SARS-CoV-2: fate in water environments and sewage surveillance as an early warning system

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ABSTRACT

Coronavirus disease has emerged as one of the greatest threats to human well-being. Currently, the whole world is fighting against this pandemic that is transmitted either through exposure to virus laden respiratory or water droplets or by touching the virus contaminated surfaces. The viral load in feces of an infected patient varies according to the severity of the disease. Subsequent detection of viral genome (SARS-CoV-2) in human feces and sewage systems is an emerging concern for public health. This also dictates to reinforce the existing sewage/wastewater treatment facilities. Rapid monitoring is the key to prevent and control the current mass transmission. Wastewater-based epidemiology (WBE) is a potential epidemiology tool that can act as a complementary approach for current infectious disease surveillance systems and an early warning system for disease outbreaks. In a developing country like India, inadequate wastewater treatment systems, low-operational facility and relaxed surface water quality criteria even in terms of fecal coliform bacteria are the major challenges for WBE. Herein, we review the occurrence, transmission, and survival of SARS-CoV-2, and disinfection and potential of sewage surveillance as an early warning system for COVID-19 spread. We also discuss the challenges of open-defecation practices affecting sewage-surveillance in real-time in densely populated developing countries like India.

Key words | open defecation, SARS-CoV-2, sewage surveillance, wastewater-based epidemiology

HIGHLIGHTS

● Fate of SARS-CoV-2 in water environment is discussed.
● Trend and reinfection in community can also be revealed by sewage-based epidemiology.
● Sewage surveillance can serve as an early warning system.
● Robust sampling strategies with subsequent rapid detection methods are crucial.
● Open-defecation activities make the available WWTPs less representative and hamper the real-time sewage monitoring.

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The severe infection of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected humans across the globe. By the first week of March 2021, over 110.7 million confirmed cases and around 2.6 million deaths had been reported worldwide (WHO 2020a). India has witnessed over 11.2 million confirmed cases, which is increasing every day with a high number of new daily infections. Consequently, India has become the second most infected country with COVID-19 after the USA (WHO 2020a). COVID-19 was first detected in Wuhan, China in December, 2019 (Wu et al. 2019; Zhou et al. 2019) and the dramatic propagation of this viral disease in European and Asian countries led the World Health Organization (WHO) to affirm COVID-19 as a pandemic in March 2020. The COVID-19 pandemic is continuously exposing innumerable unanticipated problems at every level of the world’s complex, interconnected society and has impacted various aspects of the natural environment involving food security, accessible healthcare, public health and safety, stability of economies and financial institutions. These problems have given rise to immense challenges that can voraciously consume capital resources and human lives.

Aerosols, respiratory droplets (WHO 2020a), and contact routes are the primary routes for the transmission of coronavirus in communities (Morawska & Milton 2020; Setti et al. 2020; Sharma et al. 2020). Various guidelines and mandates have been issued by Governments and regulatory bodies to reduce the virus spread. The viral RNA was detected in feces or anal swabs of infected persons (Park et al. 2020; Wang et al. 2020b), even when they tested negative for nasopharyngeal samples or after the resolution of clinical disease (Gu et al. 2020; Olusola-Makinde & Reuben 2020; Xiao et al. 2020). This certainly warns of the possible chances for the origination of fecal transmission; however, it is a less acknowledged route that also remains under-investigated in community settings. Additionally, inadequate practices of human waste disposal during pandemics have caused community-acquired infection, outbreaks, and superspreading events (Gormley et al. 2017). In the latest public guideline, the World Health Organization (WHO 2020a) emphasized the significance of safe human sanitary waste management, which included the fecal contamination of hands, prevention of aerosolized fecal matter, and fecal sludge management during such pandemics. SARS-CoV-2 is being shed as excrement, which is further disposed in the sewerage or in other components of the environment due to lack of poor hygiene and basic sanitation. Recent works on ‘SARS-CoV-2 detection in wastewater’ demonstrate that monitoring SARS-CoV-2 in wastewater/sewage could offer an economical solution for COVID-19 surveillance (Lodder & de Roda Husman 2020; Randazzo et al. 2020). However, the successes of such studies are mostly related to developed countries and limitations of surveillance have still not been reflected in developing countries. Therefore, the major objective of this perspective is to focus on sewage treatment, healthcare infrastructure and sanitation status in densely populated countries like India. Further, the study also highlights the possible risks associated with the fecal-oral transmission of coronavirus and limitations of COVID-19 surveillance through sewage monitoring in developing countries. Only a few studies are available for the detection of SARS-CoV-2 virus/RNA in sewage/wastewater from developing and
low-income countries currently suffering from COVID-19. Recently, SARS-CoV-2 viral RNA was also detected in municipal wastewater treatment plants (WWTPs) of India (Arora et al. 2020; Kocamemi et al. 2020; Kumar et al. 2020). The genetic material of coronavirus in sewage was (Ahmedabad city of India) found to be increased linearly with the increase in COVID-19 confirmed cases (Kumar et al. 2020). Limited studies of SARS-CoV-2 detection in sewage from developing countries along with poor sanitation and inadequate wastewater treatment and disposal practices put them at great risk for potential sewage-related exposure to SARS-CoV-2 (Usman et al. 2020).

