Journal of Water & Health

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Journal of Water and Health Vol 20 No 8, 1223 doi: 10.2166/wh.2022.115

# Effectiveness of environmental surveillance of SARS-CoV-2 as an early warning system during the first year of the COVID-19 pandemic: a systematic review

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## ABSTRACT

Since infected persons shed SARS-CoV-2 in faeces before symptoms appear, environmental surveillance (ES) may serve as an early warning system (EWS) for COVID-19 and new variants of concern. The ES of SARS-CoV-2 has been widely reviewed; however, its effectiveness as an EWS for SARS-CoV-2 in terms of timeliness, sensitivity and specificity has not been systematically assessed. We conducted a systematic review to identify and synthesise evidence on the ES of SARS-CoV-2 as an EWS to evaluate the added value for public health. Of 1,014 studies identified, we considered 29 for a qualitative synthesis of the timeliness of ES as an EWS for COVID-19, while six studies were assessed for the ability to detect new variants and two for both aims. The synthesis indicates ES may serve as an EWS of 1–2 weeks. ES could complement clinical surveillance for SARS-CoV-2; however, its cost–benefit value for public health decisions needs to be assessed based on the stage of the pandemic and resources available. Studies focusing methodological knowledge gaps as well as how to use and interpret ES signals for public health actions are needed, as is the sharing of knowledge within countries/areas with long experience of such surveillance.

**Key words:** COVID-19, early warning system, environmental surveillance, public health, SARS-CoV-2, wastewater-based epidemiology

## HIGHLIGHTS

- Evidence on surveillance of SARS-CoV-2.
- Early warning system for COVID-19.
- Systematic review of environmental surveillance of SARS-CoV-2.

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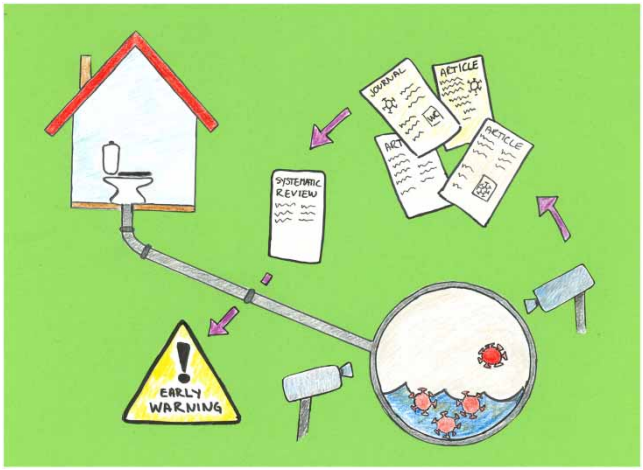
## GRAPHICAL ABSTRACT




## INTRODUCTION

In the context of the COVID-19 pandemic, environmental surveillance (ES) of microbial monitoring has been used for the detection of SARS-CoV-2 shed into wastewater from the respiratory system and via faeces (World Health Organization 2020b). ES by means of detecting pathogens in wastewater has a long history in public health, particularly for monitoring poliovirus (World Health Organization 2003; Asghar *et al.* 2014) and, more recently, antimicrobial resistance (AMR) (Aarestrup & Woolhouse 2020). In some countries, ES has been proposed to support COVID-19 response activities by detecting signals of SARS-CoV-2 transmission in communities and monitoring trends in defined population areas to inform testing policies and mitigation measures (Centers for Disease Control and Prevention (CDC) 2021).

ES can detect SARS-CoV-2 community transmission earlier than clinical surveillance systems based on reported COVID-19 cases in the community, thus serving as an early warning system (EWS), since an infected person sheds SARS-CoV-2 in faeces and respiratory droplets from 3 to 5 days before symptoms appear (Jones *et al.* 2020). Moreover, clinical surveillance typically only captures individuals that have been tested for SARS-CoV-2 and therefore depends on testing policies and practices, which generally address symptomatic cases at the more severe end of the spectrum and their close contacts as they are the ones accessing healthcare. ES could therefore complement the under-reporting that characterises clinical surveillance and aid the monitoring of trends of COVID-19 incidence and transmission in a community through wastewater-based epidemiology (WBE) (Choi *et al.* 2018). ES could also be applied in the monitoring of SARS-CoV-2 trends among travellers at points of entry, such as international airports, passenger aircraft and cruise ships (Ahmed *et al.* 2020a; Medema *et al.* 2020b). Moreover, some studies have demonstrated that community-wide molecular analysis of wastewater samples can provide a comprehensive snapshot of the SARS-CoV-2 variants in circulation and support the identification of potential new emerging variants (Kirby *et al.* 2020; Izquierdo-Lara *et al.* 2021). SARS-CoV-2 is an RNA virus with the potential to mutate into new variants that can establish themselves in the human population (Hirabara *et al.* 2022). Variants of concern (VOCs) are groups of variants with similar genetic changes (a lineage or clade) which have demonstrated increased transmissibility or virulence, a change in epidemiology or clinical disease, and/or a decrease in the effectiveness of diagnostics, vaccines and therapeutics. Variants of interest (VOIs), on the other hand, constitute a possible emerging risk for global public health due to increasing prevalence (World Health Organization 2022b).

The ES of SARS-CoV-2 has been widely reviewed. In particular, several reviews have addressed the challenges in detecting SARS-CoV-2 in wastewater due to the lack of standard protocols for sampling and analysis for optimised performance (Ahmed *et al.* 2020b; Corpuz *et al.* 2020; Kitajima *et al.* 2020; Lu *et al.* 2020; Michael-Kordatou *et al.* 2020; Alygizakis *et al.* 2021; Buonerba *et al.* 2021; Cervantes-Aviles *et al.* 2021; Eftekhari *et al.* 2021; Hamouda *et al.* 2021; Li *et al.* 2021; Mohan *et al.* 2021; Yao *et al.* 2021). Other authors have reviewed ES in a broader perspective, merely stating its potential to support the clinical testing regime, yet have pointed out a range of challenges, such as viral load, practical implementation, interpretation, modelling and legal and ethical aspects, including sampling and analysis (Aguiar-Oliveira *et al.* 2020; El-Baz

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*et al.* 2020; Foladori *et al.* 2020; Kumar *et al.* 2020; Mandal *et al.* 2020; Ali *et al.* 2021; Jordhøy *et al.* 2021; Patel *et al.* 2021; Saawarn & Hait 2021; Suthar *et al.* 2021; Zahedi *et al.* 2021). Some studies have also suggested the value of using biosensors in combination with WBE as a part of ES for the detection and management of COVID-19 outbreaks (Bhalla *et al.* 2020; Tetteh *et al.* 2020). The challenges of using ES in undeveloped areas of the world, where sanitation services are poorly implemented, have also been reviewed (Panchal *et al.* 2021; Pandey *et al.* 2021). Alongside high interest in the ES of SARS-CoV-2 as a means to manage COVID-19 outbreaks, the performance of such surveillance has been systematically reviewed, with findings suggesting that positive signals typically anticipate clinically confirmed cases by a minimum of 10 days (Shah *et al.* 2022). The implementation and institutionalising of ES have also been discussed, and the importance of bringing together stakeholders to support public health decisions has been highlighted (Medema *et al.* 2020a; Takeda *et al.* 2021). In addition, the terms used for ES have been reviewed, due to the lack of a common terminology (Larsen *et al.* 2021). Based on a growing body of documentation of the ES of SARS-CoV-2, the World Health Organization (2022a) has developed interim guidance in regard to the situations in which it may have an added value and its implementation requirements (World Health Organization 2022a).

