Estimation of the number of SARS-CoV-2 Infections in a Sewershed Based on Wastewater-Based Epidemiology

Yifan[1] Z, Wakana[2] O, Chikako[2] M, Masaaki[3] K and *Daisuke[1,2] S

[1]Department of Frontier Sciences for Advanced Environment, Graduate School of Environmental Studies, Tohoku University, Japan

[2]Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Japan

[3]Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, Japan

*Corresponding author – daisuke.sano.e1@tohoku.ac.jp

Abstract

The importance of wastewater-based epidemiology (WBE) has recently gained global attention, since scientific reports indicate that the concentration of viruses in wastewater is closely linked to the number of patients in a sewershed. WBE is thus expected to be a powerful tool in the current COVID-19 worldwide pandemic for epidemic surveillance and decision-making support. However, the feasibility of this approach remains unclear due to the inherent uncertainty and insufficient understanding of the factors involved. In this study, by taking aspects such as population size, sewage network layout, environmental conditions, and virus detection efficiency into consideration, we developed a mathematical model for estimating the number of infected individuals in the sewershed based on WBE. To test the sensitivity of the model, the number of infected individuals required for successful detection was determined using the Monte Carlo simulation.

Keywords: Epidemic surveillance, Biomarker, Viral shedding, Monte Carlo simulation

1. Introduction

The COVID-19 global pandemic, resulted from the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused tremendous losses in both global public health and the economy. As of August 18th, the pandemic has claimed the mortality of 767,158, and 21,549,706 confirmed cases have been reported worldwide, according to the WHO database [1].

Since the beginning of the COVID-19 prevalence, the importance of a proper surveillance system in combating an emerging epidemic has been picked up by authorities, and various actions on different scales have been implemented for that purpose. Epidemic surveillance can serve multiple functions, including

prevalence anticipation, early warning, and policy-making support [2].

However, conventional approaches, such as symptom-based screening and large-scale clinical testing, are not efficient enough when the society faces the unprecedented COVID-19 pandemic due to their inherent limitations. For symptom-based screening, one big issue is its tendency to overlook asymptomatic SARS-CoV-2 carriers [3], which counted for 56% of the patients tested positive in one study [4]. Also, it was estimated that the median incubation period of COVID-19 is 5.1 days [5], meaning that there will be a 5-day lag for symptom-based screening, which makes it significantly less responsive given the highly contagious nature of COVID-19 [2,6]. Although the insufficient coverage over asymptomatic and presymptomatic carriers can be overcome by large-scale clinical testing, financial, logistics, and sociological problems may become the new concerns. Hart and Halden (2020) estimated that USD 3.5 billion worth of reagents alone would be needed just to test the entire U.S. population once by real-time RT-PCR (RT-qPCR). Not only is the testing cost beyond the reach of many less-developed countries, but the derived logistic problems during the execution are also a huge challenge to tackle. Besides, high-strength individual clinical testing can cause some secondary problems, like the drastically increased exposure risks of frontline health workers [8], and the ethical concerns about the anonymity of the test [9]. There is an urgent need for an epidemic surveillance method that can complement the clinical testing and provide timely, open, and reliable information about the population-level epidemic status, which is of vital significance in eliminating misinformation and taking effective countermeasures.

Wastewater-based epidemiology (WBE) aims at extracting population-wide health information at a low cost by measuring chemical or biological agents, also called biomarkers, in wastewater. It was originally proposed for tracking illicit drug usage [10,11], but the applications have recently been expanded to lifestyle factors such as alcohol [12], caffeine [13], and pharmaceutical products [14]. Seeing the capability, the potential of WBE in tackling the challenge of COVID-19 surveillance has been under active discussion among the researchers specializing in water-related topics [8,9,15]. Despite mainly being recognized as a respiratory tract disease, COVID-19 can cause persistent shedding of viral RNA via the digestive system, symptomatically and asymptomatically alike, which may even predate the onset of symptoms and outlast the resolution of them, making it a promising biomarker [16,17]. As a proof of concept, the viral RNA of SARS-CoV-2 has been detected in the sewage samples from many parts of the world [18,19], and a correlation between the detected SARS-CoV-2 concentration and clinically confirmed cases in the sewershed has been found [20].

In this study, we evaluated the applicability of WBE to COVID-19 epidemic surveillance. We first tested the detectability of viral RNA in wastewater, then performed a Monte Carlo simulation to estimate the sensitivity of the detection (i.e. the threshold number of infected individuals for successful detection), and through sensitivity analysis, factors having the most significant impact on the result were determined as well.

