WATER RESEARCH COMMISSION

WORKING PAPER

State of knowledge on SARS-CoV-2 and wastewater

by
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Abstract

A large majority of studies on the environmental sources, fate, and transport of viruses have focused on non-enveloped viruses such as norovirus and enteroviruses despite recent global outbreaks of viral diseases having been caused by enveloped viruses including the Coronavirus family. Lack of knowledge on the presence of infective enveloped viruses in human waste, the environmental fate and transport of enveloped viruses, best practices to disinfect surfaces and water, wash contaminated body parts, and treat wastewater and faeces to removal enveloped viruses, has generally hampered outbreak response. Limited direct research is therefore available on the environmental persistence of viruses in the Coronavirus family, with majority of research having been conducted on enveloped 'surrogate' viruses. This knowledge review therefore attempts to provide some insight on SARS-CoV-2 and wastewater, particularly its persistence and behaviour as an enveloped virus in the environment. The review further explores surrogate enveloped viruses in wastewater as much of the research conducted to date have used surrogate enveloped viruses that have similar properties to the human enveloped viruses currently of urgent interest. The review outlines the required outbreak response and highlight key areas of research that require further attention.

1. Introduction

The current outbreak of novel coronavirus diseases (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in the World Health Organisation (WHO, 2020) declaring this outbreak a global pandemic. Human-to-human transmission of the SARS-CoV-2 virus occurs when individuals are in the incubation stage or showing symptoms, while some individuals remain contagious and asymptomatic (super spreaders). Transmission can occur through several means. Firstly, it is thought to occur via touching infected surfaces (skin-to-skin, touching infected inanimate objects) then mediating the SARS-CoV-2 infection through the mouth, nose, or eyes. It has been reported that infectious viruses, including coronavirus, can survive for long periods outside of their host organism (Weber et al. 2016). In this regard, SARS-CoV-2 virus is thought to survive for several hours on surfaces such as aluminium, sterile sponges, or latex surgical gloves, increasing the opportunity for transmission via touch (Ye et al., 2016). Secondly, transmission via the inhalation of small, exhaled respiratory droplets may occur as the aerosol droplets remain airborne for prolonged periods, mediating long-range human-to-human transmission via air movement (Ashour et al., 2020; Wigginton & Boehm, 2020).

Thirdly, faecal transmission routes have also been considered, as the SARS-CoV-2 genomic RNA has been detected in faeces (Holshue et al., 2020) of infected patients. Although infective SARS-CoV-2 has not yet been confirmed in stool samples, possible replication in the gut has been demonstrated in one patient in Germany. In this regard, studies suggest that SARSCoV-2 may survive in stool samples for 4 days (Weber et al., 2016) while coronaviruses have generally been reported to remain infectious in untreated sewage for days to weeks (Casanova et al. 2009). This adds another potential transmission route if the quality of personal hygiene is poor. Infected stools in wastewater can generate further transmission routes through the generation of virus-laden aerosols during wastewater flushing which is a possible fourth transmission route. It has been reported that a contaminated faulty sewerage system in a high-rise housing estate in Hong Kong in 2003 was linked to the SARS outbreak of many residents living in the surrounding buildings (Peris et al. 2003). The potential for a substantial viral load within the wastewater plumbing system (and therefore the main sewer system), in combination with the potential for airborne transmission due to aerosolisation of the virus, calls for wastewater plumbing systems to be considered as a potential transmission pathway for SARSCoV-2. The interconnectedness of the wastewater plumbing network can therefore facilitate exposure to SARS-CoV-2 within, or even between, buildings. This is of particular concern in high-risk transmission settings such as hospitals and health-care buildings. Therefore, the role of aerosol from contaminated sewage in the transmission of SARS-CoV-2 would require investigation, considering that SARS-CoV-2 RNA fragments have been detected in sewage influent (Ahmed et al., 2020; Medema et al., 2020) before any COVID-19 cases were reported.

Removing and inactivating infectious viruses in wastewater is critical in controlling waterborne diseases. Studies on the presence of viruses in wastewater and their fate through wastewater treatment plants have focused primarily on enteric viruses, which transmit gastrointestinal diseases via water. Most enteric viruses are nonenveloped, consisting only of proteins and nucleic acids. Enveloped viruses contain an outer lipid membrane in addition to proteins and nucleic acids. Enveloped viruses have often been assumed to be absent in wastewater and considered rapidly inactivated when they are released to wastewater. However, recent studies (Pinon & Vialette, 2018; Holshue et al., 2020) suggest that certain enveloped viruses can enter wastewater and may survive for long periods of time. Our current state of knowledge on enveloped viruses in wastewater has been limited due to, inter alia, a lack of appropriate methods for capturing and detecting infectious enveloped viruses in water.

