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Where do we stand to oversee the coronaviruses in aqueous and aerosol environment? Characteristics of transmission and possible curb strategies

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**ABSTRACT**

By 17 October 2020, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) has caused confirmed infection of more than 39,000,000 people in 217 countries and territories globally and still continues to grow. As environmental professionals, understanding how SARS-CoV-2 can be transmitted via water and air environment is a concern. We have to be ready for focusing our attention to the prompt diagnosis and potential infection control procedures of the virus in integrated water and air system. This paper reviews the state-of-the-art information from available sources of published papers, newsletters and large number of scientific websites aimed to provide a comprehensive profile on the transmission characteristics of the coronaviruses in water, sludge, and air environment, especially the water and wastewater treatment systems. The review also focused on proposing the possible curb strategies to monitor and eventually cut off the coronaviruses under the authors’ knowledge and understanding.

1. Introduction

The new coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the latest complex coronavirus disease and an ongoing pandemic in the world. According to the World Health Organization (WHO) report, there have been 39,196,259 confirmed cases including 1,101,298 deaths worldwide from January 17 to October 17, 2020 (Fig. 1). This situation is as profoundly alarming with enormous consequences worldwide, and had a significant effect on global public healthcare systems and restructured society and economics [2–4]. The United Nations (UN) estimated global trade to fall between 13 and 32 per cent this year [5]. It

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Since only in the 21st century, the world has been repeatedly challenged by respiratory diseases caused by the emerging coronavirus, such as the SARS-CoV outbreak in China in 2002–2003 [11,12], and MERS-CoV in Middle Eastern countries in 2012 [13,14]. The coronavirus is a kind of enveloped, positive-sense and single-stranded RNA viruses that can be divided into alphacoronavirus(α-CoV), betacoronavirus(β-CoV), gammacoronavirus(γ-CoV), and deltacoronavirus(δ-CoV) [15]. The SARS-CoV-2 is closely related to SARS-CoV and MERS-CoV, they are genetic clusters within β-CoV [16,17]. Moreover, the coronaviruses can infect more than one host species, including humans, birds, pigs, bats, camels, and other wildlife [18,19], meaning that both SARS and MERS belong to zoonotic viruses. More importantly, 10 out of the 11 diseases at high risk for severe outbreaks designated by WHO in 2015 have zoonotic diseases or transmission vectors [20]. Moreover, the monitoring on epidemiology and evolution of species jumps study showed that about 70% of the novel human infectious diseases were original from livestock or/and wildlife [21].

Looking past, the publications about coronavirus are mainly focused on veterinary medicine, human medicine, infectious disease, and public health field [22–24], while the natural environment vectors (water, air, soil) and social network are important vital mediators of zoonotic disease’s evolution into epidemics and have relatively rarely been explored and investigated. Recently, large numbers of investigations have reported that the RNA of SARS-CoV-2 has been detected in raw sewage, secondary treated wastewater (normally biological wastewater treatment process being applied), and even the non-potable water used for urban irrigation (Fig. 2) [25–44]. More importantly, the infectious virus has also been detected in feces and urine of patients [45–47], which triggered the public’s high concern about fecal-oral transmission and/or wastewater transmission. The virus that has been found can remain stable for up to a few days at 20 °C in wastewater [48]. On the other hand, a recent study reported that COVID-19 patients could exhale millions of SARS-CoV-2 RNA copies into the air per hour during the earlier disease stages [49], while most of the particles in the exhaled breath were smaller than PM 2.5 [50]. The limited but significant potential evidence supported that SARS-CoV-2 could survive in aerosols [51–55]. The presence of coronaviruses in the environment raises concerns about other possible transmission routes besides respiratory droplets and direct contact.

As such, wastewater and aerosols may contain some viable and infective coronaviruses, there is an urgent need to review the current knowledge for helping to understand the exposed and transmitted pathways of the SARS-CoV-2 in these environment vectors, and exploring the use of environmental engineering approaches to monitor and control it. The purpose of this review is to provide systematic while updated information based on various studies of SARS-CoV-2 from water, air, and environmental science and technology perspectives. Special emphasis is given to the occurrence, transmission, stability and persistence of SARS-CoV-2 for water and aerosol. Discussion on the possible prevention and control strategies of the SARS-CoV-2 is also presented. It is important to note that this review is not focused on COVID-19 itself, but it is the review related with the water and wastewater and their treatment processes under the effect of COVID-19. Fig. 3 presents the framework for this study.

2. Characteristics of coronaviruses in aqueous environment

2.1. Overview of transmission via water-related pathways

The discovery of SARS-CoV, SARS-CoV-2, and MERS-CoV in feces,
urine, and vomit has widely been reported [45,56–63]. Although there is currently no evidence of fecal-oral transmission of SARS-CoV-2, some studies supported the possibility of this transmission route, for example, the virus has been isolated from infected individuals’ feces [64,65]; virus RNA was recovered from untreated wastewater and secondary-treated wastewater [30,31,33,37,38]; and virus could highly be stable.
under certain conditions (at 20 °C in wastewater and tap water) [48]. It is also worth noting that saliva and various respiratory secretions from an infected individual may also be released into the collection system in wastewater [66,67]. This implies that the viruses would spread with human or/and animal waste in sewage and hospital wastewater and then transfer into the aqueous environment [18,68–70].

2.1.1. Urban/centralized water system

Global urbanization is accelerating with over half of people living in urban areas. However, the centralized living also creates a favorable environment for the rapid spread of the virus. The coronaviruses-containing waste has various routes to enter the aqueous environment and cause risks to public health (Fig. 4a). The foremost among pathways are: 1) inadequate collection and treatment of wastewater; 2) untreated sewage flowing directly into receiving aquatic bodies; and 3) even contaminated water production of industry and agriculture [35,71–73]. It is well accepted that the major exposure of human to water-related viruses is through unsafe water treatment [74,75]. If wastewater treatment plants (WWTPs) is insufficient to inactivate/remove coronaviruses, or combined sewer overflows/bypasses are operational during heavy rainfalls, the viruses may enter the natural water body [76,77]. A report has claimed that the infectious COVID-19 virus was found in the sewage overflow of a private rainwater/sewage pipe and caused community transmission in Guangzhou, China [78]. Similarly, burst sewer networks may be another mode of transmission [79]. In addition, due to the direct discharge of wastewater, the virus presence in river was detected in Ecuador with low sanitation [43]. In some cities, the non-potable water is directly from the river and canal to clear streets and to water the greenery. Clearly, this action is closely related to the public. Notably, the new coronavirus has been found in Paris’s non-potable water [80].

