a, 2005b

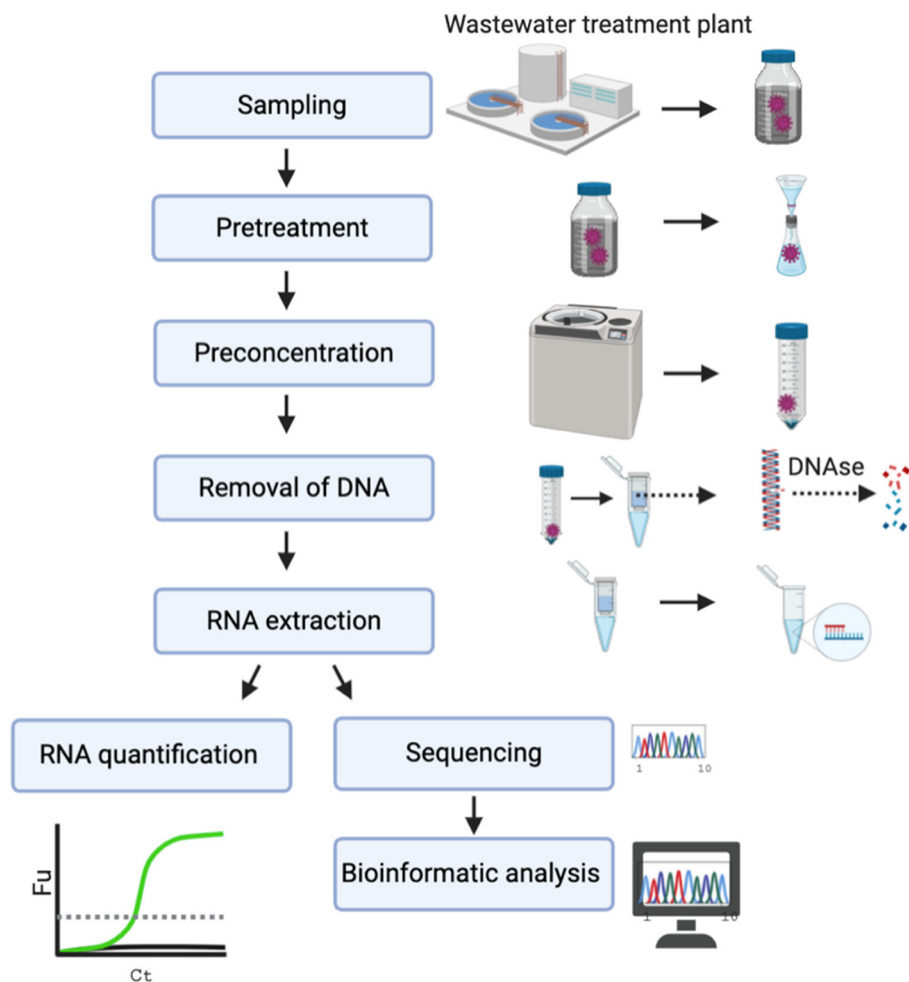


Fig. 5. Schematic representation of sample preparation steps of SAR-CoV-2 RNA in wastewater.

To further enhance the concentration factor, the first preconcentration step can be followed by another concentration step based on ultrafiltration using electronegative membrane, ultracentrifugation, or PEG (8000) precipitation (Wu et al., 2020). Among PEG precipitation and membrane filtration, the former was reported to be more effective in concentrating SARS-CoV-2 genetic material before RNA extraction (Wu et al., 2020). Although the volume used during preconcentration affects the detection of the virus, accurate quantification is often not possible due to limited knowledge on virus recovery during concentration. For enteric viruses, some studies have recommended preconcentration from <100 mL of untreated wastewater (Haramoto et al., 2018; Kitajima et al., 2020). Recently, Sherchan et al. (2020) reported the presence of SAR-CoV-2 RNA in wastewater preconcentrated using ultrafiltration and an adsorption–elution method. Subsequently, the copy number of SAR-CoV-2 RNA was determined using RT-PCR. However, concentration methods and RT-qPCR assays need to be assessed further and validated for increased sensitivity of SARS-CoV-2 RNA detection in wastewater. The electronegative membrane was chosen for sample concentration since greater adsorption of enveloped viruses is reported on such membranes (Ahmed et al., 2020b). Further unwanted DNA in the filtrate was removed using DNase, and enzymatic activity was stopped by heat treatment at 60 °C for 10–15 min. Viral RNA extraction from the filtrate can be performed using Trizol (Wu et al., 2020) or viral RNA extraction kits as per the manufacturers' protocol (Wu et al., 2020; Ahmed et al., 2020a, 2020b, 2020c). While Medema et al. (2020) reported a wide variation in the recovery values for F-specific RNA phages (70 ± 50%), most studies have

not provided adequate information on the percent recovery of SARS-CoV-2 from wastewater. For studies reporting SARS-CoV-2 viral RNA in river water, the recovery values are often not documented (Guerrero-Latorre et al., 2020). Possibly such investigations were avoided due to the risk associated with handling SARS-CoV-2 and the requirements for a BSL-3 facility. Additionally, BSL-3 facilities are lacking in developing countries, where higher viral loads are expected in sewage and waterbodies polluted by sewage. Recently, Ahmed et al. (2020b) explored the potential of seven combinations of pretreatment, and preconcentration options for MHV, a potential surrogate of SARS-CoV-2 seeded in municipal wastewater, employing adsorption-extraction method and a centrifugal filter device coupled with various pretreatment, polyethylene glycol (PEG 8000) precipitation, and ultracentrifugation. The mean recovery values ranged from 26.7 to 65.7%, with the highest and lowest recovery observed for adsorption-extraction methods with MgCl₂ pretreatment and PEG precipitation, respectively. Similarly, a wide variation in recovery values for enveloped viruses was also reported for SARS and influenza viruses. The recovery percent has been reported to range from 1% using electropositive filtration and aluminum hydroxide precipitation method (Wang et al., 2005a) to 8% with glass wool filtration and polyethylene glycol (PEG) precipitation method (Deboosere et al., 2011). Similarly, the %recovery for hepatitis virus, adenovirus, and norovirus are reported to vary from 4.5 to 71% (Table 2) (Adefisoye et al., 2016; Alexyuk et al., 2017; Blanco et al., 2019; Masclaux et al., 2013; Wang et al., 2005a). Although there exists a wide variation in virus recovery based on the pretreatment and preconcentration techniques used, the volume of water

used during concentration is one of the critical factors affecting the detection and recovery of viruses (Haramoto et al., 2018). Wastewater contains two phases, the liquid and the solid phase (dewatered sludge). Hence, the concentration of viruses present in both the phases needs to be estimated accurately.