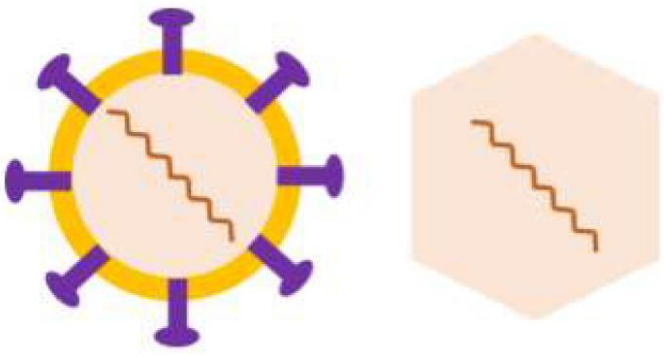
Furthermore, only a few studies attempt to provide a better understanding of the presence, survival, fate, behaviour, impact and risk of enveloped viruses in wastewater (Ye, 2018). Despite the paucity of data on enveloped viruses' behaviour in wastewater, available studies have suggested the survival of enveloped viruses in wastewater at cooler temperatures, with a larger fraction of enveloped viruses generally partitioning to the wastewater solids (Ye et al., 2016).

The knowledge gained from viral survival studies to date has shed some light on the methods for recovering and characterising infectious enveloped viruses from wastewater. The development of better characterisation techniques such as the integrated cell culture-mass spectrometry for detecting infectious viruses in wastewater has further provided a promising tool for monitoring infectious enveloped or nonenveloped viruses in wastewater samples. Furthermore, improved characterisation would provide a better understanding of the enveloped virus behaviour during the disinfection process. In this regard, protein reactions are generally believed to drive the inactivation of enveloped virus when free chlorine is used, while genome reactions would drive the inactivation of the enveloped virus when UV_{254} is used.

2. Background

2.1 Virus transmission in wastewater environments

Waterborne viruses are responsible for spreading several human diseases. Enteric viruses, for example, cause infections in human gastrointestinal system and are primarily transmitted via the faecal-oral route (Fannin et al., 1985; Fong & Lipp, 2005; Boone & Gerba, 2007). Enteric viruses such as norovirus, coxsackievirus, echovirus, and reovirus have been frequently detected in untreated municipal wastewater with infectious concentrations ranging from 10_0 to 10_8 gene copies/L (Fong & Lipp, 2005). In this regard, if wastewater is insufficiently treated, the infectious enteric viruses in the final effluent can contaminate surface waters that are used for recreation, agriculture irrigation, or serve as drinking water sources (Okoh et al., 2010; Borchardt et al., 2004; Gallimore et al., 2005). Enteric viruses are mostly non-enveloped and consist of nucleic acids and protein capsids (Figure 1), with diameters ranging in size from 20 - 100 nanometres. Generally, previous research on wastewater treatment and monitoring efforts have focused primarily on removing and inactivating non-enveloped enteric viruses (Ye, 2018).



Enveloped virus **Non-enveloped virus**

Figure 1. Structural illustrations of enveloped and nonenveloped viruses (Ye, 2018).

Unlike non-enveloped viruses, the presence and fate of enveloped viruses have not been broadly studied. Enveloped viruses contain a lipid membrane outside of their nucleic acids and protein capsids (Figure 1). Enveloped viruses are responsible for several high-profile diseases in humans, such as SARS, MERS, avian influenza and the current COVID-19. They are also responsible for less dangerous illnesses such as the common cold. Enveloped viruses have widely been assumed to be absent in wastewater environments, mainly because of the limited number of studies available (Ye, 2018). However, enveloped viruses do enter wastewater, but methods for their detection and an understanding of their mechanistic fate is currently lacking.

Currently available clinical and epidemiological evidence suggests that wastewater environments can be reservoirs for enveloped viruses. This highlights the importance of expanding our knowledge on the presence and fate of enveloped viruses in wastewater. To successfully do this, there is need to develop reliable methods for capturing and monitoring infectious enveloped viruses or their signatures in wastewater. There is also need for evaluation of the survivability of enveloped viruses that enter municipal wastewater in support of better understanding of possible risks that wastewater may present as potential transmission route of the novel SARS-CoV-2.

2.2 Enveloped viruses transmittable through wastewater

The emergence of new or re-emergence of previously known viral infections is often followed by concerns about the risks of environmental transmission. This potential exposure path was identified in the epidemic of SARS infection (McKinney et al., 2006), for avian influenza (Brown et al., 2007), the Ebola epidemic in West Africa (WHO, 2014) and more recently the COVID-19 (WHO Website, 2020). In terms of its genome, this virus is closely related to the viruses responsible for SARS in 2003 and Middle East Respiratory Syndrome (MERS) in 2012. However, there are important differences affecting the global spread of SARS-CoV-2 and the unprecedented actions that have been taken to mitigate public health impacts and ensure continuity of critical infrastructure.

