



Review article

A scoping review of global SARS-CoV-2 wastewater-based epidemiology in light of COVID-19 pandemic

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ABSTRACT

Recently, wastewater-based epidemiology (WBE) research has experienced a strong impetus during the Coronavirus disease 2019 (COVID-19) pandemic. However, a few technical issues related to surveillance strategies, such as standardized procedures ranging from sampling to testing protocols, need to be resolved in preparation for future infectious disease outbreaks. This review highlights the study characteristics, potential use of WBE and overview of methods, as well as methods utilized to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) including its variant in wastewater. A literature search was performed electronically in PubMed and Scopus according to PRISMA guidelines for relevant peer-reviewed articles published between January 2020 and March 2022. The search identified 588 articles, out of which 221 fulfilled the necessary criteria and are discussed in this review. Most global WBE studies were conducted in North America (n = 75, 34 %), followed by Europe (n = 68, 30.8 %), and Asia (n = 43, 19.5 %). The review also showed that most of the application of WBE observed were to correlate SARS-CoV-2 ribonucleic acid (RNA) trends in sewage with epidemiological data (n = 90, 40.7 %). The techniques that were often used globally for sample collection, concentration, preferred matrix recovery control and various sample types were also discussed. Overall, this review provided a framework for researchers specializing in WBE to apply strategic approaches to their research questions in achieving better functional insights. In addition, areas that needed more in-depth analysis, data collection, and ideas for new initiatives were identified.

1. Introduction

Coronavirus disease 2019 (COVID-19) has emerged as a serious infectious disease outbreak caused by the etiologic agents known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 infection was observed for the first time in Wuhan, the capital of Hubei Province, China [1]. Subsequently, the virus transmission led to a global outbreak creating a pandemic declaration in

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March 2020 by the World Health Organization (WHO) [2,3]. This virus belongs to the family of ribonucleic acid (RNA) based coronaviruses (CoV) [4]. It contains membrane, spike (S), nucleocapsid (N) and envelope (E) proteins similar to family Coronaviridae. It uses the host's receptors to bind their spike proteins and ultimately infect the host [5,6]. The SARS-CoV-2 is an enveloped virus and harbours a positive-sense single-stranded RNA with a genome similarity of 82 % when compared to severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) [7]. Current tests for SARS-CoV-2 rely on monitoring respiratory samples and saliva through clinical polymerase chain reaction (PCR) tests or rapid test kits that provide syndromic surveillance of symptomatic patients and their close contacts. COVID-19's infectiousness prior to the onset of symptoms is estimated to account for roughly 40–50 % of disease transmission [8,9]. As the pandemic progressed, it became clear that the vaccination status does not influence the ability to transmit COVID-19 before symptoms appear. In other words, even if a person is vaccinated, they can still unknowingly spread the virus before showing any signs.

Decentralized individual testing, asymptomatic transmission and viral shedding despite of vaccinations highlight the importance of wastewater surveillance (WS) as an indicator of infection trends as the virus can be detected in the early stages of infection, before any clinical manifestation [10]. Wastewater samples are independent of these variables and able to provide rapid results. The mix of health system limitations of access, turnaround time and biological hyper-shedding periods at the beginning of infection course have rendered wastewater data to be a leading indicator. This has been shown by trends from wastewater monitoring that preceded clinical reports by 4–10 days, suggesting its effectiveness as an early warning indicator of impending outbreaks [11].

Recent findings [12–15] along with other studies on wastewater-based epidemiology (WBE) have described reliable a discovery of SARS-CoV-2 gene fragments in sewerage across the world, including Australia [16], Bangladesh [17], Brazil [18], China [19], France [20], the Netherlands [21], Italy [22], Spain [23–25], and the U.S [26–28]. The “COVIDPooPs19” and NORMAN SCORE “SARS-CoV-2 in sewage” databases were established to serve as platforms for sharing data rapidly and openly, enabling the dissemination of information on the involvement of universities, research institutions, private laboratories, and location in WBE for SARS-CoV-2 surveillance. The development of these databases signifies the growth and recognition of WBE as a valuable tool for SARS-CoV-2 monitoring [29,30]. In addition, the Global Initiative on Sharing All Influenza Data (GISAID) has also published entries from SARS-CoV-2 WS. It includes entries with various sequence coverage for variant clades, and amino acids substitution data from the global WS that was made available for the first time.

However, despite the appealing side of WBE, its usage for detecting newly introduced virus carriers and initial virus circulation in a low-prevalence community, largely leans on the lowest possible detection level of viral RNA in sewage. The accuracy and representativeness of any measurement acquired from wastewater is subject to a number of factors derived from the source (population, shedding), in-network (i.e. the pipe network acting as a wastewater collection system) characteristics, sampling strategy and sample analysis. To ensure that WBE can function as an effective leading indicator and a reliable alternative to clinical disease surveillance, it is crucial to address various obstacles and challenges. This will enhance its practical application by aligning it with the prevailing conditions and requirements. These hurdles include the absence of unified standards, uncertainty and variation in sampling strategies, the approach to concentration, matrix recovery control (MRC), and detection. Overcoming these challenges will be essential for future optimization of WBE.

Additionally, several reviews on SARS-CoV-2 WBE provided valuable insights. Ferraro et al. (2022) discussed the use of wastewater surveillance as a tool for detecting and tracking the spread of the SARS-CoV-2 virus [31] whilst Daughton (2021) stressed on global collaboration, standardization, and data sharing [32]. Shimoni et al. (2022) on the other hand, discussed on the effectiveness of using WS as an early warning system for COVID-19 community transmission [33] and Wu et al. (2022) shared international experiences, addressing challenges, opportunities, and future directions for WBE in COVID-19 tracking [34]. These reviews collectively demonstrated the effectiveness and applications of WBE in monitoring and combating the spread of COVID-19.

An overview of research on SARS-CoV-2 wastewater analysis is presented in this scoping review. The domains emphasized in this review were on the current global experience on SARS-CoV-2 WBE in distinct continents, the variability and gaps in methodologies employed by various research organizations, potential use of WBE in investigating SARS-CoV-2 diversity, including novel variants and new mutations within communities. This review will complement other scoping reviews on COVID-19 WBE with a view to support the need for further research on WBE for COVID-19 and other infectious diseases.

