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REVIEW ARTICLE

SARS-CoV-2 surveillance in medical and industrial wastewater—a global perspective: a narrative review

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Abstract

The novel coronavirus SARS-CoV-2 has spread at an unprecedented rate since late 2019, leading to the global COVID-19 pandemic. During the pandemic, being able to detect SARS-CoV-2 in human populations with high coverage quickly is a huge challenge. As SARS-CoV-2 is excreted in human excreta and thus exposed to the aqueous environment through sewers, the goal is to develop an ideal, non-invasive, cost-effective epidemiological method for detecting SARS-CoV-2. Wastewater surveillance has gained widespread interest and is increasingly being investigated as an effective early warning tool for monitoring the spread and evolution of the virus. This review emphasizes important findings on SARS-CoV-2 wastewater-based epidemiology (WBE) in different continents and techniques used to detect SARS-CoV-2 in wastewater during the period 2020–2022. The results show that WBE is a valuable population-level method for monitoring SARS-CoV-2 and is a valuable early warning alert. It can assist policymakers in formulating relevant policies to avoid the negative impacts of early or delayed action. Such strategy can also help avoid unnecessary wastage of medical resources, rationalize vaccine distribution, assist early detection, and contain large-scale outbreaks.

Keywords SARS-CoV-2 · Wastewater-based epidemiology · Wastewater surveillance · PCR · Early warning

Introduction

In late December 2019, a novel coronavirus causing severe pneumonia in patients spread at an unprecedented rate, leading to the current COVID-19 pandemic. On February 11, 2022, the virus was identified and officially named SARS-CoV-2. The other two preceding human coronaviruses that cause severe diseases beyond the "common cold" syndromes are SARS-CoV found in 2002 and MERS-CoV identified in 2012 (Hu et al. 2021). Compared with the previous two human coronaviruses that usually cause severe outcomes, SARS-CoV-2 has a higher mutation rate and stronger transmissibility (Petersen et al. 2020). As of August 26, 2022, there were about 600 million confirmed COVID-19 cases and about 6.5 million deaths worldwide (https://covid19.who.int/). There are 4 proposed ways for SARS-CoV-2 to spread among humans: first, exposure to droplets generated by infected patients; second, close contact with infected individuals; third, contact with objects contaminated by SARS-CoV-2; and fourth, the newly proposed airborne transmission through aerosols (Bchetnia et al. 2020). Severe air pollution, low wind speeds, low temperatures, and other climatic conditions, as well as high population densities, aggravate the spread of the virus in the air and increase the number of infections (Coccia 2020). In addition, asymptomatic infected individuals and those with mild symptoms can also contribute to the spread of the virus (Kumblathan et al. 2023). The government's policy of relaxing COVID-19 restrictions and not regulating gatherings or festivals can result in high transmission of SARS-CoV-2 in population (Zhao et al. 2023), followed by the virus being discharged into wastewater, leading to the spread of the virus in the aqueous environment. Highly contaminated hospital wastewater, if left untreated, can lead to fecal–oral and fecal-respiratory transmission of infectious diseases to the community through wastewater,

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increasing environmental exposure (Amin et al. 2023; Núñez-Delgado et al. 2021). In addition, the presence of viruses or their genetic material in wastewater is diluted by other sources besides domestic wastewater, such as rainwater, groundwater, and melted snow from sewer pipes (Langeveld et al. 2023; Saingam et al. 2023). In this regard, the viral spread can be prevented and minimized by achieving certain protection for the human population by wearing masks, keeping social distance, restricting air travel, reducing the number of people gathered, and allocating COVID-19 vaccine.

However, SARS-CoV-2 is not static but mutable. It is an RNA virus with a higher mutation rate than a DNA virus, and mutations mainly occur in the spike protein at the surface of the virus, resulting in changes in viral infectivity and transmissibility (Alkhatib et al. 2022; Tian et al. 2022). At present, there are 5 SARS-CoV-2 variants of concern (VOC), defined as Alpha, Beta, Gamma, Delta, and Omicron. The Omicron variant, first discovered in South Africa in November 2021, has a large number of mutations and higher infectivity than the other VOCs and is currently becoming the dominating variant in the world (Tian et al. 2022). The high immune evasion capacity of the Omicron variant and the changes in cellular tropism lead to about 3 times higher transmission rate than that of the Delta variant (Fan et al. 2022). In addition, SARS-CoV-2 Omicron keeps evolving and will not likely stop.

During the pandemic, the main task of health workers is to perform laboratory-based diagnostic tests on individuals; however, it is relatively time-consuming and labor-intensive. Therefore, wastewater monitoring is often recommended as an early warning system to monitor the emergence and resurgence of outbreaks, while government officials can use it to identify target populations to test and develop measures to contain and mitigate outbreaks (Aguiar-Oliveira et al. 2020). In addition, wastewater monitoring has the potential advantage of easy sampling and the ability to estimate the overall status of the catchment area (Shah et al. 2022). The ongoing mutation of SARS-CoV-2 is leading to more asymptomatic infections, and wastewater surveillance can detect asymptomatic infections with less effort than clinical testing. Wastewater monitoring can complement clinical testing by providing large-scale monitoring through non-invasive, efficient, and cost-effective methods (Shah et al. 2022). Therefore, environmental monitoring and water testing are becoming more and more important for SARS-CoV-2 detection in public health alarming. Wastewater-based epidemiology (WBE) is also being developed as a novel tool for the analysis of biomarkers in wastewater pipelines.

