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Prevalence of human pathogenic viruses in wastewater: A potential transmission risk as well as an effective tool for early outbreak detection for COVID-19

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ABSTRACT

Millions of human pathogenic viral particles are shed from infected individuals and introduce into wastewater, subsequently causing waterborne diseases worldwide. These viruses can be transmitted from wastewater to human beings via direct contact and/or ingestion/inhalation of aerosols. Even the advanced wastewater treatment technologies are unable to remove pathogenic viruses from wastewater completely, posing a serious health risk. Recently, coronavirus disease 2019 (COVID-19) has been urged globally due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which has resulted in >4.1 million deaths until July 2021. A rapid human-to-human transmission, uncertainties in effective vaccines, non-specific medical treatments, and unclear symptoms compelled the world into complete lockdown, social distancing, air-travel suspension, and closure of educational institutions, subsequently damaging the global economy and trade. Although, few medical treatments, rapid detection tools, and vaccines have been developed so far to curb the spread of COVID-19; however, several uncertainties exist in their applicability. Further, the acceptance of vaccines among communities is lower owing to the fear of side effects such as blood-clotting and heart inflammation. SARS-CoV-2, an etiologic agent of COVID-19, has frequently been detected in wastewater, depicting a potential transmission risk to healthy individuals. Contrarily, the occurrence of SARS-CoV-2 in wastewater can be used as an early outbreak detection tool via water-based epidemiology. Therefore, the spread of SARS-CoV-2 through fecal-oral pathway can be reduced and any possible outbreak can be evaded by proper wastewater surveillance. In this review, wastewater recycling complications, potential health risks of COVID-19 emergence, and current epidemiological measures to control COVID-19 spread have been discussed. Moreover, the viability of SARS-CoV-2 in various environments and survival in wastewater has been reviewed. Additionally, the necessary actions (vaccination, face mask, social distancing, and hand sanitization) to limit the transmission of SARS-CoV-2 have been recommended. Therefore, wastewater surveillance can serve as a feasible, efficient, and reliable epidemiological measure to lessen the spread of COVID-19.

1. Wastewater recycling and its complications

Rapid expansion in industrialization and swift urbanization have increased world wastewater production by many folds, consequently leading to the contamination of freshwater resources around the world (Chen, 2004; Fang et al., 2017; Lu et al., 2017). Therefore, recycling wastewater is of great worth to compensate global water requirements of the elevated population on one hand, and to evade contaminating the freshwater resources on the other hand. Further, it would contribute to water conservation via utilization in irrigation, washing, and domestic purposes, other than drinking purposes. Therefore, the recycling of wastewater and utilization of treated water has been amplified globally in recent times. For instance, recycled water is being used in Florida and California states in U.S and also being used in San Diego, Singapore, and Namibia cities to meet the requirements of agriculture, industries, environmental increment, and to recharge the underground water level (Florida Department of Environmental Protection, 2007; Ghernaout, 2018). However, owing to the occurrence of pathogenic agents in
wastewater, there are increasing concerns about the waterborne in human being worldwide. Therefore, reducing the levels of pathogenic agents in recycled wastewater before its consumption is of critical importance to avoid any outbreak of disease. Nevertheless, the majority of the conventional wastewater treatment techniques are inefficient for maximum removal of biological contaminants from wastewater, consequently posing a serious risk for human health. Generally, wastewater is comprising of a complex mixture of both chemical and biological contaminants, such as phenols, salts, polycyclic aromatic hydrocarbons (PAHs), ammonia, metals/metalloids, thiocyanate, cyanide, personal care products, bacteria, protozoa, mycoplasmas, and helminths (Lu et al., 2017; Letzel et al., 2015; Prasse et al., 2010; Verlicchi et al., 2012; Bacnik et al., 2020). This complex mixture of chemical and biological contaminants results in inefficiency in wastewater recycling processes because of the multiple nature of contaminants (Lu et al., 2017).

Biological contaminants in wastewater, due to their unique physicochemical characteristics, are the major concern for spreading waterborne disease worldwide. Biological agents, especially viruses, are more resistant to the disinfection technologies such as chlorination, filtration, UV radiation, and ozonation, and therefore, have frequently been detected in recycled water (WHO, 2011). Even, the advanced multiple-barrier techniques could not remove the viral pathogens from wastewater (Kitajima et al., 2014; Tandukar et al., 2020). As a result, these contaminants are responsible for the highest number of deaths worldwide (Gavrilescu et al., 2015). In general, these biological agents are introduced into wastewater through human excreta, and subsequently transmit into another human being via direct contact or through aerosols (Hammer et al., 2006). Aerosols, which are the liquid or solid particles suspended/dispersed in the air, and can travel up to 100 m depending upon the size of aerosols, are considered as an important pathway for the transmission of waterborne disease. Various researches have confirmed that aerosols are the basic transmission route for severe acute respiratory syndrome coronavirus (SARS), swine flu (H1N1), Middle East respiratory syndrome coronavirus (MERS), and several other diseases (Mathai et al., 2015; Yu et al., 2004). Moreover, wastewater treatment plants (WWTPs) are the major source for the production of aerosols with bacteria, viruses, and fungi (Kowalski et al., 2017; Han et al., 2020). Therefore, the population around the vicinity of WWTPs has been reported infected with hypersensitivity, allergies, gastrointestinal, and respiratory tract infections due to ingestion, inhalation, and dermal contact with the wastewater aerosols (Masclaux et al., 2014; Thorn et al., 2002). Therefore, the risks of waterborne disease can be reduced by appropriate disposal, treatment, and provision of safe water to the communities.

