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Hospital Wastewater Surveillance of SARS-CoV-2 RNA: Association With COVID-19 Cases and Insights into Environmental Persistence

Rozhan Feizi [1,2], Neematollah Jaafarzadeh [3], Masoud Panahi Fard [2]*, Niloofar Neisi [4], Abdollah Dargahi [5], Maryam Mehrbakhsh [1]

[1] *Department of Environmental Health, Behbahan Faculty of Medical Sciences, Behbahan, Iran.*
[2] *Student Research Committee, Department of Environmental Health Engineering, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
[3] *Environmental Technologies Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
[4] *Department of Virology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
[5] *Department of Environmental Health, Khalkhal University of Medical Sciences, Khalkhal, Iran.*

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***Corresponding Author:**
Masoud Panahi Fard

Email:
masoudpanahifard@gmail.com

Tel:
+98 912 0795168

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ABSTRACT

Introduction: Analyzing municipal wastewater for the presence of SARS-CoV-2 RNA serves as a cost-effective and timely tool for epidemiological surveillance to mitigate virus-related health risks. Therefore, this study assessed the presence of SARS-CoV-2 RNA in untreated hospital wastewater and its correlation with COVID-19 case numbers over six months at Shahidzadeh Hospital in southwest Iran.

Materials and Methods: In this analytical investigation, a total of 24 grab samples of untreated hospital wastewater were systematically collected over six months, spanning from September 2020 to February 2021. Each sample was subsequently processed and analyzed using a reverse transcription quantitative polymerase chain reaction (RT-qPCR) approach, with specific amplification targeting both the RNA-dependent RNA polymerase (RdRp) gene and the nucleocapsid (N) gene of SARS-CoV-2.

Results: All 24 wastewater samples tested positive for SARS-CoV-2 RNA. Concentrations ranged between 130 to 490 gene copies/100 mL, with mean values increasing from 247 (September) to 425 (February). RNA levels were strongly correlated with hospitalized COVID-19 cases (Spearman's $p < 0.05$, $R^2 = 0.87$).

Conclusion: These findings highlight the value of hospital wastewater surveillance as a cost-effective epidemiological tool, particularly in settings with limited diagnostic capacity. Future research should investigate viral viability and optimize disinfection strategies to reduce potential environmental risk.

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Introduction

Among coronaviruses, only a limited number are known to infect humans and often cause respiratory issues. These viruses primarily lead to mild upper respiratory tract infections, with severe cases being uncommon and typically observed in

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infants, young children, and elderly individuals [1]. SARS-CoV-2 is classified within the Beta coronavirus genus as part of the Middle East respiratory syndrome-related coronavirus species [2]. The illness caused by SARS-CoV-2 is referred to as Coronavirus Disease 2019 (COVID-19) [3]. SARS-CoV-2 presence in indoor air, wastewater, and environmental surfaces [4-6]. Both the virus and its genetic material are excreted through bodily fluids, such as saliva, sputum, and feces, which eventually find their way into wastewater [7]. Previous studies have indicated that SARS-CoV-2 is unlikely to be transmitted via wastewater because of the absence of infectious virus particles, wastewater remains a valuable tool for epidemiological monitoring [3]. The virus has been found to remain viable on different surfaces for periods between 3 and 14 days [8, 9]. Additionally, SARS-CoV-2 has half-lives between 1.7 and 2.7 days at 20°C, which decrease significantly at higher temperatures, such as 40°C [10, 11]. The RNA of SARS-CoV-2 has been identified in human stool samples and subsequently in wastewater from municipal and healthcare facilities [12,13]. A comprehensive review of the presence of viruses in water and wastewater suggests that while RNA can be detected, the infectious potential in such media remains unclear, necessitating further research to understand its survivability [14]. Coronaviruses can persist in wastewater environments for several hours up to 2 days at ambient temperatures, depending on environmental conditions [15,16]. Wastewater has been identified as a potential source of SARS virus transmission [17, 18]. One recent report speculated that COVID-19 transmission might occur through a building's wastewater collection system, although this hypothesis has not yet been substantiated [19, 20]. Disinfection of municipal wastewater is effective for virus elimination [21]. In many developing countries, hospitals discharge untreated wastewater into the environment because of a lack of adequate treatment facilities [22]. Given the limited evidence of SARS-CoV-2 presence in hospital wastewater, this study aimed to evaluate the relationship between SARS-CoV-2 RNA levels in wastewater and the number of hospitalized COVID-19 cases. This approach highlights the potential of wastewater-based epidemiology (WBE) as a valuable public health tool, particularly in clinical settings with limited diagnostic tests. So far, various studies have reported that SARS-CoV-2 RNA virus is present in various municipal and hospital wastewaters. (e.g., Italy [23], Spain [24], Netherlands [25], Australia [6], India [26], and Iran [27]), confirming the feasibility of WBE. Implementing WBE in low- and middle-income countries faces important challenges, including the high costs of advanced molecular methods, lack of standardized protocols, limited laboratory infrastructure, and variable wastewater treatment practices. These constraints highlight the importance of evaluating simpler and more cost-effective surveillance strategies, particularly in high-prevalence settings such as hospitals and nursing homes. Addressing these challenges provides a rationale for the present study. However, little is known about hospital wastewater, which may show higher viral loads due to concentrated patient shedding of the virus. The central question of this study is not simply whether SARS-CoV-2 RNA levels correlate with clinical cases but whether such a correlation can be reliably detected using a low-cost surveillance approach in a high-prevalence hospital setting. Demonstrating feasibility under these conditions is important for establishing wastewater-based monitoring as a practical complement to clinical surveillance in resource-limited settings. It should be noted that the type and dosage of disinfectants can significantly affect the persistence and detectability of viral RNA in wastewater. Residual disinfectants, such as chlorine-based compounds or peracetic acid, may accelerate RNA degradation, whereas insufficient or irregular application can allow viral fragments to persist. In our study, since the hospital wastewater was discharged without chemical treatment, the direct effect of disinfectants on SARS-CoV-2 RNA was not assessed. Nevertheless, future research should systematically investigate this issue by combining (i) controlled laboratory experiments with different disinfectant