To review wastewater monitoring in several regions of the world and techniques for the detection and differentiation of SARS-CoV-2 in wastewater samples, we demonstrated the recent findings and novel applications by using advanced techniques to detect SARS-CoV-2 in environmental water samples in this narrative review. Technological advances in the detection of pathogens in the aqueous environment and prospects are discussed with details. Recommendations are made for how we can apply WBE to unknown pandemics that might take place in the future.

Materials and methods

This review covers the topic of the detection of SARS-CoV-2 in wastewater with molecular detection methods. Data of results in this review were collected using PubMed for searching relevant articles published in 2020–2022, using the terms "SARS-CoV-2," "COVID-19," "PCR," "dPCR," and "LAMP." Irrelevant articles and reviews that did not include complete data were filtered manually. Selectively, 39 research articles were included in this study, and selected results from these studies are summarized.

Results and discussion

SARS-CoV-2 detection in wastewater samples worldwide

Wastewater epidemiology was first proposed by Dr. Christian G. Daughton, a scientist from the National Environmental Protection Agency of the USA. The principle is to analyze the concentration of chemical substances in the wastewater treatment plant (WWPT) and to deduce the consumption of this substance in specific areas by combining information such as human metabolic mechanism, influent flow, and the number of people served, to explore the relevant public dynamic information such as drug abuse, disease, and health. Later, it was popularized to monitor drug abuse, infectious pathogens, and other fields (Hernández et al. 2018; Zuccato et al. 2005). Regarding the global epidemic, WBE is used to monitor SARS-CoV-2 and the VOCs in wastewater with the ability to control and mitigate the outbreak of the epidemic.

Many countries have detected SARS-CoV-2 from wastewater to develop and integrate such strategies into their early health alert system (Table 1, Fig. 1). In Australia, Ahmed et al. first detected SARS-CoV-2 in untreated wastewater and estimated approximately 171 to 1090 infected individuals based on calculated SARS-CoV-2 RNA copy numbers (Ahmed et al. 2020a). Ahmed et al. detected SARS-CoV-2 RNA in wastewater from commercial airliners and cruise ships, which could be used as a complement to clinical swab testing to take appropriate precautions against the ongoing COVID-19 pandemic and could help these industries resume their entire operations rapidly (Ahmed et al. 2020b). In South

Table 1 Detection of SARS-CoV-2 nucleic acid in sewage in different countries

	Country	Region	Time	Concentration method	Detection method	Detection gene	Max. detection concentration	Application	Ref.
Oceania	Australia	Queensland	2020/02–2020/04	Method A and method B	RT-qPCR	N	12 copies/100 mL	Estimation of the number of infected persons	Ahmed et al. (2020a)
			2020/04–2020/05	Method A and method B	RT-qPCR, RT-ddPCR	N1, N2, E	596 copies/100 mL	Tracking infected individuals	Ahmed et al. (2020b)
South America	Brazil	Minas Gerais	2020/08	PEG method	RT-qPCR	N1, N2	4.3×10^4 GC/mL	Early warning	Fongaro et al. (2022)
		São José do Rio Preto	2020/07–2021/07	Ultracentrifugation	RT-qPCR	N1, N2	1.3×10^5 copies/L	Early warning	Martins et al. (2022)
Northern America	America	Virginia	2020/03–2020/07	Method B	RT-ddPCR	N1, N2, N3	1×10^4 copies/100 mL	Tracking epidemiological trends	Gonzalez et al. (2020)
		Ohio	2020/07–2021/01	Adsorption-precipitation-based method, organic flocculation and centrifugal ultrafiltration	RT-qPCR	N1, N2, E	1×10^5 copies/L	Tracking epidemiological trends	Ai et al. (2021)
		Nevada	2020/03–2021/04	Ultrafiltration	RT-qPCR WGS	ORF1a, E, N1, N2	8.7 $\log_{10}$ GC/L	Assess disease incidence, early monitoring of variants	Vo et al. (2022)
	Mexico	Tapachula	2020/09–2021/01	PEG method	RT-qPCR	N1, N2	7.38×10^2 copies/mL	Early warning	Zarza et al. (2022)
Asia	Japan	Ishikawa	2020/03–2020/06	PEG method	RT-qPCR	N	3.5×10^4 copies/L	Early warning	Hata et al. (2021)
	India	Gujarat	2020/05	PEG method	RT-qPCR	ORF1ab, N, S	3.5×10^2 copies/L	Understand epidemiological trends	Kumar et al. (2020)
	Iran	Tehran	2020/06–2020/07	Membrane filtration and PEG perception	RT-qPCR	ORF1ab, N	1.09×10^3 GC/mL	—	Tanhaei et al. (2021)
	China	Hong Kong	2020/06–2020/09	Method A	RT-qPCR	N	1975 copy/mL	Early warning	Xu et al. (2021)
Europe	France	Greater Paris	2020/03–2020/04	Ultracentrifugation	RT-qPCR	E	2.5×10^6 UG/L	Tracking epidemiological trends	Wurtzer et al. (2020)
	Italy	Lombardy	2020/03–2020/06	PEG method	RT-qPCR	N	2.1×10^5 copies/L	Tracking epidemiological trends	Castiglioni et al. (2022)
	Germany	Frankfurt	2020/04–2020/08	Method B	RT-qPCR	ORF1ab, N, S	1×10^{15} copies/day	Early warning	Agrawal et al. (2021)
		Saxony	2022/01–2022/05	PEG method	RT-qPCR	E, S	3.2×10^6 GC/L	Track the epidemiology	Dumke et al. (2022)
	Spain	Murcia	2020/03–2020/04	Aluminum hydroxide adsorption-precipitation concentration method	RT-qPCR	N1, N2, N3	5.4 ± 0.2 log10 copies/L	Early warning	Randazzo et al. (2020)

