Translating SARS-CoV-2 wastewater-based epidemiology for prioritizing mass vaccination: a strategic overview

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Abstract

The inception of the novel coronavirus has forced the world into despair. Rapid progress has been made in addressing the situation, and various clinical diagnostic methods were developed for early detection of transmission. However, with a rapidly increasing number of infected populations worldwide, the testing of each individual was impractical. The wastewater-based epidemiology (WBE) has been implemented to evaluate disease outbreaks as an early warning system for pandemic preparedness. Numerous studies reported the presence of SARS-CoV-2 in the open drains and STPs across the globe via recovery efficiency of surrogate virus from existing virus concentration protocols. However, the such reported studies did not justify the use of WBE to identify or pinpoint the specific hotspots of transmission which could be prioritized for rapid efforts to contain or accelerate active vaccination efforts. Identifying precise locations of hotspots could be an essential aspect in controlling the outbreak and surge of wave by prioritizing the region for primary outbreak response. This article focuses on the issues relating to the primary focus for WBE that can be adapted, and its suitability for utilization in the mass vaccination program is discussed. Effective use of WBE information in terms of source tracking might be crucial as we move towards mass vaccination to control outbreaks of COVID-19 pandemic.

Keywords
Wastewater · Epidemiology · SARS-CoV-2 · Vaccination · Policy making

Introduction

An outbreak of disease from pathogenic viruses has always caused human hardship. Mitigation of these diseases requires the combined action of their detection and prophylaxis.

Highlights
• Introduction to the wastewater-based epidemiology strategy
• Assessment of WBE strategy for SARS-CoV-2
• Proposition of SARS-CoV-2 WBE approach in mass vaccination program
• Back tracing approach to identify potential hotspots

Viruses have caused outbreaks of public disasters, such as the 1920s’ “Spanish” pandemic influenza (H1N1) which devoured the life of more than 50 million people (Russell and Webster 2005). Several threatening viral outbreaks have also occurred, such as Ebola, influenza, Zika, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and Crimean-Congo hemorrhagic fever (CCHF).

The world has recently shattered into confusion and disruption over a novel respiratory distress virus that originated in China and triggered a cascade of reactions resulting in a pandemic (Chan et al. 2020). In the last few months, the minds of humanity as a whole have had a sense of immense fear. Scientists are vying, health workers have sleepless nights, and the governments worldwide are on their feet to tackle the outbreak of a novel virus. The causative agent is a positive-sense single-stranded enveloped RNA virus classified later as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or 2019-novel coronavirus (2019-nCoV) (Gorbunova et al. 2020). Coronavirus have the largest genomes among RNA viruses that possess a high risk of broadening pathogenicity due to their distinct replicative
mechanism of obtaining part of the host genome (Woo et al. 2005). Zhu et al. (2020) initially characterized 2019-nCoV from the initial patient samples through multiplex reverse transcriptase polymerase chain reaction (rt-PCR), virus isolation, transmission electron microscopy (TEM), and genome analysis identifying the novel species with distinct RdRp gene of pan β-CoV. Consequently, various assays were developed worldwide from accessible knowledge facilitating its clinical detection worldwide. Tang et al. (2020) suggested the presence of 2019-nCoV in the stool samples from symptomatic and asymptomatic patients, which had a concern about the communicable nature of the virus entering the sewage. The viral load in feces was also higher than respiratory track; however, their infectivity was ambiguous. Pandemic declaration meant the spread of the virus throughout the world, but the clinical assessment of each individual became increasingly impractical. Additional diagnostic tools were therefore needed to evaluate the spread of the virus. The epidemiological wastewater approach (WBE) to illicit drug use was studied in Italy by Zuccato et al. (2005) for surface water to monitor public drug abuse. It was suggested that the work should recognize the limitations of self-reporting the illicit drug use among the public. Chopra and Arora (2020) pointed out that COVID-19 acted similar to tuberculosis in the case of social stigma, where public fear of an unexplored virus leads to discrimination against infected individuals and health professionals. This has led to people hiding their infections, fluctuating in the dynamics of infection, where the actual number of infected individuals in society can remain undisclosed, increasing the potential risk to society as a whole. Hence, WBE work was started quickly, and the research community was flooded with multiple studies detecting 2019-nCoV in wastewaters.

Although several studies have detected 2019-nCoV using the WBE approach, backtracking to an affected precise geographic location still remains a mystery. This raises the question of whether the current approach to the WBE is rationalizing. This article reflects instrumentality of WBE around the globe while trying to ascertain the benefits to society, especially concerning the mass vaccination program.

**Wastewater-based epidemiology for virus surveillance**

Several institutions worldwide have adapted a wastewater-based monitoring tool (WBE) for the analysis of outbreaks involving poliovirus, hepatitis virus, and other enteric viruses, where a proportion has also analyzed the recovery efficiency of their protocols by sampling quantified surrogate viruses. Heijnen and Medema (2011) worked towards influenza A viral detection in sewage where pandemic influenza A (H1N1) was not detected in the samples. However, it was also discussed how a wastewater-based tracking tool is essential and has currently been neglected. Work with several other enveloped and non-enveloped viruses have also been carried out significantly in different parts of the world. La Rosa et al. (2010) carried out a follow-up of the hepatitis E virus (HEV) from wastewater while using feline calcivirus as process control for the entire study to evaluate the recovery of samples and detected the presence of HEV throughout Italy.