Accurate quantification required the use of appropriate primers, probes as well as standards (positive and negative controls) and analysis of replicate samples (e.g., triplicate) to minimize experimental error (Corman et al., 2020; Medema et al., 2020). Recently, the WHO has approved target genes of SARS-CoV-2 for the detection of the virus in environmental samples. These include the nucleocapsid genes (N1, N2, and N3) and the genes coding for the spike proteins. These QA/QC measures are essential for ensuring representative analyses. Additionally, Ahmed et al. (2020a) conducted PCR sequencing of the virus RNA and further purified and sequenced it via Sanger's method. In such an analysis, it is recommended to remove low quality read mappings. Different samples from different areas can be tested in a similar manner, and phylogenetic analysis can be done by Next-Generation Sequencing by comparing and aligning the reference SARS-CoV-2 strains using various bioinformatics tools, such as MEGA and FigTree. These software tools were found to be helpful in understanding the origin of the strain.

Corman et al. (2020) developed a robust diagnostic workflow for SARS-CoV-2 using synthetic nucleic acid technology. This technology could detect SARS-CoV-2 and further discriminate SARS-CoV-2 from SARS-CoV with good reliability. It can be particularly useful in public health laboratory settings where the viral genetic material is not available or is present at a very low concentration, such as in the environmental matrices. Biosensor based detection techniques may also be developed for the detection of SARS-CoV-2. Sensors are easy to use, cost-effective, and provide high sensitivity and real-time monitoring ability. Altintas et al. (2015) provide a detailed review of the application of biosensor technology for virus detection. Water and wastewater quality parameters, such as pH and turbidity, may influence the sensitivity of biosensors. Culture-based techniques may not be suitable for common human viruses. For these viruses, molecular detection techniques are the best alternative. Most of the current diagnostic methods for detection of SARS-CoV-2 genetic material have relied on time-consuming RT-qPCR based methods (WHO, 2020b) and comparatively less reliable serological tests. Although some of the rapid detection kits highlighted by the WHO can detect the virus within 15 mins, there is a shortage of diagnostic kits and necessary reagents. Thus, current research should focus on quick detection and mass production of reliable kits to test SARS-CoV-2 in various environmental matrices.

The prevalence of SARS-CoV-2 infection within a sampling area can be predicted by developing a correlation-based approach. The total number of RNA copies in wastewater each day determined using RT-qPCR may be correlated with the number of SARS-CoV-2 RNA copies shed in faeces by an infected individual each day (Ahmed et al., 2020a; Hart and Halden, 2020). Further research to improve the limit of detection and quantification in complex environmental matrices with low viral loads is required. In some studies, the detection limit was reported to be 10 copies of the control plasmid (Nemudryi et al., 2020). Although previous studies highlighted the significance of monitoring of wastewater samples as a potential early warning sign of virus transmission, further refinement with regards to both molecular process controls (MPCs) and predictions are needed for its robust and reliable application. No consensus on MPCs has been established as yet for enveloped viruses. Also, the concentration method employed for virus recovery is another essential factor that requires optimization for improving the sensitivity of detection of SARS-CoV-2 in wastewater/natural water samples. Most of the studies have reported virus detection and quantification from the aqueous phase. Copies of the genetic material of the virus adsorbed on the suspended solids have not been measured. Therefore, it is not easy to interpret the results regarding the spread of the virus in a community, unless appropriate corrections are made for virus adsorption on suspended solids.

5. Can wastewater and water treatment techniques eliminate SARS-CoV-2?

5.1. Persistence and infectivity of corona viruses

It is commonly believed that the persistence of infective CoVs in wastewater and water is not likely as these are enveloped retroviruses (Jia and Zhang, 2020). Enveloped viruses differ structurally from non-enveloped viruses as the former contains a lipid bilayer membrane (envelope) surrounding the protein capsid (Ye et al., 2018, 2016; Kumar et al., 2020e). This makes enveloped viruses more susceptible to changes in pH, salinity, and temperature. Stallknecht et al. (1990) examined the persistence of the avian influenza virus at varying temperatures, pH, and salinity. The infectivity of the virus was found to be inversely proportional to the salt content. Moreover, compared to freshwater samples, virus persistence was lowest in brackish water. The survivability of viruses is often defined by the time required to reduce infectivity to 90% of the original value (i.e., t_{90} value). Some CoVs, such as SARS-CoV and human CoV 229E exhibited t_{90} values of more than a day in urine and several days in filtered wastewater samples (Ye, 2018; Ye et al., 2016). Studies by Gundy et al. (2009) indicated that CoVs might remain stable in wastewater for a longer period than in tap water. They calculated $t_{99.9}$ for the human and feline CoVs and concluded that these are inactivated faster in tap water at 23 °C (<10 days) than in tap water at 4 °C (>100 days). Available data from hospital effluents suggested that the RNA of SARS-CoV can survive for 10 days in the primary treated effluent and 5 days in the secondary treated effluent (Gundy et al., 2009), and disinfection may result in variable effects (Wang et al., 2005a, 2005b). However, these viruses were killed more rapidly in wastewater, with a $t_{99.9}$ range between 2 and 4 days. The persistence of enveloped Ebola virus in sterilized wastewater has also been documented (Bibby et al., 2015b). Ahemed et al. (2020) recently studied the persistence of RNA of SARS-CoV-2 and its potential surrogate murine hepatitis virus (MHV) in untreated wastewater, autoclaved wastewater, and dechlorinated tap water stored at four different temperatures, i.e., 4, 15, 25, and 37 °C (Fig. 6). With an increase in temperature from 4 to 37 °C the average t_{90} values for SARS-CoV-2 in untreated wastewater, autoclaved wastewater, and dechlorinated tap water was found to decrease from 28 to 8, 43 to 6, and 59 to 9 days, respectively, suggesting the persistence of SARS-CoV-2 RNA for several days in untreated wastewater. Additionally, Chan et al. (2020b) reported that the virus remained viable for up to 7 days and 14 days in solution when experiments were conducted at room temperature ranging from 20 to 25 °C and 4 °C, respectively. However, the virus lost its viability within 1 to 2 days when the temperature was in the range of 37–33 °C. Solution pH of 5 and 9 resulted in 2.9 and 5.33 log unit loss of infectivity in SARS-CoV-2, respectively, within 6 days (Chan et al., 2020). At extreme pH values of 2–3 and 11–12, the virus lost infectivity completely within 1 day. To cause infection, viruses must retain their infectivity until they come in contact with the next host. Based on studies conducted on the several animals, the minimal 50% infectious dose (TCID₅₀) of SARS-CoV-1 and 2 for ferrets were found to be 10⁶ (Martina et al., 2003) and 10^{5.5} (Kim et al., 2020), respectively. When experiments were conducted on cats infected with SARS-CoV-1 (Martina et al., 2003) and Rhesus macaques infected with SARS-CoV-2 (Munster et al., 2020), the TCID₅₀ for both were found to be 10⁶. A recent study highlighted that the infectious SARS-CoV-2 could persist in tap water and wastewater up to 7 days when the experiments were conducted with high-starting titer (Bivins et al., 2020).