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types and doses, (ii) field comparisons of treated and untreated effluents, and (iii) monitoring of residual disinfectant concentrations alongside viral RNA quantification. Such efforts would clarify how disinfection practices influence wastewater surveillance outcomes and the associated environmental risks.

Materials and Methods

Study area

Shahidzadeh Hospital, situated in Behbahan city of Khuzestan Province, represents one of the principal referral and treatment centers for COVID-19 in Iran during the pandemic. This medical facility, with a capacity of approximately 178 active beds, has played a pivotal role in the timely diagnosis, hospitalization, and management of individuals infected with SARS-CoV-2. Data on patient numbers and related clinical activities were systematically obtained from the Vice-Chancellor of Treatment at Jundishapur University of Medical Sciences, which officially oversees healthcare services in the region. Since the initial emergence of COVID-19 cases in Iran, this hospital alone has provided diagnostic evaluation and therapeutic interventions for more than 2300 confirmed patients, thereby contributing significantly to regional pandemic control efforts. Furthermore, Shahidzadeh Hospital generates an estimated 500 liters of wastewater per day. This effluent, which potentially contains pathogenic microorganisms and chemical residues, is currently discharged into the surrounding environment without undergoing any preliminary treatment or disinfection. Such direct disposal not only contravenes national environmental protection standards and public health guidelines but also highlights an urgent need for implementing sustainable wastewater treatment strategies to mitigate possible ecological and epidemiological risks.

Sample Collection

As presented in Table 1, a total of 24 untreated hospital wastewater samples were systematically and consistently collected throughout the study, with exactly four representative samples obtained during each month over the six-month period extending from September 2020 through February 2021. In order to accurately capture temporal variability while at the same time maintaining strict comparability between individual sampling events, the sampling days were very carefully scheduled at regular and pre-defined intervals of approximately seven days, corresponding to one sample per week within each month of the study duration. This deliberate and structured approach was specifically intended to provide a comprehensive and representative overview of monthly wastewater trends while simultaneously minimizing potential bias or uncertainty that could otherwise arise from short-term or episodic fluctuations in the overall composition of hospital effluent. Grab samples were manually obtained in the field following strict and well-documented aseptic procedures, using only pre-cleaned and sterile 1-liter Nalgene bottles that were completely filled at the time of collection in order to maintain consistent sampling volumes and to prevent any potential headspace contamination or unintended exposure to the external environment. In this investigation, no automated sampler or mechanical device was employed at any stage, and reliance on direct manual collection was intentionally chosen to allow closer supervision and more precise control of sample handling and transfer, thereby ensuring that each individual sample was obtained under highly comparable and reproducible field conditions. Sampling was consistently performed during dry daytime hours, a deliberate and precautionary choice intended to avoid any possible dilution effects that could be associated with rainfall events, stormwater inflow, or other irregular hydrological inputs. Furthermore, normalization for flow rate or precipitation was not considered necessary within this research framework, since the hospital wastewater system under investigation exhibited relatively stable discharge patterns throughout the period of interest, and the study interval coincided with a season characterized by minimal and infrequent rainfall. This combination of stable flow and dry weather conditions effectively reduced confounding variability and strengthened the

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reliability of the dataset generated during the six-month monitoring program [28]. The samples were analyzed in the virology laboratory.

