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Editorial perspective: Viruses in wastewater: Wading into the knowns and unknowns

The COVID-19 pandemic has posed immense challenges to the fields of public health, economy and education worldwide. Yet, its transmission and attenuation in the environment are not fully elucidated. What we do know is that the water and wastewater treatment plants (WTPs/WWTPs) are most susceptible to viral contamination specifically during the current COVID-19 outbreak. Although knowledge about SARS-CoV-2 in sewage and WWTPs is limited (Foladori et al., 2020), SARS-CoV-2 is likely to be rapidly inactivated under increased temperature and by disinfectants such as bleach, ethanol, benzylalkonium chloride, povidone-iodine and chloroxylenol (Chin et al., 2020). The application of secondary disinfection measures, like dosing of chloramines to maintain a certain residual chlorine level in the distribution network adds to further protection from contamination (Bhowmick et al., 2020). However, as municipal network receives huge amount of wastewater from asymptomatic patients and treated sewage from hospitals, SARS-CoV-2 from improperly disinfected wastewater might persist for a prolonged time in pipelines, in turn becoming a secondary source of transmission (Zhang et al., 2020). Therefore, we must make sure the wastewater coming out of the SARS-CoV-2 infected areas should be properly disinfected in order to reduce the impact on the receiving water bodies. This brings in the need for careful consideration of disinfection and removal strategies for SARS-CoV-2 from contaminated waters (Kitajima et al., 2020).

In terms of pathogen removal in WTPs and WWTPs, there are two factors affecting the efficacy, namely how long the pathogen stays in the system and how quickly it dies (Curtis, 2003). The former is governed by the hydraulic flow regime and the latter depends on factors such as ecology in the engineered system or persistence of the pathogen strain itself. An effective treatment method should be one that has the potential to destruct pathogenic cells and remove the nutrients to prevent regrowth or recontamination. This requires an enhancement of traditional water and wastewater treatment units including coagulation, filtration, activated sludge and biofilms to remove particles and facilitate adsorption, as well as disinfection strategies such as chlorination and ultraviolet. Regardless of the strategy used, these multibarrier treatment approaches need to be well-operated to prevent dissemination of SARS-CoV-2 into the environment, in turn safeguarding public health.

Since viruses such as SARS-CoV-2 can be shed through feces to enter into wastewater, continuous and systematic monitoring of wastewater may provide early warning signs and will potentially identify undiagnosed or successive disease at the population level, thus alerting public health officials on the on-going or future viral disease outbreaks. This is often termed as wastewater-based epidemiology (WBE), which is defined as an approach initially focused on the analysis of chemical pollutants, and subsequently including also the biomarkers in raw wastewater (Choi et al., 2018). WBE has been recognized as a promising tool for the rapid, non-invasive mass surveillance of infectious diseases at the population level with minimum costs. WBE can be used as “early warning signs” of the presence of infected individuals in the area of interest (Asghar et al., 2014; Hellmér et al., 2014). Fecal shedding of SARS-CoV-2 RNA from COVID-19 patients has been widely reported (Holshue et al., 2020; Wu et al., 2020; Xiao et al., 2020). It can persist in stool for up to 31 days (Zheng et al., 2020), and has been detected in both hospital sewage (Wang et al., 2020) and raw wastewater (Ahmed et al., 2020; Randazzo et al., 2020). The lowest number of infected individuals in a community detected by WBE is expected to correlate with the size of the community being served by the waste treatment plant/sewage line. However, the lowest proportion of population infected and is detectable by the test is expected to vary across waste treatment plants/sewage lines, with variability explained by the additional dilution effects arising from rainwater and industrial waste input. Once the lower limit of detection for the prevalence of fecal shedders is determined, the total prevalence of infected individuals in the community (i.e., including those not shedding the virus in feces) could be calculated. Additionally, the number of copies of the viral particles enumerated in sewage samples needs to be correlated with the prevalence of infected individuals in the population.

Monitoring for SARS-CoV-2 directly in wastewaters was proposed as a means to complement the current clinical surveillance, and/or to serve as an early warning of (re)emergence of Covid-19 in cities (Medema et al., 2020). Many studies have reported the sporadic detection of SARS-CoV-2 in wastewater (Ahmed et al., 2020; Haramoto et al., 2020; La Rosa et al., 2020; Randazzo et al., 2020; Sherchan et al., 2020), correlated the virus concentration with the known number of clinical cases, and made claims of WBE being a sensitive tool for early outbreak detection. However, the definition of “early outbreak detection” is still debatable since there is still no actual information of all the infected cases present in the community. Without knowing the actual detection limits of WBE, and by that, we mean the minimal number of positive cases needed in each community to achieve a confident detection of SARS-CoV-2 from the sewage networks; we are handicapped by our abilities to determine how early into the outbreak WBE can truly tell us.