2. Methodology

2.1. Scoping review strategy

A literature search was carried out in April 2022, using two databases PubMed and Scopus to identify eligible articles on wastewater epidemiology or surveillance. The search strategy was narrowed down to publications starting from January 2020 until March 2022 with the keywords related to “RNA concentration”, “COVID-19”, “SARS-CoV-2”, “wastewater”, “surveillance/epidemiology” and “variant of concern” ([Supplementary Table S1](#)). The articles were imported and managed in Zotero version 6.0.23 during the screening process. A screening criteria was developed a priori for each of the three stages: title, abstract, and full text. Two groups of researchers screened the articles independently. Disparities at the full-text stage were resolved by discussion.

2.2. Study selection

This study was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) framework [35]. All prospective papers (title, authors' names, and publication year) were managed on Microsoft Excel spreadsheet

during the search strategy phase. All eligible papers were filtered by titles, and duplicates or irrelevant articles were excluded by using Zotero review platform. The exclusion criteria for this review are as listed in [Table 1](#).

In between writing and gathering material for this review, all team members received a few training sessions. This was necessary to ensure that all authors complied with the structures and not to skip any procedures.

The eligibility form was created prior to the article search using Google form (Supplementary S2). Peer-reviewed original studies with the following criteria were included 1) Studies that are descriptive, experimental, cohort, cross-sectional and case-control; 2) Population-based communities or public/private institutions (such as hospitals and cruise ships) in geographic areas with sewerage; 3) Employs detection method of collected sample type regardless of sampling and laboratory techniques; 4) has statistical analysis (for example, correlation, association, sensitivity, and specificity) or reporting of epidemiological trends' results (including graphical); 5) Estimation of the infection prevalence, trends in prevailing outbreaks (above/below the limits, stagnation/increase/decrease in the number of cases), or early warning indicators to identify outbreak signals and epidemiological case counts or trends within specific communities and analysis to determine genetic diversity and variants of SARS-CoV-2 in the geographical area.

2.3. Graphical illustration

Graphs were created using Microsoft Excel (XLS) and Adobe Illustrator Creative Cloud software.

3. Results

3.1. Overview of study characteristics

Based on the search criteria, it resulted in 588 hits: 345 from PubMed, and 243 from Scopus. After removing duplicates ($n = 191$), 397 articles remained and underwent manual eligibility screening by two separate teams of reviewers. During primary screening of title and abstract, another 130 articles were excluded which included eight non-English articles. Full-text screening of the remaining 267 articles resulted in elimination of an additional 46 studies, which were generally (i) independent to the detection of SARS-CoV-2 in sewage (e.g., articles dwelling with actual human as well as animal samples) or was not relevant to WBE; (ii) reviews that incorporate information already obtained from other studies; (iii) press releases, technical/government reports, and unpublished (non-peer-reviewed, or preprints) materials. Finally, only 221 articles were deemed eligible for this review. [Fig. 1](#) shows a flow chart illustrating the steps of paper selection.

Out of the 221 selected articles, 204 articles (92.3 %) did not provide information on their study designs. Study design for the remaining articles were as follows: longitudinal observational studies ($n = 9$), time-series ($n = 6$), case control and case study (1 article each). WBE was utilized in almost 46 countries across the world and geographic distribution of all 221 articles are as shown in [Fig. 2](#). Majority of studies ($n = 75$, 33.9 %) were conducted in North America, followed by Europe ($n = 68$, 30.8 %), Asia ($n = 43$, 19.5 %), Oceania ($n = 16$, 7.2 %) South America ($n = 12$, 5.4 %), and Africa ($n = 7$, 3.2 %). Out of the three pandemic years, 2021 witnessed the most studies conducted, with 134 papers (60.6 %), especially in North America, Europe, and Asia as illustrated in [Fig. 3](#). For 2022, 67 papers (30.3 %) were published within just three months. While the lowest number of papers ($n = 20$, 9.0 %) were published in 2020, which was probably due to the establishment of method development and commencement of WBE for COVID-19.

3.2. The potential utilization of SARS-CoV-2 wastewater-based epidemiology

Applications of WBE reported by the selected articles were categorized into four main domains – (i) early warning system, (ii) estimation of COVID-19 cases based on SARS-CoV-2 in sewage, (iii) comparison of trends of viral RNA with epidemiological data and (iv) genetic diversity of SARS-CoV-2 and variants. However, most of the articles ($n = 86$, 39.0 %) highlighted the importance of WBE for more than one domain in their publications. All articles reported the detection of SARS-CoV-2 in sewage except for one [\[36\]](#) which did not recover viral RNA from the wastewater samples. WBE was primarily used to correlate SARS-CoV-2 RNA trend in wastewater with epidemiological data ($n = 90$, 40.7 %). Only eight articles reported either a lack of correlation between these variables or a significant increase in viral RNA levels compared to confirmed cases in the studied area.

The second highest potential use of WBE among the reviewed articles ($n = 81$, 36.7 %) was identifying the genetic modification and viral recombination. A total of 51 articles (23.1 %) highlighted WBE as a tool for early detection of COVID-19 occurrences circulating in the population. Before clinical cases were discovered, viral RNA had been detected in wastewater in eight papers (15.7 %). Whereas, the rise of viral RNA in wastewater prior to increment of clinical cases were reported in 25 articles. Moreover, 20 articles reported the presence of SARS-CoV-2 virus in areas with relatively few cases. About one fifth of the reviewed articles reported on estimation of

Table 1
Exclusion criteria for study selection.

Item	Exclusion Criteria
Type of articles	Review paper, abstracts only, theses, case report, technical report, conference proceedings, book or book chapters
Language	Other than English language
Year of article published	Articles published before January 2020
Sampling criteria	Studies that do not emphasize on wastewater epidemiology and surveillance sampling