SARS-CoV-2 RNA Detection

In this investigation, samples were processed directly for RNA extraction without any prior concentration step. This choice was based on two considerations: (i) untreated hospital effluent was presumed to contain high viral loads because of the dense presence of symptomatic patients, and (ii) one of the study aims was to assess the practicality of a simplified and low-cost monitoring strategy suitable for environments with limited resources. Although skipping concentration may have lowered recovery efficiency, this trade-off was accepted in order to maintain RNA integrity and to minimize losses caused by additional handling. RNA was extracted manually from the prepared solution. For this purpose, 200 µl of each sample was separated, and viral RNA was isolated using the SinaPure™ Viral RNA Extraction Kit (Sinaclon, Iran; Cat. No. EX6101) [29] following the manufacturer's protocol. The final elution volume was 50 µL, and no automated extraction system was utilized. Subsequent RT-qPCR analysis was carried out with the PishtazTeb COVID-19 One-Step RT-qPCR Kit (Cat. No. PT-COVID19) [29, 30]. Each 20 µL reaction mixture was prepared with 5 µL of RNA template, 10 µL of master buffer, 1 µL enzyme mix, 1 µL RdRp/N primer-probe set, 1 µL of RNase P primer-probe set (internal control), and 2 µL of RNase-free water. Strict quality assurance measures were applied, including negative extraction controls, no-template controls, and use of RNase P as an internal amplification control to detect PCR inhibition. All reactions were performed in duplicate, and assays with inconsistent Ct values were repeated. Primers and probes for the RdRp and N genes were verified against a reference RNA control to ensure specificity. Viral RNA detection was performed using a Light Cycler real-time PCR system (Roche, Germany). Quantification was based on Ct values, with Ct < 40 regarded as positive. Viral copy numbers were determined per 100 mL of wastewater using the standard curve method. The kit employed a dual-target approach covering conserved regions of both RdRp and N genes, and included internal probes for RNase P. Fluorescent signals were monitored through the FAM, HEX, and ROX channels for RdRp, N, and RNase P, respectively. Thermal cycling involved the following steps: reverse transcription for 20 minutes at 50 °C, initial denaturation for 3 minutes at 95 °C, and 45 amplification cycles, each consisting of 10 seconds of denaturation at 94 °C and 40 seconds of annealing/extension/fluorescence detection at 55 °C. The assay ended with a cooling phase of 10 seconds at 25 °C. A sample was considered positive when its amplification curve crossed the threshold with a typical sigmoidal shape across all three channels [30]. For each sampling time, one grab sample was collected, while RNA extraction and RT-qPCR runs were performed in duplicate to confirm reproducibility of results.

Statistical Analysis

The relationship between SARS-CoV-2 RNA concentrations in untreated wastewater and the number of COVID-19 cases was assessed using Spearman's rank correlation test in SPSS version 22 [31]. To further explore this relationship, linear regression analyses were also conducted. The regression model produced an R^2 value of 0.87, demonstrating a strong association between case numbers and RNA levels. Additional validation with Mann–Whitney U tests revealed statistically significant differences ($p < 0.05$) between viral RNA concentrations measured during periods of high versus low patient counts.

Results

Patients with COVID-19

The number of patients diagnosed with COVID-19 and receiving hospital-based treatment during the monitoring period is illustrated in Figure 1. This figure provides a clear representation of monthly variations in patient admissions, allowing for a direct comparison with the corresponding wastewater surveillance data obtained in the study.

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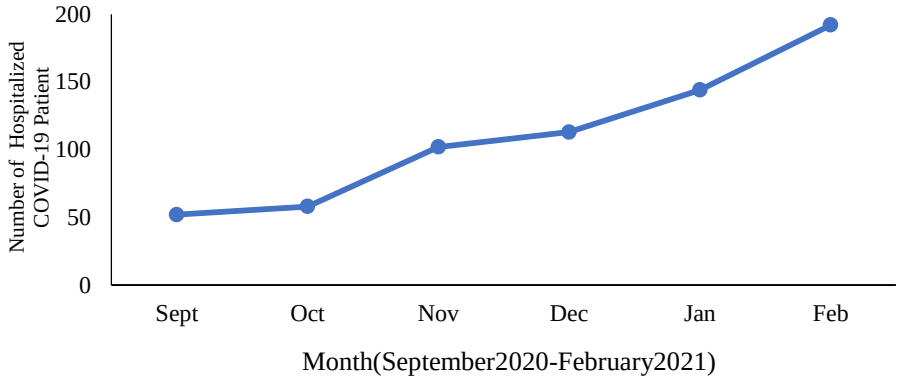

Figure 1: Temporal variation in the number of hospitalized COVID-19 patients.

