Possibility of Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) through Wastewater in Developing Countries

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Abstract: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was detected in Wuhan, China. The clinical manifestation of COVID-19 varies from asymptomatic to severe infection. The World Health Organization has reported over 248 million cases and more than 5 million deaths worldwide due to COVID-19. Additionally, developing countries are still struggling to achieve the rapid diagnosis of suspected cases to break the chain of transmission of COVID-19. There may be undiagnosed or missing cases in these countries. Various studies have reported that the SARS-CoV-2 can be discharged in the stool and other bodily fluids. Moreover, it is possible that SARS-CoV-2 can be transmitted in the environment via municipal wastewater. In developing countries, such as Nepal, the molecular-based diagnosis of SARS-CoV-2 for the entire population is not practical owing to insufficient diagnostic material and budgetary constraints. Wastewater-based epidemiology (WBE) is a promising public health surveillance tool for the early warning of disease outbreaks and the understanding the prevalence of viruses for the development of intervention measures. This study highlights the importance of the utilization of WBE as an alternative tool for the mass detection of SARS-CoV-2 RNA in wastewater.

Keywords: COVID-19; Nepal; SARS-CoV-2; wastewater-based epidemiology; wastewater treatment plant

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified by the World Health Organization (WHO) as the causative agent for the coronavirus disease 2019 (COVID-19) following genome sequencing performed by the Chinese authorities [1–3]. Owning to its rapid transmission, on 11 March 2020, the WHO declared the outbreak as a global pandemic [4]. Over 248 million people in more than 221 countries/regions have been impacted, and approximately 5 million deaths have been confirmed, as of 4 November 2021 [5]. It was reported that the virus is transmitted solely via humans by means of respiratory droplets and close contact [2,6–8]. Nevertheless, a few studies have reported the possibility of fecal-oral transmission [9–11]. In case of the COVID-19, the major symptoms are associated with the respiratory and gastrointestinal tract [12,13]. Furthermore, the virus was also found in the stool of an asymptomatic child in China with a negative laboratory result from nasopharyngeal specimens [14] Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is the best method for the detection of

SARS-CoV-2 RNA in oral swabs as well as in stool samples. Despite the development of vaccine and prevention strategies for COVID-19, approved clinical treatments are still a matter of further investigation [15].

Nepal is a landlocked country with China on the northern side and India on the east, west, and south, with an estimated 1800 km of open border. The country is considered to be one of the least developed countries, with poor healthcare and sanitation metrics. It is considered to be among the highest risk areas for the pandemic. In Nepal, the first case of COVID-19 was recorded on 13 January 2020, when a Nepalese student at Wuhan University of Technology, Wuhan, China, with no previous history of comorbidities, returned to Nepal [16]. By 4 May 2020, the total number of cases reached 75, which was validated from 14 different districts of the geographical regions of Nepal. After receiving a huge number of test kits and RT-PCR reagents from foreign countries, the government strategies for the diagnosis of SARS-CoV-2 expanded from the affected areas to the entire country. After this alteration in diagnostic approach, the number of infected cases increased slowly. However, the number of diagnostic materials is still insufficient owing to the lack of manufacturing companies in the country.

Studies have indicated that intestinal infections are common in patients infected with SARS-CoV-2 and MERS-CoV [17,18]. Similarly, recent studies showed that SARS-CoV-2 RNA was also detected in stools of patients with COVID-19 in China [10,11,19–22]. These results were validated by the use of RT-PCR, with no indication of whether the virus was infectious. The virus was isolated from a stool specimen of a laboratory-confirmed case of COVID-19 in China [19–21]. and was further validated by isolation with the use of Vero cells from stool samples. Viral particles with the typical morphology of a coronavirus could be seen under an electron microscope [19]. In a similar manner, the study of Zheng et al. (2020) found SARS-CoV-2 in 59% of stool samples and identified a higher viral load in stools than in respiratory samples [23].

In another study, stool samples from 53.42% of the patients were positive for SARS-CoV-2 RNA as determined by RT-PCR [24] with 23.29% of the patients testing positive in the feces even after the viral RNA lessened to an undetectable level in the respiratory tract [24]. Another study also reported that the detection rate of SARS-CoV-2 from stools was higher than that of other clinical specimens [25] Thus, the possibility that SARS-CoV-2 may be spread via fecal excretion cannot be disregarded. Therefore, the importance of high-sensitivity detection in stool samples in the prevention and control of the SARS-CoV-2 epidemic requires comprehensive and careful assessment.