Method A: ultrafiltration; method B: adsorption elution method using an electronegative membrane

RT-qPCR reverse transcription-quantitative polymerase chain reaction, *RT-ddPCR* reverse transcription-droplet digital polymerase chain reaction; *WGS* whole genome sequencing

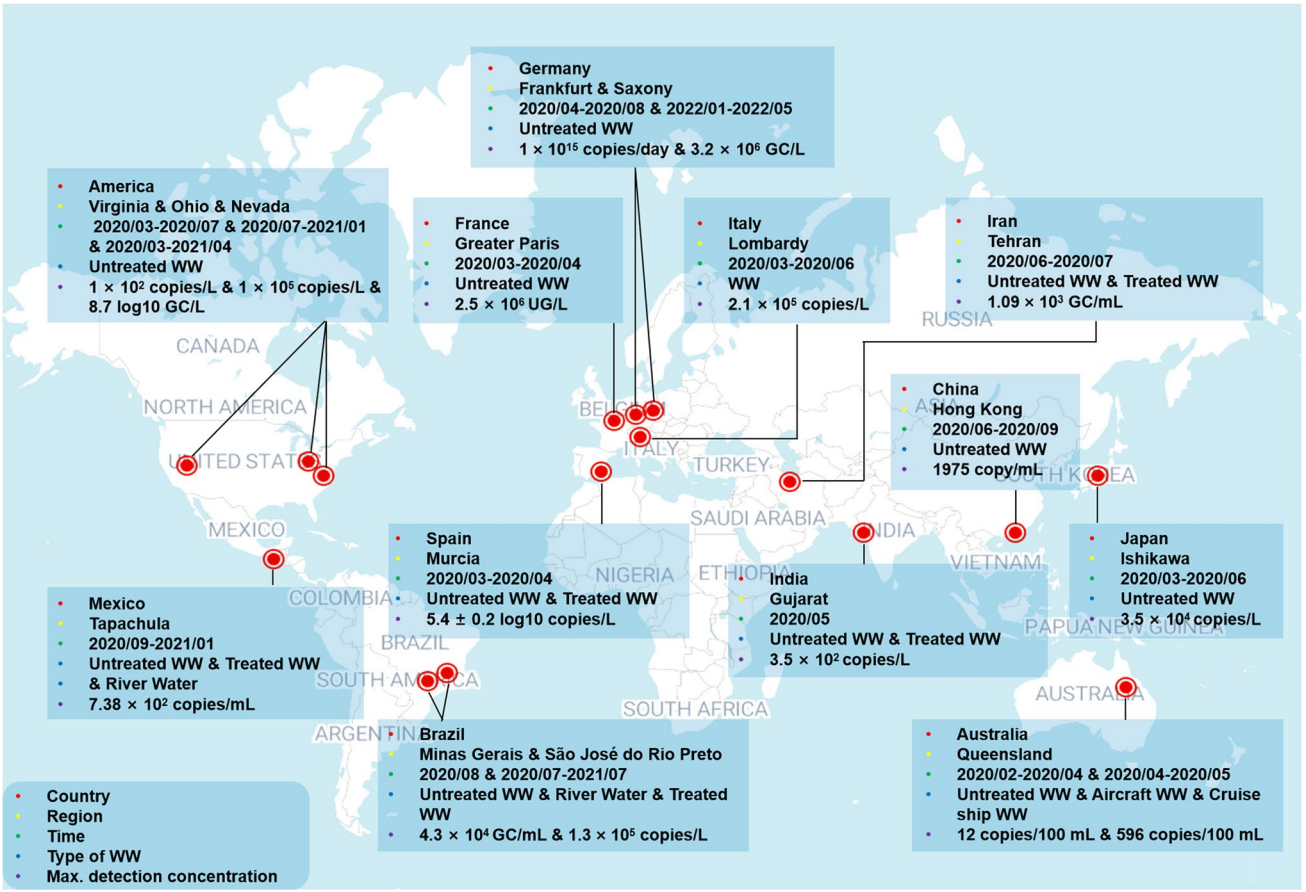

Fig. 1 Detection of SARS-CoV-2 in wastewater in different countries. Presentation of the detection of SARS-CoV-2 in wastewater in 16 different regions between 2020 and 2022 (Germany (Agrawal et al. 2021; Dumke et al. 2022), America (Ai et al. 2021; Gonzalez et al. 2020; Vo et al. 2022), France (Wurtzer et al. 2020), Italy (Castiglioni et al. 2022), Iran (Tanhaei et al. 2021), China (Xu et al. 2021), Mexico (Zarza et al. 2022), Spain (Randazzo et al. 2020), India (Kumar et al. 2020), Japan (Hata et al. 2021), Brazil (Fongaro et al. 2022; Martins et al. 2022), Australia (Ahmed et al. 2020a; Ahmed et al. 2020b)). WW, wastewater