Although it has been reported that ES could serve as an EWS, its effectiveness in terms of early warning of SARS-CoV-2-waves and possible new variants – and as a tool for public health decisions – has not been systematically reviewed. Relevant core assets in the effectiveness of a surveillance system include timeliness, sensitivity and specificity; the ability to detect a true outbreak is a balance between such assets (World Health Organization 2006; European Centre for Disease Control (ECDC) 2014). In addition, the increasing interest in ES for SARS-CoV-2 during the COVID-19 pandemic has contributed to the rapid development of scientific knowledge in this field, which highlights the need for a systematic literature review. Therefore, the aim of this systematic review is to identify and synthesise the evidence on the ES of SARS-CoV-2 as an EWS to evaluate its effectiveness and added value as a public health tool. The main aim includes the following objectives:

- To assess the effectiveness of ES as an EWS for SARS-CoV-2 in terms of timeliness, sensitivity and specificity.
- To assess ES' ability to detect the early introduction of new variants into wastewater.
- To evaluate the public health impact and control measures related to ES at national and international levels.

## MATERIALS AND METHODS

### Study protocol

The protocol of this systematic review was approved by the National Institute of Health Research with the registration number PROSPERO CRD42021261383 and is available online (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021261383).

Search strategy, information sources, data management and extraction, along with the literature search strategy, are described in the Supplementary material, Additional file 1.

### Data synthesis

The data were summarised using a template form including the following information from publications fulfilling the aim of the review: country, study population, study period, data source for clinical data and SARS-CoV-2 in wastewater, sampling method in wastewater, gene targets and estimated timeliness as an EWS. The extracted data providing information about the effectiveness of ES of SARS-CoV-2 used for EWS was not suitable for pooling due to heterogeneity; therefore, a meta-analysis could not be performed. The information was synthesised in a tabular form with a narrative summary of the findings of timeliness only, since sensitivity and specificity were not reported, of detection of SARS-CoV-2 in the population. To evaluate whether sewage screening could work as an early warning of VOC or VOI in a population, SARS-CoV-2 variants in sewage and clinical samples were compared across time and place. Information given in the included studies relating to ES' ability to detect variants and public health action is presented as narrative. Two researchers were involved in the data synthesis of the effectiveness of the ES of SARS-CoV-2 as an EWS, two reviewers assessed the ability of such surveillance to detect variants, and one environmental expert assessed all studies according to the sampling methods of the wastewater and environmental aspects. Two co-authors assessed the grey literature for information on public health action and guidelines.

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### Risk of bias in the individual studies and cumulative evidence

The ROBINS-I assessment tool, version 1 August 2016 (Sterne *et al.* 2016) was applied to address the risks of bias in individual (i.e., non-randomised) studies. The body of evidence comprised by the cumulative results was assessed by the Project on a Framework for Rating Evidence in Public Health (PRECEPT), which was developed by the ECDC in 2012 (Harder *et al.* 2017).

### Ethical considerations

The current study did not require ethical approval because we did not collect any sensitive personal data or health information. The analysis only included published articles on the research topic.

## RESULTS

### Descriptive summary of study characteristics

Of the 1,014 publications resulting from the literature search, 160 were assessed through a full-text reading according to the aim of the study. Of these, 31 publications were included in the review to assess the effectiveness of ES as an EWS (Table 1). In addition, seven publications on early detection of SARS-CoV-2 variants were assessed and synthesised as a narrative summary, as presented in a dedicated section below (Martin *et al.* 2020; Agrawal *et al.* 2021b; Crits-Christoph *et al.* 2021; Hillary *et al.* 2021; Izquierdo-Lara *et al.* 2021; La Rosa *et al.* 2021a; Wilton *et al.* 2021). Two of these publications were included for evaluation of both EWS and early detection of variants (Martin *et al.* 2020; Hillary *et al.* 2021). The search for the grey literature resulted in 18 hits, of which two were fact sheets (Global Water Research Coalition 2020; Water Research Australia 2020b) on SARS-CoV-2 in drinking water and wastewater; however, surveillance of wastewater was not mentioned, and the fact sheets were therefore excluded (Figure 1). A list of excluded studies with reason for non-inclusion and a summary of the extracted data is available in the Supplementary material, Additional file 1.[1]

### Effectiveness as EWS

Information on the effectiveness of ES as an EWS for SARS-CoV-2 is summarised in Table 1. We grouped the identified publications into the following three categories: (i) EWS at the population level, (ii) early warning at 'hot spots' (such as buildings, facilities, limited areas) and (iii) publications reporting detection of SARS-CoV-2 in wastewater before clinical detection of first case of COVID-19. Each category was sub-grouped based on the duration of the EWS (Table 1).

All included publications on ES as an EWS ($n = 31$) compared environmental data on SARS-CoV-2 detection to official health data on COVID-19 cases. The included publications were from different geographical areas: 15 from Europe (Martin *et al.* 2020; Medema *et al.* 2020b; Randazzo *et al.* 2020b; Trottier *et al.* 2020; Wurtzer *et al.* 2020; Agrawal *et al.* 2021a; Chavarria-Miro *et al.* 2021; Davo *et al.* 2021; Goncalves *et al.* 2021; Hillary *et al.* 2021; La Rosa *et al.* 2021a; Roka *et al.* 2021; Rusinol *et al.* 2021; Saguti *et al.* 2021; Wurtz *et al.* 2021), seven from North America (Kaplan *et al.* 2020; Nemudryi *et al.* 2020; Peccia *et al.* 2020; Betancourt *et al.* 2021; Colosi *et al.* 2021; D'Aoust *et al.* 2021; Graham *et al.* 2021), six from Asia (Arora *et al.* 2020; Hata *et al.* 2021; Kumar *et al.* 2021; Saththasivam *et al.* 2021; Wong *et al.* 2021; Xu *et al.* 2021), two from Australia (Ahmed *et al.* 2021; Black *et al.* 2021) and one from South America (Fongaro *et al.* 2021). All publications were carried out in 2020, and the study period ranged from 14 days to 8.5 months, with an average of 2–4 months.