2. Wastewater-borne viral diseases

Despite scientific advancements and surveillance technologies in the preceding century, the outbreaks of infectious diseases are increasing rapidly. Most of water-borne diseases are transmitted through the fecal-oral mode of transmission, which usually happens by the ingestion or inhalation of the contaminated food and water, mainly due to improper sewage management and sanitation (Manusz and Karanja, 2021). World Health Organization (WHO) has reported that 4 out of 10 serious threats to global health are infectious diseases such as influenza pandemic, human immunodeficiency virus (HIV), dengue, and Ebola (WHO, 2020). Increasing concerns about new infectious diseases due to novel pathogenic agents are noteworthy. Since 1970s, more than 1500 pathogens have been identified, and 40 novel diseases are recognized (WHO, 2018). Among these infectious diseases, viral-based diseases have infected the communities on large scale in the form of the outbreak in last two decades, including SARS, H1N1, Ebola, Zika virus, and coronavirus (CoV) disease of 2019 (COVID-19) in the years 2002–2003, 2009–2010, 2014–2016, 2015–2016 and 2019–2020, respectively (WHO, 2020). Owing to smaller size, stability in the range of environmental conditions, resistance to antibiotics, and the tendency for mutation, viruses are highly dangerous biological agents for disease-causing in human beings (Habibi-Yangjeh et al., 2020).

Among the different human pathogens, viruses are of critical significance, as they are responsible for many diseases (Table 1) and cause thousands of deaths each year. Various reports have demonstrated a frequent occurrence of enteric viruses in wastewater streams (Hovi et al., 2001; Carter, 2005; Baggi and Peduzzi, 2006; Okoh et al., 2010). The viral infections are quickly transmitted in the urban areas where population density is high and thus infections could disseminate to a greater number of habitants before it gets notified by the health departments (McCall et al., 2020). It has been reported that viruses do not replicate outside the host, therefore, the prevalence of the viruses in wastewater is directly linked to the infected individuals associated with the production of this wastewater (Habibi-Yangjeh et al., 2020). Hence, wastewater and recycled water have been recognized as critical sources in transmitting infectious diseases (Pal et al., 2019). Enteric viruses such as adenovirus, rotavirus, enteric virus hepatitis E and A, caliciviruses, and some other viruses have been detected in the wastewater. Hepatitis A and E, CoV, norovirus, and rotavirus are considered to be responsible for wastewater-borne viral diseases worldwide (McCall et al., 2020). In most of the WWTPs, the viruses are not removed efficiently owing to the resistance of viruses to ultraviolet radiations and chemical disinfectants, and eventually enter into the water bodies as well as other environments (Sano et al., 2016). Therefore, viruses have frequently been detected in the wastewater streams, treated wastewater, and in the recipient water bodies. The major source of viruses in wastewater is fecal excretion to the water specifically from the infected individuals. According to an estimate, a single infected individual excretes approximately $10^5–10^12$ viral particles per gram of fecal excretion (Gerba, 2000). Likewise, another study reported that viruses are released into wastewater by more than $10^{12}$ viruses $g^{-1}$ of feces from the infected individuals (Drexler et al., 2011; Kirby et al., 2014). Additionally, viruses from other sources such as food, animal husbandry, surface runoff, weather changes, and any kind of outbreaks are also found in the wastewater. Therefore, the elevated levels of viruses in the wastewater is a matter of concern as the number of infectious waterborne diseases is increasing rapidly (Naddeo and Liu, 2020). These viruses can infect healthy individuals either by direct contact or through the ingestion/inhalation of aerosols produced by the aeration and diffusion in WWTPs (Bibby and Peccia, 2013). Aerosols were found to be a pathway for the transmission of SARS-CoV-1, previously (Bogler et al., 2020; Ding et al., 2020; Gormley et al., 2020; Yu et al., 2004). It has been reported that human CoV was infectious for a time of six days when it was in the aerosolized form at 25 °C with 50% relative humidity (RH) (Ijaz et al., 1985). Therefore, maximum removal of enteric viruses is of prime importance for the supply of clean treated water (Khan, 2013).

A large number of waterborne viruses causing various disorders in human beings have been detected in wastewater (Table 1). However, the detection of novel viruses in human excreta and wastewater by using advanced genome sequencing techniques has become a major concern for human health in last 2 decades (Ng et al., 2015; Kapoor et al., 2010). In this way, the source of microbial contamination, especially in the fecal excretion can be detected. Therefore, monitoring and surveillance of wastewater for potential waterborne viruses is of critical importance for the early detection of a possible outbreak.

3. COVID-19: a global pandemic

The occurrence of SARS and MERS by coronavirus in last ten years has gained global attention as deadly groups of viral infections (Zhang and Liu, 2020). A recent outbreak of SARS-CoV-2 has been recognized as a deadly human pathogen. More than 190 million confirmed COVID-19 cases and 4.1 million deaths have been reported until July 2021 due to SARS-CoV-2. The infection and death rate among humans due to COVID-19 is directly related to cardiovascular disorders (Mehra et al.,
### Table 1

List of viruses responsible for causing diseases in human beings.

| Sources          | Diseases                  | Symptoms                                                                 | References                                                                 |
|------------------|---------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Rotavirus        | Gastroenteritis           | Dehydration, nausea, diarrhea                                            | (Kitajima et al., 2014; Van Maarseven et al., 2010)                        |
| Adenovirus       | Respiratory disease       | Cough, respiratory problems                                              | (Jewitt et al., 2011; La Rosa et al., 2010)                                |
| Hepatitis A      | Infectious hepatitis      | Jaundice, malaise, fever, abdominal pain                                 | (Prado et al., 2011; Tsai et al., 1996)                                    |
| Poliovirus       | Poliomyelitis             | Nausea, paralysis, vomiting, fever, abdominal pain                       | (Inoue et al., 2011; La Rosa et al., 2010)                                |
| Astroviruses     | Gastrointestinal disease  | Diarrhea and vomiting                                                    | (Ashley et al., 1978; Hata et al., 2013; Mori et al., 2013; Cruz et al., 1992) |
| Enteroviruses    | Respiratory diseases      | Respiratory tract infection and pneumonia                                 | (Jewitt et al., 2011; La Rosa et al., 2010; Tsai et al., 1994)             |
| Hepatitis virus  | Hepatitis A virus         | Jaundice, anorexia, fever, discomfort, malaise, nausea, abdominal pain   | (Chitambar et al., 2001; Morae et al., 2002; Prado et al., 2011; Koslap-Petraco et al., 2008) |
| Noroviruses      | Liver disease             | Nausea, vomiting, abdominal pain and dark urine                          | (Clayson et al., 1995; Masclaux et al., 2013; Orró et al., 2004; Khuroo, 2011) |
| Aichi virus      | Gastroenteritis           | Fever, diarrhea, respiratory symptoms and purulent conjunctivitis        | (Ambert-Balay et al., 2008; Kitajima et al., 2014; Nielsen et al., 2013)   |
| Polyomaviruses   | Polyomavirus nephropathy  | Fever and respiratory tract disorder                                      | (Iata et al., 2013; Kitajima et al., 2014; Wattier et al., 2009)          |
| Salivirus        | Gastroenteritis           | Fever, diarrhea and vomiting                                             | (Iyamot and et al., 2013; Nielsen et al., 2013; Boro et al., 2016)         |
| Sapovirus        | Gastroenteritis           | Diarrhea, abdominal cramps, vomiting, and fever                          | (Iata et al., 2013; Kitajima et al., 2014; Wu et al., 2008)                |
| Torque teno virus| –                         |                                                                          | (Izama et al., 2011; Hata et al., 2013)                                    |
| Coronavirus       | COVID                     | Sore throat, cough, fever, breathlessness, malaise and fatigue            | (Chen et al., 2004; Poon et al., 2004; Singhal, 2020)                      |
| Influenza        | Flu                       |                                                                          |                                                                            |