At present, no studies have explored the infectivity of SARS-CoV-2 in WWTPs as a function of time, and there are no available estimates on the minimal infectious dose (MID) of SARS-CoV-2 in wastewater required for causing infection in humans. The presence of other microbes, nucleases, and proteases in sewage may also enhance the inactivation of viruses (Gundy et al., 2009). Since bacterial and other microbial loads are much higher in sewage compared to

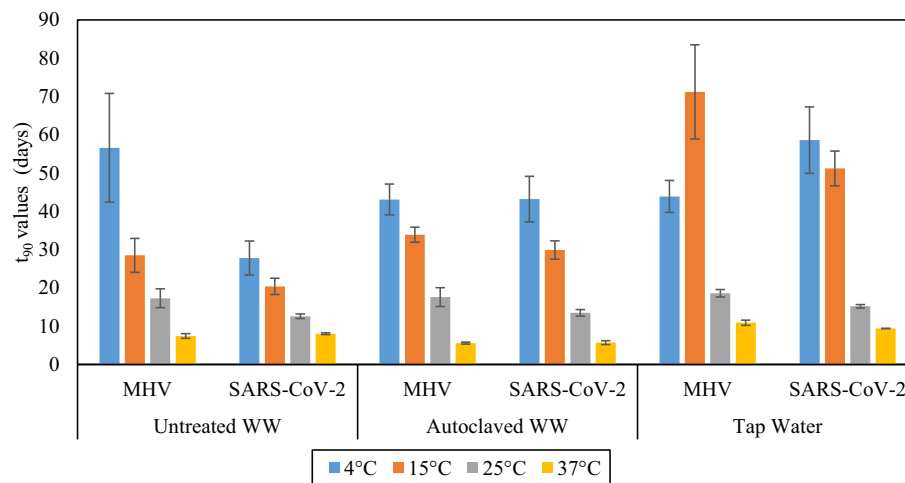


Fig. 6. Persistence of MHV and SARS-CoV-2 genetic material in various sample matrices at varying temperature. The error bars represent standard deviation. t_{90} represents the time required for 1 \log_{10} unit reduction. MHV refers to murine hepatitis virus

the viral load, survivability, and infectivity of CoVs may be limited in real wastewater systems.

5.2. Wastewater treatment and viral elimination

SARS-CoV-2 was reported to be present in the faeces of infected patients (Table 1). Thus, SARS-CoV-2 is likely to enter the sewerage system, and viral load is also expected in the WWTP influent. Recently, preliminary attempts were made to detect RNA of SARS-CoV-2 virus in composite municipal wastewater (sewage) samples from the Netherlands, Australia, United States, France, and many other countries across the globe (Ahmed et al., 2020a, 2020b; Medema et al., 2020; Nemudryi et al., 2020; Wu et al., 2020a; Wurtzer et al., 2020). The importance of composite sampling over grab sampling for SARS-CoV-2 was highlighted in the literature (Nemudryi et al., 2020). Concentration of the viral genome in the influent stream of wastewater was found to range from 120 to 10^5 copies/L, which is comparable to the concentration of other reported enveloped viruses (Table 1). In France, a total of 23 raw and 6 treated wastewater samples collected from 3 major WWTPs located in Paris city were found positive for SARS-CoV-2 viral RNA. While the increase in genome units in raw wastewater were positively correlated with increase in the number of fatal and/or infected cases in the study area, 100% removal of viral load was seen during wastewater treatment (Wurtzer et al., 2020). However, this study did not provide details on the treatment units employed at the WWTP. Although viruses are extremely diverse, with a range of genome types, structures, replication cycles, and pathogenicity (Wigginton and Boehm, 2020), in this study, the fate of SARS-CoV-2 during water and wastewater treatment is inferred based on the mechanism of removal of other viruses.

