Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Recent progress on wastewater-based epidemiology for COVID-19 surveillance: A systematic review of analytical procedures and epidemiological modeling

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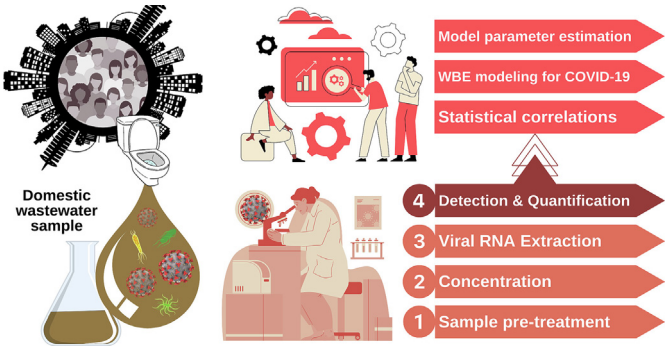
HIGHLIGHTS

- COVID-19 community cases can be predicted by wastewater-based epidemiology (WBE).
- WBE correlates SARS-CoV-2 levels in wastewater with COVID-19 clinical cases.
- Six major contribution areas to the development of WBE for COVID-19 surveillance were identified.
- Standardization of analytic procedures for SARS-CoV-2 detection is urgently needed.
- Opportunities to improve accuracy in WBE for COVID-19 are emphasized.

GRAPHICAL ABSTRACT



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ABSTRACT

On March 11, 2020, the World Health Organization declared the coronavirus disease 2019 (COVID-19), whose causative agent is the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a pandemic. This virus is predominantly transmitted via respiratory droplets and shed via sputum, saliva, urine, and stool. Wastewater-based epidemiology (WBE) has been able to monitor the circulation of viral pathogens in the population. This tool demands both in-lab and computational work to be meaningful for, among other purposes, the prediction of outbreaks. In this context, we present a systematic review that organizes and discusses laboratory procedures for SARS-CoV-2 RNA quantification from a wastewater matrix, along with modeling techniques applied to the development of WBE for COVID-19 surveillance. The goal of this review is to present the current panorama of WBE operational aspects as well as to identify current challenges related to it. Our review was conducted in a reproducible manner by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews. We identified a lack of standardization in wastewater analytical procedures. Regardless, the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) approach was the most reported technique employed to detect and quantify viral RNA in wastewater samples. As a more convenient sample matrix, we suggest the solid portion of wastewater to be considered in future investigations due to its higher viral load compared to the liquid fraction. Regarding the

Abbreviations: WBE, Wastewater-based epidemiology; SLR, Systematic literature review; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19, Coronavirus Disease 2019; RT-qPCR, Reverse transcription-quantitative polymerase chain reaction; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; WWTP, Wastewater treatment plant; VOC, Variants of concern; Ct, Cycle threshold; PEG, Polyethylene glycol; PCR, Polymerase chain reaction; CDC, Centers for Disease Control and Prevention; RT-LAMP, Reverse transcription loop-mediated isothermal amplification; RT-ddPCR, Reverse transcription droplet digital polymerase chain reaction; PMMoV, Pepper Mild Mottle Virus; ARIMA, Autoregressive Integrated Moving Average; VAR, Vector Autoregression; SEIR, Susceptible-exposed-infectious-recovered; ANN, Artificial Neural Networks; ANFIS, Adaptive Neuro-Fuzzy Inference System; GAM, Generalized Additive Model.

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epidemiological modeling, the data-driven approach was consistently used for the prediction of variables associated with outbreaks. Future efforts should also be directed toward the development of rapid, more economical, portable, and accurate detection devices.

Contents

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused a major pandemic where millions of people have been infected globally. It belongs to the *Coronaviridae* family and comprises spiked glycoproteins (S) on the surface of a spherical virion that varies from 60 to 140 nm in diameter and is surrounded by a lipid envelope (Zhu et al., 2020). Particularly, SARS-CoV-2 is the causative agent of the coronavirus disease 2019 (COVID-19), which is a life-threatening disease that represents a major threat to public health (Bar-Or et al., 2021; Flood et al., 2021; Li et al., 2020). SARS-CoV-2 is predominantly transmitted via respiratory droplets, which are generated during sneezing, breathing or coughing, and direct or indirect contact through different secretions (Tanhaei et al., 2021; van Doremalen et al., 2020). In this regard, this virus has not only been detected in sputum and saliva but its RNA has been also found in stools and urine, as well as in anal/rectal swabs (Cheung et al., 2020; Mesoraca et al., 2020; Peng et al., 2020). Rather than testing individuals, wastewater-based epidemiology (WBE) has been applied to detect viral pathogens in sewage shed from stool and urine, thus representing a viable alternative to estimate the infection prevalence in the community. WBE was theorized in 2001 (Jones-Lepp, 2001) with the original purpose of monitoring the use of illicit drugs at the community level (Claro et al., 2021). Recently, it has been successfully applied for the detection and monitoring of several viral pathogens in the population (e.g., poliovirus, enterovirus, norovirus, and hepatitis) (Barbosa et al., 2022; Hellmer et al., 2014; Medema et al., 2020; Nasseri et al., 2021; Robotto et al., 2022).

It has been proven that SARS-CoV-2 can be shed in feces after its replication in human intestine enterocytes (Ding and Liang, 2020; Haramoto et al., 2020; Lamers et al., 2020; Lescure et al., 2020), even when the patient had no gastrointestinal symptoms (Xiao et al., 2020a; Zuo et al., 2021). The shedding of this virus from stools can occur after becoming undetectable in the respiratory tract (Wu et al., 2020). Thereby, the SARS-CoV-2 shedding period was found to be longer in fecal than in upper respiratory samples, but its RNA is generally detected earlier in the latter (Zhang et al., 2021a). Additionally, it has been reported that SARS-CoV-2 RNA could be shed through respiratory and fecal routes before the infected individual exhibits symptoms (Buscarini et al., 2020; He et al., 2020; Zhang et al., 2021b). According to the above-mentioned insights, shedding in feces, sputum and saliva contributes to the SARS-CoV-2 load in wastewater (Markt et al., 2022). Interestingly, however, the analysis of wastewater performed through cell culture indicated that the SARS-CoV-2 particles were found non-infectious (Tiwari et al., 2022), bringing evidence to previous observations suggesting that SARS-CoV-2 is not potentially associated with a waterborne transmission risk in community wastewater influents (Rimoldi et al., 2020; Westhaus et al., 2021). The detection of SARS-CoV-2 in wastewater (or, interchangeably, sewage), even at low COVID-19 prevalence, makes sewage surveillance a sensitive tool to monitor its circulation in the population (Prakash, 2021). Quantifying a specific genome of an enteric virus in wastewater is an indirect, noninvasive form of assessing the current health status of the local population (Prevost et al., 2015). Moreover, wastewater surveillance enables both providing early notice of the SARS-CoV-2 (re) emergence in a population when applied routinely (Karthikeyan et al., 2021; Zhao et al., 2022), and supplementing clinical testing by assessing temporal and spatial trends, evaluating asymptomatic and symptomatic individuals, and observing the efficiency of public preventive strategies (Castiglioni et al., 2022; Gupta et al., 2020; Tomasino et al., 2021).

The development of analytical methods for WBE purposes, starting from sampling and viral detection to RNA quantification, emerges as an important research theme that has been approached by a considerable number of studies in the last two years (Carducci et al., 2020; Kabdasli and Tunay, 2021; Kitajima et al., 2020). In this context, a dearth of standardization in the sample analysis methodology was identified (Ahmed et al., 2021; Calderon-Franco et al., 2022; McMinn et al., 2021; Peinado et al., 2022), which has been characterized by the use of a myriad of methods to concentrate, extract, detect, and quantify SARS-CoV-2 RNA (de Sousa

et al., 2022; Pillay et al., 2021; Xie et al., 2022). Furthermore, the normalization of quantitative information has not been addressed: for example, standard units to express viral loads in wastewater have not been established so far (Shah et al., 2022). Regarding the output of the laboratory analysis, the accurate estimation of viral genomic concentration in wastewater is an issue that must be addressed in future COVID-19 surveillance research since this variable has been used to estimate the number of COVID-19 cases when confronted with clinical testing data (Ahmed et al., 2020a; de Sousa et al., 2022; Pillay et al., 2021). Analytical accuracy is imperative for building the path toward understanding the infection dynamics through WBE by designing trustful correlations and mathematical models relating sewershed viral concentration and epidemiological clinical data. To address this issue, we elevated the need to systematize the available knowledge on the technology for wastewater analysis as well as the scientific effort to unravel and model COVID-19 infection dynamics through existing or developed WBE mathematical models.

The purpose of this systematic review is twofold: identify the reported methodology of techniques/procedures to quantify SARS-CoV-2 viral RNA in domestic wastewater, and the mathematical methods and models by which viral loads have been associated with epidemiological data. It should be noted that this integrated approach has not been considered by any previously published review on the field. Thus, this study may serve as a reference for upcoming research that requires detailed information on these subjects, thus readers interested in one or both operational aspects of WBE for COVID-19 can find this study relevant given the exposition of methods and findings from a total of 158 studies. This review is structured as follows. After this introduction, Section 2 brings a comprehensive description of the systematic search method and selection process for evidence-based publications discussing analytical methods and mathematical modeling for COVID-19 surveillance up to August 2022. Next, in Section 3, we report our findings from the selected literature regarding in-lab and computational works that have been performed. Section 4 critically discusses our findings in terms of current issues, gaps to fill, and promising alternatives to treat wastewater toward the refinement of WBE for COVID-19 surveillance. Finally, we finish with the main conclusions drawn from this analysis and directions for further research.

2. Methods

2.1. Search strategy

This systematic literature review (SLR) was conducted by following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method (Page et al., 2021). The SLR is a reliable way to acquire a consistent overview of a specific research theme in an organized and replicable manner (Denyer and Tranfield, 2009; Tranfield et al., 2003). Before engaging in the systematic collection of studies, we conducted a non-structured search to identify regularly associated keywords and concepts about the subject. Keywords such as “COVID-19/SARS-CoV-2”, “wastewater”, “surveillance”, “methods”, “analysis”, “modeling”, and “correlation” were commonly used to identify records of peer-reviewed articles in the multidisciplinary literature. Next, we chose the following databases: ISI Web of Science (www.webofscience.com) and Scopus (www.scopus.com) given their relevance in the academic literature (Wang and Waltman, 2016), along with Engineering Village (Elsevier's Compendex) (www.engineeringvillage.com) due to its importance in the interdisciplinary engineering field (Cusker, 2013), and PubMed (MEDLINE) (www.pubmed.ncbi.nlm.nih.gov) for its reliability as a medical database for evidence-based studies such as systematic reviews (Falagas et al., 2008; Gusenbauer and Haddaway, 2020). As presented above, to address the current panorama of WBE for COVID-19 surveillance to a fuller extent, we considered a conjoint exploration of two pillars: laboratory procedures to quantify viral RNA in wastewater, and modeling (computational) methods to, among other goals, predict outbreaks in the community and city levels. The latter pillar is fed with input generated by the former. To fulfill this purpose, we performed two independent collections of records from the databases (one for viral RNA analysis and quantification and another for epidemiological modeling), with each one adopting suitable search strings as shown in Table 1. We decided to approach the search in this manner due to the extensive number of studies returned upon conducting a single search for both subjects. Then, we were able to significantly reduce the initial number of records found in the databases and work with a reasonable sample of articles. Table 1 summarizes the keywords used as search strings and how they were combined to compose each search.

2.2. Selection of studies and filtering

The academic coverage and analysis of the COVID-19 pandemic and its consequences demand trustable data to mitigate the risk of misconceptions in matters of public health information and public policies (Davenport et al., 2020; Tagliabue et al., 2020). Accordingly, we favored the side of selecting records from trustworthy sources when pondering the trade-off that exists between considering a high-quality level of discussion and broadening the information basis, with the latter often associated with doubtful reliability (Tranfield et al., 2003). Thus, we decided to include only peer-reviewed, original articles, therefore excluding other types of studies and publication formats, such as reviews, short communications, technical reports, letters, notes, abstracts, and surveys. Any available but unpublished work was excluded as well. Studies published between January 2020 and August 2022 were included in the sample. The deduplication, screening, filtering, and application of inclusion criteria were performed in EndNote 20 to reduce the original sample of studies to a trustful and representative collection of knowledge in the field. The initial search using the terms expressed in Table 1 returned a total of 2400 articles, most of them unrelated to our subjects. Next, we engaged in the screening phase as described: collected records were primarily screened for their title only, and subsequently, for their abstracts and content in full. Throughout this phase, we considered ineligible any publication that addressed topics outside our focus, such as other types of viral pathogens in wastewater, COVID-19 diagnosis and treatment, other matrices such as soil, leachate and air, elimination of various pathogens in water, water quality, wastewater from aircraft and ships, drug detection, and biosensors, to name a few. About their content, we included publications that presented (1) clear and concise descriptions and/or comparisons of analytical methods, protocols, and technologies currently used for pre-treatment, concentration, extraction, and quantification of SARS-CoV-2 nucleic acid in a wastewater matrix, and/or (2) precise information on the characteristics of studied wastewater, study location, time range, application of statistical tests for correlating WBE variables, as well as any used or developed mathematical model toward exploring COVID-19 infection dynamics.

Regarding the filtering procedures, we first used EndNote 20 to detect and exclude duplicated records independently for each search, then we applied time range and language filters, followed by the last filter regarding the type of publication from an initial total of 1106 identified literature records. After this screening step, we assessed the remaining studies through the lens of the established inclusion and exclusion criteria, thus excluding 558 and 84 records by title-only and abstract, respectively; these studies were considered out of the scope of this review, and thus deemed ineligible. Besides, 21 studies were unclear about their methodological procedures or had not presented any type of wanted information, therefore excluded. Last, we combined the two groups into a single pool and ran a second deduplication, thus excluding another 7 studies. Finally, we finished with a list of 96 studies from the first group and 62 studies from the second group as presented in Fig. 1, amounting to a final pool of 158 works.