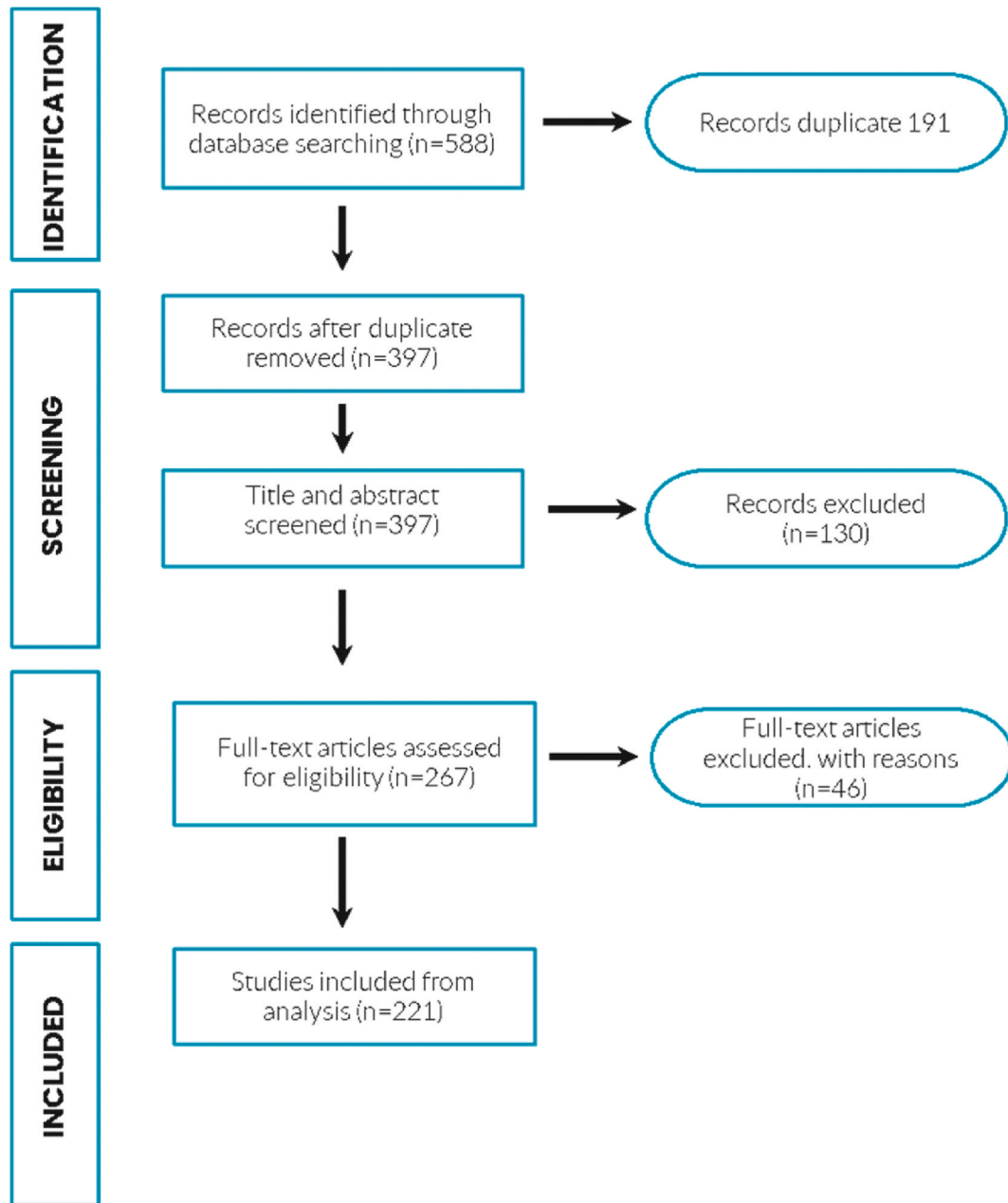


Fig. 1. PRISMA flow diagram for search strategy.

COVID-19 cases in a given community based on the viral RNA load in wastewater (n = 46, 20.8 %).

3.3. Wastewater samples and testing protocols

3.3.1. Sampling location

Globally, diverse sampling locations were opted for determining SARS-CoV-2 RNA concentration in wastewater (Supplementary S3). Majority of articles (n = 175, 79.2 %) conducted their studies at wastewater treatment plant (WWTPs) with 139 (62.9 %) focusing solely at this site. A total of 76 articles (34.4 %) were found to have taken upstream samples which implied collection from smaller sewer sheds. Other upstream sampling location included airport (n = 1), aircraft and cruise ships (n = 4), commercial and light industry (n = 2), office (n = 1), hotel (n = 1), markets (n = 2), nursing home (n = 1), aged care facility (n = 1), quarantine centre (n = 1) and workers' dormitory (n = 1). WBE from congregate living systems were studied in 28 articles. Other sampling locations reported were transfer stations (n = 1) and open drains, canals and rivers (n = 16).

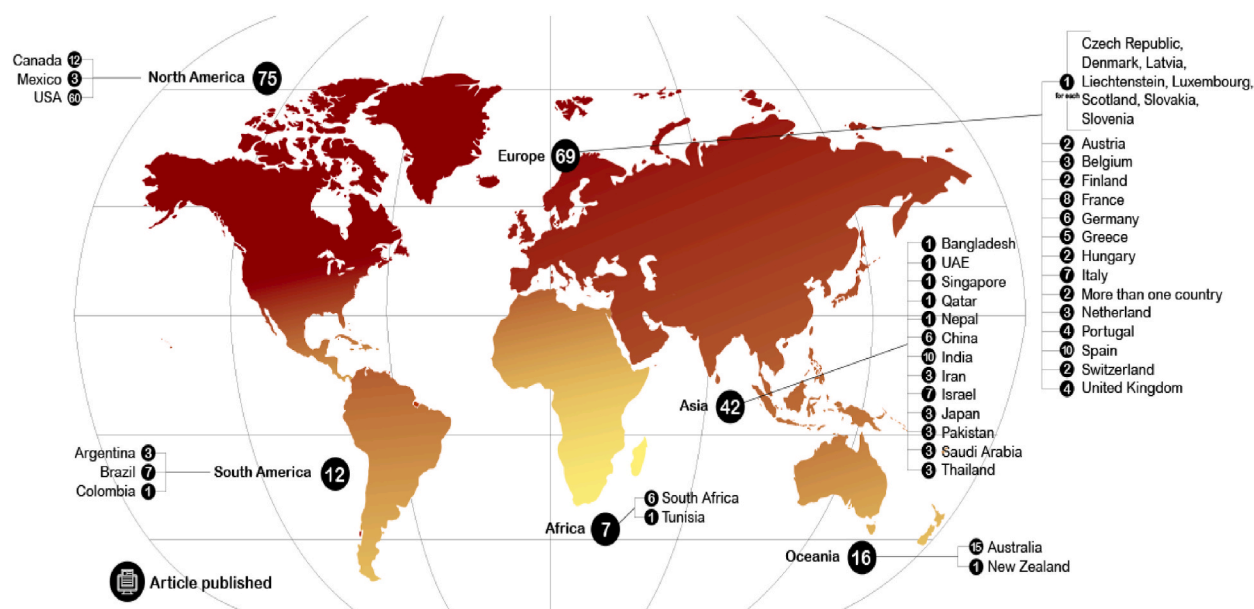


Fig. 2. Map of articles according to continent from year 2020–2022. The intensity of the brown color on the map represents the number of published papers in each area. Darker brown areas indicate a higher number of published articles, while lighter brown areas indicate a lower number of articles. The lowest number of articles published in an area is represented by the color yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

