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Review

Establishing early warning systems by monitoring Covid-19 (SARS-CoV-2) in wastewater

Covid-19 (SARS-CoV-2)’un Atıksularda İzlenmesi ile Erken Uyarı Sistemlerinin Oluşturulması

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Abstract: **Objective**: Wastewater based epidemiology studies are a complementary approach used to measure and monitor the presence and prevalence of infectious diseases when clinical testing capacity is limited. It can also help with the detection of coronaviruses in wastewater and how they spread in the society. In the COVID-19 pandemic, SARS-Coronavirus-2 (SARS-CoV-2) is excreted with the feces of infected people and mixed with wastewater. Most people infected with viruses that infect enterically spread their feces and virus into their sewage systems both for the days or weeks before and after symptoms begin to appear. Through the detection of Covid-19 in wastewater, the number of asymptomatic people who do not represent any indication related to diseases but are carriers can be determined, and the total number of people infected in that society can be estimated. Therefore, an early warning system can be created, and it will be possible to take the necessary precautions before the second or third wave occurs.

Keywords: Wastewater; SARS-CoV-2; Covid-19; Pandemic; Wastewater based epidemiology

Öz: **Amaç**:Atıksu epidemiyolojisi çalışmaları, klinik test kapasitesi sınırlı olduğunda bulaşıcı hastalıkların varlığını ve hatta yaygınlığını ölçmek ve izlemek için tamamlayıcı bir yaklaşımdır. Bu epidemiyoloji koronavirüslerin atık sularda tespiti ile toplumda nasıl bir yayılım gösterdiği hakkında da yardımcı olabilmektedir. COVID-19 pandemisinde, SARS-Coronavirus-2 (SARS-CoV-2) enfekte kişilerin dışkılarıyla birlikte atılmakta ve atık sulara karışmaktadır. Enterik olarak bulaşan virüslerle enfekte olan çoğu kişi, semptomlar başlamadan önce ve başladıktan sonra, günler veya haftalar boyunca dışkıları ile virüsü hem yaymakta ve kanalizasyon sistemlerine atmaktadırlar. Atıksularda Covid-19’un tespiti ile hastalık belirtici göstermeyen ancak taşıyıcı olan asemptomatik kişilerin sayısı da belirlenerek o toplumda enfekte olan toplam kişi sayısı belirlenebilmektedir. Bu sayede erken uyarı sistemi oluşturularak ikinci veya üçüncü dalga gerçekleşmeden, gerekli önlemlerin alınması sağlanabilecektir.

Anahtar kelimeler: Atıksu; SARS-CoV-2; Covid-19, Pandemi; Atıksu epidemiyolojisi

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Introduction

Coronaviruses (CoVs) are a family of pathogenic viruses for humans and animals associated with respiratory and gastrointestinal infections. CoVs are considered small pathogens for humans since they are responsible for common colds or mild respiratory infections in people with a weak immune system. Likewise, the emergence of new and highly pathogenic and zoonotic diseases such as Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and the latest coronavirus 2 (SARS - CoV - 2) caused by CoVs, also reveals problems that need to be addressed in order to guide public health response (1).

The emergence of severe acute respiratory syndrome SARS - CoV - 2 in the city of Wuhan, China, quickly led to a global pandemic scenario with more than 2.6 million COVIDs - 19 confirmed cases worldwide as of April 23, 2020. Symptoms of COVID - 19 are varied and often non-specific, including fever, cough, and diarrhea. A non-negligible percentage of infected people develop pneumonia, which can lead to severe respiratory distress, and then mechanical ventilation, organ failure, viral sepsis, and finally, death occur. Stiff and extremely costly epidemiological control measures, including worldwide limitations, were introduced due to the collective nature of pandemics and easy diagnosis based on symptoms as well as due to lack of treatment or vaccine. While RT-qPCR test campaigns are applied in many countries to assess the actual prevalence of the virus, this is not a viable surveillance strategy for the general population in the long run.

Although SARS - CoV - 2 is primarily a respiratory virus, previous studies with relevant SARS - CoV - 1 (the causative agent of the 2003 SARS outbreak) demonstrated the possibility of fecal-oral contamination in patients' feces based on the detection of viral RNA by RT - qPCR. Recent studies also show that SARS - CoV - 2 can be excreted in asymptomatic carriers and with feces of recently recovered patients (2–5). Specifically, viral RNA, regardless of disease severity, demonstrates that even after viral clearance from the respiratory tract, it is detected in feces for up to 10 days (6). This means that wastewater may contain viral particles or viral RNA, and therefore it is possible to utilize wastewater as an epidemiological surveillance tool. Wastewater can also collect viruses from the oral cavity and upper respiratory tract that spilled during personal hygiene. Compared to the systematic testing of individuals, wastewater analysis is clearly less invasive, more straightforward, and cheaper. However, studies on the sensitivity and reliability of this method are still ongoing.