### Table 1 (continued)

| Sources          | Diseases                  | Symptoms                                                                 | References                                                                 |
|------------------|---------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------|
|                   |                           |                                                                          |                                                                            |
| Dengue virus      | Dengue hemorrhagic fever  | Sore throat, cough, headache, malaise and gastroenteritis                | (To et al., 2010; Vester et al., 2015; Monto et al., 2000)                 |
|                  |                           |                                                                          |                                                                            |
| West Nile virus  | Neuroinvasive disease     | Headache, fever and muscle weakness                                       | (Barzon et al., 2013; Toory et al., 2005; Jean et al., 2007)               |
|                  |                           |                                                                          |                                                                            |
| Zika virus       | Zika fever                | Fever, rash, and arthralgia                                              | (Faye et al., 2008; Gourinat et al., 2015; Duffy et al., 2009)             |
|                  |                           |                                                                          |                                                                            |
| Yellow fever virus| Yellow fever              | Fever with jaundice                                                       | (Bae et al., 2003; Domingo et al., 2011; Johansson et al., 2014)           |
|                  |                           |                                                                          |                                                                            |
|                   |                           |                                                                          |                                                                            |
|                   |                           |                                                                          |                                                                            |
|                   |                           |                                                                          |                                                                            |
|                   |                           |                                                                          |                                                                            |

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Moreover, neurological disorders such as aortic ischemic stroke, polyneuropathy, and encephalitis can be encouraged by SARS-CoV-2 (Tsai et al., 2005). With a recent pandemic, it has been seen that COVID-19 has not only infected a great number of population, but also caused a higher death rate. In accordance to World Health Organization (WHO), depending upon the deaths reported, the mortality rate of COVID-19 could be estimated 5.6% for China and 15.2% outside China, which may increase if a longer delay between onset of illness and death is considered (Baud et al., 2020).

As COVID-19 is an emerging disease, thus there are fewer specific medical treatments and vaccines for its control. Although, there are vaccines, as due to the development of different variants from different regions of the world, there are uncertainties in the acceptance and applications of the medicals treatments and vaccines. Some of the side effects including the site of injection pain, fatigue, headache, muscle pain, including oral side effects such as chills, blisters, halitosis, ulcers, bleeding gingiva, and white/red plaque are observed in individuals after vaccine administration (Riad et al., 2021). Likewise, severe side effects such as heart inflammation and blood-clotting followed by death have also been reported in many individuals (Wise, 2021). Due to this, a lot of people are hesitant in getting vaccinated. Therefore, the COVID-19 surge is still increasing despite the development of vaccines.

A rapid transmission among humans, non-specific symptoms (often fever, diarrhea, cough, short-breathing, and others), and un-know behavior of SARS-CoV-2 has headed towards a pandemic situation with the large-scale spread of COVID-19 globally (Randazzo et al., 2019). Hence, the widespread nature, fewer diagnostic tools, complex symptoms, and discrepancies in vaccine administration have forced to implement of costly epidemiological control measures including lockdown, air-travel suspension, and closure of public and private institutions, subsequently disturbing the world economy. Even with all these measures, the actual figure about the infected individuals is very difficult to obtain owing to silent transmission, higher infected population, and complex symptoms (Li et al., 2020a, 2020b, 2020c; Mizumoto et al., 2020). The incorrect numeral of infected individuals is a huge hurdle in planning the most appropriate epidemiological control measures. Therefore, alternative low-cost and effective epidemiological control measures are needed to reduce the break out of COVID-19.

The infection and onset of COVID-19 include slight, self-restraint illness of respiratory tract to extreme situation pneumonia, multi-organ disorder, and death (Lei et al., 2020). This pandemic disease usually spreads through respiratory droplets, direct contact, and waste excreta (Fig. 1). Additionally, the respiratory droplets may land on the surface of various objects, where the virus remains viable for a certain period. Heller et al. (2020) proposed a fecal-oral transmission framework. According to this framework, there are three ways of fecal viral transmission; water, surfaces, and places. From these pathways, the virus can cause intestinal and respiratory infections. According to some reported researches, the viability of SARS-CoV-2 varies between the range of several hours up to 7 days in various ecological matrices (Table 2). For instance, the viability was found to be 3 h in aerosol and papers, whereas, it was found 168 h for plastic and steel surfaces (Chin et al., 2020). According to Hart and Halden (2020) in Detroit city of USA, there would be a chance for virus detection after 100 h sewer travel time in winter and about 20 h in the summer. The viability of the virus on different materials is shown in Table 2.

| Sources | Diseases | Symptoms | References |
|---------|----------|----------|------------|
| viruses characterized in fecal or environmental samples | Human Cosavirus (HCoV-V-A) | – | Kapoor et al., 2008 |
| | Human Bovavirus (HBV) [HBV-1, HBV-2, HBV-3, HBV-4] | Sore throat, cough, fever, rhinitis, and pneumonia | Allander et al., 2005; Arthur et al., 2009; Bastien et al. (2006) |
| | Human cyclovirus 1, 2 and 3 | – | Li et al., 2010 |
| | Human feces associated circovirus (HuFaCV) | | |