2.3. Data extraction

To properly organize the data extraction process, we used a MS Excel spreadsheet with designated columns to include the following

Table 1
Search strings and Boolean operators used for each search.

	Boolean operator	Search strings	Category
Analytical methods		*COVID?19* OR *SARS-CoV-2* OR *coronavirus*	Topic
	AND	*wastewater* OR *ww* or *sewage*	Topic
	AND	*"SARS-CoV-2 RNA"* OR *RNA* OR *"ribonucleic acid"* OR *"nucleic acid"* OR *genet**	Topic
	AND	*analy** OR *method** OR *procedure* OR *protocol* OR *techn**	All Fields
	AND	*detect** OR *concentrate** OR *quantif** OR *estimat** OR *measur**	All Fields
Mathematical modeling		*COVID?19* OR *SARS-CoV-2* OR *coronavirus*	Topic
	AND	*wastewater* OR *ww* OR *"wastewater-based epidemiology"* or *WBE*	Topic
	AND	*surveill** OR *monitor** OR *track**	All Fields
	AND	*predict** OR *forecast** OR *foreshadow** OR *model** OR *correlate** OR *relation**	All Fields

Note: "?" denotes a wildcard and was used due to different spellings of the term adopted in the literature. The "*" symbol allows variations of the search string. Quotation marks strictly limit the appearance of the word as it is input.

information reported by the selected studies: study location, sample collection period, wastewater characteristics (wastewater treatment plant (WWTP) influent, sewage, or treated), sample pre-treatment, concentration/extraction methods, gene targets, quantification method/technology, initial sample volume processed, lowest and highest viral concentrations recovered in both solid and liquid phases, the estimated time offset (lag) between sample analysis and epidemiological reporting, statistical test to correlate wastewater viral load and clinical data and its result, and mathematical modeling strategy. Not all the studies were thorough in reporting this set of systematized categories, nevertheless, we reasoned that these categories were potentially discussed at some level in our sample of studies, and thus every publication on the selected portfolio should be able to contribute within the scope of this review.

3. Results

3.1. Scientific contribution of the selected studies to SARS-CoV-2 WBE

A meaningful result from the analysis of the reviewed publications was the identification of six main types of contributions in the WBE for COVID-19 surveillance field from 2020 to 2022, which are listed next and depicted in Fig. 2: (1) quantitative comparison of concentration, extraction or quantification methods through parametric studies, (2) local reporting of SARS-CoV-2 detection in wastewater and the respective methodology, (3) development, adaptation and/or optimization of analysis protocols, (4) building correlations between viral concentration levels and clinical testing data, (5) mathematical modeling, simulation or parameter estimations for SARS-CoV-2 WBE, and

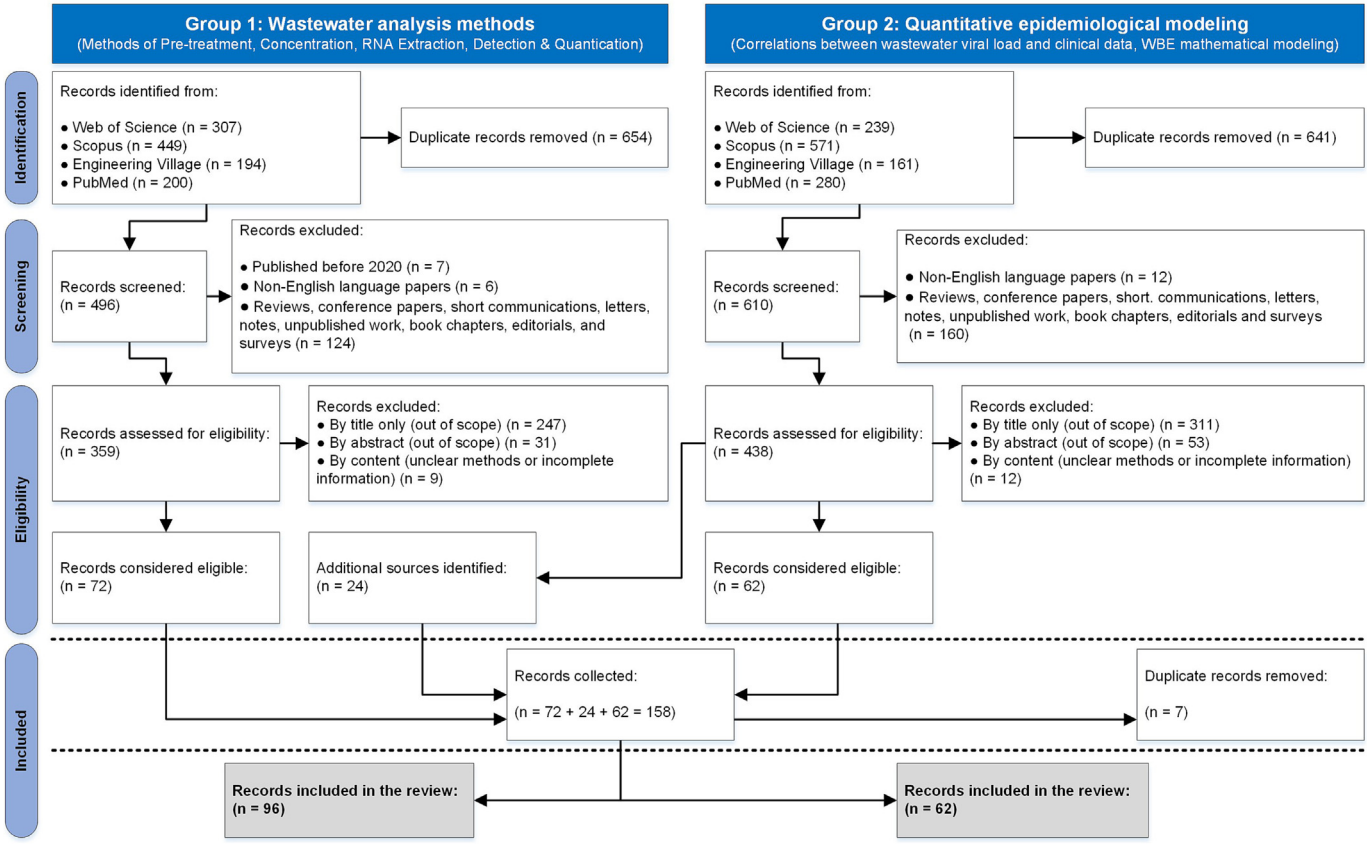

Fig. 1. Publication selection process: PRISMA-based flowchart for evidence-based research.

(1) Operation of wastewater analysis: quantitative comparisons between concentration methods, RNA isolation kits, and different PCR approaches in terms of viral recovery, sensitivity, and specificity.

(2) Reporting SARS-CoV-2 detection in wastewater: description of analytical procedures toward detection of SARS-CoV-2 genomic material in wastewater worldwide.

(3) Development, modifications and/or optimization of analysis protocols: detailed descriptions of analytical procedures for concentrating, isolating, detecting, and quantifying SARS-CoV-2 RNA in wastewater samples.

(4) Building statistical correlations between viral concentration signals and clinical testing data: development and application of correlations to assess how strongly temporal series of wastewater viral concentrations and disease prevalence are associated.

(5) WBE modeling, simulation and/or parameter estimation: details of different modeling techniques and data analysis toward understanding the spread of SARS-CoV-2 in domestic wastewater, and building complex models to predict outbreaks based on viral concentration signals.

(6) Phylogenetics, genotyping and/or identification of variants of concern (VOC): descriptions of genetic sequencing to support the hypothesis of VOCs prevalence in domestic wastewater, as well as tracking the same VOCs through the WBE approach.

Fig. 2. Types of contributions of the selected studies to SARS-CoV-2 WBE: findings from the analysis of the selected pool of publications.

(6) phylogenetics, genotyping and/or identification/quantification of variants of concern (VOC).

3.2. Aspects of wastewater analysis for SARS-CoV-2 detection and quantification

SARS-CoV-2 RNA can be found thermically stable in untreated wastewater at temperature values ranging from 4 to 37 °C (Ahmed et al., 2020c). This particularly wide range allows laboratory operations to reliably preserve and detect the virus, even having passed through sample collection and processing. The general methodology to generate a quantified viral concentration, in terms of cycle threshold (Ct) units or genomic concentration, from a wastewater sample, follows a sequential procedure of well-defined steps, namely sampling, pre-treatment, concentration, extraction (or isolation), and detection followed by quantification. This framework is depicted in Fig. 3. We found that the operationalization of these steps is well diverse, containing different technologies and protocols that use a wide range of reagents (Kaya et al., 2022). A complete list of the procedures and technology used can be found in Table 2. Next, we describe the general aspects of each step of the current paradigm of wastewater testing for SARS-CoV-2 WBE.

3.2.1. Pre-treatment

Pre-treatment of the wastewater samples has been ignored as a step of the laboratory analysis process in previous review articles, even though several procedures preceding the concentration step were found in roughly 80 % of the studies in our pool. Nevertheless, the term "pre-treatment" was employed only in a few studies (Torii et al., 2021; Zhang et al., 2022). Common pre-treatment procedures involve viral inactivation, pre-centrifugation, pH adjustment, and filtration through a single or a sequence of membrane filters. Pre-treatment serves the purpose of removing coarse solid material (Jmii et al., 2021), separating fine solids, and further purifying against bacterial beings (Reynolds et al., 2022). For the inactivation, we found that it can be performed through thermal treatment (Calderon-Franco et al., 2022; McMinn et al., 2021), UV light (Castiglioni et al., 2022; Pellegrinelli et al., 2022), or chemically (Tomasino et al., 2021). Filtration was done at the micrometer level (maximum pore size of 2 μm), and pre-centrifugation was performed at a minimal value of 1500 g but not exceeding 6000 g for a minimum duration of 5 min and a maximum of 45 min. Adjustment of sample pH was done when required for the following concentration step by using negatively charged membranes or precipitation using polyethylene glycol (PEG) (Farkas et al., 2021; Hasing et al., 2021).

3.2.2. Concentration

Concentration methods should ideally fulfill some features, including but not limited to being sensitive, reproducible, simple from a technical point of view, economical, rapid, and provide high viral recoveries (Prakash, 2021). A single standardized method for SARS-CoV-2 concentration from sewage has not been reported (Wehrendt et al., 2021). However, several methods have been described in the literature for that purpose (Prakash, 2021). Following the criteria of Birnbaum et al. (2022), these methods can be classified into two categories: (i) size-based techniques, such as ultrafiltration (Dumke et al., 2021; Hasing et al., 2021), ultracentrifugation (Zheng et al., 2022), centrifugal ultrafiltration

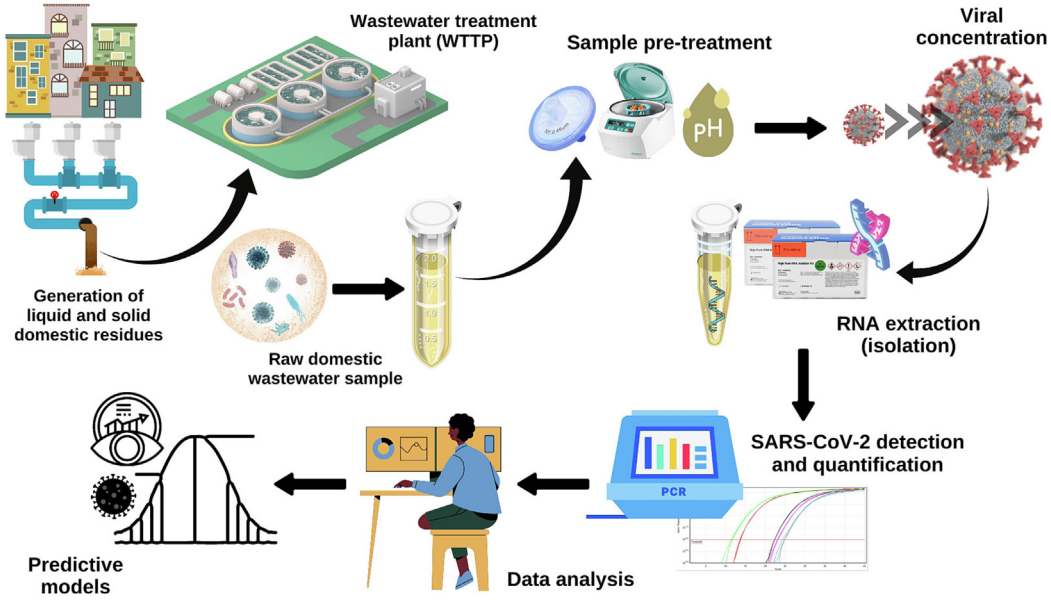

Fig. 3. SARS-CoV-2 WBE: Overall framework for sample analysis and epidemiological modeling.

Table 2
Description of analytical methods from selected literature and their contributions.