Samples consisted of influent sewage in almost all included studies ($n$=28), while three studies analysed sludge samples (Kaplan *et al.* 2020; Peccia *et al.* 2020; D'Aoust *et al.* 2021), and Graham *et al.* (2021) analysed both influent sewage and sludge (Graham *et al.* 2021). The sampling modes were heterogenous, with composite sampling most used ($n = 21$), followed by grab sampling ($n = 6$). Composite sampling was either flow-, time- or volume-proportional. Four studies applied both composite and grab sampling procedures (Black *et al.* 2021; Graham *et al.* 2021; Hillary *et al.* 2021; Roka *et al.* 2021). Sampling frequency varied between studies, being daily, weekly, biweekly or at a defined sampling time. When reported, the transport temperature condition of wastewater samples was mostly 4 °C, while the storage conditions of samples before being analysed varied between 4, −20 and −80 °C.

[1] A total of 178 publications were identified through the screening in Rayyan; however, after full-text assessment eight publications were excluded due to duplications that were not detected during the check in the literature search, retraction of articles, etc. resulting in 160 publications (see Additional file 1).

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**Table 1** | Synthesis of included publications to evaluate the effectiveness of environmental surveillance as an EWS for COVID-19 ($n$=31)

| Study characteristics | | | | Data source | | Wastewater/Sewage | | | Early warning signal compared to clinical surveillance (timeliness) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Reference | Country | Study population (~estimated) | Study period (~time) | Detection of COVID-19 cases | Detection of SARS-CoV-2 (location) | Sample type, sampling mode and storage conditions until analysis (temperature and time)[a] | Sampling frequency | Target gene(s), data normalisation | | |
| **Early warning at the population level** | | | | | | | | | | |
| Agrawal *et al.* (2021a) | Germany | Frankfurt am Main (~1,820,000) | April–August 2020 (~5 months) | Confirmed/ reported cases | 2 WWTPs in Frankfurt. | Influent sewage. 24-h time-proportional or flow-proportional. Cold – 24 h | 2 samples per week | N, S and ORF1ab. Flow normalised: copies/day | 10–14 days | Between 1 and 4 weeks |
| Arora *et al.* (2020) | India | Jaipur city (NA) | May–June 2020 (~1 month) | Confirmed/ reported cases | 6 WWTPs and 2 hospitals in Jaipur. | Influent and effluent sewage. Grab sampling. NR – NR | 1–6 samples per site | S, E, ORF1ab, RdRp, N. Not normalised: copies/ml | 6–14 days | |
| Kumar *et al.* (2021) | India | Gandhinagar (NA) | August–September 2020 (~2 months) | Confirmed/ reported cases | 4 WWTPs in Gujarat | Influent sewage. Grab sampling. Cold – NR | 1 or 2 samples per week | S, N, ORF1ab. Not normalised: copies/ml | 1–2 weeks | |
| Roka *et al.* (2021) | Hungary | Budapest (~1,800,000) | June–November 2020 (~6 months) | Confirmed/ reported and hospitalised cases | 2 WWTPs in Budapest | Influent sewage. Composite and grab sampling. Cold – 6 h | 1 sample per week | N1. Flow normalised: copies/day | 2 weeks | |
| Saguti *et al.* (2021) | Sweden | Gothenburg (~800,000) | February–July 2020 (~6 months) | Hospitalised COVID-19 cases | 1 WWTP in Gothenburg | Influent and effluent sewage. Mostly 24-h flow-proportional sampling. Frozen – NR | Pooled daily samples | RdRP. Flow normalised: copies/day | 3–4 weeks | |
| Trottier *et al.* (2020) | France | Montpellier (~470,000) | May–July 2020 (~2 months) | Confirmed/ reported cases | 1 WWTP in Montpellier | Influent sewage. 24-h composite sampling. Cold – 24 h | 7 samples per site over study period | N1, N3. Not normalised: copies/day | 2–3 weeks | |
| Wurtzer *et al.* (2020) | France | Paris (~3,000,000) | March–April 2020 (~1 month) | Confirmed/ reported and hospitalised cases | 3 WWTPs in Greater Paris | Influent sewage. 24-h flow-proportional sampling. Cold – 24 h | 1 sample per day | E, RdRP. Not normalised: copies/ml | 8 days | |
| Black *et al.* (2021) | Australia | State of Victoria (~4,336,600) | August–October 2020 (~3 months) | Confirmed/ reported cases | 46 WWTPs in State of Victoria | Influent sewage. Grab or composite sampling. Cold – 24 h or next business day | 2–15 samples per site | N, ORF1ab. Not normalised: copies/ml | 2 days | Within 1 week |
| D'Aoust *et al.* (2021) | Canada | Ottawa (~1,000,000) | June–August 2020 (~1.5 months) | Confirmed/ reported cases | 1 WWTP in Ottawa | 6-h sludge composite samples. Cold – 8 h | Every 2 days for 6 weeks | N1, N2. Normalised faecal marker: copies/copies PMMoV | 2–4 days | |
| Hillary *et al.* (2021) | United Kingdom | Six major urban centres (~3,000,000) | March–July 2020 (~5 months) | Confirmed/ reported cases and deaths | 6 WWTPs in Wales and England | Influent sewage. Grab and 24-h composite wastewater sampling. Cold – 24 h | 1 sample per week | N1, E Not normalised: copies/ml | 5 days | |
| Kaplan *et al.* (2020) | United States | New Haven, Connecticut (~200,000) | February–April 2020 (~3 months) | Hospitalised COVID-19 cases | 1 WWTP in Connecticut | Sludge. Composite sampling. Frozen – NR | 1 sample per day | N1, N2. Not normalised: copies/ml sludge | 3–5 days | |

(*Continued.*)