Fig. 1. Transmission routes for the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from infected to healthy individuals.
3.1. Symptoms and health risks of COVID-19

SARS-CoV-2 carrying individuals are the major source for spearing COVID-19 infection. Contacting the contaminated surfaces and infected individuals, as well as respiratory droplets from patients may transmit the virus to healthy individuals. Smaller aerosol particles (<5 μm) may remain suspended in the air for a longer time and eventually get inhaled by healthy individuals. Thus, aerosols particles, which are produced from sneezing or coughing, are among the main sources for COVID-19 spread (WHO, 2020). Additionally, urine, feces, and spitting of the infected individual could also transmit SARS-CoV-2 to other human beings (Jagtap et al., 2020). The presence of SARS-CoV-2 in human fecal samples depicted that the human digestive tract could be a significant source for SARS-CoV-2 transmission; however, limited research is conducted on this aspect.

Symptoms of COVID-19 at the inception of the infection are fever, fatigue, cough and, myalgia. However, available data indicated that about 50% of the patients are feverish at the time of admission (Kluymans et al., 2020). Difficulty in breathing was found as a common symptom in COVID-19 patients (Huang et al., 2020; Lippi et al., 2020). Gastrointestinal disorders were also reported in approximately 40% of the patients, including anorexia, nausea, vomiting, and diarrhea (Li et al., 2020a; Zhang et al., 2020). Moreover, about 10% patients were presenting gastrointestinal symptoms without showing any symptoms of respiratory disorder or fever and thus these patients were termed as asymptomatic (Wang et al., 2020a, 2020b, 2020c, 2020d). COVID-19 is linked with a hypercoagulable state with a higher threat of venous thromboembolism (Danzi et al., 2020). Neurological manifestations include headache, dizziness, consciousness, ischaemic, hemorrhagic strokes, and in some studies the muscle injury has been reported (Mao et al., 2020a, 2020b). Additionally, loss of taste and olfactory disorders, including anosmia have also been reported owing to COVID-19 (Giaconelli et al., 2020). Ocular manifestations consistent with conjunctivitis have also been reported in 32% of patients in a Chinese case study (Wu et al., 2020a, 2020b). Contrarily, it has been reported that >81% of the patients show minor indications (slight to no pneumonia) from COVID-19 (Clerkin et al., 2020). Therefore, accurate and quick screening of infected asymptomatic individuals and diagnosis of virus carriers is a critical step to limit the transmission of COVID-19 (Mao et al., 2020a, 2020b).

A quick, in addition to, a well-coordinated immune system, respond as a first defense line against viral infections. Male patients of intermediate life ranging between 34 and 59 years with a weaker immune system are more susceptible to COVID-19 (Bai et al., 2020; Chang et al., 2020; Huang et al., 2020; Wang et al., 2020a, 2020b, 2020c, 2020d). Moreover, people having problems of cardiovascular, cerebrovascular, and diabetes have more chances of infection (Chen et al., 2020). Alike, it was reported that people of more than 60 years of age were infected more, especially when they already have the above-mentioned disorders (Bai et al., 2020; Wang et al., 2020a, 2020b, 2020c, 2020d). Conversely, in some cases, the children with an age less than 15 were also detected positive (Bai et al., 2020; Chang et al., 2020; Huang et al., 2020; Wang et al., 2020a, 2020b, 2020c, 2020d). Up to January 20, 2020, in China 425 cases were reported in Wuhan but there was no patient of age less than 15 years (Li et al., 2020a, 2020b, 2020c; Tang et al., 2020). However, 28 pediatric individual’s infection was reported by January 2020 (Shen and Yang, 2020). The clinical characteristics of the infected individuals differ; however, most of them were infected with slight symptoms having neither fever nor pneumonia (Shen and Yang, 2020). In another case study, a child with radiological ground glass lung opacities was found asymptomatic (Chen et al., 2020a). Thus, children have fewer chances as compared to the older ones to be infected by COVID-19 overall, and if in case they got infected, represent milder manifestations than adults.

3.2. Current epidemiological measures to control COVID-19

Although, there are few emerging medical treatments ((Hydroxy-) Chloroquine, Azithromycin, Remdesivir, Lopinavir/ritonavir, Tocilizumab, Baricitinib, Anakinra) (Felsenstein et al., 2020; Stasi et al., 2020) and vaccines (Moderna/NIAID; BioNTech/Fosun/Pfizer; AstraZeneca/University of Oxford; CanSino Biological inc./Beijing Institute of Biotechnology; Sinovac) (Sharma et al., 2020) for the COVID-19 but there are still some uncertainties about their effectiveness. Implementation of different strategies is underway on local and global level. Keeping in view the infection of MERS-CoV and SARS-CoV, the only way which can be adopted to disrupt this chain of spread in present scenario is, to stop the transmission of disease. To reduce the spread of COVID-19, wearing a mask to avoid the spread of the virus through droplets, and maintaining a distance of 2 m among the people has been advised (Harapan et al., 2020). The isolation of the confirmed cases has been recommended. People are advised not to travel unnecessarily, and not to be gathered at public places. Moreover, frequent hand-washing, as well as sneezing and coughing in sleeves are recommended (Singhal, 2020).

In case of infection, boosting of immunity can be the first step to treat this disease. Interferon produces an innate and adaptive immune response that can reduce viral infection. In the clinical trials, synthetic recombinant interferon α proved to be effective for treating infected individuals (Wang et al., 2020a, 2020b, 2020c, 2020d). Further, for the patients of various age groups, for long-term use, intravenous immunoglobulin is the safest immune-modulator, which enhances the inflammatory mediator’s production (Gilarдинi et al., 2015; Wang et al., 2020a, 2020b, 2020c, 2020d). Moreover, the immune system can be boosted by proper diet, exercise, and sleep.