Furthermore, for WBE to detect future outbreak of new viruses, the current approach of quantitative PCR may not be entirely suitable. Quantitative PCR is a targeted approach which requires viral targets to be known first so that primers/probes can be designed accordingly to detect them. Instead, non-targeted approaches, for instance omics-based sequencing, may be needed to infer the unknown. However, omics-based approaches will need to be improved for their bioinformatic databases and analytical pipelines to enhance both the qualitative and

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quantitative detection of low abundance pathogens (Hong et al., 2020). Most importantly, WBE needs to be a long-term continuous effort to determine first, the baseline abundances of pathogens, and then monitored for any deviations from baseline. In this manner, WBE can serve as a warning mechanism to relevant stakeholders.

After being shed into feces and urine, coronaviruses are exposed to the wastewater environment for hours to days before they reach wastewater treatment facilities. It is important to understand how persistent the viruses or the viral RNA are in the wastewater environment so that the total viruses shed by the infected population can be estimated based on the viral load in the samples. Appropriate epidemic models that capture key transmission processes and the most sensitive factors impacting the fate and transport of viral particles are needed for hazard identification and risk assessment. Temperature, average in-sewer travel time and per-capita water use were identified as three parameters that have significant impact on virus detectability (Hart and Halden, 2020). Lagging indicators of infection are important for policy makers to make inferences about the next stage of the pandemic. Kaplan et al. developed a transmission epidemic model for the estimation of COVID-19 incidences based on the detected concentrations of SARS-CoV-2 RNA in municipal sewage sludge by incorporating probability distributions for indicator-specific time lags from infection into the transmission dynamics, and estimated that the hospital admission lags the detection of viral RNA in sewage sludge by 4.6 days on average (Kaplan et al., 2020). Researchers in Paris found that the decrease in confirmed COVID-19 cases lagged that of the wastewater viral RNA loads by about eight days (Wurtzer et al., 2020). A study in the United States also reported that the concentrations of SARS-CoV-2 RNA in sewage sludge reached peak three days before that of hospital admission and seven days before that of community cases (Peccia et al., 2020).

Considering the time lag from infection to detecting virus in wastewater, WBE would significantly benefit if monitoring can be conducted *in situ* and in real time. However, due to the difficulty of real-time *in situ* monitoring of pathogens such as virus and bacteria in water and wastewater, monitoring of general wastewater quality parameters including oxygen, pH, redox potential (ORP), conductivity (salt content), nutrient and turbidity could serve as indirect indicators for WBE. For centralized municipal water and wastewater treatment facilities, different types of monitoring technologies have been developed, including colorimetric, chromatographic, biometric and electrochemical sensors (Ahmad et al., 2016; Ensafi et al., 2009; Kalluri et al., 2009; Xu et al., 2016). However, all these sensors require frequent calibration, maintenance and replacement. For decentralized and distributed onsite water and wastewater facilities, sensors capable of accurate and continuous monitoring of water quality are needed for long-term maintenance free application (Crespo, 2017; Lee et al., 2006). Existing electrochemical and optical sensors still have lifespan problems with the longest duration of less than one month in real wastewater (Huang et al., 2019). In addition, wireless sensor networks should be developed for water infrastructure to assure early warning of water quality abnormity across networks and execute swift control and decision making (Bourgeois et al., 2003; Kadir et al., 2020).

In conclusion, this editorial perspective asks that future research in these areas be considered:

- We currently have limited data on SARS-CoV-2 removal and/or inactivation by wastewater and water treatment processes. In the future, one research need is a better understanding of drinking water disinfection efficacy and stability in inactivating and removing SARS-CoV-2 to help minimize waterborne viral infection (Bhowmick et al., 2020).
- Another research need is to investigate the persistence and inactivation mechanisms of SARS-CoV-2 including predation, UV, sunlight, and disinfection in wastewater and receiving waters.
- Nationwide and international wastewater surveillance campaigns should be carried out to better understand temporal and spatial dynamics of disease prevalence, molecular epidemiology and evolution of the virus, and efficacy of public health interventions.
- Development of new tools that facilitate *in-situ* real time monitoring of pathogens in wastewater and water matrices.

Author's contribution

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