Study	Location and sampling period	Wastewater type and sources	Sample pre-treatment	Concentration methods	RNA extraction Kit/protocol	Quantification method and gene targets	Analyzed initial sample volume (mL)	Type of contribution (Fig. 2)
Anderson-Coughlin et al. (2021)	USA August 2020–March 2021	Raw (sewage)	Filtration using a 0.22 μm polyethersulfone (PES) membrane	Centrifugal ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	40 – 45	3
Ahmed et al. (2020a)	Australia March 2020 – April 2020	Raw (influent)	(1) pH adjustment to 3.5/4 using HCl, (2) Centrifugation at 4750 *g* for 30 min	(1) Adsorption-elution with electronegative membranes, (2) Ultrafiltration	QIAgen RNeasy PowerWater Kit and QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N	100 – 200	5
Ahmed et al. (2020c)	Australia NR	Raw (influent)	(1) Acidification to pH 4 using 2 N HCl, (2) NR, (3) $MgCl_2$ addition to a final concentration of 25 mM $MgCl_2$, (4,5) Centrifugation at 4500 *g* for 10 min at 4 °C, (6) Centrifugation at 10000 *g* for 20 min at 4 °C, (7) Centrifugation at 10000 *g* for 1 h at 4 °C	(1,2,3) Adsorption-elution using electronegative membranes, (4,5) Ultrafiltration, (6) PEG precipitation, (7) Ultracentrifugation	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR NR	50	1,5
Ahmed et al. (2021a)	Bangladesh July 2020 – August 2020	Raw (sewage)	Centrifugation at 4500 *g* for 30 min, filtration using 0.22 μm filters	PEG precipitation	Favor Prep Viral Nucleic Acid Extraction Kit	RT-qPCR N and ORF1ab	50	1,5
Ahmed et al. (2021b)	NR	Raw (influent)	(1) Centrifugation at 4000 *g* for 30 min at 4 °C, (2) NR	(1) Concentrating pipette (InnovaPrep), (2) Adsorption-elution with electronegative membranes	QIAgen QIAamp® Viral RNA mini Kit and RNeasy PowerWater Kit	RT-qPCR N1	NR	1
Ahmed et al. (2021c)	Australia February 2020 – May 2020	Raw (influent)	NR	Adsorption-elution using electronegative membranes	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1, N2 and N3	100 – 200	2
Ahmed et al. (2022b)	Australia June 2021	Raw (influent)	Centrifugation at 3000 *g* for 5 min	Concentration Pipette (InnovaPrep)	QIAgen QIAamp® Viral RNA mini Kit and QIAgen RNeasy PowerMicrobiome Kit (for the solid phase)	RT-qPCR and RT-dPCR N1 and N2	50	4,6
Ai et al. (2021)	USA July 2020–January 2021	Raw (influent)	Centrifugation at 2500 *g* for 10 min at 4 °C, filtration using a 0.45 μM sterile filter unit	Sequential concentration using adsorption-elution with positively charged membranes, organic flocculation, and centrifugal ultrafiltration	QIAgen RNeasy PowerMicrobiome Kit	RT-ddPCR N1, N2 and E	100 – 200	4,5,6
Amereh et al. (2022)	Iran September 2020 – April 2021	Raw (influent)	Centrifugation at 4000 *g* for 10 min	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N and ORF1ab	50	4
Amoah et al. (2021)	South Africa NR	Raw (influent)	Heat inactivation 60 °C for 90 min	Centrifugal ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-ddPCR and RT-LAMP E, N, ORF1ab, RdRP and S	250	3
Anneser et al. (2022)	USA March 2020 – March 2021	Raw (influent and sludge)	NR	(1) PEG precipitation, (2) Spectrophotometry	TRIzol-chloroform protocol and RNeasy PowerSoil Total RNA Kit	RT-qPCR N1, N2 and N3	NR	4.5
Arora et al. (2020)	India May 2020 – June 2020	Raw (influent)	Heat inactivation 60 °C for 90 min, filtration using a 0.45 μm membrane	PEG precipitation	Allplex 2019-nCoV Assay Kit	RT-PCR N, S, E, ORF1ab and RdRp	50	2
Bagutti et al. (2022)	Switzerland July 2021–December 2021	Raw (influent)	NR	NR	Maxwell® RSC Environ Wastewater TNA Kit	RT-qPCR N1, N2 and E	40	4
Baldovin et al. (2021)	Italy April 2020 – May 2020	Raw (influent) and treated (effluent: activated sludge, peractic acid and UV lamps)	Filtration using a 0.22 μm PES membrane	Ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N and ORF1ab	100	1,6
Barbosa et al. (2022)	Brazil May 2020–October 2020	Raw (influent, sewage)	NR	Ultracentrifugation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	40	2,4,5
Bar-Or et al. (2021a)	Israel August 2020–February 2021	Raw (influent)	Centrifugation at 4696 *g* for 5 min	Adsorption-elution with electronegative membranes	NucliSENS EasyMAG	RT-qPCR E	25	2
Bar-Or et al. (2021b)	Israel March 2020–April 2020	Raw (sewage)	NR	(1) PEG precipitation, (2) Skimmed milk flocculation, (3) Ultrafiltration	QIAgen RNeasy mini Kit and NucliSENS EasyMAG	RT-qPCR N and E	250 – 1000	1,2,6

Barril et al. (2021)	Argentina March 2020–October 2020	Raw (influent)	NR	A total of 11 different methods were evaluated.	Maxwell RSC 48 Extraction System	RT-qPCR N1 and N2	Varied from method to method	2,4
Barrios et al. (2021)	Argentina June 2020 – April 2021	Raw (influent)	Heat inactivation 60 °C for 90 min	PEG precipitation	TRIzol-chloroform protocol	RT-qPCR N1	200	4
Barua et al. (2022)	USA June 2020 – November 2020	Raw (influent)	Heat inactivation 75 °C for 40 min	Electronegative filtration (HA)	QIAgen QIAamp® Viral RNA mini Kit and NucliSENS EasyMAG	RT-qPCR and RT-ddPCR N1 and N2	20	1
Bertrand et al. (2021)	France April 2020–May 2020	Raw (influent after decantation)	NR	(1) Ultrafiltration, (2) PEG precipitation	Phenol-chloroform-isoamyl alcohol protocol	RT-PCR and RT-ddPCR E and RdRp	50	1,2
Bivins et al. (2022)	NR	Raw (influent, sewage)	NR	Centrifugal ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit and AllPrep PowerViral DNA/RNA Kit	RT-ddPCR and RT-LAMP N2 and E	NR	1,3,4
Boogaerts et al. (2021)	Belgium August 2020 – January 2021	Raw (influent)	(1) Centrifugation at 4600 *g* for 30 min at 4 °C, (2) Centrifugation at 4654 *g* for 30 min at 4 °C	(1) Ultracentrifugation, (2) PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit, RNeasy plus miniKit and QIAgen RNeasy PowerMicrobiome Kit	RT-qPCr and RT-dPCR N1, N2, N3 and E	20 – 90	1,4,5
Boogaerts et al. (2022)	Belgium September 2020 – November 2021	Raw (influent)	Centrifugation at 4000 *g* for 30 min	Ultracentrifugation	Maxwell® RSC PureFood GMO and Authentication Kit	RT-qPCr and RT-dPCR N, S and E	20	3,6
Calderon-Franco et al. (2022)	Netherlands July 2020 – December 2020	Raw (influent)	Heat inactivation 65 °C for 30 min	(1) Adsorption-elution with electronegative membranes, (2) Polyethersulfone membranes, (3) Anion-exchange diethylaminethyl cellulose columns	Fast RNA Blue Kit, FAST RNA Kit and MagMax CORE Nucleic Acid Purification Kit	RT-qPCR S, N and ORF1ab	50 – 550	1,3
Canh et al. (2021)	Japan January 2021 – February 2021	Raw (influent)	Centrifugation at 3500 *g* for 15 min	(1) Ultrafiltration, (2) PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1	100	1,2
Carrillo-Reyes et al. (2021)	Mexico April 2020–July 2020	Raw (influent, sewage) and treated (secondary sludge, effluent)	Filtration using a 0.2 μm PES membrane	(1) Ultrafiltration, (2) Adsorption-elution with electronegative membranes	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR RdRp, S and E	(1) 120, (2) 30 – 100	1,2
Castiglioni et al. (2022)	March 2020–June 2020	Raw (influent)	Under UV light for 30 min, Centrifugation at 4500 *g* for 30 min at 4 °C	PEG precipitation	QIAgen QIAamp® MinElute Virus Spin Kit	RT-PCR N1 and N3	45	1,2,5
Chakraborty et al. (2021)	India September 2020	Raw (influent) and treated (primary sludge, effluent)	NR	Composite, Supernatant, Sediment and Syringe Filtration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	250	1
Chavarria-Miro et al. (2021)	Spain April 2020 – July 2020	Raw (influent)	NR	PEG precipitation	NucliSENS miniMAG	RT-qPCR N1, N2, RdRp, IP2 and IP4	800	5
Claro et al. (2021)	Brazil June 2020 – April 2021	Raw (influent)	Centrifugation at 8000 *g* for 120 min at 4 °C	PEG precipitation	PureLink™ Viral RNA/DNA mini Kit	RT-qPCR N1 and N2	40	4
D'Aoust et al. (2021)	Canada April 2020–June 2020	Raw (post-grid influence) and treated (primary clarified sludge)	Decantation and serially filtered through a 1.5 μm glass fiber filter followed by a 0.45 μm GF6 mixed cellulose-ester filter	PEG precipitation	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR and RT-ddPCR N1 and N2	32	3,5
de Freitas et al. (2022)	Brazil January 2021 – January 2022	Raw (influent)	NR	PEG precipitation	PureLink Viral RNA/DNA mini Kit	RT-qPCR N1 and N2	40	4.5
de Sousa et al. (2022)	Brazil January 2021 – August 2021	Raw (influent) and treated (effluent)	pH adjustment to 3.5 using 1 M HCl, shaken at °4C for 30 min, Centrifugation at 2474 *g* for 30 min at 4 °C	PEG precipitation	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	RT-qPCR N1 and N2	50	5
Dimitrakopoulos et al. (2022)	Greece November 2021–December 2021	Raw (influent)	NR	(1) PEG precipitation, (2) PEG precipitation with glycine, (3) Direct capture, (4) Adsorption-elution with electronegative membranes, (5) Ultrafiltration	Water DNA/RNA magnetic bead Kit, QIAgen RNeasy PowerMicrobiome Kit, AllPrepPowerViral DNA/RNA Kit and Manual EnviroWastewater TNA Kit	RT-qPCR and RT-ddPCR N1, N2 and N3	50	2,6
Dumke et al. (2021)	Germany, NR	Raw (influent)	Centrifugation at 3300 *g* for 30 min at 4 °C	(1) PEG precipitation, (2) Centrifugation with Vivaspin columns	QIAgen RNeasy kits (not specified what series)	RT-qPCR and RT-ddPCR S and E	40	2
Farkas et al. (2021)	NR	Raw (NR)	Centrifugation at 3000 *g* for 30 min at 4 °C or 1000 g for	PEG precipitation	NucliSENS lysis buffer and NucliSENS miniMag extraction	RT-qPCR N1 and N2	50	1,3

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Table 2 (*continued*)

Study	Location and sampling period	Wastewater type and sources	Sample pre-treatment	Concentration methods	RNA extraction Kit/protocol	Quantification method and gene targets	Analyzed initial sample volume (mL)	Type of contribution (Fig. 2)
			10 min at 4 °C, pH adjustment of supernatant to 7–7.5 using 1 M NaOH		system			
Feng et al. (2021)	USA August 2020–January 2021	Raw (influent)	Filtration using 0.8 μm cellulose-ester filters	Bashing Bead Lysis	QIAgen RNeasy PowerMicrobiome Kit	RT-ddPCR N1 and N2	25	1
Fernandez-Cassi et al. (2021)	Switzerland February 2020–April 2020	Raw (influent)	Filtered using 2 μm glass fiber filters	Centrifugal ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	50	5
Fitzgerald et al. (2021)	Scotland April 2020 – January 2021	Raw (influent)	Centrifugation at 4000 *g* for 30 min at 4 °C, filtration using a syringe filter	(1) Ultracentrifugation, (2) PEG precipitation, (3) skimmed milk flocculation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and E	20 – 40	1,2
Flood et al. (2021)	USA March 2020 – September 2020	Raw (influent, sewage)	(1) Centrifugation at 2500 *g* for 5 min at 4 °C, (2) Centrifugation at 4654 *g* for 30 min at 4 °C, (3) Centrifugation at 4700 *g* for 45 min at 4 °C	(1,2) Ultrafiltration, (3) PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR, RT-ddPCR N1, N2 and E	(1,2) 100, (3) NR	1
Fongaro et al. (2021)	Brazil October 2019–March 2020	Raw (sewage)	NR	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1, S and RdRp	25	4
Fonseca et al. (2022)	Brazil March 2021	Raw (influent, river)	Heat inactivation 60 °C for 90 min, filtration using 1.2 μm pore size microfiber filters, Centrifugation at 4500 *g* for 30 min at 4 °C	(1) Ultrafiltration, (2) Adsorption-elution with electronegative membranes, (3) Aluminum hydroxide precipitation, (4) PEG precipitation	MagMax Viral/Pathogen II Kit and KingFisher Duo Purification System	RT-qPCR N1 and N2	40	4,6
Galani et al. (2022)	Greece August 2020 – March 2021	Raw (influent)	Centrifugation at 4700 *g* for 30 min at 4 °C	(1) PEG precipitation, (2) centrifugal ultrafiltration	Water DNA/RNA Magnetic Bead Kit, QIAgen RNeasy Power Microbiome Kit and QIAgen RNeasy Serum/Plasma Advanced Kit	RT-qPCR N1 and N2	50	4,5
Gerrity et al. (2021)	USA March 2020–May 2020	Raw (influent)	(1) NR, (2,3) Centrifugation at 3500 *g* for 15–30 min at 10 °C	(1) Hollow-fiber ultrafiltration, (2) Centrifugal Ultrafiltration, (3) PEG precipitation.	Purelink Viral RNA/DNA mini Kit	RT-qPCR N1, N2, E and ORF1a	50	1,3
Giraud-Billoud et al. (2021)	Argentina July 2020–November 2020	Raw (influent)	Heat inactivation 60 °C for 90 min	(1) PEG precipitation, (2) Polyaluminum chloride (PAC) flocculation	NucleoZOL	RT-qPCR N1 and N2	300	5,6
Gonçalves et al. (2021)	Slovenia June 2020	Raw (sewage)	Filtration using a 0.70 μm glass fiber filter membrane	Ultracentrifugation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR E and RdRp	100	1
Gonzalez et al. (2020)	USA March 2020 – August 2020	Raw (influent)	(1) Centrifugation at 10000 *g* for 10 min, (2) NR	(1) Concentration Pipette (InnovaPrep), (2) Adsorption-elution using electronegative membranes	NucliSENS Easy Mag TNA Extraction Kit	RT-ddPCR N1, N2 and N3	125	2
Gonzalez-Reyes et al. (2021)	Mexico June 2020–July 2020	Raw (influent, sewage)	Heat inactivation 60 °C for 90 min, filtration using a 0.2 μm membrane	PEG precipitation	TRIzol protocol	RT-qPCR N1, N2 and N3	150	2
Haramoto et al. (2020)	Japan March 2020–May 2020	Raw (influent) and treated (activated sludge before chlorination)	NR	(1) Adsorption-elution with electronegative membranes, (2) Direct adsorption	QIAgen QIAamp® Viral RNA mini Kit and QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1, N2, S and ORF1ab	200 – 5000	2
Hasan et al. (2021)	UAE May 2020–June 2020	Raw (influent) and treated (effluent)	(1,2) Heat inactivation 60 °C for 90 min, filtration using a 0.22 μm PES membrane	(1) Ultrafiltration, (2) PEG precipitation	ABIOpure Viral DNA/RNA Extraction Kit and TRIzol-chloroform protocol	RT-qPCR RdRp	(1,2) 50	1,4
Hasing et al. (2021)	Canada October 2020–December 2020	Raw (influent)	pH adjustment to 9.6–10 using 5 N NaOH, Centrifugation at 4500 *g* for 10 min	Ultrafiltration	MagMAX96 Viral RNA Isolation Kit and King Fisher Flex Purification System	RT-qPCR N2 and E	100	3
Hata et al. (2021)	Japan March 2020 – April 2020	Raw (influent)	NR	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N2 and N3	80	2
Hemalatha et al. (2021)	India July 2020 – August 2020	Raw (influent)	Gravity filtration with 1 mm thick blotting sheets to remove debris and larger particles followed by	Centrifugal ultrafiltration	QIAamp® Viral RNA isolation Kit	RT-qPCR N, E and ORF1ab	100	2,3

