Wastewater Surveillance of SARS-CoV-2 as Monitoring Tool for COVID-19

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ABSTRACT

Background: The COVID-19 pandemic, caused by SARS-CoV-2, has significantly impacted global health, requiring effective surveillance methods. Wastewater surveillance has emerged as a cost-effective and non-invasive tool to detect viral RNA from both symptomatic and asymptomatic individuals, allowing early detection of outbreaks.

Objective: This study analyzes the effectiveness of wastewater surveillance in detecting SARS-CoV-2 RNA in untreated wastewater, comparing it with clinical surveillance and evaluating its role in public health monitoring.

Method: A literature review was conducted using PubMed, Google Scholar, and Scopus, focusing on studies published between 2015–2025. Articles were selected based on relevance, availability, and originality, applying the PICO strategy to refine the search. From ten initially identified studies, two were selected for in-depth analysis.

Result: The findings demonstrate that wastewater surveillance effectively tracks SARS-CoV-2 trends in communities. A study in Brazil showed a strong correlation between viral loads in wastewater and clinical case trends, proving its potential as an early warning system. Meanwhile, research in Australia confirmed the presence of SARS-CoV-2 in wastewater and estimated infection prevalence using Monte Carlo simulation, aligning with reported clinical cases. These studies highlight wastewater surveillance as a reliable tool for population-wide monitoring.

Conclusion: Wastewater surveillance is a proven method for detecting SARS-CoV-2, complementing clinical surveillance by enabling early detection, wider coverage, and cost

efficiency. Challenges such as RNA degradation and detection standardization require further research, but integrating RT-qPCR and genomic sequencing enhances its accuracy for future epidemiological monitoring.

Keyword: Monitoring; SARS-CoV-2; Surveillance; Wastewater

Introduction

The COVID-19 pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has significantly impacted daily life worldwide with historic case numbers and mortality rates[1]. This pandemic has driven extensive efforts to detect and monitor the spread of the virus. Several studies have demonstrated that SARS-CoV-2 viral RNA are present in stool samples, not only in symptomatic patients but also in asymptomatic patients[2,3]. The presence of viral RNA of SARS-CoV-2 in stool and other human excreta has enabled the detection of the virus in domestic wastewater[4].

One proven and effective method for detecting SARS-CoV-2 in wastewater is wastewater surveillance. Wastewater surveillance is a type of environmental surveillance that has historically been used to track waterborne or fecal-orally transmitted pathogens[5]. This method allows for the early detection of SARS-CoV-2 in a population without the need for direct individual testing. Untreated wastewater samples can contain viral RNA fragments excreted through the feces and urine of infected individuals[6]. Wastewater surveillance offers a cost-effective strategy for rapid response and can be integrated with other public health interventions, serving as an additional tool in decision-making[4].

The success of this approach has also led to its adoption for monitoring new SARS-CoV-2 variants, contributing to global public health strategies. By integrating molecular technologies such as RT-qPCR and genomic sequencing, wastewater surveillance provides a more comprehensive overview of the virus's spread within a given region[7.] This literature review aims to analyze the effectiveness of wastewater surveillance in detecting SARS-CoV-2 viral RNA in wastewater, covering aspects such as sampling methods, comparisons with clinical surveillance, and overall efficiency.

Method

This study is a literature review focusing on the detection of SARS-CoV-2, the virus responsible for COVID-19, in untreated wastewater using wastewater surveillance. The literature search was conducted using specific keywords, including SARS-CoV-2; Wastewater;

and Surveillance. The sources of literature were obtained from several electronic databases, namely PubMed, Google Scholar, and Scopus. Only articles published in English were considered. The search was restricted to publications within the last 10 years (2015–2025). The selected articles were original research articles, available in full-text, open-access, and not systematic reviews or literature reviews. To ensure the relevance of the literature, we applied the Population, Intervention, Comparison, and Outcome (PICO) strategy:

1. Problem: Presence of SARS-CoV-2 viral RNA in untreated wastewater
2. Intervention: Wastewater surveillance
3. Comparison: Clinical surveillance
4. Outcome: Detection of the virus

Using this search methodology and PICO strategy, we initially identified 10 journals. These were further filtered based on correspondence with the research criteria. Ultimately, we selected 2 English-language journals that met all the inclusion criteria from the initial 10 journals.