America, Fongaro et al. successfully detected SARS-CoV-2 in wastewater from a remote area with poor sanitation in Brazil, demonstrating that wastewater monitoring can be a powerful surveillance tool in remote areas where personal testing is difficult to implement (Fongaro et al. 2022). Martins et al. tested viral RNA concentrations in the San Jose-Duriopreto area for 1 year and found that viral RNA was detected in wastewater 5 days before a positive confirmed case, indicating that wastewater monitoring can be used as a powerful early surveillance tool (Martins et al. 2022). The relationship between virus concentration and environmental factors was also explored, and the findings suggest that temperature was negatively correlated with virus concentration and that rainfall weather reduced the quantification of viral RNA (Martins et al. 2022). In North America, Gonzalez et al. showed trends in virus concentrations over time, which could facilitate targeting medical resources by public health workers (Gonzalez et al. 2020). Ai et al. found trends of RNA concentration of SARS-CoV-2 in wastewater were consistent with the daily trendencies of new confirmed cases in Ohio (Ai et al. 2021). Correspondingly, Vo et al. found that trends in viral RNA concentrations were consistent with changes in COVID-19 incidence in southern Nevada, suggesting that wastewater monitoring could be used as a surrogate tool to assess disease incidence (Vo et al. 2022). Zarza et al. detected SARS-CoV-2 RNA in Mexico, indicating that wastewater monitoring in the tropics is still feasible even though virus concentrations can be affected by temperature (Zarza et al. 2022). In Asia, Hata et al. investigated the presence of SARS-CoV-2 RNA in wastewater and compared it with the number of confirmed cases during the epidemic outbreak in Japan, suggesting that wastewater monitoring can be an early surveillance tool for outbreaks (Hata et al. 2021). Kumar et al. first extracted genetic material of SARS-CoV-2 from wastewater in India and detected

viral RNA during pandemic period (Kumar et al. 2020). Tanhaei et al. detected the presence of SARS-CoV-2 RNA for the first time in raw as well as treated wastewater from Tehran, Iran (Tanhaei et al. 2021). In Hong Kong, Xu et al. detected large amounts of SARS-CoV-2 RNA in sewage from isolation wards in hospitals (Xu et al. 2021). In addition, viral RNA was also detected in sewage from residential areas 2 days before the first confirmed case was reported, demonstrating the effectiveness of wastewater monitoring as an early warning tool (Xu et al. 2021). In Europe, where the outbreak was severe, countries have also been monitoring SARS-CoV-2 in wastewater to monitor the prevalence of the virus. Wurtzer et al. quantified SARS-CoV-2 concentrations in wastewater in Greater Paris and found that virus concentrations in wastewater increased (or decreased) as the number of confirmed cases increased (or decreased) (Wurtzer et al. 2020). Castiglioni et al. quantified SARS-CoV-2 RNA in wastewater from the Lombardy region to track epidemiological trends (Castiglioni et al. 2022). In Germany, Agrawal et al. monitored the time course of viral RNA concentrations in untreated sewage in Frankfurt and demonstrated the potential of WBE as an early surveillance system for SARS-CoV-2 infection to identify global COVID-19 hotspots (Agrawal et al. 2021). Dumke et al. frequently detected SARS-CoV-2 and influenza viruses in 2 German WWTPs and concluded wastewater monitoring can be used to track disease epidemiology (Dumke et al. 2022). Randazzo et al. tested viral levels of SARS-CoV-2 in 6 WWTPs for the Murcia region and found that SARS-CoV-2 RNA could be detected in wastewater before municipalities reported confirmed cases, and argued that municipalities can use this environmental monitoring to make decisions to gradually lift the blockade measures during COVID-19 pandemic (Randazzo et al. 2020).

Molecular techniques for the detection of SARS-CoV-2 and its variants in wastewater

Fever, cough, sputum production, shortness of breath, etc. are common clinical manifestations of patients infected with SARS-CoV-2 (Guo et al. 2020). In addition, SARS-CoV-2 will also infect the host's intestinal cells causing nausea, vomiting, diarrhea, as well as other symptoms (Zhong et al. 2020). Viral RNA was detected in the stool of some patients infected with SARS-CoV-2 even after their pharyngeal swabs had turned negative (Chen et al. 2020). SARS CoV-2 can be detected in stool samples for a long time; even after 3 weeks of illness, high viral load can be detected (Cevik et al. 2021). SARS-CoV-2 RNA will not be degraded in the environment to a large extent. SARS-CoV-2 RNA can be quantified without significant loss in wastewater samples for up to 7 days at 4 °C or 20 °C (Wurtzer et al. 2021). After the infected person excretes, the complete or degraded SARS-CoV-2 and RNA fragments arrive at the WWTPs through the sewer network. The wastewater monitoring process of SARS-CoV-2 RNA includes sample collection, virus enrichment, RNA extraction, RNA detection, analysis, and data interpretation. Molecular diagnostic techniques are now increasingly being used for the rapid and reliable detection of pathogenic microorganisms in wastewater. Continuous technological advances have allowed for continuous improvement and expansion of molecular diagnostic techniques, and cutting-edge molecular methods have largely contributed to the monitoring of SARS-CoV-2 even novel pathogenic coronaviruses in wastewater (Table 1, Fig. 2).