Disposal of treated and partially treated or untreated sewage in the water body is a common practice followed in many countries. The huge gap between the generation and treatment of sewage is one of the most common causes for surface and groundwater pollution in a country like India. Factors like economic aspects, social behavior of the community, dynamism of industries located in the collection area, climatic conditions, their water consumption, and type and conditions of the sewer system affect the flow rate and composition of sewage. Approximately 80% of the wastewater generated (>95% in developing countries) is directly disposed into the environment without adequate treatment (WWAP 2017). For example, ~70% of the sewage generated in urban India remains untreated. The service level of wastewater treatment facilities and removal efficiency at every stage need to be evaluated quantitatively for each inactivation strategy performed in actual WWTPs. Generally, the efficiency of wastewater disinfection processes in unit operations is monitored using their reactivity against indicator bacteria. However, it does not provide confirmation that other microbial contaminants will show the same response towards the same level of inactivation (Yang & Zhang 2016). There are other issues associated with the traditional disinfectants, such as reduced efficiency at higher organic load in wastewater, formation of toxic, persistent and bio-accumulative metabolites or intermediates, and extra care during transportation, storage and handling. Therefore, a number of alternative disinfection strategies with reduced application concerns are being employed that also have lesser environmental impact compared with the traditional disinfection system. The association of enveloped viruses (such as SARS-CoV-2) with organic material provides a surface which acts as a physical barrier during the process of disinfection (Geller et al. 2012; Ye et al. 2016). Hence, it is likely that in a sewage sample abundant in organic matter such as secondary effluent, the enveloped SARS-CoV-2 virus would be less sensitive to many disinfectants. In the case of an outbreak, when there is huge viral load in untreated sewage, inadequate dose of disinfectants may lead to viral transmission via treated reuse. There, the advanced filtration systems, which have a very high efficiency in separating viral cells from aqueous media, such as microfiltration (MF) and ultrafiltration (UF), could stop SARS-CoV-2 dissemination. Furthermore, the modular structure of filtration systems could facilitate upgrading of existing WWTPs to reduce effluent concentrations of SARS-CoV-2.

Currently, the epidemiological state of countries varies according to their epidemic phase and quick mitigation measures. Clinical indications of SARS-CoV-2 infections can be asymptomatic or mild disease or can lead to severe pneumonia, with further multiple organ damage. Assessing the initiation and infection rate of COVID-19 at large scale is challenging; especially, mapping the distribution and its magnitude in near real-time is intimidatingly tough. Other than therapeutic treatments or pre-exposure prophylaxis, ability to quickly identify infected individuals is most important tool. Wastewater-based epidemiology (WBE) has been effectively used for detection and monitoring of various viral pathogens (La Rosa et al. 2014; Prevost et al. 2015) and has also been found significant in the context of the current COVID-19 pandemic (Medema et al. 2020a). The current review summarizes sewage surveillance as an early warning system for SARS-CoV-2 using WBE that helps in detecting viral genome in contaminated surface water. The review also attempts to highlight some of the possible disinfection methods along with the challenges and advantages from this strategy as a complementary surveillance tool in developing countries.

MATERIALS AND METHODS

A thorough literature survey was carried out and the manuscript is written based on the existing knowledge.