As illustrated in Figure 1, the month of February recorded the highest number of hospitalized COVID-19 patients compared to all other months within the study period. This temporal pattern corresponded closely with the sixth nationwide wave of coronavirus cases in Iran, which extended from November through February and was characterized by a sharp escalation in overall infection rates. Conversely, the lowest number of patients receiving hospital-based treatment was documented in September, reflecting a relative decline in disease prevalence at the beginning of the monitoring period. These contrasting trends highlight the clear seasonal and epidemic-related dynamics of COVID-19 burden during the study timeframe.

SARS-CoV-2 RNA Concentration

Data related to the exact sampling dates, together with the corresponding quantitative measurements of SARS-CoV-2 RNA concentrations, are comprehensively summarized in Table 1. This table provides a structured overview of the temporal distribution of samples and their associated viral RNA levels, thereby facilitating interpretation of patterns and comparison across the study period.

Table 1: Sampling date, RNA concentration, and standard error

Month	Sample ID	Sampling Date	RNA Concentration (Gene copies/100ml)	Standard Error (SEM)
September	1	9/2	330	12.1
	2	9/10	320	11.8
	3	9/16	130	8.6
	4	9/23	210	10.3
October	5	10/7	360	13.4
	6	10/14	290	12.0
	7	10/20	220	9.7
	8	10/29	250	10.2
November	9	11/4	390	14.1
	10	11/13	320	12.6
	11	11/20	330	11.5
	12	11/28	390	14.3
December	13	12/3	390	13.9
	14	12/10	230	10.7
	15	12/18	310	12.1
	16	12/26	260	11.2
January	17	1/5	320	11.6
	18	1/13	490	15.2
	19	1/20	370	13.7
	20	1/27	480	14.9
February	21	2/3	410	13.4
	22	2/12	460	14.6
	23	2/19	350	12.9
	24	2/26	480	14.8

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As presented in Table 1, SARS-CoV-2 RNA was consistently detected across all wastewater samples analyzed during the six-month monitoring period. The calculated mean concentrations of viral RNA in September, October, November, December, January, and February were 247, 280, 332, 357, 415, and 425 gene copies per 100 milliliters, respectively. Among these values, the lowest average concentration was recorded in September, whereas the highest concentration occurred in February, reflecting a nearly twofold increase over time. The results demonstrated a progressive and continuous rise in SARS-CoV-2 RNA levels throughout the study duration, which paralleled the gradual increase in the number of hospitalized COVID-19 patients. This association became particularly evident during the sixth national wave of infections in Iran, spanning from November to February, when both wastewater viral concentrations and hospital admissions reached their maximum levels. The temporal alignment between wastewater RNA concentrations and the abundance of patients admitted with COVID-19 is clearly illustrated in Figure 2, emphasizing the strong epidemiological linkage between environmental viral signals and clinical case trends.

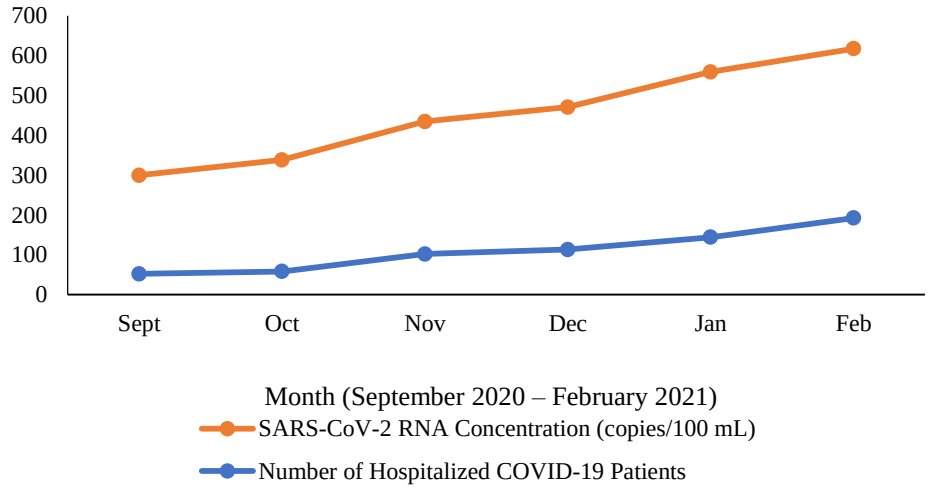

Figure 2: Temporal relationship between RNA concentrations and the number of patients.