2. Methodology

2.1 Wastewater treatment facility

In this case study, we selected a wastewater treatment plant located in Sendai, Japan for its representativeness and data availability. The plant uses a conventional activated sludge process and the average daily flow is about 400,000 m^3/day, covering an area of 109 km^2 and a population of about 750,000.

2.2 Calculation of detection sensitivity

The following equations are used to calculate the sensitivity of the method used in this study.

The initial viral load is calculated by dividing the estimated total amount of viral RNA discharged from all individuals infected by COVID-19 in the sewershed by the flow rate at the treatment plant.

$$C_0 = \frac{N_I \cdot S_1 \cdot S_2 \cdot E_f}{Q} \qquad (1)$$

, where C_0 is the initial viral load in wastewater, N_I is the total number of infected individuals, S_1 is the ratio of shedding among infected individuals, S_2 is the shedding rate, E_f is the average mass of feces excreted by one person per day, and Q is the average daily flow rate.

The viral RNA is expected to go through degradation and decay during the travel in sewage pipes, the extent of degradation is determined by the half-life of RNA at the estimated wastewater temperature, and the average travel time wastewater spends in the sewage network until it reaches the sampling point, in this study, the treatment plant.

$$C_t = C_0 \cdot \left(\frac{1}{2}\right)^{\frac{t}{t_{1/2}}} \qquad (2)$$

, where C_t is the viral load in wastewater adjusted for degradation, t is the average travel time of wastewater in the sewer line, and $t_{1/2}$ is the half-life of viral RNA in wastewater.

The degradation rate of the biomarker is generally related to temperature, thus the half-life is adjusted using the following equation obtained from Hart and Halden, (2020).

$$t_{\frac{1}{2},T} = t_{\frac{1}{2},0} \times \frac{\ln(2)}{\ln(2) \times Q_{10}^{\left(\frac{T_2 - T_1}{10}\right)}} \qquad (3)$$

, where $t_{1/2,\,T}$ is the half-life of viral RNA adjusted for wastewater temperature, $t_{1/2,\,0}$ is the half-life of viral RNA at benchmark temperature, T_1 is the benchmark temperature, T_2 is the wastewater temperature, and Q_{10} is the factor of rate change.

Finally, the concentration of viral RNA determined by RT-qPCR is the product of viral RNA concentration (adjusted for degradation) and the recovery efficiency of the experimental method, which is a multi-step process including concentration, RNA extraction, cDNA synthesis, and qPCR.

$$C_d = C_t \cdot R \qquad (4)$$

, where R is the recovery efficiency, and C_d is the viral load measured by RT-qPCR. By determining the lower limit of C_d using experimental methods, the threshold value of N_I can be calculated.

2.3 Calculation of detection sensitivity

The Monte Carlo simulation was performed using the R statistical software. The distributions and fixed parameters used in the simulation are specified in Table 1 and Table 2.

Table 1: Distributions used in Monte Carlo simulation

Parameter	Distribution type	Coefficients	Reference
S_1	Uniform	Low 40.5%, high 53.9%	[21,22]
S_2, copies/g	Log_{10}-normal	Mean 7.47, sd 0.25	[23]
E_f, g/day	Log_{10}-normal	Mean 2.11, sd 0.25	[24]
Q, m³/day	Normal	Mean 400,000, sd 20,000	Sewage work utility
T_2, °C	Normal	Mean 25, sd 1	Sewage work utility
t, h	Normal	Mean 3.3, sd 0.3	[25]
R	Uniform	Low 1.39%, high 4.12%	Experimental result

Table 2: Fixed parameters used in the Monte Carlo simulation

Parameter	Value	Reference
$t_{\frac{1}{2},0}$, h	8.53	[26]
T_1, °C	23	[26]
Q_{10}	2.5	[27]
C_d, copies/mL	1.8	Experimental result

The simulation was run by 100,000 times. The recovery efficiency of viral RNA from sewage was determined by experiment using spiked heat-inactivated SARS-CoV-2 via PEG precipitation method.

3. Results
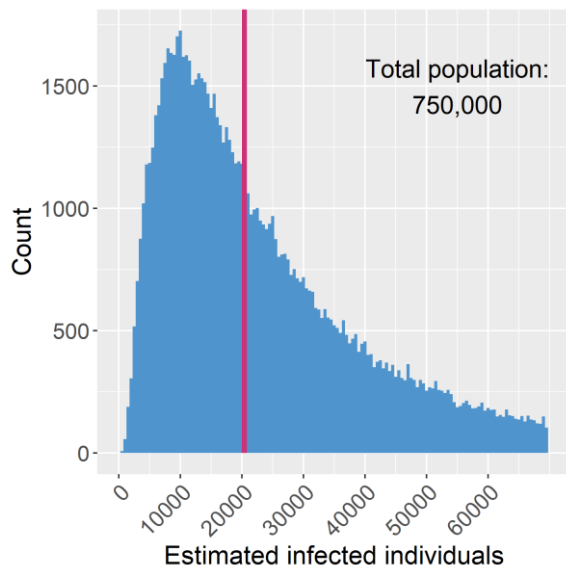
Figure. 1: The Monte Carlo simulation of the infected individuals needed for a positive qPCR detection under the current experimental setting. The cut-off value of estimation results is set to 70,000 as ~90% of the results fall in the range of 0-70,000. The median value is marked by the red vertical line.