			filtration using 0.2 μm filtration units					
Hoar et al. (2022)	USA April 2020 – February 2021	Raw (influent)	Heat inactivation 60 °C for 90 min, Centrifugation at 5000 *g* for 10 min at 4 °C, filtration using 0.22 μm acetate-cellulose membrane	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1	40	4.5
Hokajarvi et al. (2021)	Finland April 2020 – May 2020	Raw (influent)	Centrifugation at 4654 *g* for 30 min	Ultrafiltration	Chemagic Viral300 DNA/RNA extraction Kit	RT-qPCR N2 and E	60	2,3
Huang et al. (2021)	Canada October 2020 – March 2021	Raw (influent)	NR	Ultrafiltration	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1, N2, N3 and E	200	2
Iglesias et al. (2021)	Argentina June 2020–September 2020	Raw (influent, surface water)	Heat inactivation 60 °C for 90 min	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	250	2
Jafferali et al. (2021)	Sweden and Italy May 2020–June 2020	Raw (influent)	(1) Centrifugation at 4600 *g* for 30 min at 4 °C, (2) Centrifugation at 1500 *g* for 15 min at 4 °C, (3) NR, (4) Centrifugation at 4600 *g* for 30 min at 4 °C	(1) Ultrafiltration, (2) Double Ultrafiltration, (3) Adsorption-elution with electronegative membranes, (4) Centrifugation combined with adsorption-extraction	TRIzol reagent and RNeasy PowerMicrobiome Kit	RT-qPCR N	40 – 50	1
Jmii et al. (2021)	Tunisia September 2020–October 2020	Raw (influent)	Coarse filtration and microfiltration, pH adjustment to 6 with aluminum hydroxide	Adsorption-elution with electronegative membranes	QIAgen RNeasy PowerMicrobiome Kit	RT-PCR N, E and RdRp	100	1
Johnson et al. (2021)	South Africa June 2020	Raw (influent)	Centrifugation at 3500 *g* for 20 min	NR	RNeasy PowerSoil Kit	RT-qPCR N1 and N2	50 – 100	2
Juel et al. (2021)	USA October 2022 – March 2021	Raw (sewage)	NR	(1) Adsorption-elution with electronegative membranes, (2) Concentrating Pipette (InnovaPrep)	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1	40 – 100	1,3
Kevill et al. (2022)	Wales October 2020 – February 2021	Raw (influent)	Centrifugation at 15000 *g* for 10 min at 4 °C	(1) PEG precipitation, (2) Ammonium sulfate precipitation, (3) Concentration pipette (Innova Prep)	NucliSENS Lysis Buffer, NucliSENS Extraction Reagent Kit and King-Fisher 96 Flex System	RT-pPCR N1	200	1,3
Kitamura et al. (2021)	Japan June 2020–August 2020	Raw (influent, sewage)	Centrifugation at 3000 rpm for 30 min	(1) Adsorption-elution with electronegative membranes, (2) PEG precipitation, (3) Ultrafiltration, (4) Solid precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	400	1,3,4,5
Koureas et al. (2021)	Greece October 2020–April 2021	Raw (influent)	NR	PEG precipitation	MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	RT-PCR N, S and ORF1ab	105	5
Krivonakova et al. (2021)	Slovakia September 2020 – March 2021	Raw (influent)	Centrifugation at 4700 *g* for 30 min	Ultracentrifugation	Direct-zol RNA miniprep Kit	RT-qPCR E, RdRp and ORF1ab	50	2.4
Kuhn et al. (2022)	USA November 2020 – March 2021	Raw (sewage)	Filtration using a 70 μm mesh cell strainer	PEG precipitation	Bio-On-Magnetic-Beads platform	RT-qPCR N1	32	5
Kumar et al. (2021)	India August 2020–September 2020	Raw (influent)	Centrifugation at 4000 *g* for 40 min, filtration using a 0.22 μm syringe filter	PEG precipitation	NucleoSpin® RNA Virus isolation Kit	RT-PCR N, S and ORF1ab	30	2
La Rosa et al. (2020)	Italy February 2020–April 2020	Raw (influent)	Heat inactivation 56 °C for 30 min	PEG-dextran two-phase separation	NucliSENS miniMAG	RT-qPCR ORF1ab, S and RdRp	250	2,5
Langan et al. (2022)	USA January 2021–March 2021	Raw (sewage)	Centrifugation at 4000 *g* for 20 min at 4 °C	Ultrafiltration	QIAgen PowerViral DNA/RNA Kit, Zymp EnvironWater RNA Extraction Kit and Monarch Total RNA miniprep Kit	RT-qPCR N1 and N2	200	1,5
Lara-Jacobo et al. (2022)	Canada October 2020–April 2021	Raw (influent)	Adding 50 mL of acetone at 4 °C and stored overnight at −20 °C to precipitate proteins, Centrifugation at 3405 *g* for 15 min	Protein Precipitation and Digestion	QIAgen PowerMicrobiome Kit	RT-qPCR N1	40	1,3
LaTurner et al. (2021)	USA October 2020	Raw (influent)	(1) NR, (2) Centrifugation at 4100 *g* for 10 min at 4 °C, (3)	(1) Direct extraction, (2) HA filtration with bead beating, (3)	Chemagic Prime Viral DNA/RNA 300 Kit H96	RT-qPCR and RT-ddPCR	(1) 1, (2,3,5) 50 (4) 200	5

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Table 2 (*continued*)

Study	Location and sampling period	Wastewater type and sources	Sample pre-treatment	Concentration methods	RNA extraction Kit/protocol	Quantification method and gene targets	Analyzed initial sample volume (mL)	Type of contribution (Fig. 2)
			Centrifugation at 3000 *g* for 1 min at 4 °C, (4) Centrifugation at 7140 *g* for 15 min at 4 °C, (5) Centrifugation at 4100 *g* for 10 min at 4 °C	HA filtration with elution, (4) PEG precipitation, (5) Ultrafiltration		N1 and N2		
Layton et al. (2022)	USA June 2020 – July 2020	Raw (sewage)	Centrifugation at 12000 *g* for 1 min	NR	MagMAX Viral Pathogen Kit	RT-ddPCR N1 and N2	30 – 40	4
Li et al. (2022)	USA June 2020 – September 2021	Raw (influent)	Heat inactivation 60 °C for 60 min, Centrifugation at 3000 *g* for 15 min, and sequential filtration using 1.5, 0.8, and 0.45 μm sterile membrane filters	PEG precipitation	AllPrep PowerViral DNA/RNA Kit	RT-qPCR N1 and N2	NR	2,4
Maida et al. (2022)	Italy September 2021–July 2021	Raw (sewage)	NR	PEG-dextran two-phase separation	NucliSENS miniMAG	RT-qPCR NR	NR	5
Mailepessov et al. (2022)	Singapore April 2020	Raw (sewage)	(1) Centrifugation at 4000 *g* for 30 min, (2) Centrifugation at 2000 *g* for 5 min	(1) PEG precipitation, (2) Ultrafiltration	Modified TRIzol-QIAgen protocol and QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR NR	45	1,3
Markt et al. (2022)	Liechtenstein Sept 2020 - March 2021	Raw (influent)	Centrifugation at 4500 *g* for 30 min	PEG precipitation	Monarch total RNA miniprep Kit	RT-qPCR N1	70	1
Masachessi et al. (2022)	Argentina May 2020–August 2021	Raw (influent)	Centrifugation at 4750 *g* for 20 min at 4 °C	PEG precipitation	MagNa Pure 96 DNA and Viral NA Large Volume Kit	RT-qPCR N and E	500	2
McMahan et al. (2021)	USA May 2020 – August 2020	Raw (sewage)	Heat inactivation 60 °C for 30 min, Centrifugation at 6500 *g* for 10 min at 6 °C	PEG precipitation	TRIzol-chloroform protocol	RT-qPCR N	225	5
McMinn et al. (2021)	USA July 2020–October 2020	Raw (influent, primary treated)	Heat inactivation 121 °C for 60 min	(1) Ultrafiltration, (2) Concentration Pipette	QIAgen All Prep PowerViral Kit	RT-qPCR N	2000	1
Mlejnkova et al. (2020)	Czech Republic April 2020–June 2020	Raw (influent)	NR	Skimmed milk flocculation	NucliSENS miniMAG	RT-qPCR NR	500	1
Mondal et al. (2021)	USA October 2020 - Jan 2021	Raw (influent)	NR	Direct Capture	NR	RT-qPCR N1, N2 and E	40	1
Monteiro et al. (2022)	Portugal April 2020–December 2020	Raw (influent)	Hollow-fiber filtration	PEG precipitation	QIAgen QIAamp® Fast DNA Stool mini Kit	RT-qPCR N, E and RdRp	1000	1
Nagarkar et al. (2022)	USA May 2020 – November 2020	Raw (influent)	NR	Ultrafiltration	RNeasy PowerWater Kit	dd-PCR N1 and N2	225	4
Nasseri et al. (2021)	Iran April 2020–May 2020	Raw (influent) and treated (effluent)	Decantation for 5 min, Centrifugation at 1500 *g* for 20 min at 4 °C, pH adjustment to 7–7.5 using HCl and NaOH	PEG-dextran two-phase separation	FastPure Viral RNA mini Kit	RT-PCR N and ORF1ab	250	2
Navarro et al. (2021)	Italy December 2020 – February 2021	Raw (influent)	Centrifugation at 4500 *g* for 30 min at 4 °C	Centrifugal ultrafiltration	Quick-RNA Fecal/Soil Microbe Microprep	RT-qPCR N1, N3 and S	100	1
Ni et al. (2021)	Australia March 2020–April 2020	Raw (influent)	Centrifugation at 9000 *g* for 20 min at 4 °C	Ultrafiltration	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1 and N2	50	3,6
Nourbakhsh et al. (2022)	Canada September 2020–June 2021	Raw (influent)	(1,2,3) Centrifugation at 4000 *g* for 20 min at 4 °C	(1) Centrifugal ultrafiltration, (2) Zirconia-silica beads in a Bead Mill 24 Homogenizer, (3) Centrifugation	MagNA Pure 96 DNA, Viral NA Large Volume Kit and QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1 and N2	15 – 30	5
Novoa et al. (2022)	Spain May 2020–May 2021	Raw (influent, sewage) and treated (effluent)	Filtration using a 20–25 μm cellulose filter, pH adjustment to 6	Adsorption-precipitation with $AlCl_3$	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1, N2 and E	150	5,6
O'Brien et al. (2021)	USA June 2020	Raw (sewage)	NR	Ultrafiltration	QIAgen All Prep PowerViral DNA/RNA KIT, Monarch RNA miniprep Kit and Zymo Quick RNA-Viral	RT-qPCR N2	250	5
Parra-Guardado et al. (2022)	Canada NR	Raw (influent)	Centrifugation at 5000 rpm for 5 min	NR	Direct Magnetic Bead Extraction	RT-qPCR NR	50	1
Peinado et al. (2022)	Spain February 2021 – June 2021	Raw (influent)	(1) Centrifugation at 4600 *g* for 30 min, pH adjustment to 6, (2) Centrifugation at 8000 *g* for	(1) Adsorption-precipitation with aluminum hydroxyde, (2) PEG precipitation, (3) Ultrafiltration	NZY Viral RNA Isolation Kit	RT-qPCR N1 and N2	100 – 200	1

