

Detection of SARS-CoV-2 genome in the air, surfaces, and wastewater of the referral hospitals, Gorgan, north of Iran

Farzad Ramezani Ziarani¹, Alireza Tahamtan¹, Hasan Safari², Alijan Tabarraei¹, Yousef Dadban Shahamat^{2*}

¹Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

²Department of Environmental Health Engineering, Faculty of Health, Environmental Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Received: December 2021, Accepted: September 2022

ABSTRACT

Background and Objectives: Coronavirus disease 2019 (COVID-19) is a pandemic caused by the novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Knowing the virus's behavior and its persistence in different environments are crucial and will lead to the proper management of the disease. In this study, air, surface, and sewage samples were taken from different parts of referral hospitals for COVID-19.

Materials and Methods: Air samples were taken with impinger, surface samples with swabs, and sewage samples were taken from the hospital wastewater treatment plant. After viral genome extraction, a real-time RT-PCR test was applied to confirm the presence of SARS-CoV-2 RNA in the collected samples.

Results: The virus genome could be traced in the wards and wastewater related to hospitalized COVID-19 patients. Overall, 29%, 16%, and 37.5% of air, surface, and sewage samples were positive for the SARS-CoV-2 genome, respectively.

Conclusion: Findings of such studies provide valuable results regarding the degree of contamination of hospital environments and the risk of virus transmission in different environments and among hospital staff and patients.

Keywords: COVID-19; SARS-CoV-2; Hospital contamination; Air pollution; Environmental contamination; Wastewater

INTRODUCTION

At the end of 2019, a new viral disease emerged from Wuhan, China, spread worldwide and became a global crisis. The Chinese center for disease control and prevention (CDC) named this virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and later the world health organization (WHO) called the disease coronavirus disease 2019 (COVID-19) (1, 2). WHO declared this disease a global pandemic

on March 11, 2020. Despite efforts to eliminate the disease, an ongoing outbreak of COVID-19 has been reported around the world, with over 580 million confirmed cases and 6.5 million deaths. The high prevalence and spread of COVID-19 have become a challenge that requires special attention.

The SARS-CoV-2 transmits through direct or indirect contact, and person-to-person spread is the primary mode of virus transmission and occurs mainly via respiratory droplets. Generally, droplets

*Corresponding author: Yousef Dadban Shahamat, Ph.D, Department of Environmental Health Engineering, Faculty of Health, Environmental Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran. Tel: +98-9111789457 Fax: +98-1732440225 Email: Dr.udadban@goums.ac.ir

cannot transfer more than six feet, remain intact in the air, and are contagious for a limited time (3). Person-to-person transmission of SARS-CoV-2 virus in disease incubation period within two to ten days previously has been confirmed. Importantly, COVID-19 infection can occur if a person touches a contaminated surface and then hands come into direct contact with mucous membranes such as the eyes, nose, or mouth (4). Several laboratory experiments showed that SARS-CoV-2 could remain infectious in aerosols for hours and on some surfaces for days (5).

Several COVID-19 patients report gastrointestinal symptoms as their usual symptoms, such as diarrhea, nausea, abdominal pain, and vomiting (6). SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE-2) as a receptor. ACE-2 distribution and frequency are varied from person to person and are expressed in several cell types, including vascular epithelial cells, kidney and heart tissue, small intestinal epithelium, testes, and epithelial and endothelial cells in the mucosa (7). Thus, the gastrointestinal tract (GI tract) is permissive to viral replication, and some studies showed that the anal or stool swabs were positive for SARS-CoV-2 presence (8).

Undoubtedly, identifying the exact extent of environmental contamination and associated potential risk of viral transmission in high-risk transmission settings, such as healthcare facilities, is essential for infection prevention and the protection of healthcare workers. Awareness about the modes of transmission and adopting the appropriate respiratory precautions are critical factors in infection control. While many studies have been conducted in this regard, further assessment of the environmental contamination associated with COVID-19 patients is needed to improve our understanding of the modes of SARS-CoV-2 transmission. In this study, air, surface, and sewage samples were taken from different parts of the referral hospitals for COVID-19 patients in Gorgan city, north of Iran, and evaluated for the presence of the SARS-CoV-2 genome.