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**Table 1** | Continued

| Study characteristics | | | | Data source | | Wastewater/Sewage | | | Early warning signal compared to clinical surveillance (timeliness) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Reference | Country | Study population (~estimated) | Study period (~time) | Detection of COVID-19 cases | Detection of SARS-CoV-2 (location) | Sample type, sampling mode and storage conditions until analysis (temperature and time)[a] | Sampling frequency | Target gene(s), data normalisation | | |
| Medema *et al.* (2020b) | The Netherlands | Six cities and airport (~4,058,000) | February–March 2020 (~2 months) | Confirmed/ reported cases | 7 WWTPs in Netherlands | Influent sewage. 24-h flow-proportional sampling. Cold – 24 h | 4 rounds of sampling | N1-N3, E. Not normalised: copies/ml | 6 days | |
| Nemudryi *et al.* (2020) | United States | Bozeman, Montana (~49,831) | March–June 2020 (~4 months) | Confirmed/ reported cases | 1 WWTP in Bozeman, Montana | Influent sewage. 24-h volume-proportional sampling. Cold – 3 h | 17 sampling days | N1, N2. Not normalised: copies/ml | 2 days | |
| Peccia *et al.* (2020) | United States | New Haven, Connecticut (~200,000) | March–June 2020 (~3 months) | Confirmed/ reported and hospitalised cases | 1 WWTP in Connecticut | Sludge. Composite sampling. Frozen – NR | 1 sample per day | N1, N2. Not normalised: copies/ml | 1–8 days | |
| Rusinol *et al.* (2021) | Spain | Catalonia (~2,011,300) | Mid-March–November 2020 (~8–9 months) | Confirmed/ reported cases | 10 WWTPs in Catalonia | Influent sewage. 24-h time-proportional sampling. Frozen – NR | 185 samples | N1, N2. Flow normalised: copies/day | 0–7 days | |
| Graham *et al.* (2021) | United States | Santa Clara, California (~1,700,000) | March–April, 2020 (~1 month) | Confirmed/ reported cases | 2 WWTPs in Santa Clara | 24-h flow-proportional for influent sewage/24-h composite or grab for sludge. Frozen – NR | Daily and three times per week | N1 and N2. Normalised faecal marker: copies/copies PMMoV | 0 days | No early warning |
| Hata *et al.* (2021) | Japan | Ishikawa and Toyama (~596,000) | March–May, 2020 (~3 months) | Confirmed/ reported cases | 5 WWTPs in Ishikawa and Toyama | Influent sewage. Grab sampling. Cold – 72 h | 1–2 samples per week | N, N2 and N3. Not normalised: copies/ml | 0 days | |
| Saththasivam *et al.* (2021) | Qatar | Qatar (~2,800,000) | June–August, 2020 (~2–3 months) | Confirmed/ reported cases | 5 WWTPs in Doha | Influent sewage. 24-h composite sampling. Cold – 24 h | 43 samples | N1. Not normalised: copies/ml | 0 days | |
| Wurtz *et al.* (2021) | France | Marseille (~1,596,746) | July–December, 2020 (~5.5 months) | Confirmed/ reported cases | 2 WWTPs in Marseille | Influent sewage. 24-h composite sampling. Cold – 24 h | 117 samples | ORF1ab. Not normalised: copies/ml | 0 days | |
| **Early warning at 'hot spots'** | | | | | | | | | | |
| Davo *et al.* (2021) | Spain | Nursing home in Valencia (~781) | October–December 2020 (~2 months) | Confirmed/ reported cases (residents) | 5 nursing homes in Valencia | Influent sewage. Grab sampling. Cold – 24 h | 5 samples per week | N1, N2, E. Not normalised: copies/ml | 5–19 days | >1 week |
| Betancourt *et al.* (2021) | United States | University of Arizona (NA) | August–November 2020 (~3 months) | Confirmed/ reported cases (students) | 13 student dormitories in Arizona | Wastewater samples from manholes. Grab sampling. Cold – 24 h | 319 samples | N1, N2. Not normalised: copies/ml | <4 days | Within 1 week |
| Wong *et al.* (2021) | Singapore | Singapore (~177) | 4–20 July 2020 (~15 days) | Confirmed/ reported cases/ symptoms | 1 residential area in Singapore | Influent wastewater. 1-h time-proportional sampling. Cold – 24 h | 159 samples | ORF1ab. Not normalised: copies/ml | 3 days | |
| Xu *et al.* (2021) | China | Hong Kong (from ~108 to ~1,152,403) | June–September 2020 (~3 months) | Confirmed/ reported cases (residents) | 20 manhole locations in Hong Kong | Influent sewage. 24-h time-proportional sampling. Cold – NR | 107 samples | N. Not normalised: copies/ml | 2 days | |

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**Table 1** | Continued

| Study characteristics | | | | Data source | | Wastewater/Sewage | | | Early warning signal compared to clinical surveillance (timeliness) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Reference** | **Country** | **Study population (~estimated)** | **Study period (~time)** | **Detection of COVID-19 cases** | **Detection of SARS-CoV-2 (location)** | **Sample type, sampling mode and storage conditions until analysis (temperature and time)[a]** | **Sampling frequency** | **Target gene(s), data normalisation** | | |
| Colosi *et al.* (2021) | United States | Hospital centre in Virginia (~143,000) | July–September 2020 (~3 months) | Hospitalised COVID-19 cases (occupants) | WWTP, hospital and dormitories | Influent sewage. 24-h composite sampling. Cold – 24 h | ~40 samples. | N1, N2. Not normalised: copies/ml | –16 days | No early warning |
| Goncalves *et al.* (2021) | Slovenia | Hospital centre in Ljubljana (NR) | 1 June–15 June 2020 (~15 days) | Hospitalised COVID-19 cases | Hospital in Ljubljana | Influent wastewater. 24-h time-proportional sampling. Frozen – NR | 15 samples | E, RdRP. Not normalised: copies/ml | –1/5 days | |
| **Detection of SARS-CoV-2 in wastewater before first COVID-19 case detected by clinical surveillance** | | | | | | | | | | |
| Ahmed *et al.* (2021) | Australia | Brisbane, Queensland (~934,000) | 24 February–1 May 2020 (~3 months) | Confirmed/ reported first cases | 3 WWTPs in Brisbane | Influent sewage. 24-h time-proportional sampling. Cold – NR | Monitoring/ Daily | N1, N2, E. Not normalised: copies/ml | 3 weeks | Detection between 3 and 8 weeks |
| Chavarria-Miro *et al.* (2021) | Spain | Barcelona (~2,700,000) | 15 January–29 June 2020 (~5–6 months) | Confirmed/ reported first cases | 2 WWTPs in Barcelona | Influent sewage. 24-h composite sampling. NR – NR | Monitoring/ Daily | IP2, IP4, E, N1, N2. Not normalised: copies/ml | 41 days | |
| Fongaro *et al.* (2021) | Brazil | Florianopolis, Santa Catarina (~5,000) | October 2019–March 2020 (~7 months) | Confirmed/ reported first cases | 1 WWTP in Santa Catarina | Influent sewage. 24-h time-proportional sampling. Frozen – 6 months | 6 sampling events | N1, S, RdRp. Not normalised: copies/ml | 56–97 days | |
| La Rosa *et al.* (2021a) | Italy | Milan, Turin and Bologna (~4,999,534) | October 2019 – February 2020 (~5 months) | Confirmed/ reported first cases | 5 WWTPs located in 3 cities | Influent sewage. 24-h composite sampling. Frozen–NR | Monitoring/ Daily | ORF1ab, E, RdRp. Not normalised: copies/ml | ~8 weeks | |
| Martin *et al.* (2020) | United Kingdom | South-East England (4,000,000) | 14 January and 12 May 2020 (~4 months) | Confirmed/ reported first cases | 1 WWTP in South-East England | Influent sewage. 24-h composite sampling. Frozen – NR | Monitoring/ Daily | E, RdRP. Not normalised: copies/ml | 3 days | Detection <2 weeks |
| Randazzo *et al.* (2020b) | Spain | Municipalities Murcia region (~750,132) | 12 March–14 April 2020 (~1 month) | Confirmed/ reported first cases | 6 WWTPs in Murcia | Influent swage, secondary/tertiary treated effluent water samples. Grab sampling. Cold – 24 h | Monitoring/ Daily | N1, N2, N3. Not normalised: copies/ml | 12–16 days | |

[a]Cold=4 °C/ice; Frozen=–20/–80 °C; time: hours until process.