RESULTS AND DISCUSSION

Wastewater generation/treatment facility

The amount of wastewater generation is proportional to the rapid growth of cities and domestic water supplies. Grey and
Black Water Recycling (GWR/BWR) systems tend to be a necessary and unavoidable component in residential and commercial facilities in the near future, considering the increasing per capita water consumption in an emerging economy like India. The treatment of wastewater in a low-income and densely populated country is a major challenge. High operating costs, rising pollutant loads, insufficient technology, and extremely high capital costs of treatment plants have proven to be inefficient for an effective sewage/wastewater treatment. In 2019, the total volume of sewage generated by households in urban India was 61,754 million liters per day (MLD), which adds up to an annual volume of 22,540 million cubic meters (Figure 1(a)). The current operational capacity for collection and treatment is about 22%, out of which only a small proportion is reused. The status of the sewage treatment system in India is also presented in Figure 1(b), showing that only 63.97% of the facilities are operational. Therefore, the room for improvement is significant. There are 35 metropolitan cities with more than 10 million people, and these cities produce 15,644 MLD of sewage. The treatment capacity exists for 8,040 MLD, i.e., only 51% of treatment capacity is created. Delhi has the highest treatment capacity among metropolitan cities, at 2,330 MLD (30% of the total treatment capacity of metropolitan cities). Mumbai, which is second only to Delhi, has a capacity of 2,130 MLD, accounting for 26% of the total capacity in metropolitan cities. As a result, Delhi and Mumbai together have 55% of the metropolitan cities’ treatment capacity. The treatment potential of some cities, such as Hyderabad, Vadodara, Chennai, Ludhiana, and Ahmadabad, matches the volume of generation. Cities like Delhi and Dhanbad have a capacity of more than 50%, while the rest of the cities have a capacity of less than 50%. This void indicates that there will be a twin-edged problem in coming years; first is how to deal with reduced freshwater availability and second is the increased wastewater generation.

**Wastewater use/disposal: a lucrative source of income**

Insufficient treatment capacity and inadequate service level for wastewater, along with elevated sewage generation, poses a high threat to disposal of wastewater. Lack of sewage treatment facilities with inadequate capacity has led to mismanagement of sewage. Consequently, a significant portion of wastewater is either not processed, i.e., bypassed in sewage treatment plants (STPs), or it is sold to the nearby farmers at a fixed cost by the Water and Sewerage Board. Surprisingly, most of the untreated wastewater is directly disposed into river basins and indirectly used for irrigation. In states of India such as Vadodara, Gujarat that lack alternative sources of water, selling wastewater and renting pumps to lift it by lower social strata is one of the most money-spinning activities. In India, ∼73,000 ha of peri-urban agriculture land is subjected to sewage irrigation. These agricultural sites are used for year-round, intensive vegetable production systems (300–400% cropping intensity) or other perishable commodities such as fodders. The farmers earn up to four times more per unit from such land irrigated with wastewater as compared with freshwater. Using sewage or industrial effluent mixed with sewage for irrigation has resulted in a saving of ∼50% of N and P fertilizer and leads to ∼27% higher crop productivity than normal water.

**Incorporation of suitable wastewater treatment facilities**

In progressive countries like India, the issues related to wastewater reprocess have intensified with the ineffective treatment process. The task is to find cost-effective,
technically sound and comprehensible solutions that do not jeopardize our substantial wastewater-dependent livelihoods as well as safeguarding our precious natural resources from degradation. Constructed wetlands are now being accepted as an effective wastewater treatment technology. Furthermore, for essential strategic, safe, and sustainable wastewater usage and coherent programs, including economical decentralized wastewater treatment technologies, bio-filters, competent bacterial strains, organic/inorganic agronomy, appropriate yield harvesting systems, lucrative non-edible crop farming and modern sewage water application methods appears to be necessary.

Open defecation: prevalence and trends

Sanitation is concerned with public health, specifically with the availability of adequate and safe sewage disposal facilities for excretion of human feces and urine. Despite the fact that sanitation encompasses four major frameworks (wastewater management, solid waste management, excreta management and sewerage system), it is most closely linked to the excreta management system. Open defecation poses a considerable threat to human wellbeing and is inextricably linked to people's daily lives, especially in India's rural areas and urban slums. The concern of origin of fecal-oral route via contaminated water is very common in a country like India, where ~17.7% of the total human population resides and millions are still deprived of clean drinking water. There are various problems that exist, such as the reluctant behavior of the community towards using private or public toilets, underutilization of toilets due to a lack of water supply, and lack of knowledge of the importance of handwashing in disease prevention. Additionally, even after the government’s financial and infrastructural support, people prefer to have a bowel movement outside (RICE 2019). However, due to constant government efforts, the population without access to toilets has been reduced significantly by an estimated 450 million people in India (WASH 2020).