MATERIALS AND METHODS

This study was conducted at 5 Azar and Shahid Sayad Shirazi hospitals in Gorgan city as referral COVID-19 hospitals in Golestan province, north of Iran, from September to December 2020. In this study, the presence of the SARS-CoV-2 genome was

investigated in the air and surface of the infectious ward, intensive care unit (ICU), emergency ward, exhaust fans, and wastewater.

Sample collection. For air samples, impingers (midget impinger, glass, 250 ml, SKCinc, USA) were used to collect bioparticles ranging from 0.1-10 μm as a standard method and efficient way to collect airborne viruses (9). The indoor air samples were taken using a high-volume air pump with an airflow rate of 20 L/min in the impingers. Before sample collection, impingers and connection tubes were disinfected using an autoclave and 70% isopropyl alcohol. For transport media, 100 ml Dulbecco's Modified Eagle's medium (DMEM) with 100 $\mu\text{g}/\text{mL}$ streptomycin, 100 U/mL penicillin, and 1% antifoam reagent (isoamyl alcohol) were used to collect samples (10). To collect samples from ICU and infection ward related to the hospitalized COVID-19 patients, the impingers were placed 1.5 meters away from the patient's bed at the height of one meter. The sampling time was one hour on different days, considering the temperature, room space, and the number of patients in each room. A total of 14 samples were collected from the infectious ward and three samples from ICU. For sampling from the emergency ward, impinger was placed in three different places: triage, admission, and doctor's room (with 1 hour sampling time), and nine different samples from this section were collected.

Environmental samples must be taken using a swab containing a synthetic tip and plastic shaft. The collection vials should have 1-3 ml of the viral transport medium (11). For surface samples, 3 ml of DMEM was used as a transport medium, and sterile Dacron swabs were drawn on a surface of approximately 25 cm^2 to collect samples from surfaces that contact patients, such as bed handles, mobile, and floor. To understand the proper performance of the exhaust fans, surface samples from the outer part of exhaust fans related to wards of hospitalized COVID-19 patients in the Shahid Sayad Shirazi hospital were taken. Thirty-one different samples were taken from the infectious ward, ICU, emergency room, and exhaust fans.

For wastewater sampling, 5 ml of treated and untreated sewage were collected simultaneously from the hospital refinery using a separate sterile pipette and pipet filler to prevent cross-contamination. A total of 8 samples of wastewater were collected from the hospital wastewater treatment plant (4 untreated and four treated). Also, in this study, air samples were

taken from the hospital wastewater treatment plant because activities lead to aerosol production in the wastewater treatment process at the sewage aeration stage. All samples were transferred on ice immediately after collection to the virology lab for genome detection.

Genome detection. Two methods were performed for genome extraction: direct genome extraction from collected samples and extraction after polyethylene glycol (PEG) concentration for wastewater samples. High molecular weight PEG has been used to concentrate virus samples such as influenza and respiratory syncytial virus (12). The virus genome was extracted from collected and concentrated samples according to the manufacturer's instructions (ROJE Technologies, Iran). After extraction of the genome, the samples were stored in a refrigerator at -20 degrees Celsius to detect the possible presence of the virus genome using the RT-PCR method.

Detection of the SARS-CoV-2 genome was performed via a real-time reverse transcription-polymerase chain reaction (RT-PCR) kit targeting the RdRp and Nucleocapsid (N) genes (Pishtaz Teb, Iran). Each reaction contained 5 µl RNA, 9 µl Master mix, 1 µl primer-probe, and 5 µl RNase-free water. Prepared reactions were run with initial conditions of 50°C for 20 min, 95°C for 3 min, followed by 45 cycles of 94°C for 10 s, 55°C for 40 s, and finally cooling 25°C for 10s. The Ct (Cycle threshold) values higher than 40 consider negative results (13, 14).

In the real-time PCR test (Pishtaz Teb, Iran), the positive control sample was the definitive positive sample of patients with COVID-19 with a CT value equal to 13. The negative control sample was distilled water with the forward and reverse primers included in the kit. Also, according to the manufacturer's instruction, the minimum number of detectable copies (limit of detection) in this test is 200 copies per milliliter.