Each individual throws about 100 g of fecal matter per day (7). An infected individual contains 10^5 to 10^9 enteric virus particles in each gram of stool (8). A live SARS-CoV-2 was detected in stool samples with high RNA copies. A recent study shows that alive SARS-CoV-2 is isolated from the feces and urine of infected people (3). This indicates that SARS-CoV-2 will eventually enter the wastewater treatment system afterward. Additionally, it is stated in another study that this virus can survive for several days in a suitable environment even after it expelled from the human body (8). As the World Health Organization reported, the lifespan of viruses in wastewater is up to 20 days at 4 ° C and 24 hours at 20 ° C (9). Although wastewater is not a critical potential contamination setting for coronaviruses such as SARS-CoV-2, increased circulation of the virus in the population is likely to increase the virus load in the sewage systems of the cities (9). To date, there is limited information on the potential for transmission of coronavirus infection through the environment (10).

Also, inevitably loosening of existing restraint measures taken to protect public health can lead to a recurrence of local outbreaks or importation of cases from other regions. Consequently, it is imperative to create viable and reliable epidemiological monitoring strategies that can enable us to be prepared in future viral emergencies. Besides, this epidemiology can be used as a tool designed to help co-ordinate virus monitoring strategies under the current circumstances when authorities begin to lift measures against coronavirus gradually.

Monitoring of SARS-Cov-2 with Wastewater Epidemiology

Human enteric viruses enter the sewage system through excretion (feces-urine). Viruses are observed in extremely high amounts in the feces of infected individuals (11). The feces and urine of humans or animals are excreted into the sewage system. Therefore, wastewater contains many biomarkers and pathogens that can enter from the carrier of the disease in the communities, from infected people and patients in hospitals. These pathogens, such as bacteria, viruses, and parasites in wastewater, are risky for humans because they will always have the possibility of causing outbreaks across the populations. However, if these pathogens can be monitored at an early stage, the dangers can be minimized. Some viruses cannot be eradicated from the effluent of wastewater treatment plants, despite decontamination processes commonly used for drinking water and sewage treatment (8).

Therefore, it is necessary to develop new analytical tools that can collect samples from wastewater treatment plants and monitor these low-level biomarkers/pathogens accurately and quickly. RT-qPCR technology plays an essential role in the quantitative analysis of biomarkers and pathogens in sewage. Detecting biomarkers of diseases in wastewater can provide support in providing real-time data for the assessment of public health. Also, these data have a clear potential for providing early warning systems (11).

Wastewater epidemiology (WBE) has been a strategy successfully used in many countries to monitor the chemical and biological markers of human activity, including illegal drug consumption, drug use/abuse, water pollution, and the formation of antimicrobial resistance genes (8,12,13). Moreover, it was proven to be an innovative and promising tool to monitor the oxidative stress biomarkers such as illegal drugs (12,14), alcohol (15), tobacco use, and F2-isoprostanes with WBE (16,17). This approach includes additional information about the lifestyle, health, and pollutant exposure of a community, which can be revealed by analyzing sewage biomarkers (11).

With the help of WBE, there is great hope for assessing the levels of specific disease biomarkers in wastewater and for monitoring infectious and non-contagious diseases (18). Studies conducted from past to the present show that with WBE, the presence of pathogens/infections such as HIV, tuberculosis, poliovirus, echovirus, hepatitis A, rotavirus, adenovirus, as well as coronaviruses are detected in wastewater (11,16,17,19). Recent research (20–22) and ongoing studies (23–26) acknowledge that studies for COVID-19 detection in sewers have already been started. Preliminary results from a limited number of samples of the studies applied in countries such as China, Australia, the Netherlands, France, and the USA demonstrate the technical possibility of detecting COVID-19 in wastewater. The importance of such analysis is emphasized by team leader Christoph Ort, the Swiss Federal Institute of Aquatic Science and Technology, with his saying, "The wastewater is not lying; it reflects what was thrown by the public within a few hours". In addition, while the results of the analysis found the high rate of the virus residue in the last samples, they stated that they could not detect the virus in samples they collected in February (27).

In the study of Medema et al. In the Netherlands, SARS-CoV-2 was detected in wastewater, and the number of infected people was calculated. Also, They also acknowledged in this study that SARS-CoV-2 was observed in wastewater, yet at that time, no cases had been reported in the Netherlands. Thus, it contributed to the establishment of an early warning system (23). In a study conducted by Wu et al. In the USA, SARS-CoV-2 was detected in wastewater and was found to be higher than the clinically determined figures (24). It can be said that these studies also contribute to the shaping of the country's policies by determining asymptomatic people.