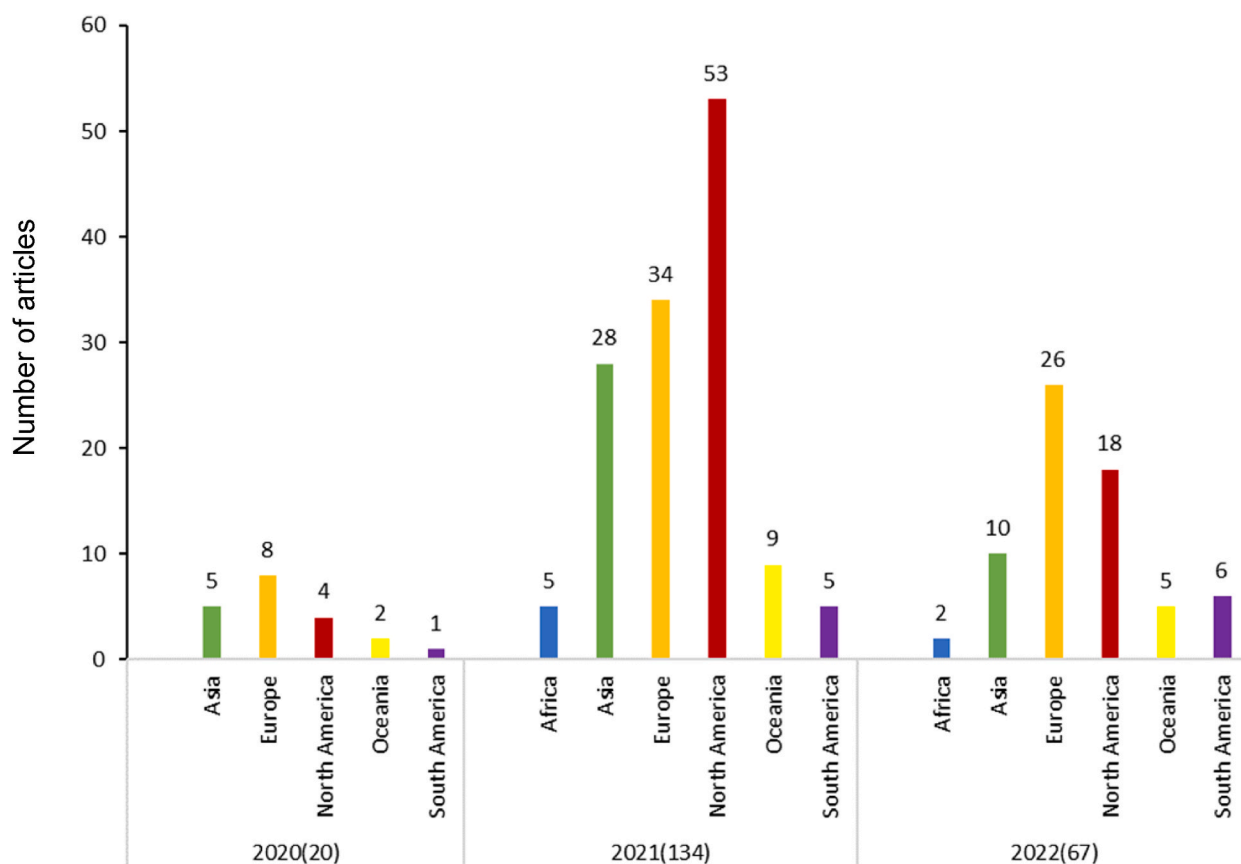
3.3.2. Variability in type of sample collected and upstream sample processing approach

While there was no standard recommended method for virus determination in wastewater due to pre-analytical and technical limitations, the common methodological framework from various SARS-CoV-2 WBE for viral surveillance in wastewater were described. Table 2 summarizes sample types, concentration methods, types of MRC used, and collection methods, that were most often used in SARS-CoV-2 WBE. In planning a study, it is crucial to consider the sample type and collection method to obtain representative samples which would reflect the characteristics of the wastewater treatment system and the location where samples will be collected. Nearly 80 % ($n = 175$) of studies took influent samples for analysis. Other remaining studies took sludge ($n = 6$) and effluent ($n = 5$) as their sample types. The most common matrix for determining viral SARS-CoV-2 in wastewater were influent ($n = 75$, 79.2 %) and composite samples ($n = 113$, 51.1 %). Whilst, virus concentration, the most common technique was precipitation ($n = 57$, 25.8 %) and ultrafiltration ($n = 38$, 17.2 %) as shown in Table 2. Also listed in Table 2 are the spiked Matrix Recovery Control (MRC) viruses that were used in studies. A single MRC was reported in 107 articles with 14 (5.4 %) studies each that used inactivated SARS-CoV-2, Pepper Mild Mottle virus (PMMoV) and Bacteriophage MS2. Other MRC's utilized included Murine Hepatitis virus (MHV) ($n = 12$, 5.4 % articles), Transmissible Gastroenteritis virus (TGEV) ($n = 3$ articles), Porcine Epidemic Diarrhoea virus (PEDV) and *Oncorhynchus keta* (O.Keta) ($n = 2$ articles), Enterovirus, Semliki Forest virus (SFV), Infectious Bronchitis virus (IBV), Feline Calicivirus (FCV), CrAssphage CPQ_064, Armoured RNA Quant IPC-1 Processing Control, and S-gene target failure (SGTF) and Human RNA (one article each). Notably there were 18 articles that reported used of multiple MRCs while 96 articles did not specifically mention the MRC used.

The commonly used sampling strategies, as shown in Fig. 4(a), focused on composite sampling, with the majority of articles being connected to influent (100/175) and sludge (3/6) respectively. As shown in Fig. 4 (b) precipitation (48/175) followed by ultrafiltration (29/175) and membrane filtration (23/175) were the most preferred methods for virus concentration from influent samples. Both precipitation and ultracentrifugation (2/6 articles each), were the preferred concentration methods used to enhance the detection of SARS-CoV-2 in sludge. Unlike influent and sludge samples, effluents were concentrated mainly using ultrafiltration (2/5), followed by precipitation (1/5). It is important to highlight that most WBE articles that focused on influent and sludge sample types opted for enveloped viruses as MRCs instead of non-enveloped viruses as shown in Fig. 4(c). The comprehensive summary of the collection methods, concentration techniques and MRC for sludge and effluents were presented in Fig. 4.