Statistical analysis. The relationship between the presence of the SARS-CoV-2 genome in the rooms of patients in the infectious department based on the number and density of patients, taking into account the temperature and ventilation conditions of the room was performed. After sampling and extracting the probable virus genome, the Ct value number from the RT-PCR test indicates the virus load in collected samples. Lower Ct values represent that the virus

load at that sample was higher. The Mann-Whitney U test is used to find the relationship between the presence of the virus genome and the parameters mentioned above.

RESULTS

Twenty-six different air samples were collected from hospital wards; 14 from the infectious ward, three from ICU, and nine from the emergency ward. After performing real-time RT-PCR, 35.7%, 67%, and 22% of the infectious ward, ICU, and emergency ward samples were positive for the SARS-CoV-2 genome, respectively. Thirty-one different environmental samples were collected from floor, surfaces, and exhaust fans with swabs from hospital wards. Three samples were collected from the floor of the infection ward and three from the ICU. After analyzing the results, all of the samples taken from the infection ward were negative, and 1 of the samples taken from the ICU was positive for the presence of the SARS-CoV-2 genome.

Also, five samples from surfaces of the infection ward, three from ICU, and nine from the emergency room were collected, and the results show that respectively 40%, 33%, and 11% of them were positive for the presence of the SARS-CoV-2 genome. Other environmental samples were the exhaust fans, and from 8 samples, none were positive for the presence of the SARS-CoV-2 genome.

To check the existence of virus genome in the hospital sewage treatment plant, two types of samples were collected from the air of the sewage treatment plant and the hospital sewage. Five air samples were collected from the hospital wastewater treatment plant, and none of these samples were positive for the presence of the SARS-CoV-2 genome. Eight samples were collected to examine the presence of the SARS-CoV-2 genome in the hospital wastewater. Out of the eight samples collected, four are related to wastewater before treatment, and four samples are related to wastewater after treatment. The samples were taken in pairs and simultaneously from this section. Of 4 untreated and four treated wastewater samples, two treated and one untreated wastewater samples were positive for the SARS-CoV-2 genome.

Genome extraction from the hospital wastewater treatment plant section was done by direct extraction and genome extraction by concentration method using polyethylene glycol. The results showed that by using

the extraction method with polyethylene glycol, the diagnostic limit of the kit is lower, and the samples become positive at lower CTs, and it is also worth mentioning that the negative samples using both methods of genome extraction remained negative. The results are shown in Table 1.

In this study, we investigated the relationship between the presence of the SARS-CoV-2 virus genome in different rooms in the infection ward based on the number of patients in the room area with considering the temperature and condition of the central air conditioning of the hospital. A total of fourteen samples with the conditions mentioned above were taken from the hospital infection ward, out of these fourteen samples, 5 samples were positive for the presence of the virus genome. The results, CT value and sampling condition mentioned in Table 2. Using SPSS software (version 16) and the Mann-Whitney U test, which is a non-parametric test for data with small size and high skewness, the result showed that, the Asymp sig was less than p-value and there were no statistically significant results between patient density and virus load in the samples. The results are shown in Table 3.

DISCUSSION

In this study, the SARS-CoV-2 genome was investigated in different referral hospital sections and confirmed the presence of SARS-CoV-2 genome in the indoor air and environments of different hospital sections. Such studies yield valuable information about the level of contamination in the hospitals and the risk of virus transmission among hospital staff and patients. There are different opinions about the transmission and presence of SARS-CoV-2 in hospital environment studies. A direct comparison between findings from this study and other studies that evaluated the SARS-CoV-2 transmissibility and presence of genome is not possible due to differences in sampling method, experimental strategies, the number of hospitalized patients, and physical characteristics of the hospital buildings, and staff approaches cleaning/disinfection protocols.

In this study, 26 air samples, 35.7%, 67%, and 28% of air samples taken from the infection ward, ICU, and emergency room, respectively, were positive for the presence of the SARS-CoV-2 genome. Other studies with methods similar to ours have different views on the presence of the SARS-CoV-2 genome.