NR, not reported; WWTPs, wastewater treatment plants.



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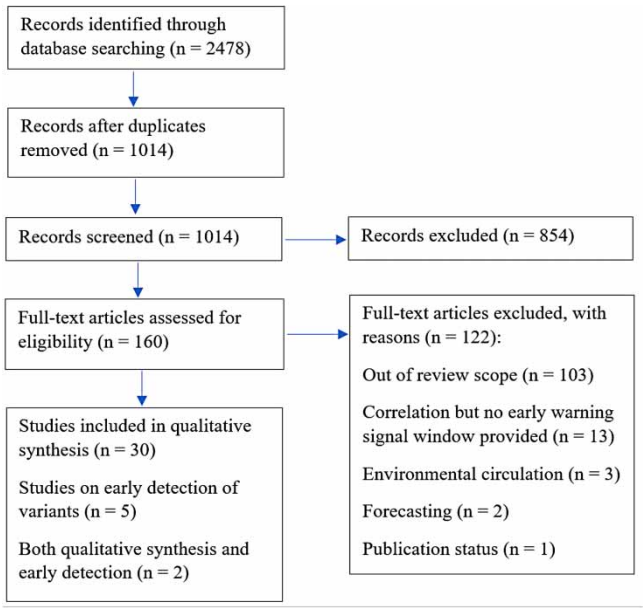


**Figure 1** | Flow of peer-reviewed studies identified and screened in the review according to PRISMA guidelines.

### EWS at the population level – reporting of timeliness

Of the 31 included publications, 19 report on ES as an EWS applied at the population level, such as metropolises/cities and urban areas. Of these, seven publications from Europe ($n = 5$) and Asia ($n = 2$) reported an EWS between 1 and 4 weeks, while eight publications from North America ($n = 4$), Europe ($n = 3$) and Australia ($n = 1$) reported an EWS within 7 days. Finally, four publications from Asia ($n = 2$), North America ($n = 1$) and Europe ($n = 1$) reported no EWS from ES. All studies compared data from ES with COVID-19 cases reported from clinical surveillance ($n = 17$) or hospitalisation data ($n = 2$). When available, the estimated population covered by the wastewater systems, including one or more wastewater treatment plants (WWTPs) varied between ~49,831 and ~4,336,600 inhabitants (Nemudryi *et al.* 2020; Black *et al.* 2021). Almost all studies ($n = 15$) used N genes ($N_1$, $N_2$, $N_3$) as molecular target for ES or in combination with *ORF1ab*, E and S genes. A few studies only used *RdRp* or *ORF1ab* genes (Saguti *et al.* 2021; Wurtz *et al.* 2021), and one study used a combination of E and *RdRp* genes (Wurtzer *et al.* 2020). Sampling frequency varied vastly across the studies, but more than half the studies of EWS ($n = 11$) processed the samples within the first 24 h.

### Early warning signal at 'hot spots' – reporting of timeliness

Six of the 31 included publications on EWS studied detection of SARS-CoV-2 in wastewater near or adjacent to facilities such as hospitals (Goncalves *et al.* 2021), nursing homes (Davo *et al.* 2021), university campuses (Betancourt *et al.* 2021; Colosi *et al.* 2021) and residential buildings (Wong *et al.* 2021; Xu *et al.* 2021). Of these, three publications reported an EWS in residential buildings and university campuses up to a week earlier than onset of symptoms or detection by clinical testing. One study reported an EWS between 5 and 19 days in two nursing home settings before cases among residents or staff (in both cases symptomatic) or outbreak declaration (Davo *et al.* 2021). Finally, two publications reported no EWS from ES in hospital settings (Colosi *et al.* 2021; Goncalves *et al.* 2021). When reported, the estimated population covered by the wastewater systems, including one or more WWTPs, varied from 108 to 1,152,403 inhabitants (Xu *et al.* 2021). Three studies used N genes (N, $N_1$, $N_2$), while two studies only used the *ORF1ab* gene (Wong *et al.* 2021) or a combination of E and *RdRp* genes as molecular targets for ES (Goncalves *et al.* 2021). As regards studies of EWS at the population level, sampling schemes varied among the six 'hot-spot' studies, with sample number ranging from 15 to 319. Four of the six studies processed the samples within the first 24 h.

### Detection of SARS-CoV-2 in wastewater before detection of the first case of COVID-19

Six of the identified publications report on detection of SARS-CoV-2 in wastewater before the detection of the first COVID-19 case in a specific area. Fongaro *et al.* (2021) reported that SARS-CoV-2 was circulating in the Americas as early as 27

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November 2019, 56 days ahead of the first reports of COVID-19 cases in the continent and more than 90 days in the case of Brazil. A study in Italy demonstrated that SARS-CoV-2 was already circulating in Northern Italy approximately 8 weeks before the first detected case in the region (La Rosa *et al.* 2021a), while in Spain Chavarria-Miro *et al.* (2021) detected SARS-CoV-2 in wastewater 41 days ahead of the first COVID-19 case in the Barcelona metropolitan area (Chavarria-Miro *et al.* 2021). In Brisbane, Australia, Ahmed *et al.* (2021) detected SARS-CoV-2 in wastewater 3 weeks before the first case of COVD-19 in the area, while it was observed 12–16 days earlier in the Spanish region of Murcia (Randazzo *et al.* 2020b). In the UK, low levels of RNA from SARS-CoV-2 were detected 3 days before the first case of COVID-19, and later in the pandemic, ES could track the effect of 'societal lockdown', demonstrating a decrease in levels in wastewater (Martin *et al.* 2020; Wurtzer *et al.* 2020). The estimated population covered by the wastewater systems, including one or more WWTPs, varied between ~5,000 and ~4,999,534 inhabitants (Fongaro *et al.* 2021; La Rosa *et al.* 2021a). Four studies used N genes ($N_1$, $N_2$, $N_3$) as molecular target for ES, alone or in combination with *ORF1ab*, E, S, *RdRp*, IP2 and IP4 genes. Two studies used a different combination of *RdRp*, E or *ORF1ab* genes.