In 2003, a study on SARS proposed that sewage was implicated in the infection of a cluster of cases in the Amoy Gardens apartment block in Hong Kong [26]. In this study, it was found that SARS-CoV could survive in sewage for 14 days at 4 °C and for two days at 20 °C and that its RNA could be detected for eight days even if the virus had been inactivated [26]. Moreover, the transmission routes of SARS-CoV-2 are still unknown, and there has been no clear determination of the length of viability and persistence in wastewater of SARS-CoV-2 [19–21,27,28]. This study focuses on the global experience of the detection of SARS-CoV-2 RNA in wastewater and potential use of wastewater-based epidemiology (WBE) as an additional diagnostic tool for an early warning system in developing countries. Considering effectiveness of WBE as an early warning system, globally, researchers and policymakers are nowadays focusing on the development of framework for the early detection of SARS-CoV-2 RNA in wastewater and its possibilities for future environmental surveillance and monitoring.

2. Basic Characteristics of SARS-CoV-2

Coronaviruses are members of the family *Coronaviridae*, which comprises four genera as follows: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. *Beta-coronavirus* is considered an important genus since all the pathogenic viruses, including SARS-CoV-2, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV, are in this group [23,29]. SARS-CoV-2 is an enveloped virus consisting of a single-stranded

and non-segmented positive-sense ribonucleic acid [15]. It ranges from 80 to 160 nm in length and has a genome of 27–32-kbp [30] With the advancement of the genetic technique, sequence analysis of newly discovered SARS-CoV-2 has almost 79.6% similarity with SARS-CoV, and whole-genome sequencing revealed 88% resemblance with two bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) [31,32]. In contrast, phylogenic analysis indicated that it was genetically different from SARS-CoV and MERS-CoV [32].

3. Brief History and Disease Mechanism

The outbreak of SARS in 2003, which was presumed to be the first global pandemic coronavirus cases in history, impacted 8096 people in approximately 30 countries, with a resulting 774 deaths [33]. Two prototypes of human coronaviruses, HCoV-OC43 and HcoV-229E, were reported prior to SARS. These strains of viruses were also considered highly pathogenic to humans [34]. This evidence indicated that the SARS viruses are not novel in humans; they have been circulating among them via several sources of animals. The outbreak of another strain of SARS-CoV was reported in 2012 from Saudi Arabia; this was later named as MERS-CoV, based on the location of where the virus appeared. MERS-CoV resulted in 856 deaths from 2494 confirmed cases of 2494 [35]. Subsequently, at the end of December 2019, an outbreak of unknown flu-like symptoms with pneumonia in Wuhan was reported; this was later named COVID-19. Studies have shown that the causative agent of this new outbreak was of the same family of SARS-CoV and that the full-length genome sequence was almost identical, sharing 79.6% sequence similarity to SARS-CoV [31]. The origin and spread of SARS-CoV-2 was initially suspected from Huanan Seafood Market, but many studies are still under progress to find the actual route of the outbreak [2].

The disease mechanisms of SARS-CoV-2 are still under investigation. Because of the structural similarly of the spike proteins of SARS-CoV-2 and SARS-CoV, SARS-CoV-2 is expected to enter human cells via the angiotensin-converting enzyme 2 (ACE2) receptors [27,31,32,36]. The protein ACE2 contains 805 amino acid units and has two functional domains [37]. The ACE2 genes provide a receptor molecule for three strains of coronavirus, including SARS-CoV-2 [27]; nonetheless, the mechanisms underlying the disease pathogenicity of COVID-19 can be studied. Viruses usually carry a spike protein (receptor-binding Domain), enhancing the binding affinity with the host cell receptor, namely ACE2 for SARS-CoV [19–21]. After complete attachment, proteolysis of the spike protein occurs. Following several steps of proteolytic cleavage [36]. SARS-CoV-2 enters host cells through endocytosis/penetration and starts to replicate. Ultimately, the infected host cell releases a large number of virions outside through exocytosis [30]. Apart from ciliated bronchial epithelial cells and type-II pneumocytes, the ACE2 receptor is abundantly expressed in the heart, kidney, endothelium, and intestine [19–21,29,38,39].