For example, Faridi et al. took samples via impingers from the indoor air of the ICU wards and all air samples were negative for SARS-CoV-2 genome presence (10). In another investigation conducted in Ardabil, Iran, Vosoughi et al. took air samples from different hospital wards. They set up their research operations in corona wards at different heights, the approximate distance from patients was 2.0 to 5.0 m,

Table 1. Results of testing for SARS-CoV-2

Area/Sample type	Number of samples/ positive/negative	Rate of positivity, %
Infection ward/Air	14/5/9	35.7
ICU/Air	3/2/1	67
Emergency room/Air	9/2/7	22
Infection ward/Floor	3/0/3	0
ICU/Floor	3/1/2	33
Infection ward/Surface	5/2/3	40
ICU/Surface	3/1/2	33
Emergency room/Surface	9/1/8	11
Exhaust Fan/Surface	8/0/8	0
Wastewater/Air	5/0/5	0
Wastewater	8/3/5	37.5

Table 2. The relationship between the presence of the SARS-CoV-2 genome in different rooms in the infection ward based on the number of patients in the room area

#Of samples	Patient density (bed/m ²)	Temperature	Ct value number	Result
1	2/30	26	-	Negative
2	2/30	26	-	Negative
3	4/30	25	-	Negative
4	4/30	25	-	Negative
5	2/30	25	-	Negative
6	2/30	25	36	Positive
7	2/30	25	34	Positive
8	3/30	25	35	Positive
9	1/20	25	-	Negative
10	4/30	25	-	Negative
11	4/30	25	-	Negative
12	4/30	25	-	Negative
13	6/30	25	34.3	Positive
14	5/30	25	34.7	Positive

* The negative samples had no or ≥ 40 Ct values

Table 3. The results of the statistical analysis of the relationship between the density of patients and the presence of the virus

Patient density	N	Mean	SD	Median	Min	Max	Results
Negative	9	3	1.224745	4	1	4	Asymp sig = -0.628
Positive	5	3.6	1.81659	3	2	4	p-value = 0.5854
Total	14	3.214286	1.423893	3.5	1	6	p-value>Asymp sig = 0.5298

and samples were taken by suction of the air into the sterile impinger. After analyzing the data, all samples were negative, suggesting that airborne transmission may not affect this pandemic (15).

Despite Faridi et al. and Vosoughi et al. studies that with the same method could not detect viruses from air samples, this study used vacuum pumps with a higher flow rate, and this could be one of the reasons why this research was able to determine the presence of SARS-CoV-2 genomes in the collected air samples. On the other hand, in research by Kenarkoohi et al. in Shahid Mustafa Khomeini hospital wards in Ilam province, east of Iran, the liquid impinger bio-sampler (liquid-phase SKC biosampler) was used to capture airborne viruses in liquid. In this study, 2 of 14 samples were positive for the presence of the SARS-CoV-2 genome (16). In Lednicky et al. study, air samplings were performed at the University of Florida Health (UF Health) Shands hospital with a VIVAS air sampler. After collecting air samples, isolation of SARS-CoV-2 was done in cell lines such as rhesus monkey kidney epithelial cells (LLC-MK2 line) and African green monkey kidney epithelial cells (Vero E6 cells). After cell growth and observation of cytopathic effects (CPE) as evident, the presence of SARS-CoV-2 was determined by rRT-PCR, and SARS-CoV-2 genomic RNA (vRNA) was detected by real-time reverse transcriptase quantitative polymerase chain reaction. This study showed that the viable virus could transmit a distance of over 2 meters (17).

Based on available epidemiological data and studies of environmental transmission factors, surface transmission is not the main route by which SARS-CoV-2 spreads, and the risk is considered low. Other studies in this field in hospitals have identified the genome of the SARS-CoV-2 virus on different surfaces. For example, in a study conducted in Turkey, Aytoğan et al. using Dacron swabs took surface samples from an ophthalmology examination room to measure the degree of environmental contamination by asymptomatic patients. After sampling and rRT-PCR tests, two of the seven samples taken from surfaces in contact

with asymptomatic individuals were positive for the presence of the virus genome (18).

In a study by Pasquarella et al., in a person's room with a positive SARS-CoV-2 test, environmental samples were taken from a bed, chair, desk, and telephone 2 hours after disinfection. After genome extraction and rRT-PCR tests, out of 15 environmental samples taken, four samples (26.66%) were positive for the presence of virus genetic material (19). In another study by Razzini et al. at a hospital in Milan, Italy, environmental samples were taken from inpatient wards of patients with COVID-19. In this study, 37 different environmental samples were taken from the wards of patients, and after RT-PCR tests, 24.3% of the samples were positive for the presence of the virus genome (20).