The provision of antibacterial therapy, nutritional supplements, oxygen therapy, external cooling, and acetaminophen helps against COVID-19 (Wang et al., 2020a, 2020b, 2020c, 2020d). Plasma therapy, has shown encouraging results against COVID-19. Plasma therapy, is an easy way to produce antibodies against COVID-19, from the individual who has been recovered from the infection of COVID-19 and then the person who is treated with plasma therapy produces the antibodies against the virus, moreover, it has reduced the mortality rate (Wang et al., 2020a, 2020b, 2020c, 2020d). The produced antibodies limit the attack of virus and cease the infection, which in turn help to treat the viral infection. The plasma can be collected from the individual who has been recovered from the infection and can be used for the production of plasma globulin. However, plasma production specific to SARS-CoV-2 needs further consideration (Li et al., 2020a, 2020b, 2020c; Wang et al., 2020a, 2020b, 2020c, 2020d). Among several treatments, auxiliary blood purification is also being performed to maintain capacity.
load, acid-base balance, electrolyte balance, cytokine storms, inflammatory factors blood purification technique is used (Lim et al., 2015; Wang et al., 2020a, 2020b, 2020c, 2020d; Zarbock et al., 2016).

Despite, all of the aforementioned measures, the spread of COVID-19 is escalating with each passing day. Lockdown is being implemented in cities and even at the country level to limit the movement of population and to ensure social distance. Almost, all the social and economic activities have been ceased. Educational institutions, including schools, colleges, and universities are closed. The air travel has been suspended, and the borders have been closed. Exports and imports activities are suspended in most of the countries of the world. As a result, the world economy has been damaged drastically leaving millions of people fighting for their lives. It has been estimated that there will be a loss of 2.4% in the gross domestic product to the majority of economies because of this pandemic (Duffins, 2020). Therefore, if the situation prevailed for a longer time with current control measures, economies of the developed countries may smash whilst most of the developing countries will be at the edge of destruction. Therefore, it is an utmost need of time to improve feasible, effective, and reliable epidemiological measures to lessen the spread of COVID-19 as well as to evade any future viral re-emergencies.

3.3. SARS-CoV-2 in wastewater: a possible transmission route

COVID-19 is a matter of global concern now-a-days. There are a lot of studies that are being carried out on the world level to be aware of the health risks, mode of transmission, and possible ways to get rid of this viral infection. The possible transmission of the viral infection happens through the droplets which are produced from the sneezing or coughing of the infected individuals (Jagtap et al., 2020). The possible ways to avoid the infection, which are being adopted worldwide include social distancing, use of masks, and sanitizers.

Recent reports have shown the viability of SARS-CoV-2 in the wastewater, which may become a possible medium of viral transmission. SARS-CoV-2 can survive in water for several hours, posing a serious risk of transmission to healthy individuals. Therefore, there is rising concern about the transmission of SARS-CoV-2 through wastewater. The occurrence of SARS-CoV-2 in the wastewater is linked with the feces, urine, and spitting of an infected individual passed into the wastewater. In the feces samples, a higher number of viral RNA copies were detected. It has been reported in the current studies that SARS-CoV-2 was identified in the human excreta such as feces, rectal, and anal swabs. Nonetheless, the occurrence of SARS-CoV-2 also has been investigated in human feces recently. Wang et al. (2020a, 2020b, 2020c, 2020d) conveyed that the feces of about 29% of COVID-19 individuals contained SARS-CoV-2, whereas, 2–10% of the patients were experiencing diarrhea as well. In another study, ~10⁸ copies/mL of SARS-CoV-2 titers were found in COVID-19 individuals having diarrhea, while ~2.5 × 10⁵ copies/mL were found in urine samples (Kitajima et al., 2020). Likewise, Xiao et al. (2020) reported that, (39 among 73), 53% of hospitalized individuals possessed SARS-CoV-2 titers in their fecal which was sampled. In some other studies, a viral concentration of 10⁸ copies gram⁻¹ was detected in the fecal samples (Wölfel et al., 2020). The recent clinical investigations reported that SARS-CoV-2 can be detected in fecal samples even after the seven weeks of the onset of the earliest symptom. In another study, it was found that in 81.8% of cases, viral RNA of SARS-CoV-2 can be identified in the feces even when the throat swab test was detected negative (Ling et al., 2020). Current investigations have shown that 17.3%–30.8% of the infected proportion is asymptomatic and RNA of the SARS-CoV-2 was still identified in the fecal samples of those patients as well (Minumoto et al., 2020; Nishiuura et al., 2020). This is also noteworthy that RNA of SARS-CoV-2 virus can be found in the individual feces after 33 days of infection, even when an individual has been confirmed negative for SARS-CoV-2 (Ling et al., 2020; Wu et al., 2020a, 2020b).

The molecular detection of SARS-CoV-2 in wastewater has been reported in Australia, France, The United States of America (USA), Netherlands, Turkey, and several other countries as shown in Table 3 (Ahmed et al., 2020; Kitajima et al., 2020; Lodder and de Roda Husman, 2020; Medema et al., 2020; Nemudryi et al., 2020; Wurtzer et al., 2020).

Table 3 shows that the RNA of SARS-CoV-2 virus concentrations were detected up to a maximum range of 10⁶ copies/liter, while in France a maximum range of 10⁴ copies per liter was investigated in wastewater (Wurtzer et al., 2020). The load of SARS-CoV-2 in municipal wastewater is assessed to be in the range between 56.6 million to 11.3 billion viral genomes per infected individual per day. This bulk load converts into concentrations of 0.15–141.5 million viral genomes per liter of waste-water produced in North America and Europe. If, for example, ten coronavirus RNA genomes/mL of sewage was detected and after that there is no addition of commercial, industrial, storm water and wastewater, it is assumed that a 0.88% population is infected (1 in 114 individuals) as few as 0.00005% (Hart and Halden, 2020).