On the other hand, an inadequate plumbing system in a residential building likely contributed to coronaviruses transmission. The coronavirus-containing water droplets have overflow from sewage pipe, causing virus transmission. For example, Hung [81] demonstrated in a survey of 321 SARS patients from Amoy Gardens in Hong Kong, two-thirds of SARS infected patients had diarrhea. A large amount of virus loads have been discharged into the sewage pipe in the block, and the water droplets containing coronaviruses spilled from the water closets, basins, bathtubs, and the bathroom floor drains which caused the spread of the virus in the community.

2.1.2. Rural/decentralized water system

Different from the city’s centralized water treatment, which builds a series of grey or technology-based infrastructure, the rural water supply and treatment are mostly decentralized and untreated (Fig. 4b), especially in developing and less developed areas [82]. In these regions, however, agricultural farming and animal husbandry are one of the main sources of potential virus. In these regions, agricultural farming and animal husbandry is one of the main sources of the potential virus. The livestock and/or poultry waste normally has not been effectively managed and disposed of, which may contain the zoonotic virus. It is worth mentioning that animal feces, during precipitation events, can be carried into surface water bodies with runoff. Although there is no report on the new coronavirus being detected in agricultural farming, caution should be taken on it as cats, ferrets and dogs [83,84], and tiger [85] have been reported to be infected by the SARS-CoV-2. The more serious information is that the coronavirus has been detected at eight mink farms in the Netherlands, and at least two workers were infected, which could be the first known cases of animal-to-human transmission [86]. Likewise, around ninety minks and seven workers have also been tested positive for coronavirus in northern Spain [87]. Some studies have reported that livestock animal waste significantly associated with drinking water contamination in low-and middle-income countries [88,89], 67% of households sampled had faecal-contaminated drinking water in peri-urban communities of Kisumu, Kenya [89]. Contaminated water by humans and animals is one of the important ways of indirect transmission of virus [90]. Therefore, it should be urgently assessed about coronaviruses transmission via water in low sanitation regions.

In addition, the lack of wastewater treatment or improper management of wastewater disposal, leakage of septic tanks, and mismanagement of animal waste disposal are also possible pathways to cause contaminated aqueous environment [76]. The wastewater treatment ponds are most applied in rural and developing countries, which need more than two weeks to get reduction one log of viruses [91]. Therefore, if the wastewater and waste from these underdeveloped regions was not treated properly, it would result in snowballing transmission of COVID-19 in wastewater, as seen in other viral diseases previously [92,93].
2.2. Survival characteristics of coronaviruses in the environment through water-related pathways

The survival and persistence of coronaviruses released into the aqueous environment determine the magnitude of human health risks [94]. Many factors affect the survival or infection of coronaviruses in the water/wastewater environment. The main effects are viral load and environmental conditions, especially water temperature and wastewater characteristics, such as type of medium, the presence of organic and inorganic matters [58,68,69,95–97]. To date, there is limited scientific evidence for the persistence of coronaviruses in water and wastewater. The latest study estimated the times for 90% reduction ($T_{90}$) of viable infectious SARS-CoV-2 with a high titer in wastewater and tap water by measuring tissue culture infectious dose per milliliter (TCID$_{50}$ mL$^{-1}$). The result has shown that $T_{90}$ was 1.6 days in wastewater and 2.0 days in tap water under room temperature (20 °C), while it will be decreased to only 15 and 2.2 min in wastewater at 50 °C and 70 °C, respectively [48].

Another study about the stability of SARS-CoV-2 in different environmental conditions reported that the virus is highly stable at 4 °C, but very sensitive to temperature, and can be inactivated in 5 min at 70 °C [95]. Likewise, the SARS-CoV-2 can remain stable and infectious for up to 25 days at 5 °C in water, but rapidly inactivated at 30 °C [73] (not an experimentally determined value, it was estimated in vitro study data). In addition, SARS-CoV-2 can be stable at pH 3–10 at room temperature [95].

During the SARS epidemic outbreak in China, the Xiaotangshan hospital and the 309th hospital in Beijing were each equipped with sewage treatment site. The behavior of the SARS-CoV in hospital sewage has been investigated and claimed that the SARS-CoV could remain infectious for 2 days in sewage, while the RNA could be detected for 8 days [58]. The survival characteristics of the isolated SARS-CoV in different media and temperatures showed that the SARS-CoV could survive for 14 days in saline and 17 days in urine at 20 °C, while in domestic sewage and dechlorinated tap water only survive for 2 days at the same temperature. While at 4 °C, the virus was found to remain infectious for over two weeks in all mediums [96]. MERS-CoV was similar to SARS-CoV, and was more favorable for survival at low temperatures and humidity [98].

It should be noted that unlike other waterborne viruses, the membrane of coronaviruses is a lipid layer, thus highly unstable [99]. Disrupting the lipid envelope can eliminate risk. For example, SARS-CoV was more unstable in wastewater containing disinfectants compared to non-enveloped viruses [96]. On the other hand, some other surrogate coronaviruses survival characteristics were also investigated. For example, the persistence of human CoV 229E, enteric feline CoV (ATCC-990), transmissible gastroenteritis virus (TGEV), mouse hepatitis virus (MHV), and Pseudomonas phage φ6 in water and wastewater had been evaluated. The variable result showed that water temperature, coexisting pollutants (organic matter, suspended solids, etc.), test media, and biological activity were together determining the survival characteristics of the coronaviruses [68,69,100].

Overall, the stability of coronaviruses in aqueous was highly variable, while increased temperature or adding some strong oxidants like free chlorine could disrupt the lipid envelope to inactivate coronaviruses. At this point, we can deduce that SARS-CoV-2 is also highly dependent on water temperature and easily inactivated by disinfectants. In other words, however, the lower temperature will be more suitable for virus survival in aqueous. The special attention is required in winter to prevent a possible “second wave” of outbreaks.