Open defecation is regarded as a significant health and environmental risk (WHO 2014). In 2016, it was estimated that 892 million people lived without access to sanitation and relied on open defecation (in gutters, behind bushes, in open water bodies etc.). The majority of people who practice open defecation (almost 90%) live in rural areas, but the vast majority of them live in two regions (Central Asia and South Asia) (World Health Organization 2017). A study carried out in the rural part of India (Amravati district) to examine the impact on the physicochemical and biological quality of water due to open defecation activities in open defecation-free (ODF) and open defecation-not free (ODNF) villages revealed that fecal pollution in drinking water was 17% in ODF villages and 48% in ODNF villages (Tambekar & Raigire 2012). According to a report, 90% of survey respondents defecate in open areas, contaminating water and increasing the chances of water-borne disease incidents. Among all 32% of cases defecate in agricultural fields, 25% near water bodies, 21% by the side of thorny trees, and the rest in streets and open drains (WHO & UNICEF 2010). Proper handling of children’s feces is also a critical sanitation and hygiene process. The feces of a child contain as many germs as those of an adult, so it’s important to dispose of them quickly and safely. The survey also revealed that respondents dispose of their children’s feces in garbage cans, toilets, streets, and drainage systems. In such cases, rains play a significant role in draining and accumulating fecal contamination in surface water bodies. Incidence of respiratory disease due to sewage-contaminated swimming pool water is a typical example of such contamination. Thus, disposal or unintentional drainage of untreated water in surface water bodies can also exacerbate the current COVID-19 situation (Kitajima et al. 2020). Therefore, such ground realities cannot be overlooked and can account for possible transmission of SARS-CoV-2 through unregulated wastewater management systems. The notion that WBE, applied worldwide for SARS-CoV-2 tracking, can be used in India is hampered by the fact that a large proportion of the population defecates in the open air, thus rendering the available WWTPs much less representative than in other countries.

COVID hits; how India is affected

In early December 2019, the first COVID-19 outbreak was discovered in Wuhan, Hubei Province, where multiple patients with viral pneumonia were found to be epidemiologically related to the Huanan seafood market in Wuhan. The World Health Organization (WHO) declared this outbreak a Public Health Emergency of International Concern (PHEIC). On 30 January 2020 and in February 2020 it officially termed this outbreak COVID-19, where CO stands for Corona, VI stands for Virus, D stands for Disease and 19 is the year it primarily occurred. Likewise, as a developing country with the world’s second-largest population, India’s rural and urban growing economy both suffered severely from this COVID-19. Coronavirus cases in India initially occurred as a result of an international link without any regional transmission. The first three cases of infection
were reported in Kerala on the 30 January and 3 February with a past travelling record from Wuhan, China (Patrikar et al. 2020). The Ministry of Health and Family Welfare (MoHFW), India issued travel advisories similar to those enforced during previous pandemics, such as SARS, bubonic plague, and Ebola, including the implementation of 14-day self-quarantine rules for all foreign travellers entering the nation. On 22nd March 2020, Janata curfew was imposed by the Prime Minister of India, urging people to stay home. In order to control the COVID-19 transmission, imposed by the Prime Minister of India, urging people to stay home. In order to control the COVID-19 transmission, a four-phase lockdown was imposed in India from 24 March to 31 May 2020 (68 days) (Pulla 2020). Meanwhile, India reached its first 1 lakh confirmed cases on 18 May 2020, and surpassed the 10 lakhs mark by end of July. India also enacted the quarantine law under the Epidemic Disease Act, 1897 to ensure effectiveness of the lockdown and social distancing. Mobility in grocery, pharmacy, retail shop, transportation, malls and parks, and workplaces decreased by 64.2%, 70.51%, 65.6%, 46.17 and 60.03%, respectively due to this lockdown (Saha et al. 2020). COVID-19 has exacerbated human misery, harmed the economy, turned billions of people’s lives upside down, and had a huge impact on the health, economic, environmental, and social spheres.

Environmental concern: wastewater scenario

The global waste generation dynamics have been altered as a consequence of the pandemic of COVID-19, demanding special attention. Moreover, the presence of SARS-CoV-2 RNA is more frequent in feces than gastrointestinal symptoms (17%) such as diarrhea in a recent study of COVID-19 patients (Cheung et al. 2020). SARS-CoV-2 has been detected in the feces of both infected symptomatic and asymptomatic patients (Foladori et al. 2020; Pan et al. 2020; Randazzo et al. 2020; Tang et al. 2020; Xiao et al. 2020; Zhang et al. 2020). It was also evident from clinical experiments and research that traces of SARS-CoV-2 are present in urine samples of infected patients (Lescure et al. 2020; Ling et al. 2020). These results suggest that open defecation practices may increase the virus in sewage/wastewater from symptomatic patients along with the asymptomatic individuals, which ultimately reaches sewage treatment plants (Haramoto et al. 2020). Even after the patient no longer has the respiratory symptoms, virus can be present in feces for several days (Wu et al. 2020). SARS-CoV-2 RNA in feces can be detected for an average duration of 22 days (Zheng et al. 2020).

Various studies have revealed that the virus may be present in wastewater systems, especially in municipal wastewater of areas affected by COVID-19. Coronavirus has recently been detected in untreated wastewater of Australia, Netherlands, the United States, and France (Ahmed et al. 2020; Medema et al. 2020b; Nemudryi et al. 2020), confirming the hypothesis that the virus may be found in wastewater (Sunkari et al. 2020).