### Early detection of SARS-CoV-2 variants

All included publications on early detection of variants ($n = 7$) compared SARS-CoV-2 sequences detected in wastewater with those from clinical samples. The included publications were from different geographical areas: six from Europe (Martin *et al.* 2020; Agrawal *et al.* 2021b; Hillary *et al.* 2021; Izquierdo-Lara *et al.* 2021; La Rosa *et al.* 2021b; Wilton *et al.* 2021) and one from the USA (Crits-Christoph *et al.* 2021).

Most studies were performed in 2020 (Martin *et al.* 2020; Agrawal *et al.* 2021b; Crits-Christoph *et al.* 2021; Hillary *et al.* 2021; Izquierdo-Lara *et al.* 2021), while two covered parts of 2020 and 2021 (La Rosa *et al.* 2021b; Wilton *et al.* 2021). The sampling periods varied from 1 day (Agrawal *et al.* 2021b) to several weeks (Martin *et al.* 2020; Crits-Christoph *et al.* 2021; Hillary *et al.* 2021; Izquierdo-Lara *et al.* 2021; La Rosa *et al.* 2021b) and 1 full year (Wilton *et al.* 2021). Wastewater sampling was 24-h composite, except for two studies in which different sampling strategies were used (Hillary *et al.* 2021; La Rosa *et al.* 2021b). Some studies state storage conditions, for example, chilled and stored at −80 °C (Martin *et al.* 2020) and no storage (Crits-Christoph *et al.* 2021). Ultrafiltration after pre-filtration or centrifugation were the main methods used for concentration of virus particles, and the volumes used were from 40 ml (Crits-Christoph *et al.* 2021) to 150 ml. In one study, 1 l of sewage was filtered using a negatively charged membrane (Agrawal *et al.* 2021b), while 250 ml and PEG-precipitation were used by La Rosa *et al.* (2021b). The total number of samples analysed varied from three samples collected in 1 day (Agrawal *et al.* 2021b) to 84 RNA extraction duplicates from 42 time-points in the UK (Hillary *et al.* 2021). Methods used for sequencing varied, and some studies used combinations of methods. Most studies included Illumina sequencing (Martin *et al.* 2020; Crits-Christoph *et al.* 2021; Hillary *et al.* 2021; Izquierdo-Lara *et al.* 2021), three studies used Nanopore (Agrawal *et al.* 2021b; Izquierdo-Lara *et al.* 2021; La Rosa *et al.* 2021b), while Sanger was used in three (Martin *et al.* 2020; La Rosa *et al.* 2021b; Wilton *et al.* 2021). All studies used amplicons for sequencing, except Crits-Christoph *et al.* (2021) who took an oligo-capture approach for target enrichment (Crits-Christoph *et al.* 2021).

Regarding the regions of SARS-CoV-2 analysed, some sequenced more than 75% of the genome or the complete SARS-CoV-2 genomes (Agrawal *et al.* 2021b; Crits-Christoph *et al.* 2021; Izquierdo-Lara *et al.* 2021), while others looked into specific regions (Martin *et al.* 2020; Hillary *et al.* 2021; La Rosa *et al.* 2021b; Wilton *et al.* 2021) – particularly the regions that code for parts of the S-protein. All studies used GISAID (https://www.gisaid.org/) to find information on SARS-CoV-2 genome sequences from clinical samples, except Agrawal *et al.* (2021b), who used COVID GC (https://covidcg.org/), which is enabled with data from GISAID (Agrawal *et al.* 2021b).

Three studies were conducted in England and Wales during January–May 2020 (Martin *et al.* 2020), March–July 2020 (Hillary *et al.* 2021)) and January 2020–January 2021 (Wilton *et al.* 2021). B.1.1.7 mutations were identified in viral sequences from sewage collected on 10 November in London, while retrospective analysis showed first detection in clinical samples on 21 November (Wilton *et al.* 2021). This demonstrates the possibility of detecting important mutations in sewage prior to the detection in a clinical sample. Martin *et al.* (2020) detected amplicons from five sewage samples containing nucleotide polymorphisms at selected positions of SARS-CoV-2 that were also detected in clinical samples from the same period (Martin *et al.* 2020). The third British study suggested that multiple genetically distinct clusters were co-circulating in the local populations and that the genetic variants observed in wastewater reflected similar single nucleotide polymorphism (SNPs) observed in contemporaneous samples from clinical cases (Hillary *et al.* 2021). One study performed in the USA in the period May–July 2020 demonstrated that sequencing of wastewater RNA can identify SARS-CoV-2 sequences already

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detected in clinical samples from the region or from the USA, together with sequences that were unique (Crits-Christoph *et al.* 2021). In a study in the Frankfurt area (Germany), three sewage samples were collected on 4 December 2020, and SARS-CoV-2 sequences were compared to sequences in clinical samples that had been registered by 7 March 2021 (Agrawal *et al.* 2021b). A total of 75 unique mutations were detected, with 18 reported in the Frankfurt region. One of the Frankfurt mutations was D614G, which was associated with the B.1.1.7, B.1.351 and P.1 variants. In The Netherlands and Belgium, the results of studied SARS-CoV-2 sewage sequences from 25 March to 3 June 2020 showed that detected variants did not appear in datasets from clinical samples (Izquierdo-Lara *et al.* 2021). However, sewage samples did group into clades of SARS-CoV-2 that were also prevalent in clinical samples from both countries, which indicated that sewage samples can be linked to specific outbreaks. The last study was performed in Italy (Rome, Abruzzo and Umbria) in three periods spanning September 2020–8 February 2021 (La Rosa *et al.* 2021b). The focus was on key spike protein mutations that had been detected in major circulating SARS-CoV-2 variants, and mutations corresponding to the UK and Brazilian VOC, as well as a Spanish variant, were detected.

### Public health action and guidelines (the grey literature)

Among the 16 identified grey literature publications (Supplementary material, Additional file 1), three were guidance (Canadian Water Network 2020; World Health Organization 2020d; Public Health Laboratory Network 2021), one was a review of the grey literature (Public Health Ontario 2021), two were research project reports (Water Research Australia 2020a; (RIVM) 2021), one was a scientific brief (World Health Organization 2020c), one was a policy brief (International Water Resource Association 2020), one was a report from an expert meeting (World Health Organization 2020a), one was a technical report (European Commission 2021b), one was a PowerPoint presentation (US Environmental Protection Agency 2020), three were reports with recommendations (Water Research Foundation 2020; Centres for Disease Control & Prevention 2021b; European Commission 2021a), and two were web pages (UK Parliament 2020; Centres for Disease Control & Prevention 2021a). In addition, a report from the EU commission is a recommendation to member countries to establish a surveillance system (European Commission 2021a).