2.3. Possible prevention and control strategies

As mentioned above, the RNA of SARS-CoV-2 has been widely detected in wastewater. However, most scientists believe that wastewater is currently not a significant transmission route [30,94,101], while WHO also stated that there is no evidence about the survival of SARS-CoV-2 in wastewater or drinking water. Indeed, some possible prevention and control strategies for coronaviruses have been adopted in the water cycle, but it is still a need to understand the characteristics of possible curb strategies for coronaviruses in water-related pathways and grasp some technologies for inactivation in daily life.

2.3.1. Wastewater treatment

To our knowledge, there are few studies on the fate and removal efficiency of coronaviruses in actual WWTs, especially to monitor the change of coronaviruses infectivity (Table 1). It is because several barriers prevent investigating viable coronaviruses in wastewater treatment, from detection technology to the special working environment. Thus, most scientific efforts were placed on tracing for coronaviruses RNA in WWTs. Randazzo et al. [33] investigated the occurrence of SARS-CoV-2 RNA in different WWTs in Spain. They collected influent, secondary, and tertiary effluent water samples to analyze SARS-CoV-2 RNA, the result showed that 83% (35 out of 42) influent samples, and 11% (2 out of 18) secondary treated water samples were tested positive for SARS-CoV-2, while none was tested positive in the tertiary effluent samples (0 out of 12). This agrees with data reported by Rimoldi et al. [35] in Italy. Likewise, although the presence of the viral genome has been confirmed in untreated wastewater at higher ambient temperature (above 40 °C), the negative results for the presence of viral RNA in the effluent from the WWTs were reported in India [36]. The conventional wastewater treatment process (normally the activated sludge process) should be good enough to inactivate SARS-CoV-2. In contrast, some studies reported that the positive results for viral RNA in effluent could still be discovered even after WWTP treatment [25,44]. The possible reasons to explain these contradictory results are the lack of a standardized procedure of SARS-CoV-2 RNA detection in wastewater, and the various treatment techniques adopted in different WWTs at reality environmental conditions. Therefore, further studies seem desirable to face the situation.

As the size of the coronavirus ranges from 60 to 220 nm [69], the conventional primary treatment (screens, grit chamber, primary clarifier) may be insufficient to remove coronaviruses by physical processes. One study reported that up to 26% of enveloped viruses were removed by solid settling [100]. However, other studies pointed out that organic matters and suspended solids could help protect and survive for coronaviruses [69,71]. A study stated that SARS-CoV-2 RNA was still detected in the wastewater after the primary settler process [34]. The non-enveloped human has been found to be more persistent in water for long periods of time than enveloped viruses [69,76,99,102]. Therefore, the fate of coronaviruses in the secondary treatment process (activated sludge process or/and biofilm process) in WWTs can be predicted by referring to the non-enveloped viruses. The enteric viruses, norovirus, and adenoviruses are commonly waterborne non-enveloped viruses and are normally used as surrogates for treatment performance evaluations in the aquatic environment [103–106]. Based on the collective data, the level of virus removal in secondary treatment has a variable feature between less than 1 log unit to greater than 4 log units, depending on the treatment process used and the virus types [107–110]. The mechanism of remove viruses in activated sludge is mainly attributed to sludge flocs adsorption and subsequent separation in secondary clarifiers [108]. The membrane bioreactor (MBR) shows a higher removal effect because it owns better solid separation than the conventional activated sludge technologies [109,111]. In a recent report, no SARS-CoV-2 genetic material in effluent wastewater was detected in either sequencing batch reactor (SBR) or moving bed biofilm reactor (MBBR) [36].

As the last step of treatment process, disinfection is a vital process for inactivating the viruses. The ultraviolet (UV) light and/or chemical disinfection are the main disinfection processes in actual WWTs. The chemical oxidants include chlorine, chloramines, ozone, and chlorine dioxide, etc. Regardless of the process applied, disinfection highly depends on wastewater physicochemical properties and operational conditions, especially the product of disinfectant concentration and contact...
time (CT value). A number of studies have been carried out and have demonstrated that the reduction in non-enveloped viruses, such as human adenovirus (HAdV), enterovirus (EV), in effluent treated with UV or chemical disinfection is more effective, with 0.1 to 5 log units, and point out coronaviruses are more sensitive to UV than non-enveloped viruses [107,109,112,113]. For the inactivation of coronaviruses in wastewater, a study to explore the inactivation of SARS virus in municipal wastewater by chlorine and chlorine dioxide showed that the chlorine dosage of 20 mg L\(^{-1}\), a contact time of over 1 min, and free chlorine residual of $>0.4$ mg L\(^{-1}\) were found to complete coronaviruses inactivation, while chlorine dioxide needs to be using a dose of 40 mg L\(^{-1}\) with a required contact time of $>5$ min to achieve the same effect [58]. However, the co-existing high concentration of organic compounds and suspended solids in wastewater would interfere with disinfection for the virus. During the COVID-19 outbreak in Wuhan, China, the treatment of medical wastewater containing SARS-CoV-2

