Detection of SARS-CoV-2 through Wastewater

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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.

SARS-CoV-2   Mechanism   Wastewater   Public Health

1. Basic Characteristics of SARS-CoV-2

Coronaviruses are members of the family *Coronaviridae*, which comprises four genera as follows: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. *Betacoronavirus* 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 [1][2]. SARS-CoV-2 is an enveloped virus consisting of a single-stranded and non-segmented positive-sense ribonucleic acid [3]. It ranges from 80 to 160 nm in length and has a genome of 27–32-kbp [4] 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) [5][6]. In contrast, phylogenic analysis indicated that it was genetically different from SARS-CoV and MERS-CoV [6].

2. 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 [7]. 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 [8]. 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 [9]. 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 [5]. 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 [10].

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 [11][5][6][12]. The protein ACE2 contains 805 amino acid units and has two functional domains [13]. The ACE2 genes provide a receptor molecule for three strains of coronavirus, including SARS-CoV-2 [11]; 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 [14][15][16]. After complete attachment, proteolysis of the spike protein occurs. Following several steps of proteolytic cleavage [12]. 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 [4]. Apart from ciliated bronchial epithelial cells and type-II pneumocytes, the ACE2 receptor is abundantly expressed in the heart, kidney, endothelium, and intestine [14][15][16][2][17][18].

3. 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 [19][20]. 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 [21]. 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 [19][20][21][22]. 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 [23][24][25][26][27][28]. Moreover, studies have been conducted to find the most suitable concentration and detection methods for the analysis of SARS-CoV-2 in wastewater [24][29][30][31].

4. 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 [23][24]. 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 [25]. The detection of the incidence of SARS-CoV-2 via molecular diagnosis in wastewater samples was also reported in the USA [26][27]. An article published in *Nature* [28] 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 [28]. 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 [27].

The detection, molecular characterization, and reduction efficiency of pathogenic viruses at WWTPs have often been reported [32][33] and a study already reported the reduction efficiency of SARS-CoV-2 during wastewater treatment [34][35]. Furthermore, prior studies indicated outbreaks of viral diseases resulting from insufficient wastewater treatment [36][37] 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 [20][22]. SARS-CoV-2 is normally transmitted either via direct contact from infected individuals or via droplets generated by them during coughing, sneezing, and talking [38]. 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 [23][24][29]. 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 [22][23][24][25][29].

5. Public Health Importance

5.1. Urgent Need for Alternative Viral Detection Methods

Worldwide, only 32% of individuals infected with SARS-CoV-2 seek medical attention [39]. 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 [40]. 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 [41]. Nepal is struggling to promote urbanization, as a

majority (79.85%) of people are living in rural areas [42]. 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 [39]. 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.

5.2. Preparing for Stronger Future Variants in the Pandemic

In COVID-19, mutation of the delta variant (K417 N) was detected in India [43] 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 [44]. 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.

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