4. Significance of Detection of SARS-CoV-2 in Wastewater

The detection of infectious viruses from wastewater is not a novel approach; this technique has been applied to trace sources of contamination and to estimate disease status in the community [40,41]. The evaluation of wastewater may provide an effective means of predicts of the potential spread of the virus. Moreover, interpretation of positivity for COVID-19 from wastewater sample is still challenging. It is more appropriate to correlate the findings with clinically confirmed cases from the same site to generate valuable information [42]. Because of this, many researchers have already applied WBE to identify the early stages of viral infection in communities, enabling public health authorities to implement effective intervention as early as possible, such as restricting the movements of the local population. Various studies have been performed to quantify as well as identify prevalent waterborne pathogens that can cause gastroenteritis from the environmental samples [40–43]. Millions of copies of viruses are excreted from the infected patient; thus, to determine the possible incidence of disease, viruses are also assessed from wastewater

treatment plants (WWTPs). The detection of SARS-CoV-2 RNA in wastewater currently consists of concentration methods, such as electronegative membrane adsorption-direct RNA extraction, ultrafiltration, polyethylene glycol precipitation, and ultracentrifugation, followed by RT-qPCR, such as N_Sarbeco, NIID_2019-nCOV, and CDC N1, N2, and N3 assays [44–49]. Moreover, studies have been conducted to find the most suitable concentration and detection methods for the analysis of SARS-CoV-2 in wastewater [45,50–52].

5. Evidence of the Presence of SARS-CoV-2 in Wastewater

A recently conducted study in Australia estimated the number of individuals infected with SARS-CoV-2 by applying Monte Carlo simulations to RNA copy numbers detected in wastewater [44,45]. In another study conducted in the Netherlands, sewage samples collected from seven cities and airports were analyzed by RT-PCR. This study reported the presence of SARS-CoV-2 RNA in sewage when there were a low number of cases in that population [46]. The detection of the incidence of SARS-CoV-2 via molecular diagnosis in wastewater samples was also reported in the USA [47,48]. An article published in *Nature* [49] emphasized the importance of the analysis of SARS-CoV-2 RNA in sewage to prevent future consequences related to COVID-19. Because SARS-CoV-2 RNA can be detected in wastewater as soon as three days after infection, assessing wastewater may be an alternative tool to extract data related to disease presence [49]. A study conducted by Wu et al. (2020) proposed that the feasibility of measuring SARS-CoV-2 RNA in wastewater could provide population-level estimates of the burden of COVID-19 [48].

The detection, molecular characterization, and reduction efficiency of pathogenic viruses at WWTPs have often been reported [53,54] and a study already reported the reduction efficiency of SARS-CoV-2 during wastewater treatment [55,56]. Furthermore, prior studies indicated outbreaks of viral diseases resulting from insufficient wastewater treatment [57,58] driving the development of more advanced treatment technology. In the Kathmandu Valley, the lack of sanitation and inadequate wastewater collection, treatment, and solid waste collection and disposal contribute to the devastating contamination of surface and groundwater contamination [41,43]. SARS-CoV-2 is normally transmitted either via direct contact from infected individuals or via droplets generated by them during coughing, sneezing, and talking [59]. There is a higher chance of the production of small droplets if these viruses are shed into feces/urine and discharged in wastewater. SARS-CoV-2 is transmitted to natural bodies of water either via human fecal matter or via animal sources. To obtain more information as regards viral etiological agents and possible transmission factors in the environment, studies should be performed with larger sample sizes and should consider a wider variety of risk factors to point out important barriers to safe water access and to act as an appropriate warning system for the government.

The examination of SARS-CoV-2 should be conducted to evaluate the potential health risk associated with the occurrence of this virus in wastewater. In developing countries, untreated raw wastewater is frequently used for irrigation in agriculture; consequently, this wastewater may be a serious threat to human health. However, a high incidence of viruses does not equal the presence of these viruses in that community, but it could provide consequences of prior or future disease scenarios. Most of the transmission of SARS-CoV-2 occurs through aerosols/droplets; nevertheless, the currently published studies proposed that wastewater may be a possible route of transmission of SARS-CoV-2 in the environment [44,45,50]. There is growing evidence of gastrointestinal symptoms due to SARS-CoV-2 infections and the presence of viral RNA not only in the feces of patients with COVID-19 but also in wastewater [43–46,50]. The studies that have reported the successful detection of SARS-CoV-2 RNA from both untreated and treated wastewater are summarized in Table 1.