Although documents in the grey literature explored different aspects, certain topics recur within them. There seems to be a consensus that ES is most useful for detecting trends and possibly as an EWS to prevent new outbreaks (International Water Resource Association 2020; UK Parliament 2020; US Environmental Protection Agency 2020; World Health Organization 2020a, 2020c; Centres for Disease Control & Prevention 2021a, 2021b; European Commission 2021a). It is also a common opinion that ES can complement, but not replace, traditional surveillance or clinical testing (World Health Organization 2020a, 2020c; Centres for Disease Control & Prevention 2021a, 2021b; Public Health Ontario 2021).

### Risk of bias – outcome of ROBINS-I assessment and cumulative body of evidence

The risk of bias of the included studies was assessed to be moderate (Supplementary material, Additional file 1). The cumulative body of evidence was graded as having a high/moderate to an overall low/moderate risk of publication bias, although the PRECEPT framework could only be partly applied due to the heterogeneity of the included studies.

## DISCUSSION

This systematic review provides a synthesis on the effectiveness in terms early warning signal compared to clinical surveillance (here applied as timeliness) of the ES of SARS-CoV-2 as an EWS during the first year of the COVID-19 pandemic and investigates its added value as a public health tool to monitor SARS-CoV-2 in different targeted areas as well as its ability to detect new variants.

The aims of the studies reviewed vary, including monitoring/surveillance of SARS-CoV-2 in wastewater or detection in targeted areas with the purpose of early detection, early warning, containing outbreaks in small settings, evaluating the effect of lockdown and merely reporting environmental detection before the first reported COVID-19 case in the same area. Therefore, studies were grouped into the following categories to analyse and summarise their results within similar study designs: (i) early warning at the population level, (ii) early warning at 'hot spots' and (iii) detection of SARS-CoV-2 in wastewater before clinical detection of first case of COVID-19. It was only possible to provide an overview of timeliness to evaluate the surveillance performance since sensitivity and specificity values were not systematically reported in the included studies.

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### EWS – timeliness

The included studies are heterogeneous in terms of number of tested sampling locations, sampling type, mode, frequency and target genes used to detect SARS-CoV-2 in wastewater, reflecting the early stage of the application of ES as a response to the COVID-19 pandemic in different countries and/or a lack of logistics in place. These factors made it challenging to compare and evaluate the outcomes of each study to support the aim of this systematic review with a meta-analysis (Gough *et al.* 2017). Protocol harmonisation would have facilitated the comparison of results across studies, allowing a meta-analysis enabling conclusions to be drawn regarding timelines and EWS performance. For this reason, a descriptive approach has been used for this review (Gough *et al.* 2017).

Despite the short average study period (~2–4 months), most of the included studies ($n = 25$, 80.6%) support the potential of ES as an EWS for SARS-CoV-2, with a window of 1 day to 4 weeks. This is in line with other findings evaluating its performance (Shah *et al.* 2022). However, four studies at the population level do not show an EWS when comparing ES with clinical surveillance. Factors potentially influencing the outcome of these four studies range from different sampling frequencies (Centres for Disease Control & Prevention 2022), use of different virus concentration methods and RT-qPCR assays (Agrawal *et al.* 2021a) and clinical testing capacity, which could vary between countries/targeted areas (Medema *et al.* 2020b; Kumar *et al.* 2021). Moreover, the early warning windows need to be interpreted based on the type of clinical data used for comparison, such as onset of symptoms and reported/confirmed positive cases or hospitalised COVID-19 cases. It is worth noting that not all the studies used multiple target genes to detect SARS-CoV-2, which could be a further factor influencing the confidence and reliability of results due to the possible presence of RT-qPCR inhibitors and different sensitivity of the assays (Agrawal *et al.* 2021a).

Similar heterogeneity is also reported between studies on early warning at 'hot spots' (Table 1). For this category, the early warning window has been estimated at between 2 and 4 days in residential buildings and university campuses and up to 19 days in nursing homes. However, in two studies monitoring wastewater in hospital settings, SARS-CoV-2 was detected in sewage between 1 and 16 days after the first reported hospitalised COVID-19 cases. In this case, too, sampling frequency could have played a role in the obtained results, as only 15–40 samples were collected during their study period. Our third study category reported results on EWS due to SARS-CoV-2 detection in wastewater before the first COVID-19 cases were detected. The early warning window reported is higher (range 3–97 days) than the study focusing on early warning at the population level and at 'hot spots'. However, all studies were carried out at the early stage of the COVID-19 pandemic, and some are the result of a retrospective analysis reporting detection of SARS-CoV-2 in wastewater dated towards the end of 2019 (Fongaro *et al.* 2021; La Rosa *et al.* 2021a). Although in some of these studies the early warning value is more theoretical than practical in terms of public health actions, they provide useful information on the potential of ES as a preparedness tool for future threats with similar biological characteristics and mode of transmission as SARS-CoV-2.

### Factors influencing the effectiveness of ES for SARS-CoV-2

The effectiveness of ES for SARS-CoV-2 is also influenced by the use of different sampling methods, namely grab sampling versus composite sampling. As reported by Hata *et al.* (2021), compared with a 24-h composite sample, SARS-CoV-2 concentration in a grab sample is expected to be variable due to the intermittent wastewater pulses (i.e., toilet flush) which compose the nonhomogeneous wastewater stream in sewers (Hata *et al.* 2021). Therefore, to see a correlation between the number of COVID-19 cases and the concentration of SARS-CoV-2 in wastewater, it is suggested that composite samples perform better (Hata *et al.* 2021). Several methods are also used for concentration and detection, along with molecular assays, of SARS-CoV-2 RNA in sewage. Although some studies compared the effectiveness of these assays, a gold standard method has not yet been determined (Hata *et al.* 2021; Pecson *et al.* 2021).

Other factors influencing the effectiveness of a surveillance system are data normalisation and the estimation of viral load. Some studies corrected SARS-CoV-2 RNA concentration for flow at the WWTP (Gonzalez *et al.* 2020) and adjusted for cases or positive tests for differences between local authority populations and catchment areas (Medema *et al.* 2020b). While, other prominent references show no benefit of correcting for the above-mentioned factors on Spearman correlation coefficients between SARS-CoV-2 RNA concentration from WWTPs and positive tests/COVID-19-related deaths. However, in such case, extensive quantitative comparisons between sites need to be carefully evaluated (Hillary *et al.* 2021). In addition, wastewater can be significantly diluted during rain events, causing a bias in the measured viral concentrations and making it difficult to obtain comparable results over space and time. It is therefore necessary to correct for dilution. Different approaches have been suggested for data normalisation (Medema *et al.* 2020a). Wastewater flow data provide direct

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information on the dilution of the sewage and can be used to calculate viral load, which was suggested to be a better predictor of COVID-19 cases than viral concentration (Westhaus *et al.* 2021). The other approach is to use faecal indicators to estimate the faecal fraction within the wastewater sample. The advantage of this method is that faecal indicators can be analysed from the same sample as the viral concentration (Hillary *et al.* 2021). For example, using a metric of Pepper Mild Mottle Virus (PMMoV) normalised with SARS-CoV-2 viral copies is described within the field of ES (Wu *et al.* 2020a; D'Aoust *et al.* 2021; Kitamura *et al.* 2021) and normalises SARS-CoV-2 viral copies to the number of copies of PMMoV in wastewater to account for variations inherent to wastewater samples, in particular variations related to the wastewater's water quality, solids and faecal matter concentrations (Wu *et al.* 2022).