The wastewater treatment train at a typical full-scale WWTP comprises preliminary, primary, secondary, and tertiary treatment units. The preliminary and the primary treatment unit comprises of screens, grit chamber, and primary clarifier. The secondary treatment unit placed subsequently consists of biological treatment units (activated sludge process or membrane bioreactors), which is sometimes followed by disinfection, i.e., chlorination. Studies with two model enveloped viruses (MHV and $\phi 6$) in raw wastewater indicated that they had a tendency to sorb more effectively on to solid residues in wastewater and could retain infectivity for several days, depending on the viral strain, compared to model non-enveloped viruses (MS2 and T3) (Ye et al., 2016). It was observed that coronavirus survival was slightly higher in primary wastewater compared to that in secondary treated wastewater. This behavior can be attributed to the presence of higher concentrations of organic matter in primary wastewater, which may have provided

protection to the viruses (Wang et al., 2005a, 2005b). Randazzo et al. (2020) reported the virus genetic material both in primary effluent and in the secondary treated effluent. When tertiary treatment processes were present in the treatment train, no genetic material was detected in the effluent stream. Suspended particles, including colloidal matter, algae, bacteria, and chemical or biological flocs present in wastewater matrices not only serve as reservoirs for several pathogens but also safeguards their activity by protecting them from the oxidants present in wastewater and ultimately, plays a major role during the treatment cycle, as has been seen for SARS-CoV (Gundy et al., 2009). The interaction between virus and suspended particles present in water matrices, in general, can be explained by the extended Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which takes into account several forces of interactions, including van der Waals forces, electrostatic double layer forces, and hydrophobic interaction (Verbyla and Mihelcic, 2015). Although osmotic interactions do not have much role to play, steric interactions and hydration forces are reported to hinder the association of virus with the particles present in the aqueous medium (Pham et al., 2009). The electrostatic interaction between viruses and particles is reported to be governed by the overall surface charge of the virus and the particle surface. Depending upon the location of carboxyl groups and amines groups, and the pH of the medium, the net surface charge of viruses are likely to vary (Templeton et al., 2008). In general, viruses experience a net positive charge and negative charge, when pH of the system in relation to the isoelectric point of the virus decreases or increases, respectively. Thus, over a suitable pH range, virus-particle interactions may be favoured and such interactions can ultimately decide the fate of viruses during wastewater treatment. By adopting modern DNA-labelling techniques and DLVO model, bacteriophages were reported to be strongly adsorbed on the model colloidal particles at a concentration ranging from 2.6 to 450 mg/L (Xing et al., 2020b). As a result, during the settling stage, a significant reduction in virus concentration was reported in WWTPs based on the activated sludge processes (Barrett et al., 2016).

Hydrophobic interactions depend on the characteristics of the virus, the particle surface, and water/wastewater constituents (Schijven and Hassanizadeh, 2000). Virus proteins contain several hydrophobic amino acids that might explain the aggregation of viruses at pH levels above their isoelectric point (pI) (Verbyla and Mihelcic, 2015). Lipid bilayer destabilization may also impact their survival and partitioning behavior in water and wastewater systems (Ye et al., 2016). The increased hydrophobicity conferred by the constituents of the lipoprotein layer of CoVs would make it more likely to interact with other hydrophobic surfaces. This was demonstrated by a reduction in viral copies in filtered wastewater compared to raw wastewater (Gundy et al., 2009). Thus,

adsorption of the virus on suspended solids followed by settling in secondary clarifier is the primary removal mechanism during the activated sludge process.

Preconcentration of organic carbon through chemically enhanced primary treatment (CEPT) processes, followed by conventional trickling filters and activated sludge process, demonstrated comparable removal for norovirus and sapovirus (Taboada-Santos et al., 2020). However, the viral reduction was higher in the high-rate activated sludge process (HRAS) originally designed for nutrient removal. The high removal was attributed to greater adsorption of the virus onto suspended particles (Campos et al., 2016; Taboada-Santos et al., 2020). A poor correlation between physicochemical parameters of the influent/effluent and concentration of the enteric virus suggests that virus removal cannot be predicted using the parameters that are monitored routinely (Aw and Gin, 2010; Sidhu et al., 2018).

In a full-scale membrane bioreactor (MBR) with a nominal pore size of 0.04 μm , Chaudhry et al. (2015) evaluated the removal of viruses through mechanisms such as attachment of the virus to mixed liquor suspended solids; virus retention on the membrane soon after backwashing; virus retention by the cake layer on the membrane; and virus inactivation. For adenovirus, norovirus, and F-specific coliphage, the just backwashed membrane provided the maximum removal, while attachment to suspended solids provided the least removal. Graft-polymerized zwitterionic SPP ([3-(methacryloylamino) propyl] dimethyl (3-sulfopropyl) ammonium hydroxide) functionalized commercial membranes reported enhanced removal of viruses compared to non-functionalized membranes (Lu et al., 2017b). While the diversity of viruses in the effluent stream of conventional activated sludge processes and membrane bioreactors were not found to vary significantly, the type of disinfection process used significantly affected their diversity in treated water (O'Brien et al., 2017). In an MBR based WWTP with provision for chlorination, the viral diversity, viral load, and infectious capacity of adenoviruses could be reduced by up to 4 log units (Jumath et al., 2017). In another study, the concentration-time value (Ct) for free chlorine disinfection of nitrified membrane bioreactor effluent to achieve >5 log unit virus inactivation with MS2 bacteriophage was found to be 3 mg-min/L (Ikehata et al., 2018). The Ct values for chlorination may vary with the type of effluent, i.e., it may differ for effluents originating from a conventional activated sludge-based plant versus nitrified effluent generated from a granular media-filter. A previous study had demonstrated that the RNA of SARS-CoV was detectable in hospital sewage samples before disinfection and occasionally after disinfection (Wang et al., 2005a, 2005b). A detailed discussion on the disinfection of coronavirus is covered in the next section.

Other treatment technologies such as wetland systems, hybrid ultrafiltration techniques, and photo-Fenton processes have also been explored for the removal of enteric viruses. The reduction in viral activity in a wetland system may be overestimated due to its long retention time. Optimized temperature mediated biological activity in wetlands could reduce enteric and polyomavirus from 1 to 3 log units and 2 to 4 log units, respectively (Rachmadi et al., 2016). A combination of treatment processes, such as coagulation-sedimentation-ultrafiltration, can enhance the removal of viruses from wastewater (Lee et al., 2017). A low-cost denitrifying reactor commonly used to reduce nitrate (NO_3^-) present in septic tank effluent and drainage water could reduce the concentration of an F-specific RNA bacteriophage by 1.3 to 2 log units (Rambags et al., 2019). For the photo-Fenton process based on Fe oxides and H_2O_2 , virus removal may be enhanced depending on the system pH and isoelectric point of the virus (Giannakis et al., 2017). The inactivation potential of MS2 bacteriophage in urine has been evaluated using UVC, UVC/ H_2O_2 , and UV/Fenton processes. The addition of H_2O_2 significantly improved the deactivation rate by 20–60% and reduced the time taken for deactivation of the virus. This was attributed to the high oxidative power of the $\text{OH}\cdot$ radicals (Giannakis et al., 2018).