Real-time quantitative PCR

The most commonly used assay for wastewater monitoring of SARS-CoV-2 is real-time quantitative PCR (RT-qPCR) (Table 1). The RT-qPCR technique has the advantages of high specificity and short detection time and is the "gold standard" for detecting and quantifying low concentrations of viral particles in complex matrices. RT-qPCR is divided into one-step and two-step methods. Qiu et al. found that the one-step method could still detect RNA-dependent RNA polymerase (RdRp) and envelope (E) genes until sample dilution of 10^{-4}, while the two-step assay could only detect RdRp and E gene at sample dilution of 10^{-3} and sample dilution of 10^{-2}, respectively, illustrating that the one-step assay has a lower limit of detection (LOD) and higher detection sensitivity compared to the two-step RT-qPCR (Qiu et al. 2022). The RT-qPCR primer–probe sets currently developed for global application target different SARS-CoV-2 RNA regions, including the nucleocapsid (N), E, RdRp, and open reading frame (ORF) (Table 1). The sensitivity measured using different primer probes is different. Xu et al. evaluated the detection performance of 7 sets of primer probes, and they showed that N1 sets (primer and probe to detect N) had the highest sensitivity and specificity with 68% and 100%, respectively (Xu et al. 2022b). Martins et al. used N1 and N2 to quantify viral RNA in wastewater and showed higher positive detection rates for N1 than for N2, with 100% for the former and 96.6% for the latter (Martins et al. 2022). Maksimovic Carvalho Ferreira et al. demonstrated experimentally that N1 and N2 assays are 10 times more sensitive than E assays and 100 times more sensitive than RdRp assays, and present the lowest LOD