Recently, it is reported that viral shedding via digestive system is long-lasting than oral transmission (Quilliam et al., 2020). Researches have presented the occurrence of culturable SARS-CoV-2 in the fecal samples; however, Wölfel et al. (2020) have stated that culturable virus cannot be isolated from the feces. The variability in the occurrence or detection of the virus may be due to the collection, storage, pretreatment of samples and protocols followed by laboratories. Nonetheless, the presence of COVID-19 in wastewater has been confirmed by many researchers and it has been described that a minor portion of viral RNA can survive from days to weeks (Qu et al., 2020). Therefore, there is a need to manage the feces discharging from quarantine centers, hospitals, infected persons, recovering and even recovered infected individuals.

3.4. Survival of SARS-CoV-2 in wastewater

The survival and viability of a virus depend on many factors such as organic compounds, pH, relative humidity (RH), sunlight, and temperature. Generally, the viruses protect themselves against sunlight by adsorbing some substances, such as organic matter, which is available in the vicinity. The presence of microorganisms influences the survival of viruses too. Recently, the environmental conditions are being correlated with the time of survival of SARS-CoV-2. A study was conducted between three environmental factors related to the total number of cases infected with COVID-19. Temperature, RH, and wind velocity are the main environmental dynamics influencing the prevalence of SARS-CoV-2. This experiment was performed in four cities of China, whilst five cities of Italy. The SARS-CoV-2 prevalence related to wind and RH was negligible; however, it was observed in some cases that an increase in RH and wind decreased the virus prevalence. The relationship between environmental temperature and viral prevalence was negligible to moderate. The evidence of prevalence with the increase in temperature has decreased according to Bhattacharjee (2020). According to Chin et al. (2020), SARS-CoV-2 can remain viable for a longer time at 4 °C, while its prevalence can reduce to just 5 min when the temperature is raised to 70 °C. Thus, it was seen that increase in temperature decreases the survival time of the virus, due to the increase in extracellular activities of enzymes and denaturation of the proteins (Gundy et al., 2009). A recent study conducted in China reporting 24,139 cases in 26 regions demonstrated that 1.0 °C rise in temperature reduced the total cases by 0.86% (Wang et al., 2020a, 2020b, 2020c, 2020d). Generally, very high acidic and alkaline pH, heat, and sunlight help killing the SARS-CoV-2 (WHO, 2020).

On the basis of afforested facts, it was reported that, SARS-CoV-2 can exist in the wastewater; however, its presence and lifespan in the wastewater depend on many factors such as temperature, sunlight, organic compounds, RH, and the presence of other microorganisms. According to Hart and Halden (2020), the half-life of SARS-CoV-2 at 20 °C ranges between 4.8 and 7.2 h. This value is approximately the same for certain hydraulic retention periods of wastewater around the world. Conditions, where the temperature of waste flow is about 20 °C
Presence of SARS-CoV-2 RNA fragments (viral genomes) in the wastewater and sludge samples.

| Source | No. of samples | Quantity of samples (mL) | Molecular detection methods | Concentration of vRNA | Area | SARS-CoV-2 concentration (gc/100 ml) | Covid-19 positive cases | Covid-19 cases | Sampling dates | References |
|--------|---------------|--------------------------|----------------------------|-----------------------|------|-------------------------------------|------------------------|----------------|----------------|------------|
| WWTPs  | 13            | 200                      | RT-qPCR                    | –                     | Murcia | 6 (46%)                             | 622                    | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
|        | 10            |                          |                            |                       | Cartagena | 6 (60%)                             | 190                    | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
|        | 13            |                          |                            |                       | Molina de Segura | 7 (54%)                             | 70                     | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
|        | 13            |                          |                            |                       | Lorca | 7 (54%)                             | 31                     | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
|        | 10            |                          |                            |                       | Cartagena | 7 (54%)                             | 49                     | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
| WWTPs  | 11            |                          | RT-qPCR and PCR primers and PCR | 5.104 genome units/L | France | 100% positive                       | 404                    | 09 March 12 | 14 April, 2020 | Wurtzer et al. (2020) |
| Suburban pumping station (PS) and two WWTPs | 100–200 |                            | RT-qPCR and PCR          | –                     | Southeast Queensland | 22.2% positive | 404 | 09 March 12 | 14 April, 2020 | Ahmed et al. (2020) |
| WWTPs  | 100–200       |                           | quantitative culturing, RNA extraction and RT-PCR | – | Netherlands | N1: 14/24 | 58% positive detected | 1413 | 09 March 12 | 14 April, 2020 | Medema et al. (2020) |
| Wastewater samples | 36–150 |                           | RT-PCR | – | Netherlands | Positive detected | – | 09 March 12 | 14 April, 2020 | Wurm et al. (2020) |
| Wastewater samples | 250 |                           | RT-qPCR | vRNA detected (Milan) IT National Institute of Health (ISS) | NA | – | – | 09 March 12 | 14 April, 2020 | Lapolla et al. (2020) |
| Wastewater samples | 250 |                           | RT-qPCR | – | National Institute of Health (ISS) | Positive detected | – | 09 March 12 | 14 April, 2020 | Lapolla et al. (2020) |
| Non-drinkable street-cleaning water | 11 |                           | RT-qPCR | – | Paris | Positive detected | – | 09 March 12 | 14 April, 2020 | Wurtzer et al. (2020) |
| WWTPs  | 250–1000      |                           | RT-qPCR | vRNA detected | Israel | NA | Positive detected | – | 09 March 12 | 14 April, 2020 | (Or et al., 2020) |
| Untreated wastewater | NA |                           | RT-qPCR | vRNA detected | Wuchang Fangcheng Hospital China | 0.05–1.87 × 10^4 | 1124 | 09 March 12 | 14 April, 2020 | Zhang et al. (2020) |
| Sewage samples | NA |                           | RT-qPCR | vRNA detected | Massachusetts, USA | 10^3.2–10^4 | 71% positive detected | 18 | 09 March 12 | 14 April, 2020 | Randazzo et al. (2020a, 2020b) |
| Sewage sludge samples | 2.5 |                           | RT-qPCR | vRNA detected | New Haven, Connecticut, USA | 1.7 × 10^3.4–6.4 × 10^4 | 100% positive detected | 2711 | 09 March 12 | 14 April, 2020 | Peccia et al. (2020) |
| WWTPs  | 500           |                           | RT-qPCR | vRNA detected | Bozeman, Montana, USA | 10^2.10^6 | 100% positive detected | 10 March | 09 March 12 | 14 April, 2020 | Nemadry et al. (2020) |
| Sludge and wastewater | 50 |                           | RT-qPCR | vRNA detected | Ourense, Spain | NA | 09 March 12 | 14 April, 2020 | Balba et al. (2020) |
| Sludge samples from WWTPs | 9 |                           | RT-qPCR | – | Istanbul, Turkey | 10^2–10^5 | Positive detected | 21 to April 25, 2020 | Kocamemi et al. (2020) |