			30 min at 4 °C, (3) Centrifugation at 4600 *g* for 30 min					
Pellegrinelli et al. (2022)	Italy March 2019–December 2020	Raw (influent)	(1) Centrifugation at 4500 *g* for 30 min, (2) Centrifugation at 4500 *g* for 30 min at 4 °C, (3) Centrifugation at 1200 *g* for 30 min at 4 °C	(1) PEG-Dextran two-phase separation, (2) PEG precipitation chloroform purification, (3) PEG precipitation with chloroform purification	QIAgen QIAamp® MinElute Virus Spin Kit and NucliSENS EasyMAG	RT-PCR N1, N3 and ORF1ab	(1,3) 250, (2) 80	2
Perez-Cataluna et al. (2021)	NR	NR	(1) NR, (2) Centrifugation at 2500 *g* for 10 min at 4 °C	(1) Aluminum hydroxyde adsorption-precipitation, (2) PEG precipitation	NucleoSpin RNA Virus Kit	RT-qPCR N1, N2, E, IP2 AND IP4	200	1
Petala et al. (2022)	Greece October 2020 – January 2021	Raw (influent)	pH adjustment to 4 using 2 M HCl, Centrifugation at 4000 *g* for 30 min	Adsorption-elution using electronegative membranes	Phenol-chloroform-based RNA extraction protocol	RT-PCR N2 and E	200	5
Philo et al. (2021)	USA March 2020–July 2020	Raw (influent after sedimentation)	NR	(1) Bag-mediated Filtration System (BMFS), (2) Skimmed milk flocculation, (3) PEG precipitation, (4) Ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1, N2 and N3	(1) 100, (2) 500, (3) 1000	1
Philo et al. (2022)	October 2020–March 2021	Raw (influent after sedimentation)	NR	Skimmed milk flocculation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	50	1
Pillay et al. (2021)	South Africa July 2020 – October 2020	Raw (influent)	Heat activation 60 °C for 90 min, Centrifugation at 3500 *g* for 10 min	Ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	dd-PCR N2	250	5
Pino et al. (2021)	Colombia NR	Raw (influent)	NR	(1) Flocculation with $AlCl_3$, (2) PEG precipitation, (3) Flocculation with skimmed milk, (4) Ultrafiltration	EZNA Total RNA Kit	RT-qPCR N, E and RdRp	200	2
Prakash (2021)	India June 2020–July 2021	Raw (sewage)	(1) Centrifugation at 4700 *g* for 30 min, (2) Precentrigufation at 5000 rpm for 30 min, (3) NR	(1) Ultrafiltration, (2) PEG precipitation (3) PEG-dextran two-phase separation	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR N1, N2 and E	200 – 550	1
Qiu et al. (2022)	Canada May 2020	Raw (influent)	pH adjustment to 9.6–10 using 5 N NaOH, Centrifugation at 4500 *g* for 10 min, Removal of supernatant and pH readjustment to 7	Centrifugal ultrafiltration	QIAgen RNeasy PowerMicrobiome Kit, MagMAX-96 Viral RNA Isolation Kit, MagMAX Viral/Pathogen Viral RNA mini Kit and ReliaPrep RNA miniprep System	RT-qPCR N1, N2, E and RdRp	100	3
Ramos-Mandujano et al. (2021)	Saudi Arabia June 2020	Raw (sewage)	NR	(1) Adsorption-elution with electronegative membranes, (2) Silica-coated magnetic nanoparticles	QIAamp® RNA mini Kit	RT-PCR N1 and N2	300 – 500	1,3
Randazzo et al. (2020)	Spain March 2020 – April 2020	Raw (influent) and treated (secondary and tertiary effluents)	NR	Aluminum hydroxide adsorption-precipitation	NucleoSpin RNA virus Kit	RT-qPCR N1, N2 and N3	200	1
Reynolds et al. (2022)	Ireland September 2020 – March 2021	Raw (influent)	Centrifugation at 3200 *g* for 5 min	Ultracentrifugation	QIAgen RNeasy PowerMicrobiome Kit	RT-qPCR and dd-PCR N1	200 – 225	2.6
Robotto et al. (2022)	Italy July 2020–March 2021	Raw (influent)	NR	NR	Wastewater Large Volume Total Nucleic Acid Capture Kit AX9550	RT-qPCR N1, N2 and E	40	1,3
Rocha et al. (2022)	USA July 2020–May 2021	Raw (influent)	2.5 M $MgCl_2$ was added at a ratio of 1:100 to a final concentration of 25 mM	Adsorption-elution with electronegative membranes	QIAgen PowerViral Kit	RT-qPCR N1 and N2	40 – 495	3,4,5
Rodriguez Rasero et al. (2022)	Spain July 2020 – February 2021	Raw (sewage)	pH adjustment to 6 using 2 N HCl	$AlCl_3$ precipitation	NucleoSpin RNA Virus Kit and QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N, E and IP4	200	5
Roka et al. (2021)	Hungary June 2020 – October 2020	Raw (influent)	(1) NR, (2) Centrifugation at 4500 *g* for 30 min at 4 °C	(1) Skimmed milk flocculation, (2) Ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N	(1,2) 50	1,4
Rondeau et al. (2021)	USA NR	Raw (sewage)	Heat inactivation 60 °C for 1 h, filtering using 0.22 μm filter	Centrifugal ultrafiltration	Quick RNA miniprep Kit	RT-qPCR N1	40	3
Rosiles-Gonzalez et al. (2021)	Mexico August 2020–January 2021	Treated (primary, biofilter and biological treatment)	(1) Filtration using a 0.45 μm cellulose-ester membrane, (2,3) Sequential filtration using 0.8, 0.65, 0.45 and 0.22 μm	(1) Adsorption-elution with electronegative membranes, (2) PEG precipitation, (3) Centrifugal filtration.	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	(1) 1.0 – 5.4, (2) 200 – 1000, (3) 0.6 – 1.3	1,3

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Table 2 (*continued*)

Study	Location and sampling period	Wastewater type and sources	Sample pre-treatment	Concentration methods	RNA extraction Kit/protocol	Quantification method and gene targets	Analyzed initial sample volume (mL)	Type of contribution (Fig. 2)
			cellulose-ester membranes					
Sapula et al. (2021)	Australia NR	Raw (influent)	(1) Centrifugation at 5000 *g* for 30 min at 4 °C, (2) adding $MgCl_2$ to a final concentration of 25 mM	(1) PEG precipitation, (2) Adsorption-elution with electronegative membranes	TRIzol-phenol extraction, NucleoSpin RNA Virus Extraction Kit and RNeasy PowerWater Kit	RT-qPCR N1 and N2	100	1,3
Saththasivam et al. (2021)	Qatar June 2020 – August 2020	Raw (influent)	Heat inactivation 56 °C for 30 min, Centrifugation 4500 *g* for 30 min at 4 °C	PEG precipitation	Quick RNA Viral Kits (Zymo)	RT-qPCR N1, N2 and RdRp	200	2,5
Scott et al. (2021)	USA August 2020 – December 2020	Raw (sewage)	NR	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR and dd-PCR N1 and N2	200	1
Sharma et al. (2021)	India May 2020 – May 2020	Raw (sewage)	Chloroform was added and mixed thoroughly using a magnetic stirrer for 30 min at 4 °C, Centrifugation at 3000 *g* for 20 min at 4 °C	PEG-dextran phase separation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR E and RdRp	500	2
Sherchan et al. (2020)	USA January 2020–April 2020	Raw (influent) and treated (secondary treatment, effluent)	(1) Centrifugation at 3000 *g* for 30 min, (2) NR	(1) Ultrafiltration, (2) Adsorption-elution with electronegative membranes	ZR Viral RNA Kit	RT-qPCR N1 and N2	100 – 1000	1
Song et al. (2021)	USA April 2020 – June 2020	Raw (influent)	(1) Heat inactivation 60 °C for 90 min, Centrifugation at 4000 *g* for 30 min, filtration using 0.45 μm sterile membrane filter, (2) NR	(1) PEG precipitation, (2) Direct extraction method	QIAgen QIAamp® Viral RNA mini Kit and Zymo Quick-RNA Fecal/Soil Microbe Microprep Kit	RT-qPCR and ddPCR N1 and N2	50	1,2,6
Tandukar et al. (2022)	Nepal July 2020–February 2021	Raw (influent, sewage)	NR	Electronegative membrane-vortex (EMV)	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1, N2 and E	100	1,3
Tanhaei et al. (2021)	Iran June 2020–July 2020	Raw (influent) and treated (effluent)	NR	Adsorption-elution with electronegative membranes	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N and ORF1ab	200	2
Tanimoto et al. (2022)	Japan February 2021 – October 2021	Raw (influent)	Centrifugation at 10000 *g* for 30 min	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N	40	4
Thongpradit et al. (2022b)	Thailand January 2021–February 2021	Raw (sewage)	Centrifugation at 3000 *g* for 10 min at room temperature	Adsorption-elution using electronegative membranes	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N, S and ORF1ab	100 – 400	2
Tiwari et al. (2022)	Finland August 2020–May 2021	Raw (influent)	Centrifugation at 3000 *g* for 25 min	Ultrafiltration	Chemagic Viral300 DNA/RNA Extraction Kit	RT-qPCR E and N2	NR	1
Toledo et al. (2022)	USA Sept 2020 - Feb 2021	Raw (influent)	Centrifugation at 4600 *g* for 30 min at 4 °C	PEG precipitation	Promega Wastewater Large-Volume TNA Capture Kit	RT-qPCR and RT-ddPCR N1 and N2	45	1
Tomasino et al. (2021)	Portugal May 2020–March 2021	Raw (influent)	pH adjustment to 3.5/4 using HCl, Heat inactivation 60 °C for 90 min	(1) NR, (2) Sequential centrifugations followed by PEG precipitation	QIAgen RNeasy Powersoil Total RNA, QIAgen RNeasy PowerMicrobiome Kit and IDEXX DNA/RNA Magnetic Bead Kit	RT-qPCR N1 and N2	(1) 10 – 80, (2) 35	3
Torii et al. (2021)	Japan NR	Raw (influent)	(1) Centrifugation at 3500 g for 15 min, (2) filtration using a through 0.45 μm cellulose-ester membrane, (3) Centrifugation at 3500 *g* for 5 min	(1) Ultracentrifugation, (2) EMV, (3) PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit and Acid guanidium thiocyanate-phenol-chloroform extraction using TRIzol protocol	RT-qPCR N1, N2 and N3	40 – 50	2
Torii et al. (2022)	Japan July 2020–October 2020	Raw (influent)	(1,2) Centrifugation at 3500 g for 5 min, (3) Centrifugation at 4700 *g* for 30 min at 4 °C, (4) filtration using a 0.2 μm hydrophilic polytetrafluoroethylene membrane (Millipore), (5) NR	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1 and N2	41	1,2
Trottier et al. (2020)	France May 2020–July 2020	Treated (effluent)	Centrifugation at 4500 g for 30 min at 4 °C,	Centrifugal filtration	NucleoSpin RNA Virus Kit	RT-qPCR N1, N2 and RLP27	50	3

Trujillo et al. (2021)	NR	NR	Heat inactivation 60 °C for 60 min, filtration using a 0.22 μm filter	PEG precipitation	TRIzol-chloroform protocol	RT-qPCR N1	40	3
Vallejo et al. (2022)	Spain March 2020 – May 2020	Raw (influent)	Centrifugation at 4000 *g* for 30 min, filtration using 0.22 μm membranes	Ultrafiltration	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N	100	4.5
Wehrendt et al. (2021)	Argentina April 2021–May 2021	NR	(1) Centrifugation at 12000 *g* for 1 h at 4 °C, (2) pH adjustment to 6–7	(1) PEG precipitation, (2) Centrifugation with PAC	High Pure Viral Nucleic Acid Kit and Viral Nucleic Extraction Kit II	RT-qPCR N and ORF1	(1) 200, (2) 40	1,3,4
Westhaus et al. (2021)	Germany April 2020	Raw (influent after sand trap) and treated (activated sludge)	Centrifugation at 4700 *g* for 30 min	Ultracentrifugation	NucleoSpin RNA virus Kit	RT-qPCR N, E and RdRp	45	2
Whitney et al. (2021)	USA NR	Raw (influent)	NR	NR	4S-column and 4S-Milk-of-Silica	RT-qPCR N1	40	1
Wu et al. (2022)	USA January 2020 – Ma 2020	Raw (influent)	Heat inactivation 60 °C for 90 min, filtration using a 0.2 μm sterile membrane filter	PEG precipitation	TRIzol-chloroform protocol	RT-qPCR N1 and N2	40	4,5
Xiao et al. (2022)	USA March 2020 – June 2020	Raw (influent)	Heat inactivation 60 °C for 1 h, filtration using a 0.2 μm vacuum-driven filter	Centrifugal ultrafiltration	NR	RT-qPCR N1 and N2	15	5
Xu et al. (2021)	Hong Kong June 2020 – September 2020	Raw (influent and sewage)	Heat inactivation 60 °C for 30 min, Centrifugation at 4750 g for 30 min	Ultrafiltration	TRIzol Plus RNA Purification Kit	RT-qPCR N	50 – 90	1
Yanac et al. (2022)		Raw (influent) and treated (primary sludge, secondary effluent, final effluent)	(1) Cheesecloth and low-protein binding 0.45 and 0.2 μm 47-mm Supor-200 membrane disc filters, (2) NR	(1) Ultrafiltration, (2) Skimmed milk flocculation	QIAgen RNeasy PowerMicrobiome Kit and MagMAX Microbiome Kit	RT-qPCR N1 and N2	120	1,4,5
Yaniv et al. (2021)	Israel November 2020–March 2021	Raw (influent)	Shaken and mixed for 2 min manually and left standing 15 min to large particle settlement	Ultrafiltration	NucleoSpin RNA Extraction Kit	RT-qPCR N1, N2, N3 and N4	2000 – 5000	1,5
Zhang et al. (2022)	Australia August 2020 – September 2020	Raw (influent)	NR	Adsorption-elution with electronegative membranes	QIAgen RNeasy PowerWater Kit	RT-qPCR N and E	100	2
Zhao et al. (2022)	USA September 2020 – August 2021	Raw (influent)	NR	PEG precipitation	QIAgen QIAamp® Viral RNA mini Kit	RT-ddPCR N1 and N2	NR	4
Zheng et al. (2022)	Hong Kong September 2020 – November 2020	Raw (influent and sewage)	Heat inactivation 60 °C for 30 min	(1) Ultracentrifugation, (2) PEG precipitation, (3) $AlCl_3$ flocculation, (4) $MgCl_2$ flocculation, (5) Ultracentrifugation 10 kDa, (6) Ultracentrifugation 30 kDa, (7) Membrane adsorption with $AlCl_3$, (8) Adsorption-elution using electronegative membranes, (9) Combination of centrifugation and ultracentrifugation, (10) $AlCl_3$ precipitation, (11) Membrane adsorption	QIAgen QIAamp® Viral RNA mini Kit and TRIzol Plus RNA Purification Kit	RT-qPCR N1	30 – 1000	1
Zhu et al. (2022)	Japan August 2020 – February 2021	Raw (influent)	NR	Ultracentrifugation	QIAgen QIAamp® Viral RNA mini Kit	RT-qPCR N1	40	4,5

Note: NR stands for Not Reported.