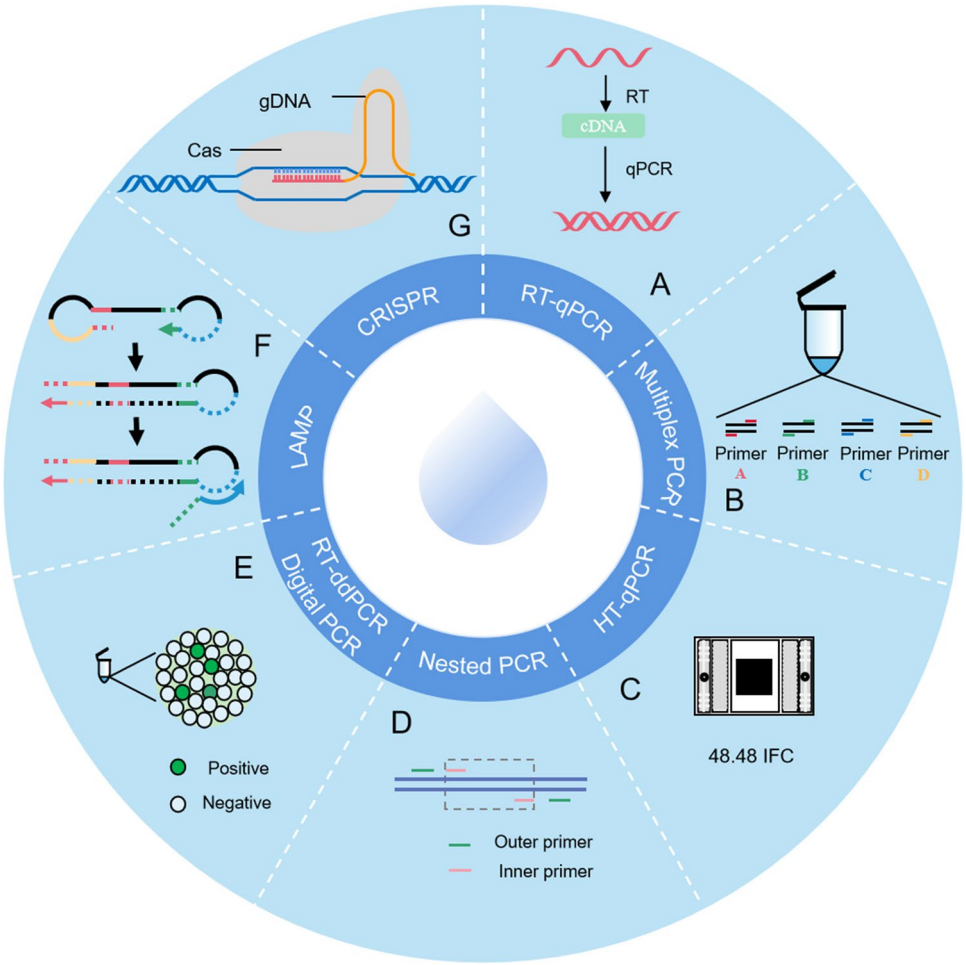

Fig. 2 SARS-CoV-2 RNA detection methods. Current methods applied to wastewater samples include RT-qPCR, multiplex PCR, HT-qPCR, nested PCR, RT-ddPCR, LAMP, and CRISPR. **A** Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is an experimental method applied to PCR experiments that uses RNA as the starting material. In this method, total RNA or messenger RNA (mRNA) is first transcribed into complementary DNA (cDNA) by reverse transcriptase. Subsequently, the cDNA is then used as a template for a quantitative PCR reaction. **B** Multiplex PCR refers to the simultaneous amplification of several different DNA sequences using the polymerase chain reaction. In an experiment, multiple target sequences are amplified in a single PCR reaction by using multiple primers in the reaction mixture. **C** The high-throughput qPCR is based on microfluidic technology and uses an integrated fluidic circuit chip for qPCR reactions. A 48.48 IFC chip allows 2304 reactions in one HT-qPCR run. The number of genes measured is higher compared to conventional qPCR. **D** Nested PCR is a modification of the polymerase chain reaction designed to reduce non-specific binding in the product due to the amplification of unexpected primer binding sites and involves 2 sets of primers for 2 consecutive polymerase chain reactions and a second set of primers for the amplification of secondary targets in the first batch. **E** Digital PCR is the third generation of PCR technology after the first generation of general PCR and the second generation of fluorescent quantitative PCR. The principle is that a sample is fully diluted and assigned to different reaction units, each containing less than or equal to one copy of the target molecule (DNA template), and a separate, parallel PCR reaction is performed in each reaction unit to achieve "single-molecule template PCR amplification". After amplification, the reaction unit containing the nucleic acid template produces a fluorescent signal and the analysis software calculates the concentration or copy number of the target molecule. Droplet-based microfluidic droplet digital PCR is one method for creating multiple independent reaction units. **F** The loop-mediated isothermal amplification technique requires the design of 3 pairs of specific primers based on 6 regions at the 3′ and 5′ ends of the target gene, including 1 pair of external primers, 1 pair of loop primers, and 1 pair of internal primers. The 3 specific primers rely on DNA polymerase with a strand displacement function, making strand-substitution DNA synthesis non-stop self-cycling. This reaction starts with the formation of a dumbbell-shaped template, which enters the cyclic amplification phase, followed by elongation and cyclic amplification, in 3 stages. RT-LAMP requires one more step of conversion from RNA to DNA. **G** The CRISPR complex requires guide RNA and a family of Cas proteins responsible for gene shearing. The guide RNA binds to the target fragment of the viral gene, at which point the Cas proteins are activated and are able to non-specifically cleave other nucleic acid sequences. When the sample is tested, probes with fluorescent and quenched groups within the reaction system are cleaved by the activated Cas proteins to produce fluorescence, demonstrating the presence of viral nucleic acids in the sample

for N1 (0.69 copies/μL) compared to N2 (1.37 copies/μL) (Maksimovic Carvalho Ferreira et al. 2022). Kaya et al. used N1, N2, E-Sarbeco, and RdRp to detect SARS-CoV-2 in wastewater and demonstrated the highest sensitivity of N1 with a 76.2% positive detection rate, followed by N2 with a 57.1% positive detection rate (Kaya et al. 2022). Pérez-Cataluña et al. used N1, N2, IP2, IP4, and E to detect SARS-CoV-2 in wastewater (Pérez-Cataluña et al. 2021). N1 had the highest positive rate of 91.2% (Pérez-Cataluña et al. 2021). Zhang et al. compared the detection performance of 4 SARS-CoV-2 RT-qPCR primer–probe sets (US CDC-N1, China CDC-N, N-Sarbeco, and E-Sarbeco), and in low-concentration simulated wastewater samples, the China CDC-N group showed relatively good linearity compared to the US CDC-N1 group. In real wastewater samples, the US CDC-N1 group had the highest detection sensitivity with a 60% positive rate, followed by the China CDC-N group with a 53.3% positive rate (Zhang et al. 2022).

Multiplex qPCR and high-throughput qPCR

Multiplex qPCR (mqPCR) is capable of detecting and quantifying multiple gene targets of viruses in a single run. Navarro et al. based mqPCR method to simultaneously detect the N1, N3, and S genes of SARS-CoV-2, and the results show that mqPCR assays can provide a SARS-CoV-2 detection method that is as powerful as a single qPCR and is rapid and low-cost (Navarro et al. 2021). Mondal et al. used mqPCR to simultaneously detect the N (N1, N2) and E gene targets of SARS-CoV-2, and found that all collected wastewater samples were tested positive for these 3 gene targets (Mondal et al. 2021). The LOD of the multiplexed assay was as low as 5 copies, and the limit of quantification (LOQ) was as low as 8 copies (Mondal et al. 2021). While mqPCR can detect up to 5 gene targets in a single run, Malla et al. used a high-throughput qPCR (HT-qPCR) based on microfluidic technology that has thousands of reaction chambers in a single run, each with a volume of only 10 nL, and can detect 22 gene targets at the same time (Malla et al. 2022). When tested with a single gene target (e.g., N1), only 3 samples from the WWPT were positive, while more than 8 samples were positive when 14 gene targets were tested simultaneously by HT-qPCR, indicating that HT-qPCR is a highly sensitive assay (Malla et al. 2022).

Nested PCR

A SARS-CoV-2 nested RT-PCR targeting ORF1ab designed by La Rosa et al. detected 6 positive specimens from 12 wastewater samples with higher sensitivity compared to the SARS-CoV-2 nested RT-PCR targeting the spiked region (2/12) designed by Nao et al., which was the first detection of a SARS-CoV-2 from wastewater in Italy (La Rosa et al. 2020). Single-tube nested PCR allows the reaction to be performed in a single PCR tube, reducing the possibility of cross-contamination. The detection performance of the single-tube one-step nested quantitative PCR (OSN-qRT-PCR) method and the RT-qPCR method was evaluated by Rusková et al. (2022). The LOD of OSN-qRT-PCR was the first order of magnitude higher than that of RT-qPCR and was able to improve virus detection, indicating that OSN-qRT-PCR can sensitively detect SARS-CoV-2 in a wastewater environment (Rusková et al. 2022).