**Wastewater epidemiology for 2019-nCoV**

Peccia et al. (2020) suggested that the presence of virus particle in wastewater could not be directly correlated to gain insight into the number of infected individuals in the population. However, a correlation of fluctuation between the 2019-nCoV virus titer and the number of infected individuals has been observed throughout the wastewater samples collected from the sewage treatment plant, indicating WBE can help understand the community transmission dynamics for better preparedness. Similarly, WBE has been employed worldwide to identify the presence of 2019-nCoV in wastewater. 2019-nCoV was recently introduced into the virus inventory, and recovery efficiency through various standard virus purification protocols had to be optimized to improve the wastewater-based monitoring tool. The significant Virus Concentration Protocol Diagnostic Candidacy was promptly initiated. Ahmed et al. (2020a, b, c) implemented seven evidence-based virus concentration protocols that revealed recovery efficacy for murine hepatitis virus (MHV) as a surrogate for 2019-nCoV. The virus adsorption protocol involving MgCl₂ and electronegative membrane filters yielded the highest recovery of MHV seed in the sample and was considered efficient and feasible with low PCR inhibition products co-precipitated.

Ahmed et al. (2020a, b, c) provided the first confirmation of 2019-nCoV detection in wastewater in Australia using the electronegative membrane filtration protocol described earlier. The RT-qPCR products were also sequenced using the sequencing platforms, and positive sample titer was accessed to the deduced correlation with the dynamics of the infected population. However, due to differences in geographic distribution and viral load shedding patterns for 2019-nCoV, this was considered problematic. It was also noted that the exact viral titer in the sample was not deductible as the concentration methods were not evaluated, particularly for 2019-nCoV.

Similarly, the 2019-nCoV WBE was carried in many parts of the world. Westhaus et al. (2020) examined both the solid and liquid phases of wastewater in Germany and the ability of the isolated virus to infect the cell line showing cytopathic effect. Nemudryi et al. (2020) examined the genomic variants using the Oxford Nanopore NGS platform and observed the phylogenetic assessment of viral strains. Rimoldi et al. (2020) in Italy, Medema et al. (2020) in the Netherlands, Gonzalez et al. (2020) in the USA, Hata et al. (2020) in Japan, Trottier
et al. (2020) in France, D’Aoust et al. (2021) in Canada, Manupati et al. (2020), Kumar et al. (2020) and Arora et al. (2020a, b) in India, and others were involved in WBE using analog virus concentration protocols, rt-PCR assays, and confirmatory sequencing the rt-qPCR products. Huang et al. (2021) tested anal swab and surrounding samples from the hospital with infected individuals to conclude patients’ release of viral particles as an indicator of the possibility of transmission. Gonçalves et al. (2020) in Slovenia examined the presence of 2019-nCoV in hospital wastewater, while Zhang et al. (2020) revealed the inefficiency of current disinfection protocols to remove 2019-nCoV from hospital wastewater. Hasan et al. (2020) conducted studies at 11 STPs and 38 wastewater access points (manholes) in the UAE, and Ahmed et al. (2020a, b, c) in Bangladesh focused on drainage water near the 2019-nCoV Isolation and Care unit to conclude the presence of 2019-nCoV.

Discussion

Monitoring and back tracing infection hotspots

Numerous articles have identified the presence of 2019-nCoV in the fecal matter of infected individuals as the virus finds a perfect niche in the human intestine owing to the presence of ACE2 receptors (Wang et al. 2020). Recently published WBE monitoring articles and research papers discuss concentration protocols suitable for concentration of viruses and molecular diagnosis focusing on 2019-nCoV. Due to the current pandemic situation of 2019-nCoV, the ubiquitous presence of 2019-nCoV in the world is not difficult to conclude. Therefore, it can easily be deciphered that most of the world’s sewage treatment plants could carry detectable virus genetic material. Thus, most of the studies detecting 2019-nCoV using WBE approach targeted sewage treatment plants (STPs) and drainage system associated with COVID-19 Care Units and Hospitals. However, the WBE articles only discussed the presence of viral genetic materials in untreated wastewater while at the same time assessing the efficacy of STP in the removal of viral particle confirmed by diagnostics. The trailing for the precise geographical locations for a higher infected population was not discussed in the best of our knowledge in any article. An advantageous result for WBE could be the backtracking of precise geographic areas.