and travel time is about 10 h, approximately 25% of the virus load should persist. Several researchers reported the presence of SARS-CoV-2 in wastewater for several weeks and reported its presence after four days, when the cases were confirmed (Nghiem et al., 2020). From the literature, it has been stated that infection due to SARS-CoV-2 virion is discharged from virus-infected gastrointestinal cells, having the capability for fecal-oral spreading. In the recent research studies, the viability of virus was reported infectious for 14 days when the temperature was 4.0 °C and it could survive for 2.0 days when the temperature was raised to 20 °C. Furthermore, it was investigated that, SARS-CoV-2 can survive for 5 days when the temperature range is 22 °C–25 °C with 40%–50% RH. Further, the survival time was reduced at 20 °C temperature and 40% RH from 48 h to 8 h when the temperature was increased to 30 °C with 80% RH (Yeo et al., 2020). Similar to temperature and RH, the prevailing pH may also pose significant impacts on the viability of SARS-CoV-2. In accordance with Chin et al. (2020), the virus can efficiently survive at ambient temperature between 3.0 and 10 pH. Likewise, Chan et al. (2020b) reported that SARS-CoV-2 can survive for several days in pH range of 4.0 to pH 11; however, the viability of the virus was lost in pH range of pH 2.0–3.0 and pH 11–12.

As the research has confirmed that coronavirus can be infectious for a longer time period in drinking water and days in the wastewater, this causes the infection to human when aerosols are produced. The virus can enter the drinking water where there is some leakage problem and causes the infection to human when aerosols are produced. The virus can infect the individual through a shower and drinking water in the community (Naddeo and Liu, 2020). Therefore, the viability of SARS-CoV-2 in water sources must be monitored to avoid further escalation in the pandemic.
4. Wastewater based epidemiology surveillance for COVID-19

The most adverse circumstances of an epidemic could be minimized by employing the appropriate, well-timed, and selected screening approaches. Wastewater-based epidemiology (WBE) is a technique that provides rapid and reliable information about a particular population to inform the community about the outbreak of a disease existing and can be used as a complementary monitoring technique. WBE is a key technique for tracking the movement of viruses in a population. It is possible to determine the outbreak of COVID-19 infection by continuously monitoring the viruses level. This tool provides an efficient and sensitive detection of the levels of virus in a community and gives a primary detection of the epidemic before its outbreak (Venugopal et al., 2020). WBE provides an unbiased method for the evaluation of transmission of infectious diseases in various zones, moreover, it works where the capabilities regarding clinical screenings are inadequate and communication sources are not available like in progressing countries (Kitajima et al., 2020).

In developed countries such as Germany, having a high capacity of screening as compared to the other European countries, approximately a time period of continuous testing for three months is required to detect 100,000 assays per day, to evaluate the infection status of about 83 million entire population for one time. This testing is not only expensive and time-consuming, but there are also chances of major changes in the prevalence of the virus. Whereas, in Germany 9636 wastewater treatment plants are situated which can be tested easily within 24–48 h with a 0.014% cost of the total cost required to test the individuals by clinical testing procedure (Hart and Halden, 2020). The WBE testing would be still cheaper if it is conducted again and again for this huge population. Monitoring of the wastewater effluent of a community is being used as an effective tool to acquire information about drugs consumption, water pollution, pharmaceutical usage, and antimicrobial resistance genes associated with the particular human population (Randazzo et al., 2020a, 2020b; Wurtzer et al., 2020). Thus, WBE is an effective tool that is being used for the investigation of transmission of infection by screening the infectious agent responsible in the wastewater. This technique can also be used as a surveillance method for the population with severe attack of disease with asymptomatic infections. The WBE can be utilized to monitor the smaller and confined populations. Major targets which can be achieved using WBE include congested housing societies, schools, universities, hospitals, prisons, airports, airlines, entertainment hubs, shopping malls, meat processors, and other confined areas like this. The focus of interest will be sub-populations where healthcare facilities are not adequate or sometimes individual avoïds to seek health care (Daughton, 2020).

The basic advantage of WBE is that, it gives epidemiological information about the viability of disease in a community by overcoming the patient’s accusation, that sometimes consequences from clinical testing of COVID-19 ongoing outbreak (Kitajima et al., 2020). By using this approach, it is possible to monitor the viral infection even when the clinical diagnostic approaches seem to be limited because of the asymptomatic nature of the viral infection. Thus, wastewater surveillance can be employed as a pre-warning sign for the emergence of COVID-19 in a particular population. Therefore, WBE can serve as an important technique over diagnostic testing as it has also the ability to detect COVID-19 before its emergence as an outbreak.

The wastewater effluent can be analyzed for the investigation of the genetic material of SARS-CoV-2 virus, which seemingly is shed through saliva, sputum, and feces, and subsequently releases into wastewater (Randazzo et al., 2019). It has been seen in the literature that the viral fragments of SARS-CoV-2 RNA have been reported in the feces of some infected individuals in their period of illness and even after their recovery (Guan et al., 2020; Woelfel et al., 2020; Xiao et al., 2020). The shedding of RNA fragments into the feces find their way into wastewater. In most of the infected cases, the patients show the symptoms of diarrhea, through which, most probably, the occurrence of the virus in wastewater is possible (Bai et al., 2020; Chan et al., 2020a; Chen et al., 2020; Wu et al., 2020a, 2020b). These results are the proofs of the environmental surveillance for the diagnosis of the current pandemic in the community. The capability of sewage surveillance to estimate the outbreak of a viral infection, after careful surveillance models, sub-clinical, mild, or asymptomatic cases can be considered a good strategy (La Rosa et al., 2020). It is better practice to assess the occurrence of SARS-CoV-2 in a population of interest rather than testing each individual.