(Anderson-Coughlin et al., 2021), and adsorption-elution with electronegative membranes (Barril et al., 2021; Jmii et al., 2021), and (ii) entrapment in chemical precipitates, namely, PEG precipitation (Alexander et al., 2020; Farkas et al., 2021), aluminum flocculation (Pino et al., 2021; Salvo et al., 2021), or skimmed milk flocculation (Philo et al., 2021; Pino et al., 2021). Explaining the fundamentals of these concentration methods is beyond the scope of this review, nevertheless the literature is rich in guidelines for the application of these methods. Readers can refer to the studies of Kaya et al. (2022), Dumke et al. (2021), Barril et al. (2021), and Salvo et al. (2021) to understand in detail these concentration methods and how they have been compared quantitatively. Overall, these studies share the conclusion that PEG precipitation, aluminum flocculation, and ultrafiltration methods favor higher viral recovery rates during the concentration step.

3.2.3. Detection & quantification

The most frequently used method in WBE for SARS-CoV-2 RNA detection is polymerase chain reaction (PCR)-based quantification (Ni et al., 2021). In this regard, real-time reverse transcription–PCR (real-time RT-PCR) has been employed for identifying SARS-CoV-2 genetic targets (Ni et al., 2021; Thongpradit et al., 2022) and is still considered the gold standard method for the detection of SARS-CoV-2 (Ambrosi et al., 2021). Regarding its genomic targets, the nucleocapsid (N) or the envelope (E) protein genes, as well as the ORF1ab gene are the most often used RT-PCR targets, as presented in Table 2 (Corman et al., 2020; Kitajima et al., 2020). The Centers for Disease Control and Prevention (CDC) indicates the use of probes targeting several loci (N1 and N2) of the nucleocapsid via separate reactions (CDC, 2020). Particularly, N1 is commonly employed as an indicator for detecting SARS-CoV-2 in wastewater (Navarro et al., 2021). Different PCR procedures form a list that encompasses the reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Amoah et al., 2021), the reverse transcription droplet digital PCR (RT-ddPCR) (Flood et al., 2021), the reverse transcription quantitative PCR (RT-qPCR) (Ahmed et al., 2020a) and its variations and improvements (La Rosa et al., 2020; Navarro et al., 2021). As can be concluded from Table 2, the RT-qPCR is the most often employed detection/quantification technology and was used in roughly 87 % of the studies in our pool.

3.3. Correlating clinical testing data to viral concentrations in wastewater

The correlation between SARS-CoV-2 viral concentration in sewage water and the number of COVID-19 cases is one of the major challenges of applying viral detection in sewage water to track the scale of SARS-CoV-2 spread in a community (Haque et al., 2021). According to Peccia et al. (2020), some studies have reported the successful correlation of viral RNA levels in wastewater and sludge with the number of reported COVID-19 cases. Such correlation is useful to predict the number of active cases in the population (Ahmed et al., 2020a; Hellmer et al., 2014; Li et al., 2021b; Saththasivam et al., 2021). Particularly, with this information, WBE models can translate viral concentrations in wastewater to the incidence of SARS-CoV-2 shedders within a community (Cao and Francis, 2021).

There are mainly two statistical-based approaches to evaluate these correlations, the estimation of Pearson's correlation coefficient (Forthofer et al., 2007), which is applied to evaluate the level of linear association between two normally distributed variables, and Spearman's rank correlation coefficient for non-normally distributed data prone to contain outliers (Schober et al., 2018). Both coefficients vary within the range from -1 to $+1$, where -1 indicates a perfect negative relationship between the variables, 0 indicates the inexistence of a linear relationship and $+1$ points to a strong positive linear association. We found rather high positive coefficients peaking at values of 0.947 (Galani et al., 2022), 0.95 (D'Aoust et al., 2021), and 0.96 (Layton et al., 2022), for instance, but we also collected moderate (Giraud-Billoud et al., 2021; Tandukar et al., 2022; Tomasino et al., 2021) and weak (Ahmed et al., 2020a) correlations when evaluated through these two statistical approaches depending on the nature of their data. Additionally, some studies in the literature have reported negative correlations (Wehrendt et al., 2021). Recently, a meta-analysis study conducted by Li et al. (2023) collected 133 correlation coefficients ranging from -0.38 to 0.99 for Pearson's or Spearman's coefficients; according to the authors, such a wide range of coefficient values is endorsed by several factors, including variations of the environmental conditions, epidemiological conditions, sampling design, air temperature, etc. This observation is consistent with the numbers found throughout our review process, which also showed a spacious range of values.

To maintain concentration levels meaningful and consistent, normalizing the viral concentration is of paramount importance due to the variability of viral levels in wastewater, which is caused by several factors (Li et al., 2023), such as wastewater flow rate, weather conditions, total suspended solids, and daily fecal discharge. This normalization has been reported in the literature to be addressed via various approaches, such as daily mass flux and/or the use of biomarkers (Qiu et al., 2022). One of these biomarkers, the Pepper Mild Mottle Virus (PMMoV), found in human fecal excreta (Rosario et al., 2009), has been used to normalize the SARS-CoV-2 signal (LaTurner et al., 2021; Qiu et al., 2022; Robotto et al., 2022), contributing to obtain strong correlations between the viral concentration level in wastewater and COVID-19 clinical cases (D'Aoust et al., 2021). When addressing the correlation between viral concentration in wastewater and COVID-19 cases it should be noted that viral RNA concentrations in wastewater can be considered a lagging indicator since the virus continues to be shed after the infected individuals have been recovered (McMahan et al., 2021). This lag time has been reported in several studies to range between 2 and 28 days (Zhao et al., 2022), but it lacks a well-accepted definition as discussed in the next paragraphs. Such variation in the lag times can be caused by multiple factors, including but not limited to, daily changes in population size, wastewater sampling methods, responses of the society to the pandemic, and variations in the time required for reporting case data (Medema et al., 2020; Peccia et al., 2020). For instance, some authors have reported that the duration of viral shedding in the stools can be extended up to 33 days after obtaining a negative nasopharyngeal swab (Gupta et al., 2020; Jones et al., 2020). From a symptom onset perspective, it has been suggested that fecal viral shedding can hold up to >20 days (Wolfel et al., 2020; Wu et al., 2020), with Miura et al. (2021) having estimated a value of 26 days. Although different lag time values have been proposed in several WBE studies, these works usually lack a definition for this term, which can be a potential source of confusion when comparing SARS-CoV-2 WBE studies. Zhao et al. (2022) consider the lag time as the temporal gap between the measured SARS-CoV-2 concentration peaks and the reported COVID-19 clinical testing cases peaks, while Omori et al. (2021) define this term as “the lag between the detection timing from wastewater and reporting by passive surveillance”; ideally, detection and reporting timing should be concurrent, however in practice that is not the case, especially in low and middle-income countries (Li et al., 2021d). Finally, lag times may also be influenced by SARS-CoV-2 incubation time and shedding duration (Zhao et al., 2022). For instance, Wu et al. (2022) explained that the lag time they reported (4 days) was consistent with the common incubation period from viral infection to symptom inception, which is considered to be between 4 and 5 days. Other studies reported lag periods similar to this value (Lara-Jacobo et al., 2022; Peccia et al., 2020; Xiao et al., 2022).

3.4. Modeling of WBE for COVID-19 surveillance

Modeling techniques for COVID-19 surveillance in wastewater comprise a rather wide spectrum, going from a plethora of regression techniques to the application of conservation principles and more elegant and contemporary data-driven methods. Comparing or ranking the results from each modeling approach is beyond the scope of this review and itself is a complex task, although several studies have presented comparisons between the performance of different predictive models as in Zhao et al. (2022), Aberi et al. (2021), and Li et al. (2021b). Table 3 brings a complete description of the methodological approaches reported in our pool of studies for modeling WBE for COVID-19 surveillance.

Table 3
Summary of variables involved in SARS-CoV-2 WBE modeling and respective modeling techniques.

Study	Location and sampling period	Lowest and highest conc. (solid phase)	Lowest and highest conc. (liquid phase)	Estimated lag period	Statistical correl. and coeff. value[a]	Modeling technique/ algorithm[b]	Type of contribution (Fig. 2)
Aberi et al. (2021)	Austria (Data collected from databases)	NR	NR	2–7 days	NR	Regression models applied to predicting the number of active cases: Linear (LR), Polynomial (PL), K-Nearest Neighbor (KNN), Multilayer Perceptron (MLP), Support Vector Regression (SVR), Generalized Additive Models (GAM), Decision Tree (DT) and Random Forest (RF)	5
Acosta et al. (2022)	Canada June 2020–May 2021	NR	NR	4 weeks	Pearson's correlation ($r = 0.70$)	NR	4
Ahmed et al. (2021c)	Australia February 2020–May 2020	NR	1.35E2 – 1.2E4 gc/100 mL	NR	NR	NR	2
Ai et al. (2021)	USA July 2020–January 2021	NR	1E2 – 1E5 gc/L	5 days	Pearson correlation ($r = 0.89$) and Spearman's rank correlation ($r = 0.88$)	Polynomial models	4,5,6
Amereh et al. (2022)	Iran September 2020–April 2021	NR	4E1 – 4.5E4 gc/L	NR	NR	Monte Carlo simulation to estimate disease prevalence, LR between estimated infected population and confirmed cases ($R^2 = 0.80, p < 0.001$)	4
Anneser et al. (2022)	USA March 2020–March 2021	NR	NR	NR	Spearman's rank correlation (r = NR)	LR ($R^2 = 0.80$), GAM ($R^2 = 0.86$), Poisson ($R^2 = 0.84$) and negative binomial models ($R^2 = 0.15$)	4,5
Bagutti et al. (2022)	Switzerland July 2021–December 2021	NR	1E2 – 4.13E5 gc/L	14 days	Spearman's rank correlation ($r = 0.9395$)	NR	4
Barrios et al. (2021)	Argentina June 2020–April 2021	NR	1E-1 – 1E3 gc/L	NR	Spearman rank correlation ($r = 0.812$)	NR	4
Cao and Francis (2021)	USA April 2020–February 2021	NR	NR	NR	NR	Vector Autoregression (VAR) model	5
Claro et al. (2021)	Brazil June 2020–April 2021	NR	2.7 – 7.7 $\log_{10}$ gc/L	2 weeks	NR	Monte Carlo simulations to estimate COVID-19 prevalence for each sampling site	4
de Freitas et al. (2022)	Brazil January 2021–January 2022	NR	NR	NR	Spearman's rank correlation ($r = 0.67$)	Monte Carlo statistical model to introduce uncertainty in the virus shedding	4,5
de Sousa et al. (2022)	Brazil January 2021–August 2021	NR	N1: 2.73 – 3.73 $\log_{10}$ gc/L; N2: 2.69 – 5.47 $\log_{10}$ gc/L	NR	NR	Prediction model for infected individuals published by Ahmed et al. (2020a) with Monte Carlo simulations to introduce uncertainties	5
Fernandez-Cassi et al. (2021)	Switzerland February 2020–April 2020	NR	NR	5.5 days	NR	Incidence estimation by the Susceptible-Exposed-Infectious--Recovered (SEIR) model with Gamma distribution to represent virus shedding and time between infection and symptom onset	5
Fitzgerald et al. (2021)	Scotland April 20202 - January 2021	NR	NR	NR	Spearman's rank correlation ($r = 0.91$)	Basic linear mixed model	1,2
Galani et al. (2022)	Greece August 2020–March 2021	NR	NR	5–9 days	Pearson's correlation ($r = 0.947$)	Distributed/fixed lag modeling, LR, and artificial neural networks (ANN) were utilized to build relationships between SARS-CoV-2 RNA load in wastewater and pandemic health indicators	4,5
Gonzalez et al. (2020)	USA March 2020–August 2020	NR	1E1 – 1E4 gc/100 mL	NR	NR	NR	2
Hemalatha et al. (2021)	India July 2020–August 2020	NR	NR	NR	NR	Predictions models for infected individuals published by Ahmed et al. (2020a) and by Hellmer et al. (2014)	2,3