Generally, when concerns are raised about the risk of environmental transmission, attention naturally turns to questions of survival and persistence of the implicated virus in the environment. One of the areas of particular interest is in the survival of virus in wastewater and latrine sludge. Although SARS-CoV-2 is primarily respiratory in nature, studies have confirmed the presence of its genetic material in the faeces of infected individuals, possibly due to co-infection of cells within the gastrointestinal tract (Zhou et al., 2017). This secondary infection may explain why the genetic material can be detected in faeces after it is no longer detected in oral and nasal swabs (Xiao et al., 2020). During the previous SARS outbreak (Wang et al., 2005) and also the current COVID-19 outbreak (WHO Website, 2020), transmission via sewage was implicated but never confirmed.

Enveloped viruses are often thought to be more fragile, but studies indicate that coronaviruses can persist on surfaces (Casanova et al., 2010) and in wastewater (Gundy et al., 2009) for days. However, research also suggests that coronaviruses are more likely to partition to solids and are more susceptible to wastewater treatment processes than their non-enveloped enteric counterparts (Wigginton & Boehm, 2020). Therefore, multi-barrier wastewater treatment processes are likely to provide adequate protection against coronaviruses (Wigginton & Boehm, 2020), so the associated public health risks for treated wastewater, and water reuse are likely negligible. However, the current aged infrastructure coupled with poor operation and maintenance practices in the South African wastewater sector are a major risk with regards to a sustainable management of SARS-CoV-2 in wastewater and a proper mitigation strategy of COVID-19 impacts in South Africa.

2.3 Coronaviruses

Different coronaviruses can cause both respiratory and gastrointestinal illnesses (Vabret et al., 2006). Some strains of human coronaviruses, such as SARS coronavirus and MERS coronavirus, are the responsible agents for epidemics of deadly acute pneumonia diseases. The overall case fatality rate for the SARS outbreak in 2003 was 10%, (WHO Website, 2004) while the accumulated case fatality rate of MERS was 35% (Alsolamy & Arabi 2015). Infected individuals shed SARS and MERS coronavirus genetic material in their stool and urine samples with high frequency, and infectious SARS coronaviruses having been isolated from human stool samples (Chan et al., 2004). A recent outbreak of SARS-CoV-2 reported by the the Associated Press in a publication of 11 February 2020, in which two residents of a Hong Kong apartment building have fallen ill with the new coronavirus despite living on different floors has prompted concerns that the virus may spread through building pipes. Viral shedding has been reported for other low pathogenic strains of human coronaviruses (Risku et al., 2010; Jevšnik et al., 2013), and infectious coronavirus has been isolated from human stool samples (Vabret et al., 2006) suggesting their possible presence in wastewater.

2.4 Influenza viruses

Infectious avian influenza viruses (AIV) are shed in an extremely high concentration in bird faeces (Webster et al., 1978) and are transmitted primarily via faecal-oral route in birds (Watanabe et al., 2014). Occasionally, humans can acquire AIV (H5N1), with case fatality rates of up to 53% between 2003 and 2017, as estimated by WHO (WHO Website, 2017). The transmission route of AIV from poultry to humans is still elusive, but several transmission routes are hypothesized, including direct contact with the infected poultry, and contact with virus-laden faecal matter (Markwell & Shortridge, 1982; Peiris et al., 2007). While human-to-human transmission has rarely been reported in human AIV cases, infected individuals can shed AIV in their stool samples (de Jong et al., 2009; Hu et al., 2013). The concentration of AIV H5N1 detected in rectal swab samples has been reported to range from 8.6 x 102 to 1.5 x 106 gene copies/mL (de Jong et al., 2006; Buchy et al., 2007). Similar to AIV, seasonal human influenza virus strains have been detected in faeces at concentrations of 104 – 106 gene copies/g of stool samples (Chan et al., 2011).

2.5 Other enveloped viruses

Zika virus is an emerging mosquito-borne human pathogenic virus, and Zika virus genetic material can be detected in urine specimens (Gourinat et al., 2015). Other mosquito-borne enveloped viruses such as dengue virus and West Nile virus have also had their genetic materials widely detected in urine (Poloni et al., 2010; Hirayama et al., 2012; Barzon et al., 2013), with infectious West Nile virus isolated from the urine of acutely infected individuals (Barzon et al., 2013). Alternatively, wastewater is a habitat for mosquito larvae with resultant adults that can carry and transmit these enveloped viruses (Duffy et al., 2009; Ponnusamy et al., 2011; Hossini et al., 2017). Cytomegalovirus is carried by people of all ages, in most cases, asymptomatically, but can be a threat to those who are immunodeficiency or immunocompromised. Infectious cytomegaloviruses can be shed in the urine from infants and children who are infected at birth (Noyola et al. 2000). Contact with urine is a suspected transmission route of cytomegalovirus. On the other hand, Ebola virus, causing deadly haemorrhagic fever, can enter wastewater when patients shed bodily fluids that contain high levels of infectious viruses (Bausch et al., 2007; Mora-Rillo et al., 2015; Bibby et al., 2017).