The result of the Monte Carlo simulation is shown in Figure 1, which shows that based on our current experimental method, the hydrological condition of the selected sewage network, and the available data about the fecal shedding profile of COVID-19 patients, to get a positive result in qPCR, the most probable number of infected individuals falls within the range of 9,750-10,250 while the median value is 20,370.76 (95% confidence interval (CI), 20,218.98 to 20,507.13). It is worth noting that this value also includes the infected individuals who do not shed viral RNA in feces. Considering that the number of residents living in the studied sewershed is about 750,000, the result indicates that if judged by the most probable value, the threshold prevalence in the sewershed is one infected individual in roughly 75 residents (1.33%).

The following sensitivity analysis reveals that among the variables that come with uncertainty or fluctuation, the viral RNA shedding rate and recovery efficiency are the two factors that have the most significant impact on the detection sensitivity. On the other hand, the impact of wastewater temperature and flow rate is of minor importance.

4. Discussion

The estimated sewershed-wide COVID-19 prevalence level needed for the detection method to be functional (~10,000 in 750,000 residents, or 1.33 %) indicates that WBE may be useful in medium and high prevalence regions. For instance, a seroprevalence surpassing 10% has been reported in some countries that advocate herd immunity [28], indicating a high prevalence level and WBE may contribute to the monitoring of the epidemic there. More optimistic estimations of the viral RNA detection sensitivity from wastewater have been reported by previous studies [7,18], but some key factors, notably the recovery efficiency, natural degradation of the viral RNA, and the fraction of infected individuals who do not shed viral RNA, were often left out for either simplicity or the lack of reliable data, making the results less realistic. In this study, by conducting

experiments using heat-inactivated SARS-CoV-2 particles, the recovery process better mimics the real-world condition.

Admittedly, there is still room for improvement in detectability and reliability. The shedding rate used in this study is the observed peak value after the onset of symptoms, but considering the presence of presymptomatic transmission, there exists the possibility that viral load early in the infection period can be higher than the recorded value. The recovery efficiency measured in our study is comparable with one previous study [29], in which the PEG precipitation method was employed for SARS-CoV-1 concentration and the average efficiency was 1.02%. However, higher recovery efficiency using other concentration methods such as ultrafiltration has been reported using murine hepatitis virus as the surrogate of SARS-CoV-2 [30]. Since recovery efficiency greatly affects the result of the Monte Carlo simulation, we expect that measures aiming at improving it will greatly enhance the detection sensitivity and eventually, the feasibility of the WBE approach. It has been stated that enveloped viruses are more likely to adsorb to wastewater solids [31], and in one of our trial experiments, viral RNA was successfully recovered from the solids pellet that was supposed to be disposed of during the concentration process, thus we are currently working on developing a methodology for recovering viral RNA from both the liquid and solid phases of the sewage sample.

It is worth noting that in a complete WBE framework, the successful detection of the biomarker is merely the first step. The ultimate goal, and also the biggest challenge, as highlighted by previous studies, is to establish a reliable quantitative relationship between the measured biomarker concentration and the number of infected individuals [8,9,15,18,20]. If we take one step further, the inferred number of infected individuals can be incorporated with statistical epidemic models such as the Susceptible-Infected-Recovered (SIR) model

to contribute to epidemic prediction. In order to get there, substantial efforts are needed to develop a much deeper understanding of the various aspects involved, including the patients' shedding profile, the environmental persistence of SARS-CoV-2 RNA, the hydrological modeling of the target sewershed. Also, advanced epidemic dynamics modeling techniques would be a critical factor. As more attention and resources are being directed to WBE, we expect the expertise and collaboration of researchers from different disciplines and regions to contribute to tackling this global health conundrum faced by humanity.

5. Conclusion

Based on our estimation of the prevalence level required for wastewater viral RNA detection (1.33%), we conclude that WBE stands a chance to complement conventional clinical testing and enable valuable population scale epidemic monitoring. However, as the current knowledge base is still at an incipient stage, more studies need to be conducted to push this approach forward.

Acknowledgments

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