Methods and Calculations Used in Monitoring SARS-Cov-2 in Wastewater

Detection of SARS-Cov-2 viral genomes by qPCR Method

The most direct method for detecting SARS-CoV-2 is a nucleic acid-based polymerase chain reaction (PCR) analysis. With this method, it is possible to develop efficient and robust analytical tools to accurately and quickly monitor low-level SARS-CoV-2 sources through WBE and also possible to verify suspicious cases and screen asymptomatic infected cases without central laboratories (28).

The limit of detection for different viruses by qPCR is 6 to 10 viral genomes / 50-μL reaction mix based on the dilution series of the pUC57cl plasmid with a certain amount of all targeted regions cloned into the EcoRV region.

In the equation below, the total prevalence of SARS-CoV-2 infection from wastewater is calculated using the mass balance of the total number of viral RNA copies measured from wastewater by RT-qPCR daily and the number of SARS-CoV-2 RNA copies spilled from the stool by an infected person (Equivalent. (1)) (26).

$$Persons\ infected = \frac{\left(\frac{RNA\ copies}{liter\ Wastewater}\right) * \left(\frac{liters\ Wastewater}{day}\right)}{\left(\frac{g\ feces}{person - day}\right) * \left(\frac{RNA\ copies}{g\ feces}\right)}$$

Calculation of the number of patients infected with SARS-Cov-2 with data from wastewater

CT values in qPCR obtained for wastewater samples are used to calculate the number of viral genomes (Ci) by performing a linear regression of CT values obtained from pUC57cl serial dilutions based on the dilutions of the plasmid.

It is assumed that an infected individual secretes 10^7 to 10^{11} norovirus, HAV, enterovirus, and adenovirus particles per day. To our knowledge, the number of virus particles excreted daily is not known to other viruses investigated. Therefore, it is assumed that an infected person secretes similar amounts of all viruses investigated. In this study, the calculation of the number of infected people is based on the maximum amount of virus (10^{11}

virus particles/day) excreted from the body by a newly infected person, thus estimating the minimum number of infected people whose viruses are mixed into wastewater. The daily number of virus particles expected to be present in wastewater from the excretion (Cexp) of an infected person is calculated according to the following equation:

$$Cexp=10^{11} / \{[\Sigma(relevant\ daily\ flow)] / 7\}$$
$$Cexp=10^{11} / average\ daily\ flow$$

Number of potentially infected individuals (Ninfected) depending on the presence of the virus in the wastewater;

$$N_{infected} = C_i / C_{exp}$$

$N_{infected}$: number of infected individuals

CT: Values obtained for wastewater samples in qPCR

Ci: Linear regression of CT values

C_{exp}: number of daily virus particles expected in wastewater

Determination of SARS-CoV-2 from Wastewater and Its Importance for Public Health

It was reported in clinical studies that some of the SARS-CoV-2 carriers show asymptomatic while some others have no fever and show only mild infection symptoms. In a study conducted in Iceland, it was stated that 50% of patients who were COVID-19 positive had no symptoms at all (29). Since it is not possible to scan asymptomatic patients quickly and effectively, these people are mostly not reflected in the total count of patients. Therefore, rapid and accurate screening of potential virus carriers and the diagnosis of asymptomatic patients are essential for establishing early intervention and prevention strategies.

Healthcare professionals continue a rather challenging process to scan COVID-19 cases practically and effectively. The availability of COVID-19 test technologies and kits are limited. However, as an alternative method, WBE has the potential to provide information about the potential spread of infection by testing infectious agents in wastewater.

By using WBE, early detection of COVID-19 in wastewater will highly likely prevent population spread by restricting population mobility.

Conclusion

With the data obtained from wastewater epidemiology, existing clinical cases will aid the public in understanding the messages of education and prevention better and will also provide an additional database including asymptomatic people as well. It will also offer further information about the spread of the virus at the regional and local scale based on the correlation between the number of infected people calculated with the help of the RT-qPCR technique applied to water collected from wastewater treatment plants and the number of people carrying the virus detected clinically via COVID-19 the detection test. Regarding the spread of the virus, a road map for Turkey will be prepared both seasonally and spatially; therefore, it will be possible to take measures before the second or third wave appear. SARS-CoV-2 monitoring in the effluent of wastewater treatment plants will also contribute to the procedures required for the elimination of the virus in the waters directed from the treatment facilities to surface waters.

Monitoring this new virus in wastewater is vital in taking protective measures for wastewater treatment plant personnel and determining whether it poses any risk for agricultural areas or surface waters irrigated with wastewater. This study should be used as an alternative study that can complement existing clinical information limited to COVID-19 patients with the most severe symptoms.

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