2.6 Virus survival in wastewater

To cause infection, viruses present in wastewater must retain their infectivity until they encounter the next host. The survivability of viruses is often measured by the length of time to lose 90% of their original infectivity (i.e., T90 value). Enveloped viruses have often been assumed to be less stable in water, but this assumption is too simplistic. The T90 values available in the literature suggest that enveloped viruses are not necessarily more susceptible to environmental conditions than

nonenveloped viruses in various water environments (Brainard et al., 2017). Some strains of coronavirus and avian influenza virus retain their infectivity as long as non-enveloped viruses. Coronaviruses, SARS and 229E for example, have a T90 greater than one day in urine and filtered wastewater samples (Brainard et al., 2017). For context, one day is the maximum retention time of wastewater in a common sewerage system. However, inactivation of enveloped surrogate viruses in human sewage has been reported by Casanova., (2015). Results suggest that enveloped viruses can undergo 6−7 log inactivation in sewage in 3−7 days, depending on temperature. In this regard, longer holding times may be desirable to accommodate lower temperature conditions.

However, the current survival studies on enveloped viruses are limited, with less reported for raw wastewater and available reports focussing on laboratory-based studies. If the viruses can survive in raw wastewater and then enter the wastewater treatment plants (WWTPs), viruses need to be removed or inactivated effectively through the treatment processes. The mechanisms and removal efficiency of nonenveloped enteric viruses in WWTPs have been reviewed in previous publications (Gerba, 1981; Hurst & Gerba., 2009). For non-enveloped viruses, the removal efficiency from wastewater depends on virus partitioning with wastewater solids in primary treatment and the adsorption to activated sludge in secondary treatment (Gerba, 1981; Ye, 2018). Ye et al., (2016) have reported partitioning behaviour of enveloped viruses to follow a similar pattern to that of nonenveloped viruses. This suggests that the fate and behaviour of enveloped viruses in wastewater should focus on their interaction with biosolids and the resultant risks. Significant and comprehensive studies are therefore required for enveloped viruses to strengthen our limited ability to predict the fate of infectious enveloped viruses in WWTPs. Currently, wastewater effluent disinfection is generally applied to inactivate viruses during wastewater treatment.

2.7 Virus inactivation by disinfection treatment

Disinfection is used in WWTPs to inactivate pathogenic viruses and other microorganisms. The disinfection efficacy of a number of disinfection methods has been widely reported for nonenveloped viruses (Aieta & Berg 1986; Kim et al., 1999; Kitis, 2004), whereas limited data is available for enveloped viruses. A recent research study identified molecular features of an enveloped virus that are susceptible to chemical oxidants or UV radiation (Ye et al., 2018). The study developed a framework for studying molecular reactivities that can be adopted to investigate enveloped virus survivability under various environmental conditions. In this regard the focus on reviewing virus inactivation mechanisms by ultraviolet 254 (UV254) and free chlorine becomes key, as these are the commonly used disinfection methods.

2.7.1 UV disinfection

UV is one of the most commonly applied disinfection methods and UV light is subdivided into three regions according to wavelength, namely UVA (320-400 nm), UVB (290-320 nm), and UVC (100-290 nm). Viruses are most sensitive to UVC due to the high photo reactivity of nucleic acids in the UVC region. Low-pressure mercury lamps emit the highest UVC intensity around 254 nm; therefore, most studies on virus inactivation by UVC focus on this specific region (i.e., UV254). Our current knowledge on virus inactivation mechanisms has been established primarily with non-enveloped model viruses. A study of bacteriophage MS2, for example, suggests that the inactivation of a non-enveloped virus by UV254 is mainly attributed to damage in the viral genome (Wigginton et al., 2012). Follow-up studies have underscored the findings that the UV254 reactivity of viral genomes correlate to virus susceptibility to UV254 (Beck et al., 2013; Sigstam et al., 2013; Ho et al., 2016; Qiao et al., 2018). Two main factors determine the UV254 reactivity of viral genomes, namely genome size and genome types (single stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and double stranded RNA (dsRNA)). Other mechanisms of virus particle damage by UV254 can also lead

to non-enveloped virus inactivation. In the MS2 model, protein damage sensitized by adjacent viral RNA sequences contributes to 20% of the observed virus inactivation (Wigginton et al., 2012), whereas in nonenveloped dsDNA viruses, the damaged genome can be repaired in the host cell and this results in higher resistance to UV254 (Sinha & Häder 2002). Although genome reactions are believed to drive the inactivation of the model enveloped virus by UV254, the comprehensive mechanisms of enveloped virus inactivation by UV254 have not been thoroughly established and therefore require further investigation.