### Table 1: Summary of detection and quantification of SARS-CoV-2 in wastewater and sludge in WWTPs.

| Location                        | Period of examination | Sample condition (type, volume, temperature) | Method of RNA extraction, detection and quantification | RNA concentration of influent (copies/L or samples positive) | Wastewater treatment technologies | RNA concentration of effluent (copies/L or samples positive) | Reference |
|---------------------------------|-----------------------|-----------------------------------------------|-------------------------------------------------------|-------------------------------------------------------------|----------------------------------|-----------------------------------------------------------|-----------|
| Spain, Region of Murcia         | March 12 to April 14, 2020 | Municipal WW; Grab sampling 200 mL; 4 °C | NucleoSpin RNA virus KIt; TaqMan real-time RT-PCR; gc | 5.1 $\pm$ 0.3, 5.5 $\pm$ 0.2, and 5.5 $\pm$ 0.3 log10 gc/L of N1, N2 and N3 primer/probe mixes | CAS, Coagulation, Flocculation, SF, Disinfection, UV, NaClO | Secondary effluent: 5.4 log10 gc/L of N2 primer/probe mixes; Tertiary effluent: No detection | [33]      |
| India, Ahmedabad                | May 8 and 27, 2020     | Hospital treating WW; 4 °C                   | NucleoSpin® RNA virus KIt; TaqPathTM Covid-19 RT-PCR KIt; Ct value | 8 May: 100% of positive rate (5.6 $\pm$ 10 copies/L); 27 May:10% of positive rate (3.5 $\times$ 10^2 copies/L) | UASB                             | 8 May: 0% of positive rate 27 May: 0% of positive rate | [41]      |
| Italy, Milan and Monza Brianza  | April 14 and 22, 2020  | Municipal WW; Grab sampling Separate stainless steel buckets | QIAMP Viral RNA mini Kit; 2019-nCoV real-time RT-PCR kit panel; Ct values | April 14: 75% of positive rate(3/4); April 22: 25% of positive rate(1/4) | CAS + peracetic acid or high intensity UV | No detection | [35]      |
| India, Jaipur                   | May 4 to June 14, 2020 | Municipal WW and Hospital WW; Sterile bottles | Allplex™ 2019- nCoV Assay Kit; TaqPath™ COVID-19 Combo Kit; Ct values | Positive | MBBR/SBR + Cl/UV | Negative | [36]      |
| Chile, Santiago                 | May 20 to June 16, 2020 | Sewage; 24 h composite sample; Sterile propylene bottles | QIAamp® Viral RNA Mini Kit; TaqMan 2019-nCoV Assay Kit v1; Ct values | 20 May: 354–628 (copies/L); 16 June: 2304–4805(copies/L) | n.a.                             | n.a. | [44]      |
| France, Paris                   | March 5 to April 17, 2020 | Municipal WW; 11 mL | PowerFecal Pro kit on a QIAampVirusesystem; Ct values | 100% of positive rate (over 10^5(copies/L)) | n.a. | 75% of positive rate (6/8); (near 10^5(copies/L)) | [25]      |
| China, Wuchang                  | February 5 to March 10, 2020 | Municipal WW; Grab sampling; 2.0 L; 4 °C | EZ1 virus Mini Kit; AgPath-ID™ One-Step RT-PCR KIt; Ct values | Non-detected (After primary disinfection tank before septic tank) | Preliminary disinfection tank + Septic tank | (7.5 $\pm$ 2.8) X 10^2 – (14.7 $\pm$ 2.2) $\times$ 10^3 copies/L in the effluents of septic tanks | [71]      |
| Spain, Ourense                  | April 6 to April 21, 2020 | Municipal WW; 250 mL; 24 h composite samples; 4 °C | STARMag 96 x 4 Universal Cartridge Kit; RT-qPCR Alplex system™ 2019-nCoV; Ct values | 100% of positive rate (5/5); | Grit and sand separator, primary settler, SBR, Microfiltration | Secondary effluent: 25% of positive rate (1/4); Finally effluent: No detection | [34]      |
| Japan, Kofu                     | March 17 and May 7, 2020 | Municipal WW; Grab sampling; 1.0 L | RNeasy PowerWater Kit; High-Capacity cDNA Reverse Transcription Kit; Ct values | 4.0 $\times$ 10^3 – 8.2 $\times$ 10^4 copies/L | CAS: primary sedimentation, aeration, final sedimentation, chlorination | Secondary effluent: 1.4 $\times$ 10^2 to 2.5 $\times$ 10^3 copies/L | [39]      |
| USA, Louisiana                  | January to April 2020  | Municipal WW; 24 h composite samples; -80 °C | ZR Viral RNA Kit; High Capacity cDNA Reverse Transcription Kit; Ct values | 13% of positive rate(2/15) | | n.a. | None of the secondary treated and final effluent samples | [40]      |
| Spain, Ourense                  | April 6 to April 21, 2020 | Sludge; 250 mL; 4 °C | STARMag 96 x 4 Universal Cartridge Kit; RT-qPCR Allplex system™ 2019-nCoV; Ct values | 41% of positive rate(14/34) in primary sludge, biologic sludge, and thickened sludge | Gravity thickening and centrifuge, thermal hydrolysis, anaerobic digestion | 0% of positive rate(0/5) in digested sludge | [34]      |
| USA, New Haven                  | March 19 to May 1, 2020 | Sludge; 0°C | RNeasy PowerSoil Total RNA Kit; Bio-Rad iTaq Universal Probes One-Step Kit; Ct values | 100% of positive rate; 1.7 $\times$ 10^3 to 4.6 $\times$ 10^3 copies/mL | Settler | n.a. | [27] |
| Turkey, Istanbul                | May 7, 2020 | Sludge; Grab sample | Roche Magna pure LC total nucleic acid isolation Kit; Ct values | 100% of positive rate in primary sludge, 1.25 $\times$ 10^4 to 2.33 $\times$ 10^4 copies/L | CAS | 100% of positive rate in waste activated sludge, 1.17 $\times$ 10^3 to 4.02 $\times$ 10^3 copies/L | [26]      |

Note: n.a.: not available.

CAS: conventional activated sludge; SF: Sand filtration; UASB: upflow anaerobic sludge blanket reactor; MBBR: moving bed biofilm reactor; SBR: sequencing batch reactor.
viral RNA located at Wuchang Fangcang hospital, China, in which the wastewater was firstly pumped from toilets and showers into the preliminary disinfection tank, while adding 800 mg L\(^{-1}\) of sodium hypochlorite for disinfecting with contacting time of 1.5 h, then the SARS-CoV-2 viral RNA was not fully detected after this kind of preliminary disinfection process [71]. Thereafter, the wastewater was pumped into the septic tank. It was surprising that the SARS-CoV-2 viral RNA was detected from the effluent sample of the septic tank. It is believed due to the suspended solids in the septic tank and thus noting that a small size of particles can shield viruses from disinfection [71]. It is noteworthy that chemical oxidants would mainly damage viral capsid proteins for disinfection, while the UV would damage the viral genome and proteins [114,115]. A separate study demonstrated that UV disinfection would reduce single-stranded RNA virus levels below detection limits [116]. Ye, et al. [117] explored the inactivation mechanism of enveloped viruses in UV and free chlorine, showing that the molecular features of an enveloped virus were susceptible to chemical oxidants or UV radiation. As the coronaviruses are enveloped viruses, it should be considered that a combination of UV and chemical oxidation disinfection is suggested to better inactivate coronaviruses, which can achieve at least 5 log units reduction of the waterborne virus [118]. Similar to chemical oxidation disinfection, suspended solids would also affect UV disinfection efficacy. Overall, the regulation running of urban WWTPs would satisfactory to remove coronaviruses in wastewater.