The principal mode by which people are infected with SARS-CoV-2 is through exposure to respiratory droplets carrying the infectious virus. In most situations, cleaning surfaces using soap or detergent and not disinfecting is enough to reduce risk. Disinfection is recommended in indoor community settings where there has been a suspected or confirmed case of COVID-19 within the last 24 hours. The risk of fomite transmission can be reduced by wearing masks consistently and correctly, practicing hand hygiene, cleaning, and taking other measures to maintain healthy facilities (21).

The SARS-CoV-2 virus can spread in the feces of people with COVID-19, whether asymptomatic or symptomatic or recovering from illness. In people who excrete the virus through the gastrointestinal tract (approximately 30-60% of people), the viral load varies greatly, ranging from 104 to 108 copies per liter of feces. Gastrointestinal transmission of the virus peaks just before the onset of clinical signs of the disease, and the spread of viral RNA from an infected person can continue for several weeks. However, the viable virus is rarely detectable more than 7 to 10 days after the onset of symptoms (22, 23).

Based on previous studies, the genetic material of SARS-CoV-2 has been found in untreated wastewater. There is little evidence of the infectious virus in

wastewater and no information to date that anyone has become sick with COVID-19 because of exposure to wastewater. Wastewater treatment plants use chemical and other disinfection processes to remove and degrade many viruses and bacteria (24). Based on the results obtained in this study, 37.5% of wastewater samples were positive for the presence of the SARS-CoV-2 genome. Studies in other places also show almost identical results in the presence of the virus genome in sewage. Giuseppina La Rosa et al. performed the study and collected twelve raw sewage samples from three wastewater treatment plants (WWTPs) in Milan and Rome. After that, Sample concentration took place using a two-phase (PEG-dextran method) separation as detailed in 2003 WHO guidelines for environmental surveillance of poliovirus protocol. 50% of the wastewater samples showed positive results for SARS-CoV-2 RNA (25). In Research by Kocamehi et al. in Istanbul, the primary and waste-activated sludge samples were collected from nine WWTPs in Istanbul, Turkey. After analyzing the data, all collected samples were positive for SARS-CoV-2 (26).

Our study had several limitations. First, RT-PCR test results did not indicate the presence of active and reproducible viruses in the collected sample. Finding the genome is not necessarily the reason for transmitting the virus. Second, the aerosol transmission distance cannot be strictly determined for the unknown minimal infectious dose. Finally, the number of samples taken from different hospital wards could be more.

CONCLUSION

This study investigated the presence of the SARS-CoV-2 virus in different parts of referral hospitals for COVID-19 patients in Gorgan city, Golestan province, Iran. Due to the rapid spread of the virus and the different behaviors of variants, our study shows that measures such as continuous disinfection of hospital environments, proper ventilation, and the use of safety equipment such as masks can help reduce the transmission of disease in hospital environments.

ACKNOWLEDGEMENTS

This research was part of M.Sc. thesis (Farzad

Ramezani Ziarani) entitled "Investigation of the SARS-CoV-2 genome in the indoor air and surfaces of rooms and wastewater treatment plant of the Gorgan city Hospitals, Northern Iran" with the ethic approval code of IR.GOUMS.REC.1399.042 and grant. No. 111591, supported by the Golestan University of Medical Sciences. The authors express their gratitude for the support and assistance extended by the facilitators during the research process.