2.7.2 Free chlorine disinfection

Free chlorine is a strong oxidant that readily inactivates microorganisms. Free chlorine is an aqueous solution of chlorine species: HOCl, OCl-, Cl2 (aq), and Cl2O(aq) (Sivey et al., 2010), with the primary oxidant species being the neutral molecule hypochlorous acid (HOCl). Based on the nonenveloped MS2 model, the reactions of free chlorine with virus proteins and genomes impact the ability of viruses to bind, enter, and replicate in the host cell (Wigginton et al., 2012). The inactivation of enveloped viruses with free chlorine have only been compared to non-enveloped viruses in one study, where the enveloped bacteriophage Phi6 and Ebola virus experienced higher levels of inactivation than non-enveloped bacteriophages MS2 and M13 in 0.5% sodium hypochlorite solution (Gallandat et al., 2017). However, that report provided limited information on the chlorine demand of samples and other important experimental conditions; consequently, it is impossible to draw general conclusions about whether enveloped viruses are more or less susceptible to inactivation by free chlorine than non-enveloped viruses. Further studies would be required to understand comprehensive mechanisms of enveloped virus inactivation by oxidants.

A bottom-up characterization of enveloped virus inactivation could help identify molecular features that drive inactivation. With this information, we would be better equipped to select and improve disinfection methods for treating enveloped viruses. This is particularly important during outbreak events, when culturing viruses to see how well disinfection are working is often not possible.

3. Established research on SARS-CoV-2 and wastewater

Research on enveloped viruses in wastewater, including coronaviruses suggest these viruses are inactivated at faster rates than most nonenveloped viruses (Gundy et al., 2009; Casanova et al., 2015; Bibby et al., 2015; Ye at al., 2016; Brainard et al., 2017). Furthermore, they partition to wastewater solids just like nonenveloped viruses (Ye at al., 2016), and wastewater temperature is positively associated with their inactivation rates (Ye at al., 2016). In wastewater treatment processes, they are generally susceptible to oxidant disinfectants (Rice et al., 2007; Ye et al., 2018) with the presence of an envelope appearing not to impact virus susceptibility to UVC light (Ye et al., 2016), since UVC targets virus genomes and lipid membranes do not shield the genomes from UVC radiation.

3.1 SARS-CoV-2 Virus and wastewater treatment plants compliance

Our wastewater treatment plants, are generally designed using microbial risk assessments and process performance data with nonenveloped enteric viruses. Based on the facts that (i) the closely related 2003 SARS was excreted in faeces at lower levels than enteric human noroviruses, (ii) model coronaviruses are inactivated at faster rates in wastewater than non-enveloped viruses, (iii) the enveloped viruses studied to-date are more susceptible to oxidant disinfectants than non-enveloped viruses, and (iv) the large single-stranded RNA (ssRNA) genome (~29.8 kb) of SARS-CoV-2 likely renders it more susceptible to UVC inactivation than enteric ssRNA viruses, the multibarrier wastewater treatment systems are likely effective in protecting against SARS-CoV-2. Nonetheless, there may still be wastewater-related exposures that need to be considered if infectious SARS-CoV-2 viruses are present in urine or faeces. Such exposures may occur in communities that experience

combined sewage overflows, that do not have sewage infrastructure or have aged infrastructure, or that use wastewater for irrigation, as well as buildings that have faulty plumbing systems and occupational exposures to wastewater and excrement. In this regard, the current aged infrastructure coupled with poor operation and maintenance practices in the South African wastewater sector becomes a major risk with regards to a sustainable management of SARSCoV-2 in wastewater and a proper risk mitigation strategy of COVID-19 in South Africa.

Enveloped viruses are extremely diverse, with a range of genome types, structures, replication cycles, and pathogenicities. For example, of the 158 identified human RNA virus species defined in 2018, 122 species from 11 virus families were enveloped and 36 species from 6 families were non-enveloped (Woolhouse & Adair, 2013). Consequently, enveloped viruses are likely to display a diverse range of environmental behaviour, persistence, and fate (Aquino de Carvalho et al., 2017). However, the limited studies on enveloped-virus fate, transport, and inactivation have focused on only a small fraction of human viruses or their proxies including animal coronaviruses and bacteriophage phi6. Although studies using animal coronaviruses outbreak (Casanova et al., 2010; Hulkower et al., 2011; Ye et al., 2016) have been valuable for the current COVID-19, it is essential to consider an expanded set of enveloped viruses that better represent human enveloped virus diversity for wastewater studies.