For decentralized rural wastewater treatment, there have been a variety of technologies existed, ranged from the simple individual septic tank to comprehensive ecological engineering systems, such as constructed wetlands, soil infiltration treatment systems, and biofilter technologies. In general, these treatment facilities have a good performance on removing COD and considerable removal of nutrients, but research on the removal of waterborne viruses is still in its infancy [119,120]. More importantly, because small decentralized wastewater treatment system may be lack of disinfection approach, and lack of public’s poor risk and threat perception of water, caution should be taken to drainage system when infected patients live in the “catchment area”. For asymptomatic individuals or mild patients, when they are quarantined at home, it is recommended to put disinfected chlorine tablets in the toilet tank, which can effectively inactivate coronaviruses and reduce the wastewater loading of viruses and secondary transmission. The septic tank system should preferably be located at a certain horizontal separation distance (least 30 m) from the water source to avoid contaminating the water supply [121].

Overall, the adsorption with solids or sludge, and filtration with sand and/or membrane could remove coronaviruses from wastewater, but the inactivation process is highly dependent on chemical oxidation disinfection and UV disinfection for wastewater treatment.

2.3.2. Drinking water and reclaimed water treatment

Urban drinking water system is composed of four important parts, which are source water, waterworks, distribution network as well as point-of-use. Management and control of each part need to pay attention to prevent and control the water-related virus spread. We must point out that, so far, SARS-CoV-2 has not been detected in drinking water, and the drinking water is safe.

The rigorous pollution control in source water is the first step in the control of the drinking water quality. Due to SARS and MERS are zoonotic viruses, the management of animal farming near source water needs to be pay attention. Waterworks and treating water treatment plants (DWTNs) are the most critical part of controlling water-related viruses in the urban water cycle. The water-related virus can be removed by physical separation and inactivation in DWTNs. In a typical conventional drinking water treatment process, chemical coagulation could achieve 0.1–2.4 log unit viruses removal range with iron or aluminum coagulants [122]. The mechanism may be attributed to the physical removal of inclusions in the floc and inactivation of chemical oxidation [123]. The effect of precipitation and filtration on virus removal highly depends on the filtration technologies. The effect of membrane filtration on virus elimination is better than trickling filtration and sand filtration [124,125]. The reverse osmosis (RO) process should be the best in theory for virus removal, but in practice, more ultrafiltration (UF) and nanofiltration (NF) membranes are adopted due to more cost-effective reason and similar removal level to be obtained (over 4 log units reduction for norovirus) [125,126]. Disinfection, as the last checkpoint of DWTNs, is the controlling point for virus inactivation. Chlorine disinfection is a common method of drinking water, the concentration of free chlorine \( \geq 0.4 \text{ mg L}^{-1} \) could completely inactivate SARS-CoV [58]. For effective centralized disinfection, the WHO has suggested free chlorine \( \geq 0.5 \text{ mg L}^{-1} \) after at least 30 min of contact time at pH \( < 8.0 \). However, the performance of this concentration of free chlorine on SARS-CoV-2 real medical wastewater is unsatisfactory [71]. On the other hand, with the high dose of disinfectant, toxic disinfection by-products (DBPs) (such as chloramine, chlorite, bromate, etc.) will also significantly increase which will deteriorate the water quality [114]. Thus, it is important to ensure that the lower concentration of influent turbidity and nutrient enter the disinfection process, which would significantly influence the level of DBPs and disinfection efficiency. Therefore, more research on the efficacy of various disinfection options is needed on coronaviruses in water and wastewater. It is highly necessary to: 1) ensure the safety of the water distribution network system; 2) reduce the network accident and cross-contamination as much as possible; and 3) ensure the residual chlorine and/or monochloramine at the terminal water supply [114].

In the rural and/or underdeveloped regions where there is no centralized water supply, people may have a higher risk to directly drink unsanitary surface water. In this situation, the prolonged boiling and/or adding bleaching powder or chlorine tablets are possible ways to inactivate coronaviruses in water based on the survival characteristics of coronavirus. SARS-CoV-2 could be efficiently inactivated by heating at 92 °C for 15 min (>-6 log units removal) [127].

As the advanced treatment process, reclaimed water is similar to that of ordinary DWTNs, the possible prevention and control strategies for the water-related virus in DWTNs are suitable for reclaimed water management [107,128]. Based on the data during the outbreak of SARS and MERS, most DWTNs systems that meet the virus removal/inactivation regulations are effective for coronaviruses control. Furthermore, some combined advanced oxidation processes have begun to be applied in the treatment of reclaimed water and advanced drinking water, which include UV-based advanced oxidation processes (UV/AOPs) and ozone/biologically activated carbon (O\(_3\)/BAC). Indeed, these will further enhance the ability to inactivate the water-related virus.

2.3.3. Sludge treatment

Like most pollutants are removed in traditional activated sludge process, virus removal can be seen as just a transfer from the aqueous phase to the solids phase, and then further into the sludge. Although the limited literature reported about the coronaviruses in sludge (Table 1), the risk of virus transmission in sludge disposal should not be ignored [26,27,34,129]. The hydrophobicity of enveloped viruses makes coronaviruses more prone to adhere to solids [69]. Therefore, coronaviruses (RNA filament or its fragments) are generally concentrated in suspended solids [100]. Once they are not inactivated in time and exposed to the open environment, the virus or virus RNA adsorbed by the sludge may be leached out when spread in agriculture [129]. The SARS-CoV-2 RNA was not detected in the effluent from WWTPs, but the primary and thickened sludge showed higher and steadier concentrations [34]. The virus RNA presented in primary and waste activated sludges in WWTPs during the COVID-19 outbreak in Turkey has been reported [26]. In another study, it was found that the SARS-CoV-2 RNA concentrations in the primary sludge showed a strong correlation with the number of confirmed cases in local communication [27]. In 2013, a survey of 10 sewage sludge samples from five WWTPs throughout the continental USA has shown that contained genes from coronaviruses were found in
more than 80% of the samples by metagenome analysis [130]. Another study also pointed out that the viruses could persist for several months in the sludge particles under a suitable environment [131]. It is important to note that the survival and fate of the virus in sludge would be difficult to exact interpreter and predict due to the various compounds mixed in sludge. Based on the currently available data, the coronaviruses transmission by sludge was not studied, especially when the viral load in the sludge is high.