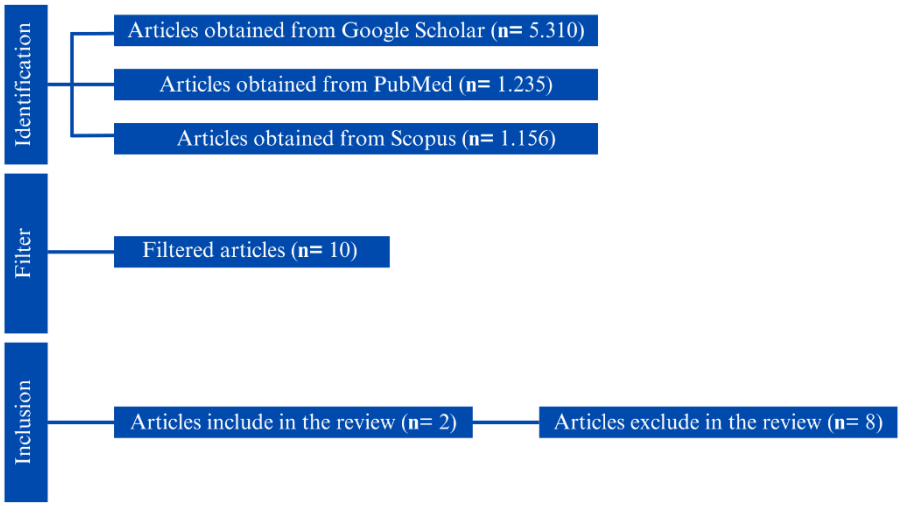

Figure 1. Flowchart of literature search strategy and screening process.

Result

In searching for this article, 2 journals were found that were relevant to the following topics:

Table 1. Summary of Selected Research Results

Title	Author and Year	Research Subject	Methods	Results
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	Warish Ahmed, Nicola Angel, Janette Edson, *et al.* 2020	Untreated wastewater (sewage) from one suburban pumping station (PS) and two WWTPs representing urban catchments in Southeast Queensland (SEQ).	Quantitative study	2 of the 9 samples tested were detected to contain the SARS-CoV-2 virus through RT-qPCR analysis confirmed by sequencing.
Wastewater-based epidemiology: A Brazilian SARS-COV-2 surveillance experience	Rodrigo de Freitas Bueno, Ieda Carolina Mantovani Claro, Matheus Ribeiro Augusto, *et al*. 2022	Sewage samples were collected from the entrance of wastewater treatment plants (WWTP), and in other locations of the sewer system, such as lift stations and sewer manholes.	Quantitative Study	A positive and significant correlation between viral load in wastewater and clinical cases was verified, with a significance level of 95% ($p < 0.05$). Spearman's rho correlation coefficient values ranged from 0.41 to 0.61.

Discussion

Taking Untreated Wastewater as The Sample

Based on research conducted by Warish Ahmed, Nicola Angel, *et al*. 2020, the samples taken were untreated wastewater (sewage) from 3 different locations, namely one suburban pumping station (PS) and two WWTPs representing urban catchments in Southeast Queensland (SEQ). The samples were stored at 4°C until analysis. In contrast to research conducted by Rodrigo de F.B., Ieda Carolina M. C., *et al*. 2022, wastewater samples were taken from the entrance of the wastewater treatment plant (WWTP) and at other locations in the sewage system, such as lift stations and sewage manholes. At the WWTP, the samples taken were 24-hour composite samples of 1000 mL using a refrigerated automatic sampling method with a storage temperature of 4°C. Meanwhile, at the sewer manhole and lift station, the samples taken were 4-hour semi-composite samples of 1000 mL and were also taken using the same sampling method. Then this sample was divided into two parts with each part containing 40 mL and stored at a temperature of 4°C for a maximum of 36 hours.

Sample Concentration and Analyzing Method

The virus was concentrated using 2 methods, namely Method A (direct RNA extraction from electronegative membranes) and Method B (Ultrafiltration)[(8)]. Another method for concentrating the virus is by precipitation[(4)]. After the wastewater was concentrated, an analysis was carried out to see if there was a virus from SARS-CoV-2 in the sample. From both studies, there were similarities in the use of methods to analyze the samples. The method used is an RT-qPCR assay, which in several publications this method has indeed been used to detect RNA from SARS-CoV-2 in wastewater samples[9] . In addition, in both studies, the RT-qPCR test was carried out using the Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories). Inhibition tests were also carried out by each study to verify the effect of inhibitors on RT-qPCR performance.