3.3.3. Methods utilized to track SARS-CoV-2 and its variants in WBEs

Overall, PCR-based assays were employed in the majority of WBE studies with (reverse transcription quantitative polymerase chain reaction (RT-qPCR) being the most widely used ($n = 182$, 82.35 %) followed by reverse transcription droplet digital PCR (RT-ddPCR) ($n = 14$, 6.33 %) (Supplementary S4). Other method used was genome sequencing with two articles (0.9 %) utilizing it for virus detection [37,38]. Other than the single method of either PCR-based assays or genome sequencing performed, 10.41 % ($n = 23$) of WBE studies performed multiple approach to detect the presence of SARS-CoV-2 [16,22,39–59]. Combinations of multiple methods included 1) RT-qPCR in combination with genome sequencing ($n = 3$), 2) a combination of multiple PCR-based assays, such as RT-qPCR, nested RT-PCR (nRT-PCR), RT-ddPCR and volcano-2G PCR ($n = 15$), and 3) RT-qPCR coupled with reverse transcription



Note: Year 2022 is only for January - March 2022

Fig. 3. Number of articles based on continent from respective pandemic year.

loop-mediated isothermal amplification (RT-LAMP) ($n = 2$) and a combination of RT-qPCR with viral culture technique ($n = 1$).

Recently, RT-ddPCR has gained increasing popularity for the detection and quantification of viral nucleic acids. A detailed inspection of the number of studies that performed comparisons between RT-ddPCR and RT-qPCR revealed that 10 out of the 23 studies conducted such comparisons [39,40,43,44,46–51]. Among these 10 studies, four demonstrated that RT-ddPCR was more sensitive, two indicated that RT-qPCR was more sensitive, and four showed that the two techniques yielded comparable or minimally different results. The performance of other PCR-based assays, such as RT-LAMP and nRT-PCR, was also evaluated against different PCR-based techniques like RT-ddPCR and RT-qPCR. One study revealed that RT-ddPCR was more sensitive than RT-LAMP in detecting SARS-CoV-2 in wastewater (Amoah et al., 2021), while another study found that RT-qPCR detected more positive samples than nRT-PCR [54]. In addition, one study concluded that the detection limits for both V2G-qPCR and RT-qPCR technologies were similar [58].

Majority of the articles ($n = 140$, 63.3 %) did not perform detection of SARS-CoV-2 variants in the samples (Supplementary S5). From the 81 articles that performed variant detection, 28 articles (34.5 %) utilized a PCR-based method [14,45,51,60–83], 42 (51.8 %) utilized genome sequencing (supplementary S6), one study (1.23 %) performed LAMP assay [84] and 10 studies (12.3 %) performed multiple methods to identify SARS-CoV-2 variants [85–94]. Out of the 10 articles that performed multiple methods, eight of them performed both PCR-based method and genome sequencing, while the other two were a combination of PCR-based method and melting curve genotyping, and genome sequencing and metatranscriptomic.

4. Discussion

Based on the number of articles, it can be concluded that countries in North America had the highest number of WBE applications compared to other countries. The United States of America (USA) had the highest number of articles followed by Australia and Canada. However, the earliest discovery of SARS-CoV-2 in wastewater originated from Europe, where 30.8 % of the studies were conducted. The research was pioneered by Dutch scientists who studied sewage samples from six cities in the Netherlands in early 2020 [21]. The WBE method was later explored in Spain, Italy, France, Germany and Turkey [20,24,95–97]. Significance of WBE was greatly

Table 2

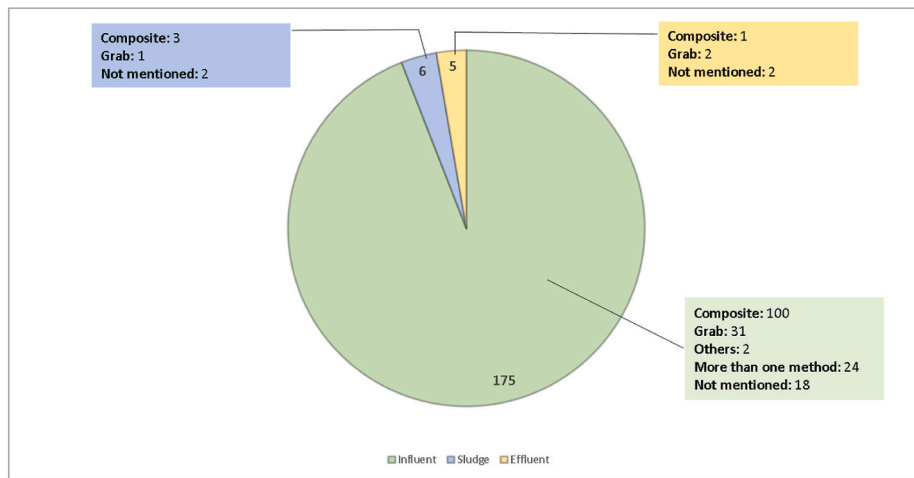
Number of articles and their percentages based on common sample types and methodologies for wastewater testing of SARS-CoV-2: (a) Sample type, (b) Concentration method, (c) Matrix recovery control, and (d) Sample collection method.

Intervention	Number of articles (N)	Percentage (%)
1. Sample Type		
(a) Influent	175	79.2
(b) Sludge	6	2.7
(c) Effluent	5	2.3
(d) More than one sample type	24	10.9
(e) Others	5	2.3
(f) Not mentioned	6	2.7
2. Concentration Method		
(a) Precipitation	57	25.8
(b) More than one concentration methods	43	19.5
(c) Ultrafiltration	38	17.2
(d) Membrane filtration	26	11.8
(e) Ultracentrifugation	19	8.6
(f) Flocculation	4	1.8
(g) Others	6	2.7
(h) Not mentioned	28	12.7
3. Matrix Recovery Control		
<i>Enveloped Virus</i>		
(a) Inactivated SARS-CoV-2	14	6.3
(b) MHV	12	5.4
(c) HCoV	9	4.1
(d) BCoV	6	2.7
(e) BRSV	6	2.7
(f) MNV	5	2.3
(g) Phi6	6	2.7
<i>Non-enveloped virus</i>		
(h) Bacteriophage MS2	14	6.3
(i) PMMoV	14	6.3
(j) MgV	6	2.7
More than one matrix recovery controls	18	8.1
Others	15	6.7
Not mentioned	96	43.4
4. Sample Collection Method		
(a) Composite	113	51.1
(b) Grab	44	19.9
(c) More than one collection method	32	14.5
(d) Others	5	2.3
(e) Not mentioned	27	12.2