REFERENCES

1. World Health Organization. Naming the coronavirus disease (COVID-19) and the virus that causes it. *Braz J Implantol Health Sci* 2020; 2: 1-4.
2. Ayatollahi AA, Aghcheli B, Amini A, Nikbakht H, Ghassemzadehpirsala P, Behboudi E, et al. Association between blood groups and COVID-19 outcome in Iranian patients. *Future Virol* 2021. 10.2217/fvl-2021-0090.
3. Teymoori-Rad M, Samadzadeh S, Tabarraei A, Moradi A, Shahbaz MB, Tahamtan A. Ten challenging questions about SARS-CoV-2 and COVID-19. *Expert Rev Respir Med* 2020; 14: 881-888.
4. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The incubation period of Coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med* 2020; 172: 577-582.
5. Li H, Wang Y, Ji M, Pei F, Zhao Q, Zhou Y, et al. Transmission routes analysis of SARS-CoV-2: a systematic review and case report. *Front Cell Dev Biol* 2020; 8: 618.
6. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; 395: 514-523.
7. Singhal T. A review of coronavirus disease-2019 (COVID-19). *Indian J Pediatr* 2020; 87: 281-286.
8. Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020; 382: 1564-1567.
9. Verreault D, Moineau S, Duchaine C. Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev* 2008; 72: 413-444.
10. Faridi S, Niazi S, Sadeghi K, Naddafi K, Yavarian J, Shamsipour M, et al. A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran. *Sci Total Environ* 2020; 725: 138401.

11. World Health Organization. Surface sampling of coronavirus disease (COVID-19): a practical “how to” protocol for health care and public health professionals. 2020. WHO/2019-nCoV/Environment_protocol/2020.1
12. Alexander MR, Rootes CL, van Vuren PJ, Stewart CR. Concentration of infectious SARS-CoV-2 by polyethylene glycol precipitation. *J Virol Methods* 2020; 286: 113977.
13. Mahapatra S, Chandra P. Clinically practiced and commercially viable nanobio engineered analytical methods for COVID-19 diagnosis. *Biosens Bioelectron* 2020; 165: 112361.
14. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert Rev Mol Diagn* 2020; 20: 453-454.
15. Vosoughi M, Karami C, Dargahi A, Jeddi F, Jalali KM, Hadisi A, et al. Investigation of SARS-CoV-2 in hospital indoor air of COVID-19 patients' ward with impinger method. *Environ Sci Pollut Res Int* 2021; 28: 50480-50488.
16. Kenarkoohi A, Noorimotlagh Z, Falahi S, Amarloei A, Mirzaee SA, Pakzad I, et al. Hospital indoor air quality monitoring for the detection of SARS-CoV-2 (COVID-19) virus. *Sci Total Environ* 2020; 748: 141324.
17. Lednicky JA, Lauzard M, Fan ZH, Jutla A, Tilly TB, Gangwar M, et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int J Infect Dis* 2020; 100: 476-482.
18. Aytogan H, Ayintap E, ÖzkalayYılmaz N. Detection of Coronavirus Disease 2019 Viral Material on Environmental Surfaces of an Ophthalmology Examination Room. *JAMA Ophthalmol* 2020; 138: 990-993.
19. Pasquarella C, Colucci ME, Bizzarro A, Veronesi L, Affanni P, Meschi T, et al. Detection of SARS-CoV-2 on hospital surfaces. *Acta Biomed* 2020; 91: 76-78.
20. Razzini K, Castrica M, Menchetti L, Maggi L, Negroni L, Orfeo NV, et al. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. *Sci Total Environ* 2020; 742: 140540.
21. Harvey AP, Fuhrmeister ER, Cantrell ME, Pitol AK, Swarthout JM, Powers JE, et al. Longitudinal monitoring of SARS-CoV-2 RNA on high-touch surfaces in a community setting. *Environ Sci Technol Lett* 2021; 8: 168-175.
22. Van Doorn AS, Meijer B, Frampton CMA, Barclay ML, de Boer NKH. Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission. *Aliment Pharmacol Ther* 2020; 52: 1276-1288.
23. Jones DL, Baluja MQ, Graham DW, Corbishley A, McDonald JE, Malham SK, et al. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. *Sci Total Environ* 2020; 749: 141364.
24. Saawarn B, Hait S. Occurrence, fate and removal of SARS-CoV-2 in wastewater: Current knowledge and future perspectives. *J Environ Chem Eng* 2021; 9: 104870.
25. La Rosa G, Iaconelli M, Mancini P, Bonanno Ferraro G, Veneri C, Bonadonna L, et al. First detection of SARS-CoV-2 in untreated wastewaters in Italy. *Sci Total Environ* 2020; 736: 139652.
26. Kocamemi BA, Kurt H, Sait A, Sarac F, Saatci AM, Pakdemirli B. SARS-CoV-2 Detection in Istanbul Wastewater Treatment Plant Sludges. *medRxiv* 2020. 10.1101/2020.05.12.20099358