Current research shows that coronaviruses can be hidden in fine particles and escape from disinfection [69,71]. If sludge is proved to host coronaviruses with infective capacity then coronaviruses removal in sludge should focus on inactivation. It is difficult to inactivate the virus by physical methods such as sludge thickening, dewatering, and drying [132]. Strong oxidative chemicals (e.g. ozone and chlorine) can effectively inactivate viruses, but disposal costs and possible carcinogens (THMs, trichloromethane) limit their practical application. Using lime to condition sludge to stabilize it is an economical and reliable method. It has been reported that a sufficient amount of lime addition to increase the pH of the sludge higher than 12 can effectively inhibit the growth of the microorganisms [95,133]. When pH is adjusted to around 13, the SARS-CoV can be inactivated immediately and efficiently (average reduction of 3.6 log units) [134]. Although there is currently no study on the stabilizing effect of lime on inactivating SARS-CoV-2, it could be a foreseeable approach to use the lime for inactivation.

Sludge heating treatment processes, such as ultra-high-temperature aerobic fermentation, temperature-phased/thermophilic anaerobic digestion, and sludge incineration may effectively inactivate coronaviruses. The mesophilic anaerobic digestion (MAD) process showed limited inactivation effectiveness for the pathogen (mean reduction of 1 log units) [132], but a study reported that no SARS-CoV-2 RNA was detected in the digested sludge, probably due to the result of the combined effect of thermal hydrolysis and long residence time [34]. Notably, sludge generated during the treatment of wastewater from hospitals and centralized quarantine centers must be disposed of in strict accordance with the guidelines of hazardous waste, and incineration is accordingly recommended.

3. Characteristics of coronaviruses in aerosol and inanimate surfaces

The facts of COVID-19 human-to-human transmission and SARS-CoV-2 viral RNA being detected on different inanimate surfaces and droplets in air in healthcare and patient’s family settings have indicated the virus may be airborne transmission [52,55,135–138]. It is widely accepted that the respiratory droplets are the main transmission way when a person has close contact with an infected person [6,139]. More and more scientists believe that the virus transmission via airborne is the dominant route for the spread of COVID-19 [7,49,52,55,140,141]. A WHO recent report pointed out that short-range aerosol transmission may occur in crowded and inadequately ventilated spaces [142]. There is little scientific information about the infectivity and load of SARS-CoV-2 viruses in the air, causing some controversy regarding the airborne transmission. The following discussion of indirect evidence may help people to realize the high risk of the airborne transmission of coronavirus and the need to take corresponding prevention and control strategies to curb it.

3.1. Spreading pathways

The possible routes of exposure to coronaviruses can be divided into direct and indirect contact transmission (Fig. 5). The direct pathway is via respiratory microdroplets, which is the dominant route of transmission. The tiny droplets from coughing, sneezing, singing, or talking/speech of infected individuals through their nose and mouth result in the spread of the virus [8,52,141,143]. It has been reported that microscopic droplets can remain aloft in air and even propagate 2.5 m away by gas cloud entrainment dynamics [144–147]. The airborne transmission of SARS-CoV proved to be the main mode of transmission in a relatively closed space [148,149]. A study about the aerodynamic nature of SARS-CoV-2 in different areas of two Wuhan hospitals has shown that the SARS-CoV-2 RNA in aerosols has been detected in some relatively unventilated areas, such as patients’ toilet and medical staff areas [137]. Similarly, Guo et al. [150] tested air samples from an intensive care unit (ICU) and a general COVID-19 ward in a hospital, the results show that SARS-CoV-2 was widely distributed in the air, and distribution characteristics of SARS-CoV-2 indicated that the transmission distance can reach 4 m. Respiratory droplets, however, generally are greater than 5–10 µm in diameter which only remains in the air for a short time and travels only a short distance (within 1 m). A recent study on the exhaled breath samples from COVID-19 infected individual has shown that the breath emission was estimated to be from 1.03 × 10^5 to 2.25 × 10^7 viruses per hour [49]. SARS-CoV-2 was detected in surface swabs and air samples, indicating that expiratory breath emissions play an important role in the emission of SARS-CoV-2 into the air [49]. Although this study was occurring in medical site, some relatively unventilated spaces, such as chorale [53], restaurant [151], fitness [152], have also reported the possibility of aerosol transmission. On the outdoor, the SARS-CoV-2 RNA has also been found on particulate matter in northern Italy [42].

The indirect contact transmission refers to the transmission of coronaviruses from contaminated dry inanimate surfaces to humans, initiate self-inoculation of mucous membranes in the nose, eyes, or mouth by contact with hands. Furthermore, the virus RNA in the table, chair, floor, and fan surfaces has been detected in the patient’s room, which may create a condition to transfer virus [153]. A number of studies have shown that coronaviruses could persist (a few hours to days) on inanimate surfaces [10,51,95,135,154].

Suspended water droplets in the air are also an important mode of viral transmission [155]. It is well known that wastewater could form virus bioaerosols after atomization, such as mechanically agitated, mixed, and aerated [156]. In oxidation ditch (OD) and anaerobic-anoxic-oxic (A²/O) process, for example, there are greatly produced aerosols with different particle size, microbial and chemical compositions [157,158]. More importantly, the aerosols with sizes under 3.3 µm have been detected which are considered to be inhalable, called comparable respirable fraction [157]. Like other common viral and bacterial pathogens found in wastewater, the SARS-CoV-2 could also spread and survive in wastewater aerosols [94]. It is worth noting that the emission of airborne aerosols was dependent on the type of reactor and aeration 

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mode [157,159,160]. Thus, it should be borne in mind that the transmission via sewage-derived aerosol in WWTP cannot be ruled out if aerosols retain the sufficient concentration of infectious virus [161]. Another overlooked transmission pathway is fecal bioaerosol transmission [162]. The toilet flushing also produces droplets that are enough small to become aerosol [163], while virus has been isolated from infected individuals’ feces.

3.2. Survival characteristics

3.2.1. Survival on aerosols

In general, the virus’s survival in the air depends on various factors, including biological stresses, light, and meteorological factors, such as ambient temperature, wind speed and direction, and relative humidity (RH) [164]. Previous experimental reports on MERS-CoV showed that it had a strong ability to survive in aerosols, and it could still maintain infectivity after 60 min of atomization at 25 °C and 79% RH [165]. Researchers using a 3-jet Collison nebulizer to generate aerosols containing the SARS-CoV-2 found that the virus can remain viable up to 3 h in aerosols, and concluded that the stability of SARS-CoV-2 was similar to that of SARS-CoV under laboratory conditions [51].