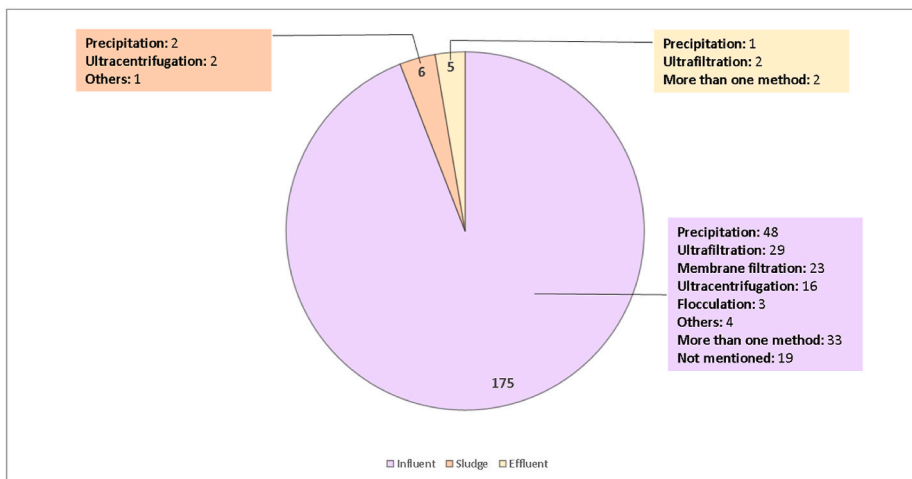
*BCoV, Bovine coronaviruses; BRSV, Bovine Respiratory Syncytial virus; MHV, murine hepatitis virus; PEDV, porcine epidemic diarrhoea virus; MgV, mengovirus; Phi6, bacteriophage; PMMoV, pepper mild mottle virus; SARS-CoV-2, inactivated Severe acute respiratory syndrome coronavirus 2; IBV, Infectious bronchitis virus; FCV, Feline calicivirus; HCoV, Human Coronavirus; Emesvirus zinderi, MS2; MNV, murine norovirus; O.keta, *Oncorhynchus keta*; SFV, Semliki Forest Virus; TGEV, Transmissible Gastroenteritis Enteric Virus.

emphasized with the successful detection of SARS-CoV-2 during low prevalence period in many European countries including Italy, Spain, and Netherlands [20–22]. In Italy and Brazil, detection of SARS-CoV-2 in wastewater was also reported retrospectively using archival samples since December 2019, months before the first case in the region was reported [98]. This showed the benefit of wastewater surveillance which was able to detect the occurrence of SARS-CoV-2 much earlier before the first clinical diagnosis. China was the first among Asian countries to conduct SARS-CoV-2 WS, in February 2020. This was subsequently, followed by Japan and India. Although China started the WBE monitoring late into the end of the first wave of the epidemic in their country, detection of SARS-CoV-2 within low prevalence areas at the time, showed promise [99]. Moreover, with the current state of recurring outbreak of new COVID-19 variants in China, the use of WBE for early warning systems in targeting local measures to contain disease spread will be feasible. Meanwhile, India has one of the highest WBE applications in the Asian continent since their first successful effort in May 2020 [100].

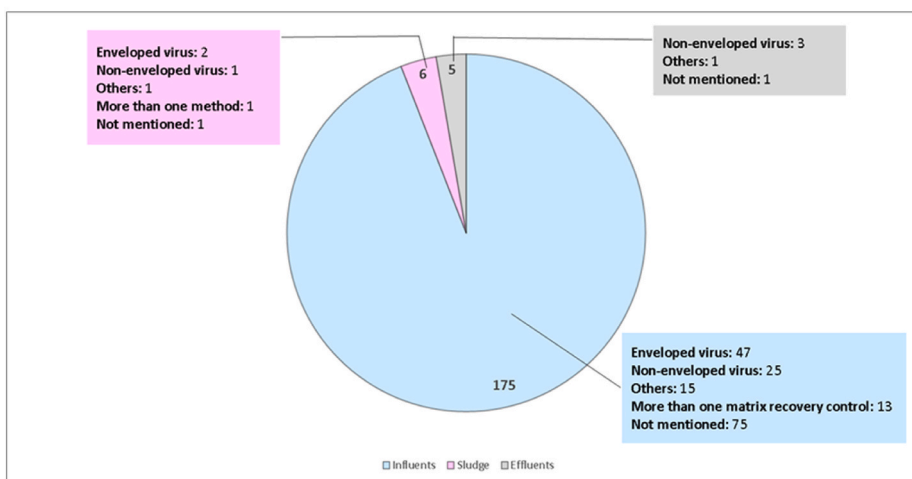
Majority of the articles in this review focused on WWTP for WBE studies. Smaller community sewage collection systems, as opposed to large WWTPs, enabled public health authorities to identify dedicated hotspots and prioritise locations for more focused clinical testing before the emergence of larger outbreaks. About one third of the articles conducted upstream sampling for targeted populations. Upstream sampling of wastewater for SARS-CoV-2 detection involved collecting samples from locations closer to the primary toilets or sources of wastewater within a targeted population. The proximity to the primary toilets is important as it can play a vital role in the detection and concentrations of viral materials in wastewater. Concentration of viral material in wastewater is typically highest the closer it is to the source, which in this case would be the primary toilets. Therefore, upstream sampling, closer to the primary



(a)



(b)



(c)

Fig. 4. Percentages of (a) collection methods; (b) concentration methods; (c) matrix recovery control according to sample types.

toilets, will allow a higher likelihood of virus detection.

Sampling locations further downstream in the WWTP may dilute concentration of viral material due to mixing with other wastewater streams. This dilution effect may pose more challenges in detecting the virus, especially when the concentration of viral material is low. By focusing on upstream sampling, public health officials can target specific populations or areas where the virus may be more prevalent. This can help in identifying COVID-19 hotspots or outbreaks early on, even before clinical cases are reported. Sewage collected upstream from different levels such as international airports, cruise ships, and aircrafts would be beneficial to observe the SARS-CoV-2 drifts among travellers [21]. The travel sector would directly profit from the ability of putting preventative measures based on WS in place. Since SARS-CoV-2 are going towards endemicity, it is imperative to concentrate on upstream wastewater sampling for upcoming studies in complementing the clinical surveillance for given catchment area.

SARS-CoV-2 in sewers could be detected from both asymptomatic and symptomatic individuals because the virus is shed through their faeces. In a given community, the quantum of asymptomatic individuals may be as high as 45 % of infected individuals [9,101]. Under reporting of the actual number of the infected cases are quite common as asymptomatic individuals usually did not undergo screening. As such surveillance of SARS-CoV-2 from sewage will overcome this issue and its surveillance is recommended to complement clinical surveillance. Another advantage of WBE is its role as an early warning system. Prado et al. (2021) reported detection of that viral RNA in sewer pipes of a community even though there were no reported clinical cases. This led to case monitoring within the community [18] and it provided insight into the circulation of SARS-CoV-2 virus within the community prior to the first confirmed clinical case.