3.2 Implications of SARS-CoV-2 detection in wastewater

Based on SARS-CoV-2 genetic material having been detected in faeces, many researchers and wastewater agencies throughout the world are now collaborating to document its occurrence in wastewater. One published study has already confirmed detection of the viral RNA in wastewater at multiple sites in the Netherlands (Medema et al., 2020) and there are ongoing studies elsewhere observing similar results (Ahmed et al., 2020). It is important to note that to date, there have not been any detections in drinking water (CDC, 2020). Given the intense interest and time-sensitive nature of this issue, researchers are now developing collaborative networks to share protocols and coordinate monitoring efforts. Although it is not necessarily surprising for the RNA of SARS-CoV-2 to be detected in wastewater, it creates additional uncertainty for the wastewater reuse industries. SARS-CoV-2 is not expected to persist through disinfection processes (Wigginton & Boehm, 2020), but the precautionary principle dictates that the industry should consider risks from aerosolization in sewers and during primary or secondary wastewater treatment.

One of the major challenges associated with COVID-19 is developing an accurate estimate of disease prevalence in various communities. This has been hindered by challenges in implementing broad clinical testing and the wide range of symptoms experienced by infected individuals, including those who are completely asymptomatic. This presents a unique opportunity for the wastewater reuse industry with respect to 'environmental surveillance' or 'wastewater epidemiology' – the study of wastewater-derived constituents as a means of characterizing levels of disease within a community. This is described in detail under section 4.

3.3 KWR Watershare webinar outcomes

The Watershare Webinar on COVID-19 in the water sector held on 30 March 2020 concluded the following based on current limited research and data;

- SARS-CoV-2 (from currently available research data) is sensitive to disinfectants and high temperatures;
- It is expected that the new coronavirus would be less abundant as an infectious virus in

sewage than known enteric viruses and less stable in currently applied WWTP or drinking water treatment plant;

- Drinking water systems are safe, based on years of research and knowledge on other viruses that are more robust than SARS-CoV-2;
- SARS-CoV-2 is not an important waterborne pathogen, the primary route of transmission being droplets through coughing and sneezing and contact with contaminated surfaces. As a result of this and with no epidemiological signals that sewage workers are at risk, the risk of SARS-CoV-2 transmission via sewage is considered low and current protective measures for these workers are considered adequate;
- Monitoring the virus in sewage is very sensitive and critical, as this serves as an early warning system of virus circulation before the Health surveillance can pick up the mild cases.

However, the above conclusions should be considered with a clear understanding of the South African wastewater sector challenges, particularly in rural areas where WWTPs do not properly function with untreated sewage flowing down the streets and into rivers. Furthermore, standards of wastewater treatment and best practices in South Africa may significantly differ from Europe.

4. Outbreak response and research needs

4.1 Wastewater-based epidemiology

It remains a highly challenging logistical exercise for medical professionals to practically and effectively screen suspected infectious cases from individual households. Such a massive undertaking is quite time-consuming and labour intensive and is constrained by the availability of testing technologies at this extremely critical time. However, an alternative method utilising wastewater-based epidemiology (WBE), may provide an effective approach to predict the potential spread of the infection by testing for infectious agents in wastewater and obtain information on health, disease, and pathogens (Yang et al., 2015). Pinon and Vialette (2018) have emphasized conducting large-scale studies in artificial environments such as WWTPs that serve the communities under investigation. The potential benefits of data from such studies for the communities are quite significant and would include, inter alia, the following:

- Measure the scope of the outbreak independent from patient testing or hospital reporting, including data on asymptomatic individuals
- Provide decision support for officials determining the timing and severity of public health interventions to mitigate the overall spread of the disease
- Better anticipate likely impact on hospital capacity in order to inform hospital readiness and the necessity of public health interventions
- Track the effectiveness of interventions and measure the wind-down period of the outbreak, and
- Provide an early warning for re-emergence of the coronavirus, if it does indeed have a seasonal cycle.

Faeces and urine from disease carriers in the community contain many biomarkers that can enter the sewer system. A recent study demonstrated that live SARS-CoV-2 has been isolated from the faeces and urine of infected people (Holshue et al., 2020), which would enter the wastewater treatment system and could typically survive for up to several days in an appropriate environment after exiting the human body. There is potential that the detection of SARS-CoV-2 in community wastewater could indicate whether there were potential SARSCoV-2 carriers in certain areas. If SARS-CoV-2 could be monitored in the community at the early stage through WBE, effective interventions could be

implemented as early as possible to restrict the movements of the local population and limit pathogen spread.

Using a WBE approach in developing an early warning system will, however, require a rapid analytical method for the on-site detection of viruses or their RNA fragments at the wastewater collection point. Currently, the most direct method for the detection of SARS-CoV-2 is a nucleic acid−based polymerase chain reaction (PCR) assay, which is also a means for confirmation of COVID-19 patients globally. Although PCR has high sensitivity and specificity, requirements for complicated sample handling in the laboratory, skilled personnel, and a long period of data processing and analysis (4−6 h) are not conducive to real-time and on-site effective monitoring of samples. Therefore, it is critical to develop efficient transportable and robust analytical tools to accurately and quickly trace low-level SARS-CoV-2 sources through WBE to confirm suspected cases and screen asymptomatic carriers without centralized laboratories.