Positive impact on water sources

Probably, the environment is likely to be the only sector that has benefited from the COVID-19 scenario. Various reports have described the improvement of physicochemical and biological water quality in different water sources during nationwide lockdown. The shutting down of Delhi-NCR factories contributes to the improvement of the Yamuna river water quality (pH, electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) improved by 1–10%, 33–66%, 51%, 45–90% and 33–82%, respectively) (Arif et al. 2020). Significant water quality improvements also were observed during the partial lockdown. Out of the parameters judged for the four rivers, the river Yamuna showed most outstanding result, with coliform content decreasing by 98% after lockdown. Similarly, for the river Gomti, the coliform content decreased by about 50%. For the river Ganga, both total dissolved solids (TDS) and coliforms showed a decline after lockdown, which is a good indicator of river health in COVID times (Ghildyal et al. 2020).

SARS-CoV-2 transmission in sewage

The outbreak of the COVID-19 pandemic has given rise to problems like management of biomedical waste and disposal of wastewater. Transmission of diseases caused due to pathogens like SARS-CoV-1, Ebola virus, and pandemic influenza has already been a topic of discussion at many recent wastewater management events (Chattopadhyay & Taft 2018). Recent literature has indicated that the coronavirus poses potential risk of waterborne transmission. Many researches have confirmed the presence of SARS-CoV-2 RNA in STPs (Quilliam et al. 2020). This has amplified the need for information about coronavirus transmission pathways in the environment, including the sewer pathways. This is due to the fact that sewage has been identified as one of the most important source of pathogen transmission. In areas without sufficient sanitation and water treatment facilities, the possibility of pathogen transmission could be a major challenge, as wastewater discharged without proper treatment could expose the
public to infection (Usman et al. 2020; Wang et al. 2020a). In areas with deficient sanitation facilities, discharge of excretion into sewage increases the possibility of exposure through the fecal-oral route (Amirian 2020; Bogler et al. 2020; Quilliam et al. 2020). Though the coronavirus’s infectivity is unknown, it can be detected in excrement up to ∼1 month after the patient has tested negative for COVID-19 (Quilliam et al. 2020). The possibility of fecal-oral transmission of the virus is officially added to the clinical guideline of ‘Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)’ in China, and it is advised to pay attention to feces- or urine-contaminated environments to search for any potential transmission through this path (National Health Commission 2020). A possibility of rapid transmission of SARS-CoV-2 in a community is shown in Figure 2.

Detection of COVID-19 footprints in WWTPs

Today the world has recognized the presence of SARS-CoV-2 in STPs. SARS-CoV-2 RNA has recently been discovered in urban wastewater samples from Chennai, India. In addition, a recent study from China suggested that the drainage system may be contaminated, as municipal wastewater from a COVID-19 designated hospital screened positive for SARS-CoV-2 RNA (Chinawaterrisk.org 2020). Presence of SARS-CoV-2 RNA in sewage has been reported from the Netherlands (Lodder & de Roda Husman 2020; Medema et al. 2020b). Further, Wurtzer et al. (2020) confirmed the relation between the increase of genome units in sewage and the number of COVID-19 patients in France. Moreover, reports published by Randazzo et al. (2020) confirmed the presence of SARS-CoV-2 RNA in six WWTPs in Spain (Nemudryi et al. 2020). Presence of the viruses in water helps the virus to become aerosolized (Casanova et al. 2009), particularly during pumping of wastewater (Figure 2) (Quilliam et al. 2020). There are no reports that confirm the presence of coronavirus RNA in aerosols near STPs. However, studies have indicated the potential risk of viral RNA in aerosols (El Baz & Imziln 2020) and infectivity for up to 16 hours (Fears et al. 2020).

Since there is a lack of knowledge on the survival of SARS-CoV-2 (viable) in sewage, the detection methods should be more aligned towards both viable and non-viable particles. Therefore, various viral components and associated debris like RNA fragments, capsid subunits, and viable virus can be targeted for detection in the sewage. A list of successful detection of SARS-CoV-2 in sewage and human excreta is given in Table 1.

Most of the methods for SARS-CoV-2 detection in sewage/feces are similar to the detection method used in humans, the Nucleic Acid Amplification Test (NAAT). Other allied approaches like enzyme-linked immunosorbent assay (ELISA) together with Most Probable Number (MPN) are used for the detection and quantification of viable and non-viable virus particles in sewage systems (Daughton 2020; Panchal et al. 2021). Some of the new approaches used for

Figure 2 | Occurrence, transmission, fate and exposure to contaminated aerosols of SARS-CoV-2 virus in water environments.
in-situ detection of SARS-CoV-2 in sewage, such as paper-based techniques (devices) and LAMP (loop-mediated isothermal amplification) are also on the market. However, their efficiency is not good as NAAT. Therefore, NAAT (reverse transcription-polymerase chain reaction (RT-PCR)) is gold-standard for the detection of SARS-CoV-2 in sewage till now.