SARS-CoV-2 WBE is able to provide evidence to support public health surveillance, spanning from the least to the most sophisticated methods for detection. At their most basic, WBE could indicate presence or absence of SARS-CoV-2 in the tested wastewater to confirm emergence or reemergence of the virus or absence of the virus in the population. In addition, quantification of SARS-CoV-2 RNA in WBE could indicate trends of COVID-19 infection by tracking the increment and decrement of viral load which corroborates with epidemiological data as evident in two fifths of articles reviewed. However, analyzing data for trends can be challenging. In order for WBE to accurately identify trends, it will require high frequency of sampling and large number of observations [102]. Therefore, research is needed to determine and recommend the frequency of sampling of wastewater for correct determination of trends. In the most advanced application, WBE could be used to monitor variants of circulating SARS-CoV-2. It could cover both known and new variants of interest or concerns and in certain situations even identify new and emerging variants which accounts for the second highest potential use of WBE.

The type of sample is another important parameter to be considered when conducting WBE. It was noted that majority of studies used influent sample followed by sludge. Use of influent at the inlet of WWTPs correlated well with the level of clinically reported infection throughout the served region in most of the studies [26,44,52,97,103]. The utilization of influent, however, still pose significant challenges, although sampling raw influent for community-level surveillance of SARS-CoV-2 is the obvious choice [26]. Virus concentrations in large volumes of wastewater can make sample processing challenging. Studies have shown that primary and secondary sludge samples could retrieve higher RNA levels compared to influent samples [104,105]. The number of COVID-19 cases for the following week and the week after were shown to be strongly predicted by primary sludge, whereas weekly cases were found to be significantly predicted by secondary sludge [104,105]. Given these discoveries, utilizing SARS-CoV-2 isolated from sludge could be a preferable option for time-lag epidemiological monitoring. However, it is important to acknowledge the limitations associated with sludge samples, such as the addition of chemicals at the treatment facility, and increased concentrations of compounds that might impede laboratory techniques or the addition of recycled waste streams from other facilities.

There has only been limited number of research done on wastewater sampling techniques for SARS-CoV-2 WBE [106,107]. Grab samples taken every 2 h for 72 h and three equivalent 24-h flow-weighted composite samples were found to have excellent agreement as reported by Curtis et al. (2020) [107]. However, the authors did caution against taking samples during minimal flow as it could result in higher concentrations of inhibitors that could block PCR reactions. Gerrity et al. (2021) confirmed a 10-fold increase in SARS-CoV-2 RNA loads in 24-h composite sampling compared to simultaneous grab sampling [108]. As a result, it is anticipated that extra data are required for standardization and validation of sampling procedures [109].

In WBE it is important to choose an efficient concentration method to accurately measure the RNA viral load in wastewater. It is noted that majority of studies used precipitation as the main concentration method for influents and sludge, followed by ultrafiltration and ultracentrifugation. A study conducted by Twigg and Wenk (2022) reported recovery efficiency values for detecting SARS-CoV-2, its surrogates, and norovirus [110]. These values typically ranged between 18 and 32 % for enveloped viruses using methods, such as polyethylene glycol (PEG) precipitation, ultracentrifugation, and ultrafiltration. There were no significant differences in recovery efficiencies observed between the different methods employed or the types of viruses tested. This could be due to the majority of viral concentration techniques discussed in the literature were first designed to concentrate non-enveloped viral particles. While many of these techniques may be effective with other non-enveloped types, applying them to SARS-CoV-2, could be challenging and may not be as efficient as non-enveloped virus. Therefore, further research need to be conducted to identify more robust concentration methods that will allow researchers to overcome the cumbersome protocols. Moreover, a concentration method that reduces turnaround time and adopting high-throughput will be of added value. By integrating the viral concentration and nucleic acid purification procedures together rather than the earlier method, which requires separate viral concentration and nucleic acid extraction steps, sample throughput can be enhanced. The technique should also be able to provide reliable recovery rates and see substantial reduction in RT-qPCR inhibitors.

For recovery control, enveloped and non-enveloped viruses had been used as surrogates for SARS-CoV-2, alongside non-enveloped viruses typically found in wastewater. However, many WBE articles on SARS-CoV-2 generally lack recovery controls and details (n = 96, 43.4 %) on recovery experiments. Feng et al. (2021) found no correlation between recovery of exogenous spikes of the bovine

coronavirus (BCoV) and recovery of SARS-CoV-2 [111]. Difficulties in assessing SARS-CoV-2 recovery from sewage were further described and recommendations for future research was suggested by Kantor et al., 2021 [112]. Another study from Spain also recommended testing actual environmental samples for native SARS-CoV-2 which was a better way to compare various concentration methods after discovering that recovery of spiked MHV and SARS-CoV-2 did not differ when tested with two concentration methods [113]. This is in agreement with the finding in this review where the second largest number of articles ($n = 14$, 6.5 %) used inactivated SARS-CoV-2 for process control. The choice of MRC is influenced by the availability of such control material, the nature of the sample, and the preferences of the laboratory [114]. This was in agreement with findings of this review in which inactivated SARS-CoV-2 was mostly chosen as MRC and this can be explained as the recovery efficiency for surrogate viruses may not accurately represent SARS-CoV-2 behaviour [115]. Surrogate viruses exhibit different structures and genome compositions than target viruses based on their specific life cycles [110].

The current gold standard for detecting SARS-CoV-2 infection in clinical samples is by real-time RT-qPCR [116,117]. This detection method can also be seen as the gold standard for detection of SARS-CoV-2 in WBEs since majority of WBE efforts performed in the period of January 2020 to June 2022 utilized this method. However, several drawbacks such as low sensitivity and the need of standard curves to quantitate virus concentration had been associated with real-time RT-qPCR [118]. Another disadvantage of using RT-qPCR to identify SARS-CoV-2 in WBE is the different sensitivity of the assays observed in different molecular targets [21,40]. Therefore researchers had ventured into using other quantification methods such as RT-ddPCR which has higher sensitivity [83]. Ahmed et al. (2022) reported that for the identification of SARS-CoV-2 in wastewater samples (eluate and pellets), RT-ddPCR gave significantly enhanced analytical sensitivity over RT-qPCR [39]. This was likely due to the partitioning of samples in RT-ddPCR to small volumes leading to increased effective concentration of reverse transcriptase or PCR inhibitors in the samples [47]. Such increased sensitivity is anticipated to be essential for WBE in areas where there are few or no COVID19 cases in the community. Given the final WBE goal of estimating the number of infected people in each community, the sensitivity bias must be accurately assessed before the quantification values are introduced into predictive epidemiological models. In this context, higher sensitivity may aid in providing more reliable estimation of the total number of infected people, allowing for more accurate comparison of infection rates in communities [119]. However, its utilization in SARS-CoV-2 WBEs is significantly lower in comparison to WBEs which utilized RT-qPCR. Factors such as higher cost of initial instrument purchase, requirement of trained personnel to perform and ability to interpret results, as well as the availability of microfluidic technologies and reagents. This resulted in significantly less usage of the platform, especially in countries with limited resources.