The effect of meteorological conditions (temperature, RH) on the viability of coronaviruses in aerosols is being debated. The stability of MERS-CoV during aerosolization has shown that the MERS-CoV was extremely stable at low temperatures (20 °C) and humidity (40% RH) [166], while more significant inactivation has occurred during experiments undertaken at 38 °C and 24% RH [164]. Researchers have proposed that viruses (bacteriophages) survived well at RHs lower than 33% and at 100%, whereas their viability was significantly decayed at 55% and 75% RHs [167]. However, SARS-CoV-2 may be resistant to high temperatures and intermediate RH [168].

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3.2.2. Survival on surfaces

Recently, a review about the persistence of coronaviruses on inanimate surfaces reported that SARS-CoV, MERS-CoV, and endemic human coronaviruses (HCoV) can remain infectious times from 2 h to up to 9 days on the different material surface [154]. A survey about virus stability during different environmental conditions has shown that SARS-CoV-2 was very stable on some inanimate surface (plastic, stainless steel, copper, and cardboard) for a few hours at 21 to 23 °C [51]. Another study investigated the stability of SARS-CoV-2 on different surfaces showed that the virus activity was dependent on material surfaces, more stable on smooth surfaces [95]. Under typical air-conditioned environments (temperature of 22–25 °C and RH of 40–50%), SARS-CoV retained viability for over 5 days on smooth surfaces, and the time is negatively proportional to temperature and relative humidity [174].

Overall, the characteristics of coronaviruses’ survival on aerosols and surfaces are similar to those in water, which can survive in a favorable environment. Therefore, surface disinfection and protective measures are particularly important.

3.3. Possible prevention and control strategies

Airborne/aerosol transmission of SARS-CoV-2 has played a potentially important role in relatively unventilated spaces [8,49,150–152]. Based on the mode of viral transmission and survival characteristics in aerosols and surfaces, it can be prevented and inactivated from several approaches. From the airborne transmission perspective, some engineering controls should be recommended in poorly ventilated spaces. These measures include increasing the ventilation rates in existing building vent, adopting air cleaning and disinfection devices for particle filtration and air disinfection, and avoiding air-recirculation as well as avoiding close contact and crowds, which have reached a consensus in the scientific communities to minimize viral transmission in semi-closed spaces [55,157,169,175–177]. On the other hand, to avoid human hand plays a major vector in the viral transmission route, some personal precautions also need to be emphasized: (1) Proper PPE: personal protection must be observed when coming into contact with possible patients, especially when taking care of infected individuals in healthcare facilities or working in water and wastewater treatment places; (2) Inactivating in time: commonly biocidal agents and disinfectants could effectively inactivate coronaviruses by surface disinfection procedures. With 67–71% ethanol, 0.5% hydrogen peroxide, 7.5% povidone-iodine, 0.1% sodium hypochlorite, and benzalkonium chloride, etc.,
Hand hygiene: unclean hands contact is the main way for indirect virus spread, such as hand-oral, hand-mucosa and food-oral transmission route. Thus, unnecessary touches must be avoided, and washing hands with soap should be done immediately when touching items that may contain viruses [95]. Overall, the evidence is emerging indicating that airborne SARS-CoV-2 may be the dominant reason contributing to the ongoing COVID-19 pandemic. The enhanced ventilation, and personal precautions are essential to minimize the risk.

4. Where do we stand?

It is quite plausible that COVID-19 will raise interest among water and wastewater industries, and atmospheric science. Given what little is known so far about how the new coronavirus might be transmitted in integrated water and wastewater systems or aerosol, additional research is desperately needed. No doubt, multidisciplinary while international collaboration will accelerate the fighting against the virus to establish control and preventing strategies from a scientific point of view. One Health approach flags the correct direction for us to work together. Indeed, there are lots of works ahead towards fully understanding the characteristics of the virus in the environmental medium, especially the survival characteristics and possible inactivation approaches. For example, so far, we do not know whether the viral particles of the SARS-CoV-2 can be aerosolized from water or suspended into air after settling and remaining infective. While such routes can occur for other coronaviruses, currently only limited direct pieces of evidence are to support the airborne transmission of the SARS-CoV-2. A precautionary approach should be taken until studies to eliminate other routes of transmission, especially in relatively ventilated areas. If the fecal-oral transmission is finally demonstrated with COVID-19, we have to design protocols to minimize risk in areas where open defecation is a fact, especially in underprivileged regions. It seems reasonable to minimize such virus load in toilet by adding chlorine tablets for disinfection. Furthermore, the safety of the drainage piping system in extra- and inter-home should also be emphasized. The sealing performance should be strengthened, especially to ensure that the toilets and kitchens are equipped with functioning U-bends. The epidemic is likely to continue into the rainy season. If the rainwater and sewage are mixed in the drainage pipeline, it will easily cause the sewage containing the virus to overflow into the environmental water bodies.

Secondarily, intensive studies should be done towards understanding the SARS-CoV-2 behavior in water and wastewater treatment to help developing the control strategies and to provide solid while enhanced technical solutions, which is also a key issue to personal safety who work for water and wastewater systems. So far, the available information supports that the current route wastewater treatment processes can provide satisfied treatment efficiency including eliminating SARS-CoV-2. However, detailed studies are desirable to confirm it regardless of the technology used for treating sewage. In activated sludge process, the wastewater derived aerosols and virus-laden sludge may be occurred, which will also pose significant potential risks associated with wastewater utility personnel, and scientists. For safety reasons, it is wiser to adopt the enhanced/advanced wastewater treatment measure and highlight the importance of wearing PPE during the outbreak period.