(continued on next page)

Table 3 (*continued*)

Study	Location and sampling period	Lowest and highest conc. (solid phase)	Lowest and highest conc. (liquid phase)	Estimated lag period	Statistical correl. and coeff. value[a]	Modeling technique/ algorithm[b]	Type of contribution (Fig. 2)
Hoar et al. (2022)	USA August 2020–April 2021	NR	NR	NR	Spearman's rank correlation ($r = 0.81$)	LR ($R^2 = 0.65$)	4,5
Jiang et al. (2022)	USA May 2020–December 2021	NR	NR	NR	NR	ANN (Best fit with $R^2 = 0.89$)	5
Karthikeyan et al. (2021)	USA July 2020–October 2020	NR	NR	3 weeks	Pearson's correlation ($r = 0.84$)	Linear Regression model with Autoregressive model (ARIMA)	3,4
Koureas et al. (2021)	Greece October 2020–April 2021	NR	NR	NR	NR	LR ($R^2 = 0.9511$) and RF ($R^2 = 0.9956$)	5
Krivonakova et al. (2021)	Slovakia September 2020–March 2021	NR	NR	2 weeks	NR	Regression models to calculate viral concentration: Simple Linear, Double Square Root, and Square Root-Y	2,4
Kuhn et al. (2022)	USA November 2020–March 2021	NR	1.6E1 – 7.3E6 gc/L	4–10 days	Pearson's correlation and Spearman rank correlation (r = NR)	General Multivariate Linear Regression, multivariate Poisson (best accuracy obtained) and Negative Binomial models	5
Layton et al. (2022)	USA June 2020–July 2020	NR	2.9 – 5.1 $\log_{10}$ gc/L	NR	Pearson's correlation ($r = 0.96$)	Monte Carlo simulation to account for the uncertainty in the point estimates for each sampling event	4
Li et al. (2021b)	Australia Used data from seven papers	NR	NR	NR	NR	Three types of data-driven models were applied to a multi-national WBE dataset: multiple linear regression (MLR), ANN and adaptive neuro-fuzzy inference system (ANFIS) to predict upcoming new cases	5
Li et al. (2022)	USA June 2020–September 2021	NR	2.76E3 – 3.86E6 gc/L	7 days	Spearman's rank correlation ($r = 0.790$)	NR	2,4
Maida et al. (2022)	Italy September 2021–July 2021	NR	NR	NR	NR	A logistic regression model was calculated to evaluate the association between the active SARS-CoV-2 incidence rates and the probability of positive PCR results of wastewater samples	5
McMahan et al. (2021)	USA May 2020–August 2020	NR	4.7E3 – 3.3E6 gc/L	NR	NR	SEIR model to predict the number of infected individuals based on the mass rate (gc/day) of SARS-CoV-2 RNA in wastewater	5
Nagarkar et al. (2022)	USA May 2020–November 2020	NR	1E3 – 1E4 gc/L for N1 and N2	NR	Pearson's correlation ($r = 0.70$)	NR	4
Nourbakhsh et al. (2022)	Canada September 2020–June 2021	NR	NR	3–20 days	NR	Viral transmission is simulated via a standard epidemiological SEIR-like model and the fate of SARS-CoV-2 in wastewater using an advection-dispersion-decay model	5
Omori et al. (2021)	USA April 2020 – June 2020	NR	~10 – ~4E2 gc/mL	8.4–11.6 days	NR	Data fitting using Poisson distribution	5
Peccia et al. (2020)	USA March 2020–June 2020	NR	1.7E3 – 4.6E5 gc/mL	6–8 days	NR	LRs were used to estimate the relationship between SARS-CoV-2 RNA copies per ml results for replicated RNA extractions of each daily sample. Estimation of primary sludge as a potential leading indicator was performed using a distributed lag measurement error time series model	4,5
Petala et al. (2022)	Greece October 2020–January 2021	NR	NR	NR	NR	Developed a set of parametric equations to estimate the evolution of global virus shedding rate in wastewater	5
Pillay et al. (2021)	South Africa July 2020–October 2020	NR	0 – 7.12E5 gc/100 mL	NR	NR	Prediction model for infected individuals published by Ahmed et al. (2020a)	5
Proverbio et al. (2022)	Luxembourg NR	NR	NR	NR	NR	SEIR epidemiological model in combination with the	5

Table 3 (*continued*)

Study	Location and sampling period	Lowest and highest conc. (solid phase)	Lowest and highest conc. (liquid phase)	Estimated lag period	Statistical correl. and coeff. value[a]	Modeling technique/ algorithm[b]	Type of contribution (Fig. 2)
	(Data collected from databases)					extended Kalman filter (EKF)	
Reynolds et al. (2022)	Ireland June 2020–August 2021	NR	NR	0 days	Spearman's rank correlation (r = 0.500)	NR	2,6
Rodriguez Rasero et al. (2022)	Spain July 2020 – February 2021	NR	NR	6 days	NR	Data fitting using quasi-Poisson modeling	5
Roka et al. (2021)	Hungary June 2020–October 2020	NR	~5E3 – ~1E6 gc/L	NR	NR	Data fitting using LR (Best fit value using a weighted average of viral load against daily new cases, R^2 = 0.720 and p < 0.0001)	1,4
Saththasivam et al. (2021)	Qatar June 2020–August 2020	NR	7.889E3 – 5.42E5 gc/L	NR	NR	Conservation principles to estimate the number of infected populations based on measuring RNA concentration	2,5
Scott et al. (2021)	USA August 2020–December 2020	NR	N1: 22.5 – 5.27E3 gc/100 mL; N2: 81.6 –3.91E4 gc/100 mL	NR	Spearman's rank correlation (r = 0.50)	MLR, Simple Logistic Regression and Multiple Logistic Regression	1,5
Song et al. (2021)	USA April 2020 – June 2020	NR	~8E0 – 9E5 gc/mL	NR	NR	NR	1,2,6
Tanimoto et al. (2022)	Japan February 2021–October 2021	1.5E7 – 2.0E8 gc/L	3.1E7 – 5.5E8 gc/L	NR	NR Solid phase: r = 0.8482, Liquid phase: (r = 0.7803)	LR	4
Vallejo et al. (2022)	Spain March 2020–May 2020	NR	1E4 – 15E4 gc/mL	NR	NR	LR (R^2 = 0.8515), GAM with a Cubic Regression Spline (R^2 = 0.8767), locally estimated scatterplot smoothing (LOESS) Linear (R^2 = 0.8685), LOESS Quadratic (R^2 = 0.8833)	4,5
Wang et al. (2021)	USA NR	NR	N1: 3.85E5 – 2.55E6 gc/L, N2: 3.79E5 – 2.15E6 gc/L	NR	Pearson's correlation (r = 0.94)	Monte Carlo simulations to estimate the number of infected individuals	4
Wu et al. (2022)	USA January 2020–May 2020	NR	NR	4–10 days	Pearson's correlation (r = NR)	Wastewater data was modeled as a convolution of new clinical cases and used Markov Chain Monte Carlo (MCMC) simulation to quantify uncertainty in the shedding model	4,5
Wurtzer et al. (2022)	France March 2020–June 2021	NR	0 – 1E6 gc/L	3 days	Spearman's rank correlation (r = NR)	LR	4
Xiao et al. (2022)	USA March 2020–June 2020	NR	NR	6.4 days	NR	Approximate Bayesian computation for estimating delay distribution, convolution to estimate the transfer function model, and MCMC simulation to quantify uncertainty in transfer functions	5
Zhao et al. (2022)	USA Sept 2020 - August 2021	N1: 7.14E2 – 7.14E3 gc/L, N2: 8.02E2 –6.2E3 gc/L	NR	5 weeks	Pearson's correlation (N1: r = 0.62 and N2: r = 0.64)	LR, ARIMA, Regression Model with Autoregressive Model with Seasonal Pattern (SARIMA) and (4) VAR	4
Zhu et al. (2022)	Japan August 2020–February 2021	NR	~10 – ~70 gc/mL	NR	NR	Generalized linear model, ANN and RF to predict the cumulative number of cases	4,5

Notes: NR stands for Not Reported;

[a] The indicated value corresponds to the best fit obtained in the respective study.

[b] The designated models aim to associate viral concentration signals from wastewater to clinical testing data.

4. Discussion

4.1. Issues and promising alternatives for SARS-CoV-2 analysis in wastewater

Quantifying low viral loads from non-clinical samples represents one of the major challenges of WBE (Calderon-Franco et al., 2022). When viral particles enter the sewage system, dilution occurs by the addition of other types of water (i.e., domestic sewage and stormwater combined or separated in the sewer), while concomitantly being exposed to a diverse range of chemical agents and physical conditions (Haramoto et al., 2020; Krivonakova et al., 2021). In this regard, a concentration step is required due to the low levels at which SARS-CoV-2 RNA is found in wastewater (Boogaerts et al., 2021; Peinado et al., 2022; Zheng et al., 2022). Several works have reported viral RNA detection in untreated wastewater (influent

of the WWTP), being its concentration in the range of 10^2–10^5 copies per liter and the maximum exceeding 10^6 copies per liter (Kitajima et al., 2020). PEG precipitation represents a simple and low-cost alternative for viral concentration in wastewater (Flood et al., 2021). In PEG precipitation, the solvent is preferentially trapped, and proteins (e.g., virion) are sterically excluded from the solvent phase by PEG. This way, proteins can be concentrated and precipitated once their concentrations surpass the saturated solubility (Torii et al., 2022). Despite being used in many laboratories, this method suffers from losing approximately half of the viral fragments bound to solid matter (Perez-Cataluna et al., 2021). On the other hand, skimmed milk flocculation can be considered a promising approach for low-resource areas since extensive laboratory resources are not needed. Additionally, this method does not require consumables that are challenging to acquire, thus enabling the performance of uninterrupted surveillance (Philo et al., 2021). It is worth highlighting that some of these methods were developed for detecting non-enveloped enteric viruses (e.g., norovirus, adenovirus, and enterovirus), which have been the focus of most studies that investigate the existence of viruses in municipal wastewater and human excreta (Ahmed et al., 2020c; Flood et al., 2021). Additionally, SARS-CoV-2 concentration methods have been assessed using surrogate viruses to mimic SARS-CoV-2, since personnel with special training and a laboratory that fulfills Biosafety Level 3 are required for the culture of this virus. Examples of these surrogate viruses include Alphacoronavirus HCoV 229E, bovine respiratory syncytial virus, bovine coronavirus BCoV, porcine epidemic diarrhea virus, murine hepatitis virus, F-specific RNA phages, avian coronavirus of infectious bronchitis virus, mengovirus or Pseudomonas phage Phi6 (Ahmed et al., 2020c; Aquino de Carvalho et al., 2017; Balboa et al., 2020; Flood et al., 2021; Gendron et al., 2010; Hata et al., 2020; Kocamemi et al., 2020; La Rosa et al., 2020; LaTurner et al., 2021; Medema et al., 2020; Randazzo et al., 2020; Torii et al., 2022).

When it comes to the precise detection and viral quantification, multiplex PCR enables multiple target detection and/or quantification with a sensitivity comparable to that of singleplex PCR. Hence, multiplex PCR is a promising technology since it is more cost-effective and time-saving, reduces the required sample volume, and minimizes the variability due to pipetting. Nevertheless, the complexity of this assay requires optimization to prevent several undesired phenomena, such as primer-probe sets interaction (Navarro et al., 2021; Xiao et al., 2020b). On a different note, the implementation of RT-qPCR to detect SARS-CoV-2 in wastewater has some limitations, as is time-consuming (it could take 24 h), is highly susceptible to the presence of inhibitors, and sample contamination may occur, resulting in false negative results (Ahmed et al., 2022a). Following this problem, it is necessary to explore new, alternative approaches for the detection of SARS-CoV-2 RNA in wastewater. Apart from PCR-based approaches, other methods can also be employed for viral detection (Lara-Jacobo et al., 2022). For instance, metatranscriptomic sequencing also referred to as Next Generation Sequencing is an alternative for SARS-CoV-2 detection and quantification. This technology allows capturing the whole virus genome, which is of paramount importance due to the incidences of mutation events that increase the virulence, thus significantly improving the sensitivity (Boogaerts et al., 2022; Ni et al., 2021). However, the low SARS-CoV-2 RNA concentration in wastewater, along with the existence of nucleic acids from bacteria, other viruses, animal products, and humans, makes conventional metatranscriptomic sequencing an inappropriate technology for WBE applications at this developmental stage (Boogaerts et al., 2022; Ni et al., 2021). To surmount the low viral RNA concentration challenge, the ARTIC Network amplicon library (Nemudryi et al., 2020) and ATOPlex (Xiao et al., 2020b) have been developed. Moreover, RT-LAMP has also been used for SARS-CoV-2 detection (Wei et al., 2021). This method is based on the amplification of the nucleic acids under isothermal conditions, thus avoiding the need for thermal cyclers. Different RT-LAMP approaches can be distinguished, such as the colorimetric or visual RT-LAMP or the fluorescent RT-LAMP. The former enables the visual reading of the results, whereas the latter facilitates the detection of positive amplification by using a fluorescent dye (Amoah et al., 2021; Huang et al., 2020).

Prominent alternatives in the field may be the use of sensors based on electrochemical principles (Chaibun et al., 2021; Kumar et al., 2022; Ramanujam et al., 2021), which have been extensively studied in terms of specificity and selectivity for different types of nanomaterials. Another plausible approach is the use of magnetic devices where magnetic nanoparticles designed for the separation and detection of the viral pathogens in water samples are employed (Gómez-Pastora et al., 2014; Materón et al., 2021; Yue et al., 2020). These devices might be a promising detection technology as they could be simpler, more accurate, economic, rapid, and portable, allowing the measurements to be performed at the WWTPs by plant technicians.