Table 1. RNA from both treated and untreated wastewater.

Sampling Location		Water Type	Virus Detection Methods			Detection Results		Reference
Country	State/City		Virus Concentration Method	qPCR Assay	Sequence Confirmation	Positive Rate	Maximum Concentration (Copies/L)	
Brazil	Niteroi, Rio de Janeiro	Raw sewage	Ultracentrifugation	CDC N2	Whole genome sequencing	188/223 (84.3%)	1.2×10^7	[60]
India	Hyderabad	Raw sewage	Centrifugation	E-gene, N-gene, ORF1ab	Not done	14/14 (100%)	2.4×10^4	[61]
		Treated wastewater	Centrifugation	E-gene, N-gene, ORF1ab	Not done	0/14 (0%)	Not available	
		Residential effluent	Centrifugation	E-gene, N-gene, ORF1ab	Not done	2/2 (100%)	Not available	
	Ahmedabad, Gujarat	Raw wastewater	PEG precipitation	S-gene, N-gene, ORF1ab	Not done	2/2 (100%)	3.5×10^2	[62]
		Final effluent	PEG precipitation	S-gene, N-gene, ORF1ab	Not done	0/2 (0%)	Not available	
Australia	Queensland	Untreated wastewater	Electronegative membrane-direct RNA extraction, ultrafiltration	Sarbeco, NIID	Sanger sequencing, MiSeq Illumina sequencing	2/9 (22%)	1.2×10^2	[44,45]
The Netherlands	Amsterdam, The Hague, Utrecht, Apeldoorn, Amersfoort, Schiphol, Tilburg Milan, Rome	Untreated wastewater	Ultrafiltration	CDC N1, N2, N3, E_Sarbeco	Not done	20/29 (69%)	2.2×10^6	[47]
Italy		Raw sewage	PEG precipitation	ORF1ab, S-gene, RdRP	Direct sequencing	6/12 (50%)	Not available	[63]
Japan	Kofu	Raw wastewater	EMV, Adsorption-direct RNA extraction	Sarbeco, NIID, CDC N1, N2	Not done	0/5 (0%)	Not available	[51]
		Secondary-treated wastewater	EMV, Adsorption-direct RNA extraction	Sarbeco, NIID, CDC N1, N2	Not done	1/5 (20%)	2.4×10^3	
		River	EMV, Adsorption-direct RNA extraction	Sarbeco, NIID, CDC N1, N2	Not done	0/3 (0%)	Not available	
France	Paris	Raw wastewater	Ultracentrifugation	E-gene	Not done	23/23 (100%)	$>10^{6.5}$	[64]
		Treated wastewater	Ultracentrifugation	E-gene	Not done	6/8 (75%)	$\sim 10^5$	
USA	Bozeman, Montana	Untreated wastewater	Ultrafiltration	CDC N1, N2	Sanger sequencing	12/12 (100%)	$\sim 10^{4.5}$	[65]

6. Importance of Monitoring at WWTPs in the Developing World to FulFill Knowledge Gap

Wastewater is a serious environmental concern because of the hazards arising from discharging poorly treated pollutants to the environment [56,66]. It is impossible to implement screening and surveillance of RT-PCR/qPCR-based assays in the entire population, especially in resource-poor countries. Nonetheless, wastewater analysis could be an effective way to manage viral transmission by carriers because the number of cases is increasing slowly, and even asymptomatic carriers or cases have been detected worldwide. These asymptomatic carriers are neither routinely tested nor kept in isolation. It can be said that the prevalence of asymptomatic infection and the detection of pre-symptomatic infection are not well understood. Owing to these extreme situations, the mechanism of disease transmission is still not well understood. Furthermore, many studies have reported the transmission of SARS-CoV-2 via asymptomatic carriers through person-to-person contact [67–70]. The asymptomatic carriers may be potential sources of free shedding of the virus into the environment and enhance the potential transmission of diseases within the community [71,72]. Therefore, identifying the patients and finding the 6 symptomaticc carriers to break the chain of transmission are essential; however, these practices are time consuming, require well-trained personnel, and require expensive reagents. A previous study reported that the load of SARS-CoV-2 detected in asymptomatic and symptomatic patients was similar, but it was higher in extreme cases than those of both asymptomatic and symptomatic patients [1].