The emergence of sublineages and variants of concern (VOC) of SARS-CoV-2 poses additional clinical and public health challenges due to their potential for increased transmissibility, disease severity, and immune evasion. Therefore, it is imperative to track circulating SARS-CoV-2 variants during the pandemic. However, more than half of the articles did not report on SARS-CoV-2 variants. From articles with SARS-CoV-2 variant detection, genome sequencing was the preferred method. This was not surprising since genome sequencing is the gold standard for comprehensive genome analysis to detect variants and lineages of viruses. PCR-based methods were another widely used technique in WBEs for detecting SARS-CoV-2 variants. PCR-based methods to detect SARS-CoV-2 variants in clinical samples were previously shown to complement well with the genomic surveillance using genome sequencing. Importantly, the data from PCR-based methods to detect SARS-CoV-2 variants preceded genomic surveillance data allowing more actionable time for public health responses [120–123]. Similarly, a PCR-based method could be a valuable tool for rapid detection of SARS-CoV-2 variants in WBE [86]. However, according to Lou et al. (2022) RT-ddPCR proved to be more effective than genome sequencing at detecting SARS-CoV-2 mutations in wastewater samples [89]. The disadvantage of PCR-based variant detection, however, is that it can only detect particular mutations related to known variants of interest and it is unable to identify new variants, making it insufficiently conclusive. In addition, studies revealed some challenges associated with PCR-based methods such as cross-reactivity between primer probes and the failure to identify SARS-CoV-2 variants in a WBE effort [86,94]. Nonetheless, genome sequencing will remain central in genomic surveillance as it can provide rich data for surveillance such as the identification of transmission networks and viral evolution [86].

4.1. Limitation and future prospects for WBE

WBE has been identified as a useful tool in monitoring the COVID-19 pandemic, but there are some limitations that should be considered. Majority of data on WBE is available in developed countries with temperate climates. More research is needed to identify the incidence, persistence, and consequence of SARS-CoV-2 in numerous environmental media in developing countries with tropical settings. In order to maximize the effectiveness of WBE, it is of utmost importance to combine it with other surveillance methods. Future studies on WBE should delve into the potential synergies between WBE and traditional clinical surveillance. Additionally, the integration of molecular techniques, including PCR-based, metagenomics and multiple pathogen target panels, with advanced data analytics should be explored. Furthermore, it is essential to discuss how the amalgamation of multiple surveillance methods can provide a more comprehensive understanding of the spread and transmission dynamics of various pathogens.

Data from WBE surveillance can be used as a public health tool in planning for more precise and targeted strategies during an outbreak or epidemic. Health authorities and stakeholders will then be able to conduct more effective disease surveillance and prepare for an impending outbreak by providing early information to district and state health departments and mobilizing frontline workers, experts and health resources in advance. Information given to health authorities and stakeholders is essential for the development of evidence-based policy decisions during an outbreak. In the case of COVID-19, real-time variant analysis which can provide information on emerging variants in a given area or district, will help in mitigation strategies in tackling the new pandemic wave. In addition, WBE studies can also be combined with spatial tools such as geographical information systems (GIS) to map potential hotspots. It is also

anticipated to see more investigation of WBE in terms of field sampling by utilizing data-driven deployment strategy or system, developed solutions to sample deep manhole and low-flow, customised autosamplers, and adaptation of Autosampler Live Monitoring System (ALMS), intelligent concentration, lysis and clean up procedures for the ease of point of care diagnostics setting in the future.

Finally, coupled with the analysis of case-based epidemiological data, the current WS methods should be expanded to include other pathogens, such as respiratory syncytial virus (RSV), influenza A and B viruses, dengue virus, Zika virus and Ebola virus. Although these pathogens are not typically transmitted by water, monitoring these pathogens in wastewater could prove to be beneficial. In fact, the success of wastewater monitoring in the surveillance of tuberculosis has been demonstrated [124], suggesting its potential use in the future for drug-resistant tuberculosis. Additionally, WS can be employed to monitor viruses or pathogens that show resistance to climate change. Given the recent COVID-19 pandemic, more research is needed to explore how WS can be utilized in monitoring other potential pandemic pathogens to guide public health interventions.

5. Conclusion

In summary, wastewater monitoring for SARS-CoV-2 in WWTPs of numerous communities around the world are being conducted. RT-qPCR was primarily used for detection, and advanced molecular sequencing were also used to identify genetic alterations of SARS-CoV-2 in sewage samples. It is envisioned that WBE will be utilized beyond this pandemic using state-of-the-art multi-omics-based technologies, which will be an effective tool for more thorough WS applications. In addition, current methods will need to be adapted and optimized to be applicable in locations without centralized sewer infrastructure. For a given sample type, these factors by themselves provide only a partial view, but when combined, they provide a plethora of information. The data generated, particularly from wastewater analysis, will serve as a valuable tool for conducting situational assessments of pathogen infection trends in the global battle against infections.

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Ethics declaration

Review and/or approval by an ethics committee was not needed for this study because this is a scoping review using existing publications without any primary or secondary data collection.

Data availability statement

Further information of this study can be found in article/supp. material/referenced.

CRediT authorship contribution statement

Siti Aishah Rashid: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization. **Sakshaleni Rajendiran:** Writing – review & editing, Writing – original draft, Resources. **Raheel Nazakat:** Writing – review & editing, Writing – original draft, Resources. **Noraishah Mohammad Sham:** Writing – review & editing, Supervision, Methodology. **Nurul Amalina Khairul Hasni:** Writing – review & editing, Writing – original draft, Resources. **Mohd Ishtiaq Anasir:** Writing – review & editing, Writing – original draft, Resources. **Khayri Azizi Kamel:** Writing – original draft, Conceptualization. **Rosnawati Muhamad Robot:** Writing – review & editing, Writing – original draft, Supervision, Resources.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e30600>.

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