Table 2 provides a comprehensive summary of the outcomes obtained from the statistical analyses performed in this study. The results demonstrated a clear and statistically significant relationship between SARS-CoV-2 RNA concentrations measured in untreated hospital wastewater and the number of patients hospitalized with COVID-19 during the same period. Specifically, correlation analysis using Spearman's rank test revealed a strong positive association (Spearman's $r = 0.76$, $p < 0.05$), confirming that higher RNA levels in wastewater were consistently aligned with increased patient admissions. In addition, linear regression modeling further supported this relationship, showing a robust predictive association with a high coefficient of determination ($R^2 = 0.87$, $p < 0.05$). These findings collectively reinforce the reliability of wastewater-based monitoring as an effective epidemiological tool, demonstrating its ability not only to reflect real-time clinical trends but also to provide quantitative insight into the scale of infection within the studied population.

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Table 2: Results of Statistical Analyses and Correlation Assessments

Statistical Test	Parameters Analyzed	Test Statistic / R^2	p-value	Interpretation
Spearman correlation	RNA concentration vs. number of patients	r = 0.76	$p < 0.05$	Positive correlation
Linear regression	RNA concentration vs. number of patients	$R^2 = 0.87$	$p < 0.05$	High capability of patient numbers on RNA concentrations
Mann–Whitney U test	RNA concentrations in low-patient vs. high-patient periods	U = 32	$p < 0.05$	Significant difference between groups

Discussion

During the study period, the concentration of SARS-CoV-2 RNA detected in untreated hospital wastewater demonstrated a clear upward trend, which closely paralleled the increasing number of hospitalized COVID-19 patients. Since the emergence of the SARS-CoV-2 pandemic, numerous studies have investigated the occurrence, persistence, and detectability of viral RNA in aquatic systems and wastewater environments, emphasizing its potential value as an indicator of community infection dynamics. The present study was designed with a specific focus on the systematic detection of SARS-CoV-2 RNA in wastewater collected from a hospital exclusively dedicated to the treatment of COVID-19 patients. In addition to quantifying viral RNA, the study aimed to evaluate the temporal association between wastewater viral load and the number of patient admissions, thereby exploring the applicability of wastewater-based epidemiology in a healthcare setting. Marked variations in RNA concentrations were observed across different sampling periods, reflecting both temporal fluctuations in patient numbers and broader epidemic trends. These findings underscore the utility of wastewater surveillance as a complementary and cost-effective approach to traditional clinical testing, offering an independent means of monitoring viral circulation at the population level. Moreover, by integrating environmental measurements with hospital admission data, the study highlights the potential of wastewater monitoring systems to provide early-warning signals and strengthen public health decision-making frameworks [32, 33]. In contrast, a survey in Japan did not identify any SARS-CoV-2 RNA in five untreated wastewater samples [34]. On the other hand, La Rosa et al. reported the presence of the virus in 6 out of 12 wastewater samples in Italy, though the concentration ranges were not specified [23]. Similarly, a Dutch investigation found SARS-CoV-2 RNA in 12 of 24 untreated wastewater samples (50%), with values ranging from 2600 to 22,000 gene copies per 100 mL [35]. In Australia, Ahmed and colleagues detected SARS-CoV-2 RNA in 22% of tested samples, with concentrations between 190 and 1200 gene copies per 100 mL [6]. Randazzo et al. documented the virus in 83% of wastewater samples from Spain, reporting concentrations ranging from 140,000 to 340,000 gene copies per 100 mL [24]. In the United States, viral RNA was identified in 28.5% of the samples, with concentrations between 3100 and 7500 gene copies per 100 mL [28]. Wurlitzer et al. found that six out of eight analyzed samples contained SARS-CoV-2 RNA; however, the viral load was markedly lower in treated samples compared with untreated ones [36]. Evidence from several developing nations has also confirmed the detection of SARS-CoV-2 RNA in raw wastewater, supporting its use as a surveillance tool in areas with limited infrastructure. In India, Kumar et al. reported viral RNA in wastewater from Ahmedabad, ranging between 2400 and 4100 gene copies per 100 mL, with levels corresponding to the number of local COVID-19 cases [26]. In Pakistan, Sharif et al. applied the polio environmental surveillance platform and identified SARS-CoV-2 RNA in 27% of samples, particularly in regions with a heavy disease burden [37]. In Iran, Tanhaei et al. detected viral RNA in wastewater collected from Tehran, with concentrations varying from 718 to 1090 gene copies per 100 mL [38]. In Egypt, Mohamed et al. found SARS-CoV-2 RNA in 62% of samples, mostly from densely populated