Effectiveness of Wastewater Surveillance in Detecting SARS-CoV-2

Both studies showed that wastewater surveillance was able to detect SARS-CoV-2 RNA before a spike in clinical cases occurred. This is because viral RNA can be excreted through the feces and urine of infected individuals, including asymptomatic and asymptomatic individuals. In a study conducted by Rodrigo de F.B., Ieda Carolina M. C., *et al*. 2022, showed that the concentration of SARS-CoV-2 RNA in wastewater followed the epidemiological pattern of COVID-19 cases, with a significant increase during the second wave in 2021 and early 2022. The study by Warish Ahmed, Nicola Angel, *et al*. 2020, also demonstrated

effectiveness by being the first evidence that SARS-CoV-2 can be detected in wastewater in the country with an estimated number of infected individuals ranging from 171 to 1,090 based on the Monte Carlo method. The main advantages of wastewater surveillance over individual testing are its wide coverage and lower cost. This method can be used to monitor an entire population in an area with just one wastewater sample, without the need to conduct thousands of individual tests.

Comparison Between Wastewater Surveillance dengan Clinical Surveillance

Wastewater surveillance can complement, or even precede, clinical monitoring systems. The following is a summary of the differences between wastewater surveillance and clinical surveillance[4,8,10]:

Table 2. Comparison Between Wastewater Surveillance and Clinical Surveillance

Aspect	Wastewater Surveillance	Clinical Surveillance
Population Coverage	Reaches entire community without individual testing	Only covers tested individuals
Early Detection	Able to detect asymptomatic individuals earlier	Depends on individuals who have symptoms and get tested
Cost	More cost-effective, simply by sampling wastewater	More expensive because it requires testing a large number of individuals
Speed of Response	Fast and can be used as an early warning system	Limited to individuals who get tested
Accuracy of Case	Estimation Can provide estimates of the number of cases based on viral RNA concentration	More accurate for confirmed cases, but often underreported

From the comparison above, it can be seen that wastewater surveillance does not replace clinical surveillance but rather as an additional tool to provide more comprehensive epidemiological information.

Implication for Public Health

Through wastewater surveillance, viral RNA from SARS-CoV-2 can be detected earlier so that it can be used as an early warning system. Thus, health authorities can respond faster

before a clinical spike occurs. For example, in Brazil, a spike in viral RNA from SARS-CoV-2 in wastewater occurred before the increase in the number of clinically reported cases. This surveillance method also allows monitoring of new variants as has been done in Brazil during the emergence of the Omicron variant. With genomic sequencing technology, genetic variations of the virus can also be identified from wastewater samples. The use of more efficient costs makes this wastewater surveillance a cheaper and more efficient solution in developing countries that have limitations.

Challenges in Implementing Wastewater Surveillance

The variability of virus concentration in wastewater can hinder the process of analyzing viral RNA. Several factors are the causes, namely changes in the pattern of virus excretion by infected individuals because it depends on the stage of infection, degradation of viral RNA in the environment, especially in areas with high temperatures, and variations in wastewater volume that can affect detection results. In a study conducted by Warish Ahmed, Nicola Angel, *et al.* 2020, differences in results were detected between the methods used to concentrate the virus before RT-qPCR was carried out. This shows that choosing the right method is very important in ensuring detection accuracy (Ahmed et al., 2020). In addition, the method used to detect SARS-CoV-2 in wastewater needs to be further validated so that the results obtained can be compared globally.

Conclusion

Wastewater surveillance is an effective tool in detecting SARS-CoV-2 and can be used as an early warning system to monitor the spread of COVID-19. Wastewater surveillance has the advantage of detecting asymptomatic cases and a wider population coverage compared to clinical surveillance. The public health implications are enormous, including early warning, monitoring of new variants, and cost savings in public health strategies. Key challenges in implementation are the variability of virus concentrations in wastewater, standardization of detection methods, and more accurate case estimates. With further technological development and research, wastewater surveillance could become an essential part of the global health system to deal with future pandemics.

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