Digital PCR and RT-ddPCR

Some factors affect the reproducibility and reliability of RT-qPCR results, such as poor protocols, reagents, sample quality, instrumentation, operators, and data analysis (Bivins et al. 2021). In addition, wastewater contains pharmaceuticals, metals, and many other chemical products. The complexity of the wastewater may have an impact on the analytical results; digital PCR and RT-ddPCR can avoid this problem (Ahmed et al. 2022). Digital PCR does not rely on standard curves for quantification. It is centered on micro drop processing, where each micro drop is an independent PCR reaction, and the copy number and concentration of the target molecule are obtained using the micro drop fluorescence signal. This improves the accuracy and reproducibility of the assay and overcomes some of the shortcomings of RT-qPCR. Flood et al. compared the detection performance of RT-qPCR and RT-ddPCR (Flood et al. 2021). RT-qPCR did not detect the E gene target in wastewater samples, while RT-ddPCR did, indicating that RT-ddPCR showed higher sensitivity in detecting the SARS-CoV-2 gene target in wastewater samples compared to RT-qPCR (Flood et al. 2021). Ciesielski et al. experimentally concluded that the LOD of RT-ddPCR was only 0.066 copies/μL of template, and there was a significant positive correlation between the measured results with a ρ value of 0.86, indicating that RT-ddPCR is a highly sensitive and reproducible assay that avoids the low reproducibility of RT-qPCR (Ciesielski et al. 2021).

LAMP and CRISPR

In contrast to conventional PCR, loop-mediated isothermal amplification (LAMP) is performed at a constant temperature. The absence of temperature cycling, rapid heating, and cooling mechanisms makes it simpler, faster, more economical, and more efficient to

perform without the need for advanced instrumentation and trained technicians. LAMP may be a better method for diagnosis and monitoring in resource-limited areas or during emergencies such as COVID-19 outbreaks. Currently, LAMP is mostly used in clinical settings, and only a limited number of studies have reported its application for the detection of SARS-CoV-2 RNA in wastewater. In Pakistan, Haque et al. have successfully detected SARS-CoV-2 in wastewater using LAMP, which is the first study reporting the presence of SARS-CoV-2 in wastewater using highly sensitive LAMP (Haque et al. 2021). LAMP also has its limitations. The detection rate of LAMP is lower than that of ddPCR or RT-qPCR, but increasing the volume of the starting RNA template can compensate for this deficit (Amoah et al. 2021; Donia et al. 2022). When the RNA template was increased from 1 to 5 µL, the detection rate of fluorescent RT-LAMP increased from 31% to 47% (Amoah et al. 2021). LAMP is best suited for qualitative rather than quantitative purposes (Amoah et al. 2021). Some research groups have improved on the disadvantage of being qualitative only. Ramírez-Chavarría et al. combined an electrochemical sensor with RT-LAMP for the detection of SARS-CoV-2 in wastewater samples and showed that the sensor was able to specifically quantify RT-LAMP amplicons at a level below 2.5×10^{-6} ng/µL, showing high reproducibility (Ramírez-Chavarría et al. 2022). Cao et al. developed a portable paper-based device based on CRISPR/Cas12a and reverse transcription LAMP for semi-quantitative analysis of SARS-CoV-2 in wastewater, and the device achieved semi-quantitative analysis from 0 to 310 copies/mL with good sensitivity and specificity, which is a method that could be a promising approach for wastewater monitoring (Cao et al. 2022).

Detecting SARS-CoV-2 mutations and distinguishing variants

Variants of SARS-CoV-2 continue to emerge, and new methods should be applied to promote the public health system. Vo et al. detected viral variants Alpha and Epsilon in wastewater samples before clinical identification (Vo et al. 2022). Heijnen et al. used RT-ddPCR for the first time to detect N501Y in mixed WT samples containing a low proportion of Beta variant (0.5%) and to accurately determine the proportion of both, showing a sensitive method to specifically detect and quantify VOC-associated SARS-CoV-2 variants in wastewater (Heijnen et al. 2021). Malla et al. established an HT-qPCR technique that allows simultaneous detection and quantification of 22 target genes in a single run, and successfully detected mutations in variants such as N501Y, del69-70, and L452R, and it is a low-cost and time-efficient method for monitoring SARS-CoV-2 with multiple mutations (Malla et al. 2022). Xu et al. used a set of allele-specific RT-qPCR (AS RT-qPCR) based assays to detect 12 mutation sites in the viral spike protein gene, which were performed in different combinations to distinguish 8 variants (Xu et al. 2022a). All assays can detect target variants in samples as low as 10 copies/µL with minimal cross-reactivity with the corresponding non-target genotype and can achieve a specificity of ~ 100% and sensitivity of > 90%, indicating that AS RT-qPCR is a rapid, sensitive, and highly specific method for monitoring variants in wastewater. Furthermore, Beta and Omicron variants have been successfully detected in wastewater in Hong Kong using this method (Xu et al. 2022a). Lee et al. targeted a segment of mutation Q493R-Q498R in the SARS-CoV-2 spike gene and used AS RT-qPCR reaction to detect this Omicron variants in wastewater (Lee et al. 2022). La Rosa et al. designed 3 nested PCR assays to detect detectable variants in wastewater samples and showed that the long-nested RT-PCR detected 3 positive samples and the 2 short-nested RT-PCRs detected 15 and 16 positive samples, respectively, indicating that the short-nested RT-PCR method was slightly more sensitive (La Rosa et al. 2021). In addition, it is also possible to screen for variants by sequencing the SARS-CoV-2 genome in wastewater. However, applying this approach to wastewater samples is challenging owing to the complexity of the chemicals in the wastewater, the fragmented viral genome, the relatively low viral titers, and the mixture of viral variants in the samples (Lou et al. 2022). Lou et al. found that 26.7% of negative results detected by sequencing were reported as positive by RT-ddPCR assays and 42.6% of positive results detected by RT-ddPCR were identified as negative by sequencing, suggesting that RT-ddPCR can monitor variants in wastewater more sensitively compared to amplicon sequencing methods (Lou et al. 2022).