Bisieux et al. (2018) and Prevost et al. (2015) reported the occurrence of human enteric viruses in wastewater and also reported that the epidemic situation was directly linked with the concentration of the viruses. The existence of these viruses in wastewater was owing to the shedding of infectious viruses into the feces, and hence, it strongly argues for surveillance of viruses in wastewater for investigating the human epidemics. Following this idea, many scientists have detected the viral RNA of SARS-CoV-2 in wastewater globally as a sensitive, non-intrusive, and premonition strategy for predicting the spatial and temporal and tendency of COVID-19 spread (Ahmed et al., 2015; La Rosa et al., 2020; Medema et al., 2020; Wu et al., 2020a, 2020b; Wurtzer et al., 2020). The RT-qPCR techniques have recently been used by many researchers for the surveillance of viral RNA of SARS-CoV-2 in wastewater as a tool to predict the actual spread of the COVID-19 for developing wastewater-based epidemiology. However, further studies are required worldwide for methodological and molecular validation for SARS-CoV-2 for enhanced wastewater surveillance.

5. Need of the time

Recent researches have investigated the existence of COVID-19 in feces samples of humans, which ultimately indicates the presence of the virus in the wastewater (Holshue et al., 2020). The foremost evidence of the SARS-CoV-2 presence in the water bodies and the wastewater was found in Australia, Czech Republic, Netherlands, Ecuador, Nicaragua, India, and The US (Ahmed et al., 2020; Mlejnkova et al., 2020; Medema et al., 2020; Guerrero-Latorre et al., 2020; Vammen and Guillen, 2020; Kumar et al., 2020; Sherchan et al., 2020). Further, the human wastewater samples were collected on February 17, 2020 from Amsterdam Airport Schiphol (Netherlands) for the virus analysis and results confirmed the presence of viral RNA with the help of quantitative RT-PCR technique, after 4 days when the first coronavirus case was identified in the Netherlands on February 27, 2020. Probably, the virus was excreted by the pre-symptomatic, asymptomatic, and symptomatic carriers passing through the airport. Lodder and de Roda Husman (2020) stated the existence of viral fragments of SARS-CoV-2 in the samples of human wastewater, collected from the Netherlands. Current researches have revealed that the prevalence of SARS-CoV-2 in human body with a suitable environment could be up to several weeks after exiting the human body. Therefore, the occurrence of SARS-CoV-2 in wastewater can be investigated by testing, and also COVID-19 can be traced through sewage pipelines. Thus, based on these facts, the availability of hygienic conditions, safe drinking water, and sanitation should be ensured for the protection of human health during the outbreaks of COVID-19. The management of waste and applied water practices are of prime importance to limit the transmission of pandemic disease. Screening of the suspected infectious persons remained a challenging exercise in the medical field. Nonetheless, clinical screening tests were not recommended for mass surveillance. The testing technique was not only expensive and time-wasting, it can infect the individuals who are dealing with this. Therefore, the conventional diagnostic testing should be increased by following pool sample analysis. Moreover, there is a need to discover an effective approach that could be used to investigate the potential spread of the pandemic. Feces and urine excreted from the infected carriers should be managed properly as it can pose a potential risk of aerosol generation, subsequently spreading the virus in atmosphere. The entrance of SARS-CoV-2 into waste treatment system via
feces and urine of the infected patients should be monitored. A decen-
tralized system could reduce the virus load and transmission in the
environment. Moreover, portable devices should be installed to disinfec-
t the drinking water, which will be useful to reduce the waterborne
infection caused by the virus. UV-based, light-emitting diode can be
used for the disinfection of decentralized systems. In those places where
a separate urine disposal infrastructure has been introduced, more
disinfectant should be added to disinfect the urine waste. If SARS-CoV-2
RNA titers are identified in the beginning in the wastewater of a com-
munity, precautionary measures should be taken to limit the movement
of native inhabitants to stop the blowout of the virus to the neighboring
communities. Physical actions (grit chambers, screening), followed by
biological and physicochemical treatments should be used to remove the
viruses from wastewater streams. In the physical actions, viruses are
adsorbed onto the suspended solids which can then be settled via
gravitational sedimentation. However, the adsorption technique does
not result in the complete removal of the virus from the wastewater.
Therefore, secondary (bioreactor, activated sludge, and aerated bi-
ological processes) and tertiary (chlorination, performic acid, ultraviolet
radiations, ozonation, and nanomaterials) treatments should be
employed where necessary (Vickers, 2017; Gerba and Pepper, 2019;
Randazzo et al., 2020a, 2020b; Teymoorian et al., 2021).

6. Conclusion

Occurrence of human pathogenic viruses in wastewater and recycled
water is posing serious health risks to human beings. Therefore, proper
management of wastewater streams through recent techniques (membrane technology, ion exchange technology, nano-
technology, adsorption, crystallization) and surveillance of pathogenic
viruses in water resources is of great worth. The available data on
the presence of SARS-CoV-2 in wastewater suggests a potential risk of
its transmission from wastewater to a healthy individual. Due to rapid
transmission, non-specific symptoms, and fewer medical treatments, the
spread of COVID-19 is still ongoing. Although, vaccines against SARS-
CoV-2 have been produced; however, there are uncertainties in the
acceptance and administration of vaccines due to the development of
different variants in different regions of the world. Moreover, the
development of some side-effects such as chills, blisters, haitosis, ulcers,
bleeding gingiva, and white/red plaque, heart inflammation, and blood
clotting in many countries after vaccine administration are reported,
which reduced the general acceptance of vaccination. Therefore, despite
the development of vaccines and medical treatment, COVID-19
pandemic has not yet been controlled. In this context, wastewater sur-
veillance could be of crucial importance as it provides water-based
epidemiology for early detection of viral outbreaks and important data
about the occurrence of infections in a community. It can be used as
a quick, cost-effective, and vigorous tool for estimating SARS-CoV-2 RNA
titers in wastewater. Tracking of wastewater can help premonition and
epidemiology for early detection of viral outbreaks and important data
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