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urban settings [39]. In Romania, Ioana et al. demonstrated that RNA concentrations in wastewater correlated directly with the number of infections [40]. Studies carried out in Qatar and Brazil also verified the presence of SARS-CoV-2 RNA in untreated sewage, with results showing consistency with reported COVID-19 cases [41, 42]. These comparative results confirm the utility of wastewater surveillance in diverse settings and support the consistency of our findings. Additionally, clinical case data in this study showed a strong correlation (Spearman's $p<0.05$, $R^2=0.87$) with viral loads in wastewater, reinforcing the potential of hospital wastewater surveillance as a complementary approach to traditional clinical surveillance systems. An important observation of the present research was that SARS-CoV-2 RNA remained detectable in samples, despite routine internal surface disinfection practices. This is explained by the continuous shedding of viral particles and RNA fragments in the feces, urine, sputum, and other bodily fluids of infected patients, which directly enter the wastewater stream regardless of surface cleaning. From an environmental perspective, this persistence highlights the limited effect of local disinfection measures on wastewater viral loads and underscores the value of wastewater-based surveillance for capturing signals of community infection that would otherwise be missed by surface hygiene practices. This finding strongly supports the main conclusion that wastewater monitoring, even with simplified methods, can serve as a complementary tool for clinical surveillance by providing a more integrated signal of ongoing viral shedding within healthcare facilities. Our findings highlight that even with simple, low-cost sampling and RT-qPCR methods, a consistent correlation between wastewater viral RNA and hospital admissions can be detected in high-prevalence environments. This emphasizes the practical value of wastewater surveillance, especially in areas where clinical testing resources are limited. Detected SARS-CoV-2 RNA in this study (130–490 gene copies/100 ml) fell within the lower to mid-range of values reported globally [39, 40]. Differences in the reported concentrations across studies can be attributed to variations in population density, detection methods, and wastewater characteristics. Disparities in the results of these studies can be attributed to variations in methodologies, including viral RNA detection techniques, RNA extraction protocols, sampling strategies, and RT-qPCR methods. Although the concentrations observed in this study are relatively modest compared to some international reports, they may reflect the specific characteristics of hospital wastewater, including a high proportion of symptomatic COVID-19 patients, low dilution, and limited exposure to disinfectants. It should be noted that direct comparison of our RNA concentration values with those reported in other studies should be interpreted with caution. In contrast to these studies, we did not apply a sample concentration step before RNA extraction. Consequently, our reported viral load values may underestimate the absolute concentrations and are not strictly comparable across studies. Our primary aim was to demonstrate the feasibility of detection using a simplified approach rather than providing directly comparable quantitative values. The samples were collected from the hospital wastewater outlet, where the wastewater produced by COVID-19 patients was proportionally higher than that produced by healthy individuals. The relatively high viral RNA concentrations observed in this study may be attributed to the untreated nature of the hospital effluents, where short hydraulic retention time, minimal solids retention, absence of settling, and lack of chemical disinfectant exposure prevented significant viral RNA reduction, unlike in studies analyzing treated municipal wastewater [43, 44]. The presence of SARS-CoV-2 RNA in hospital wastewater strongly reflects localized viral shedding, particularly from symptomatic or severely affected patients, and highlights the role of healthcare-based surveillance as an early warning system. While these data provide critical insights into localized outbreaks, direct scalability to community-wide monitoring requires additional sampling points from municipal wastewater systems. Nevertheless, hospital-based surveillance could serve as an early warning tool for localized

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infection spikes, particularly in regions with limited access to mass testing, thus complementing broader community-monitoring strategies. In addition to methodological differences, environmental and health-related factors may influence the persistence of SARS-CoV-2 RNA in wastewater. Temperature, pH, hydraulic retention time, and the presence of disinfectants have been shown to affect the stability of viral RNA in aquatic environments [45]. These aspects suggest that the relatively high RNA concentrations in untreated hospital effluents may reflect both concentrated viral shedding and favorable conditions for RNA to remain stable. RNA fragments can persist in wastewater even when the virus is no longer capable of causing infection, which is an important limitation in interpreting the public health risks of such findings. This study had some limitations. First, the viability and infectivity of the detected SARS-CoV-2 RNA in wastewater samples were not assessed [3]. Second, clinical viral load data from individual patients were not available for direct comparison with the viral concentrations in wastewater [13]. Third, environmental factors such as temperature, pH, suspended particles (which may provide a protective layer enhancing viral persistence), and residual disinfectants in wastewater were not systematically evaluated in the studies. These parameters can strongly influence viral RNA stability and should be considered in future investigations to better understand the environmental behavior and public health implications of SARS-CoV-2 in wastewater treatment.