**SARS-CoV-2 fate and anatomical configuration**

The SARS-CoV-2 virus’s genome is phylogenetically similar to that of bats’ SARS-related coronaviruses (84% nucleotide similarity with bat-SL-CoVZC45 coronavirus), and the spike protein is 78% identical to human SARS-CoV-1 (Chan et al. 2020). As a result, SARS-CoV-2 should be vulnerable to environmental conditions or disinfectants used during the SARS epidemic (Wang et al. 2020a). Coronaviruses are enveloped viruses having a viral protein capsid (containing) proteins or glycoproteins inside a lipid bilayer membrane. The functional groups on the lipid bilayer membrane of enveloped viruses compared with nonenveloped viruses are likely to impact the survival and partitioning behavior of the enveloped viruses in aqueous environments (Ye et al. 2016; Gundy et al. 2009). Coronavirus dies very rapidly (2–3 days) in sewage, due to the action of solvents and detergents. The genetic fragments remain detectable in sewage, but the virus becomes non-viable when the envelope is damaged (Ye et al. 2016; Nghiem et al. 2020).

**Elemental variation: factors affecting COVID-19**

Survival of viral RNA in wastewater depends on the physiochemical (including temperature, light exposure (solar or UV inactivation), organic matter, TDS, hardness, turbidity, pH, nitrate concentrations) and biological (antagonist microorganisms) parameters of the wastewater (John & Rose 2005; Gundy et al. 2009). Temperature is the most important factor that influences the survival of coronavirus, as elevated water temperature suppresses the viral survival rate by protein denaturation and extracellular enzyme activity. This was verified by an experiment in which filtered tap water was kept at room temperature and at 4 °C, and it was noted that 99.9% reduction of coronavirus was exhibited within 10 days at room temperature and needed over 3 months for tap water at 4 °C (Gundy et al. 2009). Further, a study (Wang et al. 2005) revealed that SARS-CoV-2 virus could survive in tap water, hospital wastewater, and domestic sewage for 48 hours at 20 °C and up to 2 weeks at 4 °C. Recently, it was demonstrated that temperature,
microbial population survival and organic matter have a huge impact on suppression of enveloped SARS-CoV-2 virus compared with non-enveloped viruses (Carducci et al. 2020). Coronavirus is considered more susceptible to elevated temperatures as it is an enveloped virus (Gundy et al. 2009; Ye et al. 2016). Coronavirus could also be removed by adsorption onto sludge and suspended solids (Gundy et al. 2009; Ye et al. 2016; Rimoldi et al. 2020; Wurtzer et al. 2020). As per WHO’s report, the enveloped coronaviruses are unstable in sewage, and chlorine based disinfectant should easily inactivate the virus at any temperature and pH (WHO 2020b). Conventional treatment processes and disinfectant processes at STP are expected to effectively remove or suppress the viral activity. SARS coronavirus was assessed to survive using two potential surrogates TGEV (transmissible gastroenteritis virus) and MHV (hepatitis virus), representing two groups of mammalian coronaviruses. Temperature, incubation period and type of water played a major role in the viral suppression kinetics. Viruses have also maintained infectivity in settled sewage for a considerable time span, displaying a 99% decline of TGEV in 9 days and MHV in 1 week. The decline was rapid at 25 °C compared with 4 °C (Casanova et al. 2009).

According to the latest guidelines released by the U.S. Occupational Safety and Health Administration (OSHA) for monitoring and prevention of SARS-CoV-2 virus, disinfection activities such as oxidation with hypochlorite, chlorine bleach, and peracetic acid, and inactivation by the use of UV radiation, are deemed adequate for SARS-CoV-2 (OSHA 2020).

Wastewater surveillance of COVID-19

Despite advancement in detection of contagious diseases for decades, these diseases appear to pose serious threats to public wellbeing. Novel pathogenic species that cause emerging infectious diseases are a major source of concern. Hence, the quick detection of diseases is essential for prevention, action and curbing them. But the existing surveillance technologies, as well as their ability to deal with huge population and environmental changes, have several limitations. With the current unprecedented population growth, rapid health surveillance and response will certainly encounter not only problems but also opportunities. Hence, a promising surveillance system is required which not only renders comprehensive and objective data but also the real-time data. The surveillance technique should be versatile and capable of tracking rare and multiple diseases simultaneously. The adopted technique should be scalable, economically feasible and work in setups with lower resources. In addition, the surveillance technique must include robust data collection systems for new disease outbreaks, the menace of indigenous diseases, and the appearance of pan-drug or multidrug resistant species. Therefore, a surveillance technique that includes environmental exposure will be extremely useful in determining overall exposure status and disease consequences.