SARS-CoV-2 genetic material was detected in primary sludge samples from USA (Peccia et al., 2020), where its concentration was found

to be two to three orders of magnitude higher compared to values reported for wastewater (Ahmed et al., 2020a, 2020b; Wu et al., 2020; Wurtzer et al., 2020). Similarly, several authors across the globe have reported their high concentration in the sludge matrix (Table 2). Due to the presence of envelope, CoVs tend to be more hydrophobic and can partition on to sludge or sediments or suspended solids. Shotgun metagenomic studies using sewage sludge from 10 WWTPs also indicated similar results, with coronaviral RNA of HKU1 being the second most abundant after adenovirus (Bibby and Peccia, 2013). It has been reported that hydrophobic interactions play a major role in the attachment of viruses to solids (Gerba, 1984; Pisharody et al., 2021). In a comparative study conducted by Ye et al. (2016), it was observed that enveloped viruses associate more strongly with wastewater solids than nonenveloped viruses. Thus, enveloped viruses would be removed to a greater extent than nonenveloped viruses in primary wastewater treatment. However, more enveloped and nonenveloped viruses have to be tested to confirm these results.

Typically wastewater exposure has been a concern for gastroenteric disease-causing viruses transmitted by the faecal-oral route. However, several studies have revealed that most of the viral load in biosolids, sludge particles, and raw wastewater were of respiratory infection-causing viruses (SARS-CoV and other coronaviruses) (Bibby and Peccia, 2013; Ye et al., 2018). Although the primary mode of transmission for these viruses is through air/aerosols, a high concentration of genetic material of such viruses was present in sludge. However, virus inactivation is expected during sludge thickening, composting, thermal digestion, and lime treatment (Bogler et al., 2020). In developing countries, poor sanitation and open defecation may cause non-point contamination of viruses in the aquatic environment, thereby posing a risk for re-emergence of the infection.

5.3. Water treatment and viral elimination

With the emergence of adenoviruses, specific human viruses have been continuously listed under the microbial water contaminant candidate list since 1998, as shown in Table A2 in ESM (Aw et al., 2009). Hence, there is growing interest in the monitoring of such pathogens in water bodies receiving untreated discharges from the various point and nonpoint sources, including discharges from WWTPs. The survival time for enveloped viruses, such as avian influenza virus (H5N1) in the surface water can vary from 19 to 61 days over the temperature range 20 to 10 $^\circ\text{C}$, and its survival was extended for several months in frozen water samples (Nazir et al., 2010). Hence, surface water pollution followed by entry of the viruses to the WTPs cannot be overlooked. The water treatment train at a typical full-scale WTP consists of coagulation-flocculation, sand filtration, and disinfection. Disinfection is achieved either through chlorination, ozonation, or UV irradiation. Virus removal in these processes may vary depending on the physicochemical properties of the virus and the choice of operating conditions. In addition to process parameters, virus removal is also significantly affected by raw water quality, which differs from season to season (Asami et al., 2016).

5.3.1. Coagulation-flocculation

One of the first processes for removal of colloidal particulate matter in a WTP is coagulation-flocculation. Depending on the choice of coagulant, this process can achieve considerable removal of human enteric viruses, such as poliovirus and coxsackievirus (Campos et al., 2016; Shamsollahi et al., 2019). However, negligible removal of enveloped viruses, H5N1 and H1N1 was observed in the presence of aluminum sulfate, ferric chloride, and aluminum polychlorosulfate at a pH higher than 7 (L  n  s et al., 2010). In the coagulation-flocculation process, in addition to pH, alkalinity, and turbidity, the chemical properties of the coagulant and nature of ions and other polymeric substances formed by hydrolysis of the coagulant determine the efficacy of viral removal. The destabilization mechanism involves coordination reactions between ionized coagulant species and the carboxyl group of the viral

capsid. The removal ratios of poliovirus and coxsackievirus in the presence of PACI with high colloidal aluminum content was found to be higher than in the presence of coagulants with high monomeric aluminum content (Shirasaki et al., 2016a). The presence of ionized Al₃₀ species, i.e. [Al₃₀O₈(OH)₅₆(H₂O)₂₄]¹⁸⁺ in PACI with high colloidal aluminum content, could achieve an effective removal of these viruses due to the stability of the polymeric substances (Matsui et al., 2003; Shirasaki et al., 2016a). Similarly, PACI could remove infectious enteric adenovirus type 40 and poliovirus type 1 and enveloped virus T4 (NBRC 20004) and P1 (NBRC 20008) virus more efficiently than conventional coagulants due to the higher charge-neutralization capability of the intermediate polymers of PACI (Matsui et al., 2003; Shirasaki et al., 2016).