Owing to the inadequate treatment plants and unorganized population planning in developing countries [73–77], the direct disposal of wastewater produced by households, industry, hospitals, and institutions to a nearby river is common. In Nepal, waste-related decisions are made without much thought, which is a major issue inducing many diseases [78]. The practice of WWTPs is still inadequate, and moreover, within the Kathmandu Valley, there are only five WWTPs [79]. In the valley, a sewerage system is provided only for 15% of the houses [80]; this has naturally resulted in major problems associated with managing the wastewater produced by the entire city [81]. Moreover, the WBE method is less approachable in these countries, with a limited number of treatment plants. Therefore, more studies are needed to predict the suitability of the WBE method before its implementation. Wastewater can transport many pathogens depending on the incidence of disease in the community and animal population [80]. These viruses, including SARS-CoV-2, can survive in wastewater systems; however, the decay rate of these viruses may vary on several environmental factors, such as temperature, pH, solids, and micropollutants, and disinfectant used [82,83]. The detection of SARS-CoV-2 RNA in influent has been reported in several studies [43–48] as has that in treated wastewater [63]. Thus, a WBE is an approach to monitor wastewater for early detection of COVID-19 infection in the particular community.

7. Public Health Importance

7.1. Urgent Need for Alternative Viral Detection Methods

Worldwide, only 32% of individuals infected with SARS-CoV-2 seek medical attention [84]. The findings of COVID-19 studies have shown that 17.6% of patients with COVID-19 patients have gastrointestinal symptoms, and 48.1% of patients have SARS-CoV-2 RNA detected in their feces [85]. Public health bodies always prioritize cost-effective interventions. The application of WBE in the handling of COVID-19 has primarily relied on the use of costly, time-consuming PCR technology to detect and quantify viral RNA in sewage. Less costly and simpler detection methods are needed, especially for rural and poorer locales in Nepal [86]. Nepal is struggling to promote urbanization, as a majority (79.85%) of people are living in rural areas [87]. Using WBE will also improve public trust in the context of the federalized government in Nepal. It will also help to sustain the present federalism in Nepal, allowing local authorities to utilize and predict COVID-19 cases. COVID-19 data generated by local authorities of Nepal may protect the public from

rampant misinformation and prevent skepticism and COVID-19 denial, which will serve to depoliticize the disease and suppress pandemic fatigue. This approach may foster a collective community consciousness leading to better engagement and greater willingness to abide by simple control measures. Overall, WBE is an effective approach that detects the virus six days before clinical symptoms are present [84]. Therefore, it is a valuable complementary surveillance tool to support the public health response to pandemics, helping tailor containment and mitigation measures and to determine the target populations for testing in rural areas.

7.2. Preparing for Stronger Future Variants in the Pandemic

In COVID-19, mutation of the delta variant (K417 N) was detected in India [88] and in Nepal after gene sequencing. The scientists believe that the delta variant is found to be more dangerous than any other variant found previously [89]. The hospitalization rate of patient infected with COVID-19 will likely increase, but the percentage is not known; however, WBE can be used to identify possible cases. The third wave of COVID-19 could be deadlier than the previous two for Nepal, given the nation's low vaccination coverage, the lack of government preparedness, complacency on both local and governmental authorities, and not providing sufficient effort into WBE. Therefore, the timely adoption of WBE methods in rural Nepal is inevitable for early diagnosis and effective control. It may be a community-centered weapon for revealing health disparities and vulnerabilities among rural communities of Nepal for all communicable diseases, including COVID-19, which will remain is a key public health issue for years to come.

8. Conclusions

The COVID-19 pandemic has affected more than 248 million people worldwide as of the end of October. It is proposed that SARS-CoV-2 is transmitted via the droplets/aerosols generated by infected patients or person-to-person contact. SARS-CoV-2 is frequently discharged in the feces of infected individuals and asymptomatic carriers, ultimately reaching the WWTP via municipal wastewater or sewage pipes. The enables various pathogens to reach the environment, and may cause serious health hazards to the community. However, this knowledge can be applied for the early diagnosis of disease incidence or to determine the major viruses circulating in the community. This manuscript highlights the importance of WBE, especially in resource-poor countries where molecular diagnosis is not affordable for the entire population. Furthermore, WBE is cost-effective, does not require medical interventions, and it is easy to obtain samples; hence, and it is an efficacious method for early warning and preparedness.

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