Finally, it has been reported that viral RNA detection can be influenced by several factors, such as the method used for RNA concentration, or the prevalence of COVID-19 infections in the community (Haramoto et al., 2020; Ni et al., 2021). As an instance of the urgent need for analytical accuracy when performing wastewater processing for WBE, we found a decent number of studies that addressed the comparison of the measured viral recovery among distinct concentration and extraction procedures; we also found studies exploring the divergences in the detection and quantification of the viral loads using variations of the PCR approach (Ahmed et al., 2022b; Flood et al., 2021). These problematics elevate the magnitude of the issue. Standardization will be a natural consequence of addressing this problem.

4.2. SARS-CoV-2 epidemiological modeling

The common assumption when using WBE for COVID-19 surveillance is that the number of viral copies observed in the wastewater samples and the reported cases from clinical sources result from the real number of infections, which encapsulates symptomatic and asymptomatic cases (Schmitz et al., 2021; Xiao et al., 2022). Following this premise, wastewater-based epidemiological models have demonstrated to be a valuable tool for estimating the number of infected individuals within a population and identifying COVID-19 infection hotspots. WBE has been hampered by the difficulty of properly correlating viral RNA measurements in wastewater to the number of infections. Also, the real number of infected individuals is generally unknown due to the limitations of the current individual testing capacity systems, especially in low-income areas. To overcome these issues, the number of infected individuals has been linked to the viral concentration (gene copies per volume) and the mass rate of viral RNA in wastewater (gene copies per day) (McMahan et al., 2021). The latter is argued to be preferable over the former because of the serial dilutions of wastewater that might occur due to rainfall, for instance, which alters the viral concentration levels along the sewer network. Using the viral mass rates can be a promising approach when developing epidemiological models given that fluctuations in flow rates compensate for the changes in viral concentrations, leaving viral mass rates unaltered. Regarding the modeling techniques, a wide range of tools has been used as demonstrated in Table 3, which can be categorized into (1) regression techniques (Krivonakova et al., 2021; Peccia et al., 2020; Tomasino et al., 2021), (2) conservation principles (McMahan et al., 2021; Saththasivam et al., 2021), and (3) data-driven methods (Aberi et al., 2021; Li et al., 2021b; Pereira et al., 2020). Different regression approaches have been explored, to name a few: simple univariate and multivariate linear regression (Kuhn et al., 2022; Roka et al., 2021; Zhao et al., 2022), logistic regression (Scott et al., 2021), Autoregressive Integrated Moving Average (ARIMA) (Karthikeyan et al., 2021; Zhao et al., 2022), and the Vector Autoregression (VAR) model (Cao and Francis, 2021; Zhao et al., 2022). WBE can be applied for the back-calculation of infection prevalence. For that purpose, regression techniques are one of the most important tools in WBE modeling. However, these methods may lead to misleading inferences, since they are proposed for independent data with linear correlations, and the WBE data are time series data (Aberi et al., 2021; Cao and Francis, 2021). In the conservation principles category, an example is the application of the

susceptible-exposed-infectious-recovered (SEIR) model, which has shown promising results to predict infection prevalence through a set of interconnected ordinary differential equations (Fernandez-Cassi et al., 2021; McMahan et al., 2021; Nourbakhsh et al., 2022; Proverbio et al., 2022). Furthermore, we found that data-driven methods have acquired considerable popularity given the number of studies that employed these approaches to address the complex task of building epidemiological models, with Artificial Neural Networks (ANN) (Galani et al., 2022; Jiang et al., 2022; Li et al., 2021b; Zhu et al., 2022), Adaptive Neuro-Fuzzy Inference System (ANFIS) (Li et al., 2021b), and the Generalized Additive Model (GAM) method (Aberi et al., 2021; Anneser et al., 2022; Vallejo et al., 2022), as examples of a larger group of techniques listed in Table 3. The common interest in using these approaches may have a root in the fact that epidemiological data are generated in large amounts with a daily frequency, and that data-driven models must be constantly fed and updated with new inputs for better prediction performance.

Ideally, WBE models should account for the changeability and uncertainty in their variables, specifically for the shedding quantities and secretion routes, such as feces, urine, and sputum (Tiwari et al., 2022). We found that the usual way to associate uncertainty appears to be through the Monte Carlo simulation, which was mostly used to associate uncertainty when estimating the infection prevalence (Amereh et al., 2022; de Sousa et al., 2022; Gonzalez-Reyes et al., 2021; Wang et al., 2021) and the shedding rates (de Freitas et al., 2022; Wu et al., 2022). Going further into modeling uncertainty, it should be noted that relevant variables are potentially able to create a certain degree of uncertainty. These variables are included as model variables or functions in different WBE models. Examples are: the number of active cases influencing viral counts in wastewater (persons) (Gonzalez-Reyes et al., 2021; Rodriguez Rasero et al., 2022), daily stool mass (g_{feces}.person^{-1}) (Ahmed et al., 2020a; Amereh et al., 2022; Claro et al., 2021; Pillay et al., 2021), shedding rate of SARS-CoV-2 RNA (gene copies. $g^{-1}{}_{feces}$ or gene copies. $g^{-1}{}_{feces}$.day^{-1}) (Ahmed et al., 2020a; Claro et al., 2021; Kuhn et al., 2022; Li et al., 2021c; McMahan et al., 2021; Pillay et al., 2021; Schmitz et al., 2021), decay of SARS-CoV-2 RNA due to storage (time^{-1}) (Kaya et al., 2022; Li et al., 2021c; Yanac et al., 2022) time-dependent RNA degradation (McMahan et al., 2021), the offset between the observed wastewater viral RNA concentration and the estimated patient viral load (Zhu et al., 2022), and RNA temperature-dependent half-life (h) (Ahmed et al., 2020b; McMahan et al., 2021). On the same note, from a clinical perspective, it is not established the influence of the severeness of the disease on the magnitude of daily shedding (genome copies per gram of stool), and this constitutes another major source of uncertainty in WBE modeling.

Apart from the aforementioned uncertainty sources, Pillay et al. (2021) reported that the variability of the WBE approach may be mainly caused by changes in the environmental conditions (e.g., the viral dilution and stability in water are influenced by rainfall events and temperature) and the unique features of WWTPs. They highlighted the major importance of accurate knowledge of the shedding pattern within the WWTP catchment. Additionally, these authors explained that the weight of stool that is daily produced per person, which is regionally dependent and may be impacted by several factors, influences the accurate estimation of the number of infected individuals. Furthermore, we found several factors that may influence the accuracy of the back-calculation of the infection prevalence, namely population size, bioindicators' stability (PMMoV), excretion rates, sampling method, and sample preparation. Additionally, several parameters, including the temperature, per-capita water, and average travel time in the sewer, represent critical variables that are needed for identifying infection hotspots when the WBE model is applied.

4.3. Current research gaps and future guidelines for SARS-CoV-2 WBE

Further clarifications on SARS-CoV-2 WBE that need to be addressed in the near future include the persistence of the virus in the wastewater, the effect of the shedding dynamics of the virus in feces, whether urban and rural wastewater systems exhibit significative differences in their characteristics, and how the normalization of viral levels in wastewater with regard to population size should be performed (Fitzgerald et al., 2021). Additionally, one must bear in mind that COVID-19 is unevenly distributed across population types so considering cross-city differences is of paramount importance. Hence, a 'one size fits all' approach should not be applied to disease surveillance (Kuhn et al., 2022). We suggest that public health information should not be predicted by wastewater analysis alone but by a combination of wastewater-derived information and other data sources. This is due to the fact that changes in factors such as local demographics along with the limitations of current clinical testing/reporting systems may affect the potential of domestic wastewater as a source of information for prediction tools (Xiao et al., 2022). Other factors that should be considered in WBE modeling are reported by Kuhn et al. (2022), including the shedding duration (i.e., how long an infected individual may shed viral particles through feces), and the relationship between the infection severity and the number of viral particles that are shed. At this point, it is not completely understood how these two variables may cause changes in the observed wastewater viral concentration.

We also suggest the utilization of the solid portion of the wastewater as an alternative matrix for the analysis. There is evidence that enveloped viruses feature a high inclination to bind to the surface of solids in wastewater in comparison to non-enveloped viruses (Ye et al., 2016). As it was previously mentioned, SARS-CoV-2 possesses a lipid outer envelope (Klein et al., 2020) whose hydrophobicity may promote greater viral binding to solids in the wastewater, thus affecting viral recovery (Ahmed et al., 2020a; Ahmed et al., 2020c; Anderson-Coughlin et al., 2021). It has been pointed out that the chain of wastewater analysis procedures should not only focus on the supernatant fraction but also on the solid portion of the wastewater (Westhaus et al., 2021; Yanac et al., 2022). Additionally, and from a WBE perspective, concentration levels from the solid portion have been correlated better with COVID-19 incidence numbers when compared to signals obtained from the liquid part of the wastewater (Tanimoto et al., 2022). For modeling purposes, normalization of the concentration levels from the solid portion of the wastewater can be performed through either total suspended solids measurements (Nourbakhsh et al., 2022) or using the concentration of PMMoV; however, comparability between the concentrations obtained from the solid and liquid phases through PMMoV normalization is still restricted (Kim et al., 2022). In this regard, different studies have reported the prevalence of viral particles in the solid phase obtained from domestic wastewater, as well as observations pointing to a significantly higher amount of viral RNA in the solid portion (Kim et al., 2022; Kumblathan et al., 2023). For instance, Kitamura et al. (2021) reported that a higher level of SARS-CoV-2 RNA, compared to PMMoV RNA, was contained in the solid fraction, whereas supernatant fractions comprised lower SARS-CoV-2 RNA levels. They reasoned that the different detection of SARS-CoV-2 RNA and PMMoV RNA in the solid and liquid fractions could result from the fact that PMMoV lacks an envelope, which is present in SARS-CoV-2. Similarly, Li et al. (2021a) found that SARS-CoV-2 RNA was considerably more abundant in the solid than in the liquid fraction. This observation was further endorsed by the studies of Ni et al. (2021) and Tomasino et al. (2021) in terms of viral recovery. The higher viral RNA concentration in the solid phase of wastewater, along with the more time-efficient processing of the solid fraction (Li et al., 2021a; Nourbakhsh et al., 2022), led us to suggest that wastewater solids may represent a more convenient sample matrix, thus being a promising approach to improve analytical accuracy in WBE for SARS-CoV-2.

As highlighted throughout the present study, WBE represents a valuable tool for predicting COVID-19 cases. To this end, WBE can be implemented via several statistical models with data gathered from wastewater (Ando et al., 2023; Anneser et al., 2022). However, establishing and standardizing protocols are still required so that worldwide conducted studies could be successfully compared (Amereh et al., 2022; Fitzgerald et al., 2021). In further words, the current lack of standardization is revealed by the wide range of sample initial volumes and concentration methods that have

been reported by the different studies. Thereby, sample initial volumes range from 2 mL to 1 L; moreover, the extensive variety of concentration methods that have been used include size-based and entrapment in chemical precipitates techniques, such as conventional filtration, ultrafiltration, ultracentrifugation, centrifugation, filtration using negatively charged membranes, precipitation, and direct extraction, as well as their combinations. Furthermore, it is still unclear how different pretreatment techniques may affect the detection performance through PCR methods. In this sense, the recognition of the pre-treatment step in the wastewater analysis process is crucial to further develop standard protocols for SARS-CoV-2 detection and quantification. Finally, quality controls, variable testing, and the optimization of the methodology are considerably lacking; however, they are required in order to provide analytical accuracy (Calderon-Franco et al., 2022).

5. Conclusions

SARS-CoV-2 will remain a constant threat to public health given the increasing infectibility of new VOCs. In this study, we reviewed the recent WBE research endeavor to mitigate the hefty burden of COVID-19 on the health systems around the globe. More specifically, this review collects and organizes the recent progress on the analytical methods reported between 2020 and 2022 to detect and quantify SARS-CoV-2 RNA from wastewater samples. We also review the methods by which SARS-CoV-2 wastewater-based epidemiological modeling has been approached to use the output of lab analysis for diverse purposes, such as predicting outbreaks in a community, estimation of active human shedders (or infected individuals), and shedding rates, to name a few. Correlating the amount of genomic material in wastewater with the number of COVID-19 cases within a community is a component of epidemiological modeling that has been tried through a wide range of mathematical methods, with data-driven models considered the most popular approach to address predictions of variables correlated to outbreaks within a certain time horizon, based on genomic viral material measurements in domestic wastewaters. We also highlight the promising opportunities to improve the accuracy and rapidness of viral detection using the solid portion of wastewater as an alternative testing matrix, and the design of novel sensors based on electrochemical or magnetic devices. However, as evidenced throughout this work, recent research has not focused on ways to standardize the analytical procedures for comparability between different locations. Implementing the WBE surveillance as a prediction tool for outbreaks and infection waves, which in turn would result in the mitigation of the COVID-19 burden, remains challenging. To promote the worldwide applicability of WBE surveillance, this lack of standardization should be managed along with the establishment of a testing framework that accounts for the different analytical sensitivities throughout the different steps of the analysis. Notably, this study contributes to future research as a reference guide for what has been proposed and worked so far to understand the dynamics of viral concentrations in wastewater. Since the effort to mitigate the effects of COVID-19 is a global one, future research must expand the scope of this review and consider the needs of low-income countries, whose health systems are often restricted and the implementation of the WBE surveillance strategy can thus become more arduous.

CRediT authorship contribution statement

Stefano Ciannella: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Cristina González-Fernández:** Writing – original draft, Writing – review & editing, Visualization. **Jenifer Gomez-Pastora:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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