Paper analytical devices have emerged as powerful tools for the rapid diagnosis of pathogens and indicators of infection transmission (Magro et al., 2017). The paper-based device is a small analytical tool with different functional areas printed with a wax printer that integrates all processes (extraction, enrichment, purification, elution, amplification, and visual detection) required for nucleic acid testing into an inexpensive paper material. The whole testing process can be completed through simple folding of a paper-based device in different ways without a pump or power supply, which overcomes the limitation of PCR and avoids multiple processes. Paper analytical devices enable multiplexed, sensitive assays that rival PCR laboratory assays and provide high-quality, fast precision diagnostics for pathogens. For example, recent work demonstrated the multiplexed determination of malaria from blood using a paper based-device in rural Uganda (Reboud et al., 2019). The test could sensitively analyse nucleic acid sequences of pathogens within 50 min, giving a high quality, faster and precision diagnosis for malaria than PCR.

In addition, paper analytical devices are easy to stack, store, and transport because they are thin, lightweight, and of different thicknesses. Visual analysis is made simple due to the strong contrast with a coloured substrate. Paper-based devices can also be incinerated after use, reducing the risk of further contamination. Although wastewater is a complex matrix, paper-based devices have shown the potential to detect pathogens in wastewater. This provides a fast “sample-to-answer” analysis method useful for quantitative monitoring of nucleic acids and genetic information in sewage (Yang et al., 2017). The method has further been confirmed using robust electrophoresis and agarose gel image assay, showing its reliability for wastewater analysis.

In summary, the paper-based device has the potential to be used as a small, portable device to detect SARS-CoV-2 in wastewater on site and to track virus carriers in the community. Such an approach could provide near real-time and continuous data and serve as an early warning sensing system to help local governments and agencies make effective interventions to isolate potential virus carriers and prevent the spread of epidemics. It is believed that in the case of asymptomatic infections in the community, rapid and real-time community sewage detection through paper analytical devices can determine whether there are SARS-CoV-2 carriers in the area in a timely manner to enable rapid screening, quarantine, and prevention. The potentially infected patients can benefit from such devices in tracing SARSCoV-2 sources based on WBE, providing information for the correct and timely treatment of COVID-19.

4.2 Research needs

Several key areas of research that require attention in terms of handling potentially highly infectious liquid wastes such as wastewater have been highlighted in literature (Bibby et al., 2017), and these include the following:

- **Move towards a mechanistic model of viral infection**

Mechanistic, rather than descriptive models will enable the rapid extension of inactivation behaviour to emerging pathogens and exposure scenarios. Historical models of environmental virus inactivation are mostly descriptive, but recent efforts should focus on providing more mechanistic models for inactivation (Decrey et al., 2015). Specific mechanisms that should be explored include the role of pH, ammonia, biological activity, temperature, and solids found in wastewater matrices. Furthermore, the impact that viral lipid envelopes have on inactivation mechanisms should be investigated as many emerging human viruses are enveloped. It should be noted, however that this task will be challenging when working with Biosafety Level (BSL) 3 and 4 microorganisms (high risk microbes), due to limited access to specialised laboratory facilities.

- **Better characterisation of exposure and transmission pathways in the wastewater environment**

Potential exposure pathways for both wastewater workers and the general public to untreated wastewater are currently poorly characterized. Specific areas of research include defining specific exposure scenarios, potential exposure following unintended releases (e.g. combined sewer overflows), the potential for aerosolization of viruses and the differential fate of structurally diverse viruses in existing sewage treatment infrastructure. Specific questions regarding the fate of structurally diverse viruses include how structure impacts virus partitioning between wastewater solids, liquid, and air, and how structure impacts viral survival in wastewater and through wastewater treatment processes. It should be noted, however, that any assessment of potential exposure routes must consider the effects of wastewater composition, dilution of contaminated wastewater, and possible inactivation of enveloped viruses during treatment and holding.

- **Reconsideration of surrogate evaluation of emerging pathogens**

Historically, surrogates (physiologically similar microorganisms) have been used to study the fate and persistence of pathogens in the environment, e.g. MS2 bacteriophage to model enteric virus fate. A recent review demonstrated that the persistence of enveloped viral pathogens in water can vary from hours to months to achieve 90% inactivation (Wigginton et al., 2015). However, it has become apparent that a limited suite of surrogates will be inadequate for a fine-scale, mechanistic understanding of viral persistence and inactivation in the wastewater environment. The variability of viral persistence of enveloped viruses (Wigginton et al., 2015) suggests that where possible, it is best practice to use the pathogen of interest. Where direct use of the pathogen is not possible, the use of multiple surrogates is ideal to capture various aspects of target pathogen physiology. Furthermore, in cases where the environmental fate of BSL3 and BSL4 viruses can be studied, experiments should include a surrogate virus in the same samples that contain the pathogenic viruses, so that the experimental results can be directly compared with other studies. It is therefore recommended that bacteriophage MS2 be included whenever possible in studies with BSL3 and BSL4 viruses as this virus has been widely used as a persistence model and would facilitate cross-lab comparison and validation of persistence studies.