Virus removal is also affected by the surface hydrophobicity of the virus and the virucidal activity of the coagulant. The irreversible adhesion of the virus to ionized aluminum species is the crucial mechanism that decides the virucidal action of aluminum coagulants. The molecular structure of the binding site (surface lipoprotein or lipopolysaccharide) of the virus, which generally adsorb onto the host cells during the beginning of infection are significantly altered due to the ionic bonding of the virus to aluminum species. Again if viral ligands get adsorbed onto the coagulants, sufficient active sites may not be available for the host cells to get attached to the virus. Hence, both ionic bonding, which may be too strong in alkaline medium, and unavailability of viral ligands, may explain the antiviral activity of enveloped virus T4 (NBRC 20004) and P1 (NBRC 20008) (Matsushita et al., 2004). Differences in physicochemical characteristics and/or shape of different viral particles also affect virus removal efficiency under similar treatment conditions. For example, the isoelectric point (pI) of a virus plays an important role during the coagulation-sedimentation process. The pI of several non-enveloped viruses lies in the range 4 to 8.3, which arises from their respective protein capsids (Asami et al., 2016). The capsid mainly consists of weakly acidic and basic functional groups, which upon ionization in the aqueous medium, results in a net surface charge that is a function of water pH. Based on the knowledge of the pIs, the pH of the system may be adjusted to enhance the removal of viruses (Giannakis et al., 2017; Lee et al., 2017; Mayer et al., 2015). The presence of other interfering charged species in the aquatic media may significantly alter the surface charge of the viruses (Matsushita et al., 2004). During electrocoagulation, physical removal of viruses primarily occurs due to inclusion in flocs, while inactivation is mainly due to ferrous iron oxidation. However, the viruses adsorbed onto floc particles can retain their infectivity (Matsui et al., 2003). Although coagulant-DOM precipitates adsorb viruses, the rate of adsorption is too low to inactivate the virus (Matsushita et al., 2004). The susceptibility to ferrous-based inactivation is governed by electrostatic attraction, virus aggregation, and capsid durability (Heffron et al., 2019). While studies pertaining to SARS-like coronaviruses are scarce, studies on other enveloped viruses (Matsushita et al., 2004; Wang et al., 2005a) may provide insights on the susceptibility of coronavirus removal through coagulation-flocculation.

5.3.2. Filtration

Straining and screening are the primary physical removal processes during slow sand filtration (SSF), when the size of the particles is larger than that of the sand grains (0.15 to 0.35 mm) (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997). However, viruses with a nominal diameter 50–200 nm (including SARS-CoV-2) are expected to penetrate deeper into the filter bed where sedimentation, interception, hydrodynamic action, and diffusion are the primary removal mechanisms (Guchi, 2015). Additionally, extracellular polymeric substances formed due to microbial activity on the surface of sand can provide binding sites for the virus. In such a scenario, virus removal can be achieved by adsorption onto biomass and subsequently through degradation by other microbes. An increase in the ionic strength of water can enhance the adsorption of the virus on the sand. Similarly, as the biomass concentration increases, higher removal of the virus is expected,

as reported for norovirus (Guchi, 2015). In general, virus removal in the rapid sand filter (RSF) and SSF varies from 0.5 to 1.3 log units and 2 to 6 log units, respectively. Virus removal in SSF is reported to increase with an increase in bed depth, an increase in water temperature, and a decrease in the rate of filtration (Asami et al., 2016; Guchi, 2015).

While several mechanisms play a crucial role in the removal of viruses, during sand filtration, the key challenge lies in developing low-cost filter media. Both copper (I) oxide and metallic copper removes viruses up to 99.99% due to a large number of adsorption sites. However, negligible removal was observed with copper (II) oxide (Mazurkiewicz et al., 2020). A higher concentration of copper could inactivate bacteriophage MS2, a surrogate enteric virus (Armstrong et al., 2017). Sand filters functionalized with chitin-binding protein extracted from *Moringa oleifera* (MO) seeds resulted in better removal (~7 log units or 99.99%) of bacteriophage, which is commonly used as a surrogate for pathogenic norovirus and rotavirus. Removal was much higher than in bare sand filters and was 3 orders of magnitude higher than the USEPA mandate for virus removal (4 log reduction) (Samineni et al., 2019; Shamsollahi et al., 2019). Coronaviruses inherently carry a negative charge at neutral pH since their pI lies in the range 5.8 to 9.6 (Ul-Qamar et al., 2019). Although good removal of CoVs may be expected through electrostatic interactions, hydrogen-bonding, and local hydrophobic interactions, only experimental investigations can confirm the removal of SARS-CoV-2 in such filters.

5.3.3. Disinfection

As a lipoproteinaceous bilayer envelops SARS-CoV-2, chlorination and UV disinfection are expected to effectively denature this virus, as has been reported for other classes of SARS viruses (Ansaldi et al., 2004; Gundy et al., 2009; Wang et al., 2005b). One study recommended free residual chlorine above 0.5 mg/L and 30 min contact time (pH < 8) and 2.19 mg/L for chlorine dioxide (ClO₂) for complete inactivation of SARS-CoV in wastewater. Free chlorine was found to inactivate SARS-CoV better than chlorine dioxide (Wang et al., 2005b). At 20 mg/L of chlorine, SARS-CoV was found to be ineffective within 1 min contact time. Free chlorine was also found to inactivate enteric viruses more effectively than ClO₂ (Rachmadi et al., 2018; López-Gálvez et al., 2018). Additionally, it was reported that free chlorine reacts with proteins to a larger extent than RNA/DNA and lipids of an enveloped virus (Ye et al., 2018). Even the enveloped bacteriophage affecting *Pseudomonas* phage (Phi6) was found to be 30× more susceptible to free chlorine than the non-enveloped MS2 (Ye et al., 2018). Free chlorine readily penetrated Phi6 with the help of three membrane proteins (P6, P9, P10, and P13) and reacted with the nucleocapsid (P8) and polymerase complex (P1, P2, and P4). Among the proteins, nucleocapsid and polymerase complex proteins were more reactive with free chlorine than the membrane protein. The most reactive peptide of enveloped Phi6 virus is 150× more reactive than non-enveloped MS2. Germicidal UV radiation (185–254 nm) could degrade SARS-CoV viral RNA under 1–2 min of exposure (Ansaldi et al., 2004; Bae and Shin, 2016). Novel pulsed UV irradiation and low-pressure UV irradiation have shown greater potential in inactivating several enteric viruses in secondary treated wastewater (Barrett et al., 2016). Hence, this method may be explored for inactivating SARS-CoV-2.