Conclusion

The present investigation clearly demonstrated the recurrent identification of SARS-CoV-2 RNA in raw, untreated hospital wastewater samples. A pronounced and statistically meaningful correlation was established between the measured concentrations of viral RNA and the number of patients admitted with confirmed COVID-19 infection during the same period. These outcomes reinforce the concept that wastewater-based epidemiology, specifically when conducted in healthcare facilities, represents a practical, reliable, and comparatively inexpensive strategy for tracking local transmission dynamics. Such an approach becomes particularly valuable in regions with limited laboratory infrastructure and insufficient access to large-scale diagnostic testing, as it provides an indirect yet robust indication of infection prevalence .It should be emphasized, however, that the identification of RNA fragments does not necessarily confirm the presence of viable or infectious viral particles. Nevertheless, the results of this study highlight the significant epidemiological contribution of wastewater monitoring as a supportive and complementary system alongside conventional clinical surveillance programs. To further strengthen this line of research, future investigations are encouraged to examine viral infectivity in wastewater matrices, assess survival under diverse environmental conditions such as temperature, pH, and organic load, and evaluate the implications for potential health risks associated with prolonged persistence.

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Conflicts of interest

The authors declare no conflicts of interest.

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Ethical Considerations

The Ethics Committee approved the study at Behbahan University of Medical Sciences.

Code of Ethics

The ethical code of the current study is IR.BHN.REC.1399.020

Author contributions

Rozhan Feizi: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Supervision. Abdollah Dargahi, Niloofar Neisi, Maryam Mehrbakhsh: Investigation, Methodology. Neematollah Jaafarzadeh, Masoud Panahi Fard: Methodology, Writing, review &

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editing, Supervision. All the authors have read and approved the final manuscript.

References

1. Hasöksüz M, Kiliç S, Saraç F. Coronaviruses and sars-cov-2. Turk J Med Sci. 2020;50(SI-1):549-56.
2. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-44.
3. Kitajima M, Ahmed W, Bibby K, et al. SARS-CoV-2 in wastewater: State of the knowledge and research needs. Sci Total Environ. 2020;739:139076.
4. Rahmani AR, Leili M, Azarian G, et al. Sampling and detection of corona viruses in air: a mini review. Sci Total Environ. 2020;740:140207.
5. Azizi Jalilian F, Poormohammadi A, Teimoori A, et al. Evaluation of SARS-CoV-2 in indoor air of Sina and Shahid Beheshti Hospitals and patients' houses. Food Environ Virol. 2022; 14(2):190-8.
6. Ahmed W, Angel N, Edson J, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ. 2020;728:138764.
7. Jones DL, Baluja MQ, Graham DW, 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.
8. Van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020;382(16):1564-7.
9. Riddell S, Goldie S, Hill A, et al. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J. 2020;17(1):1-7.
10. Riddell S, Goldie S, Hill A, et al. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J. 2020;17(1): 145.
11. Aboubakr HA, Sharafeldin TA, Goyal SM. Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: a review. Transbound Emerg Dis. 2021;68(2):296-312.
12. Tang A, Tong Z-d, Wang H-l, et al. Detection of novel coronavirus by RT-PCR in stool specimen from asymptomatic child, China. Emerging Infectious Diseases. 2020;26(6):1337.
13. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581(7809):465-9.
14. Tran HN, Le GT, Nguyen DT, et al. SARS-CoV-2 coronavirus in water and wastewater: a critical review about presence and concern. Environ Res. 2021;193:110265.
15. Carraturo F, Del Giudice C, Morelli M, et al. Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces. Environmental Pollution. 2020;265:115010.
16. Julian TR, Boehm AB. Advances in wastewater-based epidemiology in the ES&T family of journals. Environ Sci Technol Lett. 2024; 11(7): 650-3.
17. Xu X, Zheng X, Li S, et al. The first case study of wastewater-based epidemiology of COVID-19 in Hong Kong. Sci Total Environ. 2021;790:148000.
18. Godini H, Hoseinzadeh E, Hossini H. Water and wastewater as potential sources of SARS-CoV-2 transmission: a systematic review. Rev Environ Health. 2021;36(3): 309-17.
19. Usman M, Farooq M, Hanna K. Existence of SARS-CoV-2 in wastewater: implications for its environmental transmission in developing communities. ACS Publications; 2020.
20. Feizi R, Jaafarzadeh N, Panahi Fard M, et al.