Wastewater risk and informing public

A well-planned drainage system leaves no trace of common public or animals coming into contact with wastewater. However, many parts of the world do not have access to clean water or appropriate civil planning where the population can come into contact with wastewater, the water which has demonstrated presence of a high 2019-nCoV viral title in a number of research articles published worldwide. Infective competence study of 2019-nCoV from wastewater samples obtained from the STP or river system is also important. There are studies showing the course of virus titer in infected patients; however, the infectivity competence study from WBE remained concerned with probable future studies. The infectivity competence study could practically help us to monitor new emerging infections, while also prioritizing the required infectivity and source-trailing studies (Walsh et al. 2020). Although Rimoldi et al. 2020 studies have shown a result of cytopathic effect of 2019-nCoV on the VERO E6 cell line, with additional infection dynamics of 2019-nCoV being obtained, regular observations are also important.

This WBE tool may also be considered to prioritize people in the vicinity of wastewater for vaccination, as the virus does not discriminate between human-made divisions or classes, but may infect any individual who may act as a future transmission agent. Therefore, the WBE tool can also be used to provide the public with sufficient knowledge that can be made aware of wastewater risks, even if the infectivity of the virus present in wastewater is unclear.

Recovery efficiency of 2019-nCoV for WBE and public health burden

The quantification of the virus titer has an important aspect since it can be linked to the increasing infection dynamics in the population. However, the recovery of the virus from wastewater through the current standard virus concentration and recovery protocols, specifically for 2019-nCoV, was not reached as the positive results obtained confirmed the presence of the virus and, taking into account the need for an hour, the quantification assessment was considered possible for future results. Thus, the recovered virus titer mentioned in the entire 2019-nCoV wastewater literature would not be sufficient to track population dynamics and can only be useful for comparative analysis of areas with the most affected population. 2019-nCoV is a newly emerged virus infection, and many long-term health effects are uncertain at this point, but long-term health effects have been suggested as infection-cured people also complain about persistent health problems (Del Rio et al. 2020). Therefore, it is necessary to use information from WBE tool to avoid future health burdens among the population and inform public about the dangers of infections arising from wastewater.

Tracking genomic variants using sequencing platforms

The articles published for 2019-nCoV WBE mention virus titer, but variants in strains have not been studied with the best of our knowledge except for Nemudryi et al. (2020).
Mercatelli and Giorgi (n.d.) suggested that 2019-nCoV had developed into competent infectivity in humans and thus showed a low variability in genome mutations, while it was also noted that the presence of multiple strains circulating in the world had been observed when their genome was studied. However, there has been a recent addition of novel strains, such as those from the UK, which caused havoc as the data predicted the presence of strains from mid-September with increased infectivity capabilities (Wise n.d.). This determines the importance of genome sequencing to track the evolution of the virus in real time while accessing the pandemic situation. However, a WBE tool, along with sequencing platform such as Oxford Nanopore Sequencing, which does not require amplification of the sample’s genomic material (Lu et al. 2016), can also be effectively included to track new variants entering wastewater. As a WBE tool can provide a pathway to infection dynamics, the virus isolated from wastewater can be traced to genomic variants and affected areas to increase our preparedness. The initiative can be used for WBE monitoring and sequencing as studies by Peccia et al. (2020) have shown that clinical data lag behind wastewater monitoring data.

**Assessment of priority areas for mass vaccination**

This current viral pandemic of 2019-nCoV calls for an emergency response to mass vaccination as an effort to better the well-being and survival of humanity. The discussed WBE strategy can be utilized to evaluate the primary concerns of the approach. The well-planned STPs have water sources from surrounding areas, and therefore, a tracking tool can be used to track the source endpoint for access to areas containing the largest number of infected individuals. However, the areas with open access to the drains can still be at considerable risk and can also be considered as a priority for vaccination. The WBE approach may be useful, especially when approaching the mass vaccination program to eradicate 2019-nCoV.

A sample of the water body and the STPs can be tested for viral presence. The source of the virus can be traced back to its origin. As shown in Fig. 1, a schematic of a fictional system can be suggested. If the sampling point 1 is positive for 2019-nCoV, the rest sampling points can be tested. In sampling points 2 and 3, if point 3 is positive, area 2 may be considered safe. However, sampling point 3 may be traced to area 1 and

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**Fig. 1** Schematic illustrating use of WBE for determination of vaccination priority area.
area 3 for a positive viral source by sampling points 4 and 5, which may conclude viral presence in area 3. Area 3 can therefore be considered a priority for mass vaccination.

**Conclusion**

The study of a wastewater-based tracking tool carried out around the world can therefore be hailed as an essential aspect of the study of population dynamics of infectivity, as it can provide real-time information on viral loads present in the wastewater system. However, it is crucial to examine the source of higher viral load and the infectivity capacity of virus entering the system through the endpoint trailing to determine the geographical locations most affected. The presence of distinct variants in wastewater can also be analyzed in order to understand genomic variations in order to track the evolutionary aspects of the novel virus, while at the same time considering active public participation offering knowledge of preventive measures. The scope of improvement in the WBE approach may also help to deliver extensive knowledge to help with prioritizing the geographical areas for vaccination. The wastewater-based tracking tool has an immense potential to deliver value-added knowledge that has barely been exploited.

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**Declarations**

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