This review provides a relatively comprehensive overview of the various molecular detection methods used for WBE. WBE can be used to monitor shed viral RNA from patients as well as viral products in environment to track the evolution of variants. Currently, collected wastewater samples need to be sent to a laboratory for testing, while paper-based analysis equipment can achieve on-site testing without the need for a professional laboratory which is a user-friendly and time-efficient method. Indeed, such paper-based analytical equipment devices have been developed for a variety of pathogens such as HIV and malaria

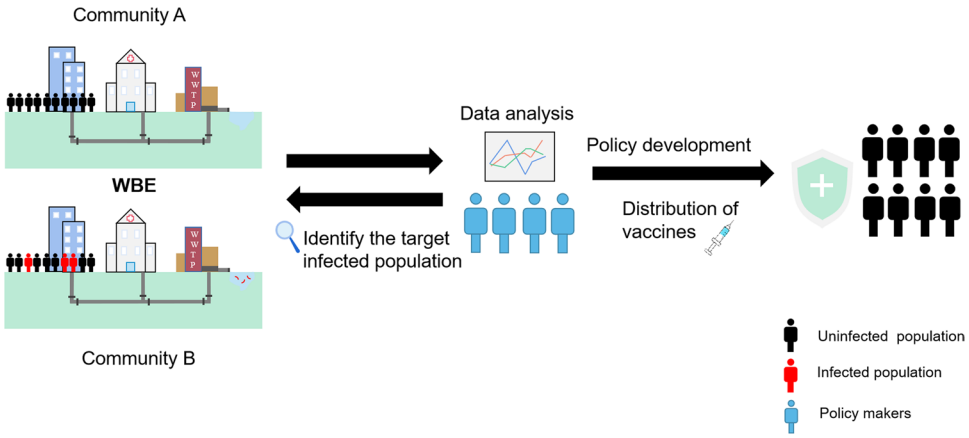

Fig. 3 Implications of WBE. Policy makers can use WBE to identify areas of infected populations early in the emergence of a pandemic in advance, and then protect more people from the viral infection by developing appropriate policies and rationalizing the distribution of vaccines to tackle the pandemic

(Mao et al. 2020), which avoids multiple processes and allows for rapid screening. Therefore, paper-based analytical equipment devices for water samples would also be a promising strategy to alarm possible SARS-CoV-2 outbreak before its massive spreading. The transmission of SARS-CoV-2 in wastewater, sludge, and air environments should be studied in more details and integrated into the public health alert system.

Innovation in technology is a problem-solving activity (Coccia 2016). Some traditional techniques have limitations in water environment testing. Traditional cell culture, for example, is time-consuming, requires more labor, and some pathogens cannot be grown on laboratory media and are not well transported from the water source to the laboratory (Gilbride 2014). Culture-based methods can be inaccurate when used in quantitative studies (Singh et al. 2022). Even though the ICC-PCR method can break the limitations of the method alone, it still cannot detect some viruses that cannot be cultured in cell culture (Corpuz et al. 2020). Molecular methods have demonstrated superior performance in terms of sensitivity, specificity, reliability, and speed and are therefore being used more frequently in wastewater monitoring studies. The classical technique of PCR is more suitable for quantification, so the use of large-scale sequencing techniques that can be used qualitatively to detect viruses in the environment is being increased (Girón-Guzmán et al. 2023). Finally, many new and innovative approaches are using the unique properties of nanomaterials to enable the detection of infectious pathogens.

Conclusion

A limitation of this review is that the virus enrichment and concentration steps prior to detection were not included in the study, although optimized enrichment and concentration methods could also further improve the final virus detection rate. The COVID-19 pandemic provides lessons for countries in dealing with future large-scale outbreaks of new pandemics. No country can be adequately prepared for an unknown pandemic (Coccia 2022b). What we can do is to detect the pandemic early and take appropriate measures to contain the widespread spread of the virus before it breaks out (Fig. 3). WBE is an implementable and effective early warning tool that can help government officials develop public health policies (Zhao et al. 2023). Rapid vaccination during the initial phase of a pandemic is the best strategy for responding to a pandemic crisis, but there are problems with vaccine distribution during the initial phase (Coccia 2022a). WBE can be used to know if there is an infected population in an area and if there is a potential epidemic (Jarvie et al. 2023). Government officials can use this as a reference for proper vaccine distribution.

More should be spent on public health to facilitate a better response to future pandemic crises (Coccia 2022b). In some countries with poor sanitation, the government should enhance public awareness of health and hygiene, improve overall sanitation, and make efforts to curb the spread of COVID-19 through multiple pathways. Treatment of hospital wastewater should also be strengthened to reduce its spread to the community and mitigate environmental pollution (Amin et al. 2023). Policy makers should make full use of the information provided by the WBE to contain the spread of the disease as quickly as possible at the most economical cost. Lifting isolation policies too early or too late can be a socio-economic burden. Site-specific and time-specific measures will effectively mitigate the negative effects of a pandemic, delaying reopening time may only defer the problem but not entirely solve it (Coccia 2021).

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Declarations

Conflict of interest The authors declare no competing interests.

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