[DOI: 10.18502/jehsd.v10i3.19791]

Integrating wastewater surveillance for future pandemic preparedness by tracking SARS-CoV-2 RNA in municipal wastewater in Southwest Iran. Sci Rep. 2025;15(1):18219.
21. Blatchley III ER, Gong WL, Alleman JE, et al. Effects of wastewater disinfection on waterborne bacteria and viruses. Water Environment Research. 2007;79(1):81-92.
22. Tariq A, Mushtaq A. Untreated wastewater reasons and causes: a review of most affected areas and cities. International Journal of Chemical and Biochemical Sciences. 2023;23(1):121-43.
23. La Rosa G, Iaconelli M, Mancini P, et al. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci Total Environ. 2020;736:139652.
24. Randazzo W, Truchado P, Cuevas-Ferrando E, et al. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 2020;181: 115942.
25. Medema G, Heijnen L, Elsinga G, et al. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ Sci Technol Lett. 2020;7(7):511-6.
26. Kumar M, Patel AK, Shah AV, et al. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Sci Total Environ. 2020;746:141326.
27. Tanhaei M, Mohebbi SR, Hosseini SM, et al. The first detection of SARS-CoV-2 RNA in the wastewater of Tehran, Iran. Environ Sci Pollut Res Int. 2021;28(29):38629-36.
28. Sherchan SP, Shahin S, Ward LM, et al. First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. Sci Total Environ. 2020;743: 140621.
29. Karami C, Dargahi A, Vosoughi M, et al. SARS-CoV-2 in municipal wastewater treatment plant, collection network, and hospital wastewater. Environ Sci Pollut Res Int. 2021:1-9.
30. Beheshti M, Neisi N, Parsanahad M, et al. Correlation of vitamin D levels with serum parameters in Covid-19 patients. Clin Nutr ESPEN. 2023;55:325-31.
31. Zhang L, Wang L. Optimization of site investigation program for reliability assessment of undrained slope using Spearman rank correlation coefficient. Comput Geotech. 2023;155:105208.
32. Wu F, Zhang J, Xiao A, et al. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. mSystems. 2020;5(4): e00614-20.
33. Qu G, Li X, Hu L, et al. An imperative need for research on the role of environmental factors in transmission of novel coronavirus (COVID-19). ACS Publications; 2020.
34. Haramoto E, Malla B, Thakali O, et al. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci Total Environ. 2020;737:140405.
35. Medema G, Heijnen L, Elsinga G, et al. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ Sci Technol Lett. 2020; 7(7):511-6.
36. Wurtzer S, Marechal V, Mouchel J-M, et al. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. medRxiv. 2020;2020-04.
37. Sharif S, Ikram A, Khurshid A, et al. Detection of SARs-CoV-2 in wastewater using the existing environmental surveillance network: a potential supplementary system for monitoring COVID-19 transmission. PLoS One. 2021;16(6):e0249568.
38. Tanhaei M, Mohebbi SR, Hosseini SM, et al. The first detection of SARS-CoV-2 RNA in the wastewater of Tehran, Iran. Environ Sci Pollut Res Int. 2021;28:38629-36.
39. Shaheen MN, Elmahdy EM, Shahein YE. The first detection of SARS-CoV-2 RNA in urban wastewater in Giza, Egypt. J Water Health. 2022;20(8):1212-22.
40. Boeraș I, Curtean-Bănăduc A, Bănăduc D, et al. Anthropogenic sewage water circuit as vector for SARS-CoV-2 viral ARN transport and public health assessment, monitoring and forecasting—Sibiu metropolitan area (Transylvania/Romania) study case. Int J Environ Res Public Health.

2022;19(18):11725.
41. Saththasivam J, El-Malah SS, Gomez TA, et al. COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar. Sci Total Environ. 2021;774:145608.
42. Claro ICM, Cabral AD, Augusto MR, et al. Long-term monitoring of SARS-COV-2 RNA in wastewater in Brazil: a more responsive and economical approach. Water Res. 2021;203: 117534.
43. Nasseri S, Yavarian J, Baghani AN, et al. The presence of SARS-CoV-2 in raw and treated wastewater in 3 cities of Iran: Tehran, Qom and Anzali during coronavirus disease 2019 (COVID-19) outbreak. J Environ Health Sci Eng. 2021;19(1): 573-84.
44. Al-Duroobi H, Vadde KK, Phan DC, et al. Wastewater-based surveillance of COVID-19 and removal of SARS-CoV-2 RNA across a major wastewater treatment plant in San Antonio, Texas. Env Sci Adv. 2023;2(5):709-20.
45. Eslami H, Jalili M. The role of environmental factors to transmission of SARS-CoV-2 (COVID-19). AMB Express. 2020;10(1):92.

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