Thirdly, the important premise, for now, is to establish a safe and reliable analysis and monitoring method for SARS-CoV-2 in water, sludge and air. So far, there is no evidence to show that the SARS-CoV-2 was detected in drinking water. Therefore, drinking water is safe unless it was polluted during the network distribution. However, more than a dozen research groups worldwide have found the traces of the SARS-CoV-2 in wastewater and sludge. These include the groups in China, the Netherlands, Switzerland, the United States, UK, Australia, Spain and Sweden, Japan, India, etc. More significantly, these studies have led to a proposal that wastewater testing could be used as an early-warning signal if the virus returns to reveal the true scale of the outbreak and help estimate the total number of infections [178]. Especially, when the manual investigation is difficult for logistical, ethical, or economic reasons, wastewater investigation may provide an alternative method [25]. As wastewater goes through the drainage system to a treatment facility, analyzing wastewater could track infectious diseases that are excreted in urine or feces (Fig. 6), such as SARS-CoV-2. Sampling and monitoring sewage in sewer network and WWTP could provide better and quicker estimates for how widespread the coronavirus is than testing individual person, because wastewater surveillance accounts for those who have not been tested and have only mild or no symptoms and thus reflects the true situation. To accurately achieve this, investigating the concentration and viable viral RNA in feces and urine from an infected person, quantifying the viral load from wastewater samples to count of infection in the population, and selecting the representative sampling sites in sewer collection network are vital. The methods for detecting the virus in air also present a challenge. So far, there is also a lack of data related to the sampling, storage, and processing of sewage sludge used to detect SARS-CoV-2 [52,179].

Fourthly, the optimization of extraction and detection methods for coronavirus nucleic acid in sewage is also worth discussing. The RT-qPCR amplification and sequencing are the main methods of confirming SARS-CoV-2 RNA in wastewater [25,31,32], but different assays and virus concentration methods may produce qualitative inconsistent RNA results (positive and negative) [32]. Furthermore, most publications focus on the qualitative and quantitative detection of viral RNA to assess the risk of viral transmission [35]. It should be noted that the detection of virus RNA by RT-qPCR provides no indication that the virus is infectious. Meanwhile, the virus infectious detection approaches face many challenges, which are also the key issues for scientists on whether acceptance of airborne and waterborne transmission of SARS-CoV-2. The factors influencing virus infectivity include the environment, host, virus properties, and transmission [180]. To further identify the infectivity of enveloped viruses, RT-qPCR should be carried out in combined with cell culture assays and transfection assays. Obviously, more research is highly desirable. In addition, fast monitoring technique and detecting the viral RNA at low levels are most important. In that context, researchers at Cranfield University (UK), and Chinese Academy of Sciences, are working on developing new paper-based rapid testing kits, which could be an effective and rapid way to be used on-site at sewer collecting point or WWTPs to trace sources and determine whether there are potential COVID-19 carriers in local areas [181]. Another issue worth exploring is that the viral RNA in the environment may be persistent than infectious viral [48], which may not reflect the actual outbreak by measuring the RNA in sewage. It requires more long term monitoring and in-depth research.

Fifthly, it is worth noting to prevent secondary environmental risks due to the overuse measures to control coronaviruses, such as the cumulative toxicity of DBPs under overdosed with disinfectant. Zhang et al. [71] pointed out that free chlorine more than 0.5 mg/L after disinfection might be not enough to completely remove SARS-CoV-2 viral RNA. On the other hand, the drugs residue and metabolite derived from the massive and unprecedented use of antiviral and anti-inflammatory drugs during pandemic events, no doubt, will eventually discharged into the environment [182]. The high concentration of by-product residuals would bring ecological risks that need special attention. Equally, the sludge generated in water and wastewater treatment needs safe disposal.

Last but not the least, in low- and middle-income countries and remote rural areas, due to the lack of adequate water and wastewater treatment facilities, the risks are more acute [72,79]. Therefore, the development of decentralized water and wastewater treatment facilities with cost-effective approaches for UV and chemically disinfecting the
coronaviruses should be strengthened. Regarding secondary treatments, it is probably useful to reduce the use of aerated systems like activated sludge where aeration-based aerosols can be formed. Alternatively, systems, where wastewater is not air-exposed, like a subsurface constructed wetland, seem to be a reasonable strategy for decentralized treatments.

5. Summary and conclusions

Based on the updated published papers, newsletters, and scientific websites, it appears that the wastewater, sludge, aerosol are potentially environmental transmission of coronavirus. Although there is lack of direct evidence to prove fecal-oral transmission during the COVID-19 outbreak, the presence and persistence of SARS-CoV-2 in water and wastewater, and the insufficient wastewater collection and treatment system of undeveloped regions/countries are drawing attention to exist virus spread via water-related pathways. There are some risks of virus transmission under the urban and rural water cycle if several possible prevention and control strategies are not adopted. Under the urban water cycle, the coronaviruses shed in the feces and urine of infected individuals can enter drainage system, and then WWTPs. So far, a few standard systems for treating wastewater have been tested and they seem to be enough for removing the presence of SARS-CoV-2. However, the diversity in existing methodologies for treating sewage suggests the need for further studies where air-exposition, temperature, hydraulic retention time, and sludge production can be key parameters to explore. It should be remembered that after understanding the fate of viruses and viral infections, in wastewater treatment, drinking water, reclaimed water, and sludge, water users and workers need to adopt targeted prevention and control strategies. According to the limited information of the virus transmission and survival characteristics, it seems that the processes in already existing DWTPs are able to reduce the total waterborne virus over 4 log units depending on the filtration removal and disinfection inactivation process. There are, however, increasing pieces of evidence that airborne SARS-CoV-2 may be the main cause of the ongoing COVID-19 pandemic. Strengthening building ventilation systems and personal protective measures are essential to minimize the risks.

To curb the coronaviruses especially the SARS-CoV-2 in integrated water and sludge, as well as airborne systems, multidisciplinary while international collaborative research is desperately needed under the novel concept of “One Health”. Indeed, a more comprehensive understanding of virus transmission and survival characteristics is a basis to establish reliable controlling strategies. Intensive studies to explore the SARS-CoV-2 behavior in water and wastewater treatment with fast while accurate virus monitoring tools are vital. If so, wastewater should be a strategic tool for following the epidemy evolution in communities, neighborhoods or even public buildings in real-time. The presence of a virus in wastewater should not be considered a real risk but a powerful ally to report its evolution. Merging accurate sensor tools in wastewater with machine learning strategies will provide a valuable scenario for making predictions way before symptoms appear in a certain community. Additional environmental risks associated with the enhanced treatment measures should be taken into account, while the development of cost-effective decentralized water and wastewater treatment facilities for low- and middle-income countries and remote rural areas for disinfecting the coronaviruses should be strengthened.

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.

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