Wastewater-based epidemiology (WBE) – new paradigm shift

WBE is a novel technique which provides detailed healthcare information on societies. The principle is mainly focused on chemical and/or biological compound extraction, identification, and subsequent study and interpretation. WBE has the ability to supplement existing infectious disease surveillance systems as well as serve as an early warning system for disease outbreaks. It could be employed to provide rapid, reliable information on the current healthcare situation in the community and may be useful in tracking disease outbreaks. With a confirmed history of polio and hepatitis A, WBE was identified as an effective population-wide monitoring technique (Asghar et al. 2014; Hellmér et al. 2014). Therefore, WBE has a lot of potential for population-wide surveillance of the pandemic caused by COVID-19. Selection of a wide range of indicators is of the utmost importance because they render a variety of information such as (i) pathogenic organisms, (ii) biochemical markers linked with physiological response, (iii) markers of biological response to intervention and (iv) markers of antimicrobial resistance.

In WBE, a high rate of RNA detection was observed in both influent and effluent of Indian hospital sewage samples which was collected between May and June 2020. The frequency of SARS-CoV-2 RNA was 75–100% in untreated sewage, which was associated with a substantial increase in the number of cases reported in every 10–14 days. However, during late sampling, this time period shrank, owing to a gradual decrease in lockdown (Arora et al. 2020). Another research showed that there was a 10 times higher viral load in between January 2020 and 27 May 2020, which corresponded to a two times increase in the number of COVID-19 cases (Kumar et al. 2020). These findings also reinforce the link between RNA identification in wastewater and trends in the numbers of new cases of COVID-19.

Viral indicators

A wide range of human pathogens are present in the surroundings. So, surrogates and markers are used to examine the fate
and spread of pathogenic strains. An indicator may be used to assess the efficacy of waste- and potable water treatment and also to analyze the presence, tenacity, sorption and transportation of pathogens in aquatic systems. For the assessment of targeted pathogens in wastewater systems, the viral indicator must have comparable inactivation and retention to the same pathogens, and the indicator must be present in the wastewater system over the years. This will lead to continuous monitoring and evaluating the contamination levels and the likelihood of pathogen presence. Furthermore, by utilizing WBE to measure the ratio of infectious citizens under a viral epidemic, like SARS-CoV-2, the indicator with steady amount of wastewater could act as surrogate for the population (Xagoraraki & O’Brien 2020). Moreover, it should be specific to source in order to differentiate between the pollution generated by animals and humans (Scott et al. 2002).

Certain enteric viruses found in wastewater may be used as indicators, but not all of them meet the criteria (Farkas et al. 2020).

Generally, nucleic acid-based polymerase chain reaction (PCR) is used for the detection of SARS-CoV-2 as well as COVID-19 infected patients throughout the world. PCR is very sensitive and precise methodology, but it entails complex sample handling, highly skilled personnel and lengthy data processing (4–6 h). Using PCR methodology, 15–83% COVID-19 infected people have detectable viral RNA in stool even though they have no gastrointestinal symptoms or diarrhea (Foladori et al. 2020). Samples can appear to be positive in feces, even though samples of the respiratory tract are negative, whereas urine is also negative.

For evaluation, accurate detection of viral activity and quantification of data are necessary. There are various available methods such as PCR, isothermal amplification of target genes, biosensors, metagenomics, microfluidics, microarrays, and culturing-based techniques for the detection and quantification of viruses in environmental samples. Most studies used real-time quantitative PCR (qPCR)-based detection method to detect and quantify enteric viruses and proposed indicators, which are fast, simple, and inexpensive methods for strain-level detection. In addition, factors like human waste association, existence and persistence in wastewater and aquatic environment, resistance to wastewater treatment process and global distribution are also accounting for the acceptability and effectiveness of viral indicators.

**Markers of pathogenic organisms**

WBE may be employed with targeted endogenous biomarkers which are significantly raised in the diseased conditions; for example, during the COVID-19 epidemic and defecated widely in the sewage through fecal matter. In view of the pandemic and the fact that SARS-CoV-2 can involve remarkable inflammatory damage, it paves the way for biomarker evaluation (Daughton 2012, 2020). In comparison to PCR, mass spectrometry (MS) or enzyme-linked immunosorbent assays (ELISA) are used to measure the majority of biomarkers, which provide many other significant advantages over its use. Hence, MS or ELISA renders more precision, better detection and more accurate data than that of PCR.

Bacterial, viral, and fungal pathogenic DNA/RNA remnants are a significant biological marker.