- **Appropriate disinfection approaches of high strength waste**

Infectious viruses are excreted from individuals in high-strength (i.e., high organic content) waste, such as blood, vomit, faeces and wastewater. Clearly, control of pathogen release at the source (i.e., disinfection) would be desirable to limit potential downstream exposure and public concern;

however, hyperchlorination of high organic content wastes insufficiently inactivates pathogens (Sozzi et al., 2015). Evaluation of alternative disinfection methods, such as pH adjustment or heat, as well as a more mechanistic understanding of chlorine and UV action in high strength waste, are necessary. A mechanistic understanding of disinfection will enable the effective extension of disinfection techniques to novel waste streams and pathogens that have not been extensively studied.

- **Communication requirements**

A critical shortcoming in the viral pandemic responses is the need for better communication between the wastewater and medical sectors, as well as with the general public. Forthcoming and accurate risk communication will be necessary to build both industry and public trust in infectious waste management. Multiple factors must be addressed, including the public's ability to understand risk, and regionally and socially appropriate risk communication.

4.3 General research needs

The emergence of multiple high consequence viral pathogen outbreaks in recent years (e.g., SARS, Ebola, MERS, SARS-CoV-2) has highlighted the value of continued investigation into viral pathogen fate and inactivation in the water environment, as well as appropriate wastewater handling and disinfection. Continued investment and attention in this critical research area are necessary to better inform future outbreak response, both minimising the potential for secondary transmission of high consequence pathogens and public concern.

Overall, the survival of the SARS-CoV-2 virus in different environmental media, including water and sewage under a variety of environmental parameters warrants systematic investigation immediately. Levels of infectious SARS-CoV-2 virus in environmental samples could be low, requiring sensitive methods with high-throughput and automated techniques to monitor viruses. In the future, this novel coronavirus may also become a seasonal infectious virus. In this regard, the occurrence, survival, and behaviour of SARS-CoV-2 virus in environmental compartments need to be determined, as part of the early warning system.

Meanwhile, to reduce the chance of infection, it is important to develop practical methods for large-scale disinfection treatment of SARS-CoV-2 virus in different environmental settings. Future studies on enveloped viruses should also seek to carefully characterize and even standardise the conditions under which measurements are conducted. Media composition, the purity of virus stock, and when possible, virus concentrations in both gene copies and infective units, should be described. When studying oxidants, the demand of the solution and change in oxidant concentration through the study should be provided. When studying radiation (UVC and/or sunlight), attenuation through the study conditions should be well characterised and incorporated into reported doses. Researchers should include a well-studied surrogate virus in their studies in addition to the enveloped virus of interest to facilitate cross-study comparisons.

It is therefore recommended to use the nonenveloped bacteriophage MS2 for this purpose, as it is one of the most studied viruses in environmental systems. Moreover, predictive models based on the underlying mechanisms controlling the persistence of enveloped viruses, and other characteristics, may reduce the need to study every virus under every condition (Brainard et al., 2017). Another promising area of research involves using sewage to monitor virus circulation in communities and detect outbreaks before clinical cases are identified. This has been to pathogenic bacteria (Diemert & Yan, 2019) and nonenveloped viruses (Berchenko et al., 2017) and more

recently to SARS-CoV-2 (Ahmed et al., 2020; Medema et al., 2020). This will necessitate a better understanding of which enveloped viruses are excreted in urine and faeces and at what levels.

The threat of COVID-19 outbreak is currently not limited to any single country or region (WHO Website, 2020). Therefore, the response, control, and prevention of novel infectious diseases require strong and sustainable international collaborative work and data sharing. In this regard, further research is imperative to fill the knowledge gaps on COVID-19 and wastewater. In addition to expertise in the fields of medicine, public health, and computer science, the contribution of environmental scientists in collaborative research is urgently required for combating the infectious disease threat at a global scale.

5. Conclusions

In conclusion, SARS-CoV-2 will certainly not be the last novel virus to emerge and seriously threaten global public health. Researchers and funding agencies tend to focus intensely on a specific virus during its outbreak, but then move on to other topics when the outbreak subsides. Environmental science and engineering researchers should take a broader, long-term, and quantitative approach to understanding viruses that can potentially be spread through the environment. However, this should not result in covidization of the research agenda. Similar to the approach for chemical pollutants in the environment, we should aim to understand and communicate to our colleagues in medicine and public health the specific characteristics that drive transport and inactivation of enveloped viruses in solutions, on surfaces, and in the air. Likewise, we should seek to understand how environmental factors shape possible virus transmission routes. That way, regardless of the identity of the enveloped virus that causes the next major outbreak, we can provide more informed descriptions of its persistence and recommendations on how to mitigate its spread.

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