Enveloped viruses such as Phi6 and influenza viruses H5N2 are susceptible to UV treatment, but their rate of inactivation with UV is much lower than with free chlorine (Lucio-forster et al., 2006; Ye et al., 2018). However, such enveloped viruses are more susceptible to UV treatment compared to MS2 bacteriophage (Lénès et al., 2010; Ye et al., 2018). Specifically, SARS-CoV is reported to be inactivated under UV-C radiation within 40 min of exposure (Darnell and Taylor, 2006) due to damage of the viral genome as has been reported for enveloped hepatitis C virus (Pfaender et al., 2015). Reactive oxygen species produced in plasma-activated water significantly inactivated viruses by damaging double-stranded DNA, single-stranded DNA, RNA, and proteins (Araud et al., 2018; Su et al., 2018; Water, 2018). However, the presence of

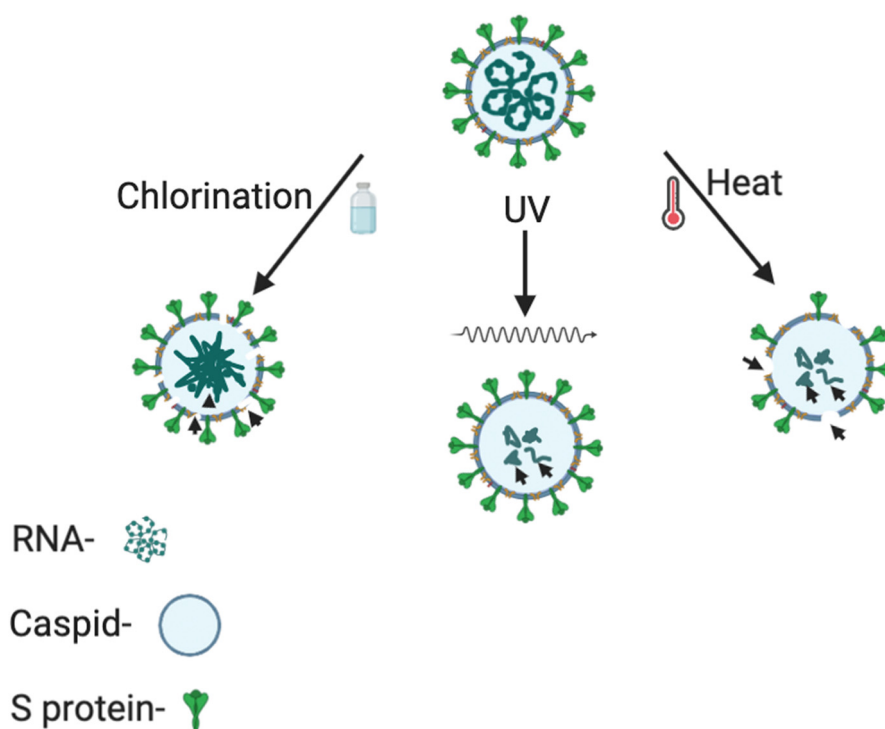


Fig. 7. Effect of chlorination, UV irradiation and heat treatment on SARS-CoV-2 viral structure.

lipopolysaccharide or peptidoglycan of bacterial origin may provide protection to viruses through the stabilization of the viral capsid (Waldman et al., 2017).

Nanoparticles (NPs), including TiO_2 and ZnO (Zhang and Zhang, 2015), $\text{Ag}_{30}\text{-SiO}_2$ particles (Park et al., 2018), activated carbon functionalized with silver and copper oxide nanoparticles (Shimabuku et al., 2017) were used to disinfect several viruses. Water quality parameters (e.g., pH, and organic matter) and nanoparticle specific parameters (e.g., specificity, size, and charge on the surface) affected the antiviral activity. Ionic silver alone and in combination with copper (Jackson et al., 2020) or ferrate (VI) ($\text{Fe}^{\text{VI}}\text{O}_4^{2-}$, $\text{Fe}(\text{VI})$) (Manoli et al., 2020) were also used for the disinfection of enteric viruses. Ozone can be highly effective as a disinfectant based on reported viral inactivation rates and typical contact time provided in water and wastewater treatment (Wolf et al., 2018).

Where centralized water treatment and piped water supply is not available, the WHO recommends high-performance ultrafiltration or nanofiltration processes, solar irradiation, and, in non-turbid waters, UV irradiation and appropriately dosed free chlorine (Kariwa et al., 2006; WHO, 2020c). Heat treatment at 60 °C for a period of 15 to 30 min could effectively deactivate SARS CoV virus (Darnell and Taylor, 2006; Kariwa et al., 2006). While thermal inactivation of SARS-CoV at 56–60 °C was highly effective in the absence of protein, the infectivity was only reduced by 2 log units at 56 °C after 30 min in 20% protein solution (Rabenau et al., 2005). Similarly, enveloped hepatitis C virus was reported to be deactivated by heat treatment due to direct damage to the viral genome (Pfaender et al., 2015). At the household level, ceramic water filters incorporating hydroxyapatite and alumina with specific surface area ranging from 3.7 to 21.0 $\text{m}^2 \cdot \text{g}^{-1}$ resulted in 99.99%, 99.97%, and 99.45% removal of *E. coli*, fluoride, and MS2, respectively (Nigay et al., 2019). Another study suggested a combined treatment for virus removal comprising of coagulation-flocculation with chitosan followed by filtration using a ceramic pot filter (Abebe et al., 2016). In contrast, a passive point-of-use treatment system, namely, a polyvinyl (alcohol) (PVA) nanofiber membrane/activated carbon column, was reported to be ineffective for the treatment of adenovirus,

possibly due to its small size (90–100 nm) and lack of other interactions between the virus and the membrane (Dobrowsky et al., 2015). However, such systems may provide adequate removal of SARS-CoV-2 due to its larger size (60 to 140 nm). While household-level bio-sand filtration (BSF) may reduce *E. coli* up to 5 log units, a reduced removal was observed for enteric viruses. However, the removal was moderately increased with the growth of schmutzdecke (Elliott et al., 2015).

In general, disinfectants can inactivate SARS-CoV-2 by targeting its various components. While UV and heat treatment is expected to directly damage the viral genome, chlorination may deactivate the virus by attacking the nucleocapsid and the polymerase complex proteins (Fig. 7). However, additional studies need to be conducted to validate the inactivation of SARS-CoV-2 and elucidate the mechanism of action.