Resistant bacteria have been highlighted as a concern in the context of emerging infectious diseases. Likewise, viruses, with their high mutation rates and easy adaptivity in new hosts, are also a major threat. This is specifically true for RNA viruses, which can lead to faster adaptation and spread in new host with their higher nucleotide substitution rate (Woolhouse & Gowtage-Sequeria 2005). Wastewater monitoring has already shown effective data with high capacity for retrospective indication of hepatitis A, norovirus, and H1N1 (swine, flu virus) outbreaks (Heijnen & Medema 2011; Hellmér et al. 2014). The complexed wastewater matrix causes trouble to extract and quantify chemical compounds, as well as biological biomarkers. Wastewater also contains various kinds of PCR inhibitors, comprising fats, proteins and humic and fulvic acid, which can induce trouble during downstream PCR processing (Mumy & Findlay 2004; Walden et al. 2017). This makes it difficult to make clear comparisons between studies and to define spatiotemporal trends. However, advances in molecular biology methods, such as digital PCR (dPCR) and next-generation sequencing technologies, have opened up new avenues for the study of genetic material. dPCR can effectively quantify the targeted genes by applying Poisson distribution statistics and by partitioning DNA/RNA samples into tens of thousands of reaction wells (Rački et al. 2014). Likewise, next-generation sequencing methods can effectively render insight on complexed microbial, pathogens and resistance genes found in samples (Fernandez-Cassi et al. 2018; Aarestrup & Woolhouse 2020).

**Key challenges for wastewater-based epidemiology**

Efficient surveillance systems are generally recognized as crucial for rapid intervention and monitoring of communicable disease outbreaks. Furthermore, population-wide surveillance data is required to supplement current clinical
data. WBE has exhibited considerable success in providing detailed and near-real time information on health condition and community exposure. In the case of COVID-19, the value of successful surveillance has been highlighted.

Several cases of pneumonia with unidentified cause were reported on 31 December 2019 in Wuhan City, China (World Health Organization 2020b). Several reports of infection with the new SARS-CoV-2 virus were reported by different countries after a few weeks. Despite early intervention initiatives such as quarantine of cities and effectuating travel bans in several countries by March 2020, the pandemic adversely affected the economy and infrastructure with a sharp rise in confirmed positive cases (World Health Organization 2020b). In addition to current routes of global virus surveillance, if the WBE was introduced to the system then it could monitor the spread of virus, and if connected to an efficient response system, then it could also aid in management. However, rapid advances are needed to overcome some of the main challenges in order to effectively implement WBE in communicable disease surveillance (Sims & Kasprzyk-Hordern 2020). The intricacy of the wastewater matrix necessitates the development of new biomarker extraction techniques, as well as challenges in accurately estimating population density to account for temporal population density differences and the lack of a biomarker discovery pipeline for chemical and biological markers.

CONCLUSIONS

The complexity of testing wastewater as a surveillance tool includes specific challenges, such as testing of viral and bacterial communities in complex matrices like wastewater, low viral concentration, presence of inhibitors (RNAase) and lack of adequate laboratory coverage. This may result in limited detection, diagnosis and treatment of human diseases. During the COVID-19 pandemic, WBE has received a significant amount of attention. The basic concept is to quantitatively assess the SARS-CoV-2 virus RNA in wastewater and then use data to assess infection prevalence. The detection method should be sensitive and effective in order to accurately represent the infection rate during an outbreak. The outbreak of COVID-19 has reflected the limited knowledge about SARS-CoV-2 in the sewage/wastewater sector. The viral or viral RNA load in the feces as well as in sewage is fundamental for WBE applications used for early-warning systems. However, there are some limitations for SARS-CoV-2 detection, such as temperature, flow rate, solids and pH. Developing countries with lack of basic sanitation and resources are at huge risk for a massive transmission of COVID-19. Further, these countries can be disproportionately affected due to weak healthcare and fragile laboratory facilities. Therefore, surveillance using WBE for prediction of COVID-19 cases is the need of the hour that can provide a true picture for disease transmission. The early detection of disease spread using WBE can facilitate identifying hot spots and can help authorities to take precautionary measures.

WBE’s future outlook may include a variety of application and research directions such as: (i) continuous data on SARS-CoV-2 RNA detection must be collected for future action plan; (ii) monitoring should not be restricted to wastewater but also include treated effluents and surface water (rivers, lakes, and streams); (iii) temporal variability in WBE data as well as epidemiological information from the community must be made accessible; (iv) the effectiveness of WWTPs in removing viruses should not be presupposed, and virus RNA accumulation or decay must be considered; (v) infectivity through viable virus estimation in natural and treated water is imperative; and (vi) WBE may assist in determining the effectiveness of the COVID-19 vaccine.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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