Until 2018 a total of 158 human RNA viruses were identified, of which 122 species from 11 virus families were enveloped, and 36 species from 6 virus families were non-enveloped (Wigginton and Boehm, 2020; Woolhouse and Adair, 2013). However, most studies on the fate of viruses in treatment plants, i.e., WWTPs and WTPs, have focused on the fate of non-enveloped viruses. Literature around the fate of enveloped viruses in such systems is still scarce and requires additional research.

6. Conclusions

The explosive spread of SARS-CoV-2 is of great concern at various levels. With an increase in the number of individuals being diagnosed as COVID-19 positive and the number of deaths associated with SARS-CoV-2, it is still unclear when the ongoing pandemic will subside. Additionally, after the pandemic subsides, the probability of a rebound outbreak cannot be dismissed. No vaccines or antiviral drugs for SARS-CoV-2 have been approved for use as yet, although several candidate vaccines and antivirals are in clinical trials.

Being an enveloped virus, SARS-CoV-2 is sensitive to environmental conditions (heat, pH, and reactive radicals), and their fragile envelope can be readily damaged by various chemical disinfectants and physical agents. Although genetic material of the virus has been detected in

faecal matter of patients diagnosed with SARS-CoV-2 and in WWTPs, data on their concentration, infectivity, and persistence in the environment is still scarce. Due to its similarity to SARS and MERS, SARS-CoV-2 is expected to behave in a similar manner. Thus, while SARS-CoV-2 specific data is lacking, data available for other coronaviruses and enveloped viruses may be used to develop initial estimates for its persistence and risk. Faecal-oral transmission of SARS-CoV-2 has not been demonstrated as yet; however, the possibility of such transmission cannot be ignored completely. Areas with poor sanitation, commonly encountered in underdeveloped and developing countries, maybe at a higher risk of faecal-oral transmission of SARS-CoV-2. However, such issues can be overcome by adopting safe hygiene practices and by using disinfectants containing chlorine, bleach, or alcohol for inactivating SARS-CoV-2. The scientific community should conduct additional research on the occurrence, fate, and transport of SARS-CoV-2 in the environment. Specifically, emphasis may be given in the following areas, some of which were previously highlighted by other researchers (Bibby et al., 2015a; Wigginton et al., 2015) during the Ebola, SARS, and MERS outbreak.

1. Occurrence and Fate: As SARS-CoV-2 may potentially enter the environment through the faecal-oral route, studies on the occurrence of this virus and its infection-causing potential when present in surface water, drinking water, wastewater, sludge, and soil should be determined. Furthermore, owing to the hydrophobicity of its envelope, the viral particles are likely to partition on to sludge and may end up in landfills. Hence, sewage surveillance could be used to monitor and locate communities with asymptomatic carriers to control virus transmission. The effectiveness of each treatment unit in WWTPs and WTPs towards the removal of this virus may be evaluated. Special attention may be given to the effectiveness of the disinfection process in these plants.
2. Development of novel detection technique for SARS-CoV-2: Rapid concentration and sensitive detection techniques may be developed for real-time surveillance in various environmental compartments for monitoring the occurrence and potential outbreak of such viruses. Specific attention may be given to sample preparation techniques, including sample pretreatment, preconcentration, extraction, and purification in various environmental matrices, such as potable water, wastewater, and sludge. Both healthcare and environmental sectors require rapid testing kits with minimal use of reagents.
3. Risk Assessment: Most of the response recommendations for the coronavirus outbreak, including disinfection, liquid, and solid waste handling, and safety of health care professionals and professionals engaged in waste management, require a systematic in-depth risk assessment. Also, modeling attempts on virus transmission and microbial risk assessment, including multiple exposure pathways, may further enhance the reliability of risk assessment. Insights may initially be drawn from existing models on non-enveloped viruses.
4. Following the current outbreak, environmental researchers across the globe may initiate research on diverse aspects of SARS-CoV-2. However, the need for biosafety level 3 laboratories may hinder further research. These problems can be overcome by developing appropriate surrogates of SARS-CoV-2, which can be handled at lower biosafety levels.
5. Nanotechnology-based disinfection techniques that can potentially remove viruses from the environment without causing chronic exposure effects in other species should be explored.
6. Inhalation of viral aerosols and exposure to contaminated waste and sludge can potentially produce infections. Hence, extra precautions must be taken to minimize the generation of aerosols during wastewater treatment and the handling of sewage sludge. The workers at hospitals, quarantine facilities, and WWTPs should be provided with appropriate personal protective equipment (PPE). Health and WWTP workers should follow the WHO and WASH guidelines to minimize the spread of this virus.

7. Cooperation among environmental researchers, environmental engineers, microbiologists, virologists, molecular biologists, epidemiologists, doctors, and veterinarians is recommended to tackle a pandemic of this magnitude.

The mounting body of knowledge demonstrated through published literature has contributed significantly to the understanding of this novel human pathogen, however, more research on the possibility of faecal-oral transmission and its possible fate and persistence in various environmental compartments is needed. Additionally, the possibility of re-emergence of a potential future pandemic due to environmental contamination needs to be carefully evaluated, and measures should be taken to prevent such a possibility. Various environmental compartments may act as sinks and future sources of this pathogen. The role of the environment in the transmission, transport, persistence, and re-emergence of SARS-CoV-2 cannot be overlooked. Future research may be directed at understanding the impact of environmental conditions on the transmission, transport, and fate of SARS-CoV-2. A greater understanding will help to control the current pandemic, as well as prevent future outbreaks.

CRedit authorship contribution statement

Sanjeeb Mohapatra: Conceptualization, Writing - original draft, Writing - review & editing. **N. Gayathri Menon:** Conceptualization, Writing - original draft, Writing - review & editing. **Gayatree Mohapatra:** Writing - original draft. **Lakshmi Pisharody:** Writing - original draft. **Aryamav Pattnaik:** Writing - original draft. **N. Gowri Menon:** Writing - original draft. **Prudhvi Lal Bhukya:** Writing - original draft. **Manjita Srivastava:** Writing - original draft. **Meenakshi Singh:** Writing - original draft. **Muneesh Kumar Barman:** Writing - original draft. **Karina Yew-Hoong Gin:** Conceptualization, Supervision, Writing - review & editing. **Suparna Mukherji:** Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.142746>.

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