The findings in this review show a great variability in the reporting on data normalisation (Table 1). It is suggested that additional work is needed to better understand when normalisation of SARS-CoV-2 RNA concentrations by PMMoV concentrations is needed. It might be useful to compare measurements made at different WWTPs, at the same plant across times when the waste stream varies in faecal strength or by using different methods that have differing recoveries (Graham *et al.* 2021; Pecson *et al.* 2021).

### Correlation and estimated prevalence of SARS-CoV-2 infections in populations and targeted areas

The ability to demonstrate a correlation between wastewater and epidemiological data is particularly desirable in relation to the surveillance of wastewater for SARS-CoV-2 in the light of the COVID-19 pandemic. As SARS-CoV-2 is shed by asymptomatic and pre-symptomatic individuals, a key driver of research on ES is to be able to predict an increase in COVID-19 cases from the level of SARS-CoV-2 detectable in sewage (World Health Organization 2022a). However, in modelling approaches, this correlation in long periods could vary, possibly due to reaching the clinical testing capacity limit, especially during acute phase (the point of an infection cycle at which a person is tested), the severity and duration of symptoms and prolonged virus shedding in the post-symptomatic phase (Ahmed *et al.* 2021; D'Aoust *et al.* 2021; Hillary *et al.* 2021; Roka *et al.* 2021). There are reports of correlations between SARS-CoV-2 RNA in sewage, with some variations (Wu *et al.* 2020a; Ahmed *et al.* 2021); on the other hand, an absolute comparison between prevalence of people infected with SARS-CoV-2 and SARS-CoV-2 RNA concentration in sewage may prove difficult since the reported prevalence depends heavily on the policy and method of clinical testing (Wu *et al.* 2020b). In addition, the size of WWTP and catchment area will influence the sewer residence time, sewage fluid dynamics and sewage composition, playing a particularly important role in the detection of SARS-CoV-2 in small areas with a low number of infected individuals (Rusinol *et al.* 2021). Overall, ES and wastewater epidemiology have been promoted as a complementary approach to estimate the presence and even the prevalence of SARS-CoV-2 in communities, including in the studies considered in this review (Table 1). However, several studies highlight the need to further explore the direct public health response, especially in cases of limited capacity for clinical testing (Randazzo *et al.* 2020a; La Rosa *et al.* 2021a).

It has been suggested that another asset related to the ES of SARS-CoV-2 is that it can target buildings or areas of interest ('hot spots'), for example, healthcare facilities. Among the studies considered in this review focused on the first year of the COVID-19 pandemic, some demonstrate a potential EWS (Table 1). However, one of the identified challenges is whether viral signals were due to new or recovered cases (Wong *et al.* 2021). Possible shortcomings of the proposed wastewater-based testing approach are that not all individuals produce a stool every day and/or building occupants may use bathroom facilities in another building. These shortcomings are potentially mitigated by frequent testing (i.e., if a positive case is missed on 1 day, it might be caught the next day) (Colosi *et al.* 2021). On the other hand, this approach might be resource-intensive and/or limited by access to suitable sampling sites to capture the targeted population (Xu *et al.* 2021). The legal and ethical implications of surveillance of smaller entities also need to be addressed (Gable *et al.* 2020; Jordhøy *et al.* 2021), although sampling from wastewater does not identify individuals, it may be possible to identify parts of the community, which can lead to stigma (Centres for Disease Control & Prevention 2021b).

### Early detection of variants

The studies included in this systematic review were conducted between 14 January 2020 and 8 February 2021; therefore, these results need to be interpreted based on the circulating VOCs during each study period. Specifically, the circulating VOCs were Alpha (lineage B.1.1.7, first documented in the UK, September 2020), Beta (lineage B.1.351, first documented in South Africa, May 2020) and Gamma (lineage P.1, first documented in Brazil, November 2020), while several variants were designated VOIs 1–2 months after the studies ended (World Health Organization 2022c). The dates for collecting

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sequencing data from GISAID spanned from 5 May 2020 to 7 March 2021. All three lineages have several mutations in the gene coding for S-protein, but also in other regions of the genome. The potential to explore SARS-CoV-2 diversity through ES was demonstrated in all seven studies. However, only one study showed that sequences identical to the B.1.1.7 VOC were detected in sewage prior to the first detection in a clinical sample (Wilton *et al.* 2021), indicating an opportunity for an EWS. Based on the results of this systematic review, reflections on the usefulness of ES for variant monitoring need to be contextualised based on the aim of such surveillance. Although it can be interesting from the research perspective to follow the evolution and monitor the diversity of circulating variants detected from wastewater over time in a specific population, it is difficult to identify which emerging variant will become a public health concern from wastewater data only. Hence, monitoring of severe and/or hospitalised clinical cases remains of utmost importance to identify new VOCs which require close monitoring in timely fashion. In this context, ES could support clinical surveillance and public health decisions through the monitoring of specific VOCs, particularly when a new VOC is identified in another country/area and is fast spreading among different populations or areas, thus serving as an EWS.

### Usefulness as a public health tool

Although the studies considered in this review suggest the potential of early detection of SARS-CoV-2, there is currently a lack of evidence in peer-reviewed studies on the usefulness of ES as a public health tool in practice (Supplementary material, Additional file 1). Initially, the usefulness of such surveillance faced obstacles such as lack of knowledge, information and communication between those performing ES and public health decision makers. Despite the assets of the surveillance systems, there is a lack of standards for the analysis and representativeness of samples, shedding rate, sampling method and frequency and interpretation of how to use the results from such surveillance (International Water Resource Association 2020; World Health Organization 2020a, 2020c; Centres for Disease Control & Prevention 2021a; European Commission 2021b; (RIVM) 2021). However, the field is in rapid development, and interim guidance highlights the importance of considering how the ES of SARS-COV-2 ES is anticipated to add value to health sector decision-making for the COVID-19 response (World Health Organization 2022a).

In light of several recommendations that ES could be a complement to clinical surveillance and based on the findings in this systematic review, we offer reflections on ES as a complement to clinical surveillance in different scenarios to enrich the discussion. To be relevant for ES, such reflections are dependent on future microbiological threats causing a massive outbreak or pandemic sharing the same characteristics of SARS-CoV-2 in terms of shedding. Different phase scenarios in which ES could play a role, based on context, time and testing capacity, are:

- Early phase of an emerging novel virus: having the sewage system infrastructure in place could play a role in preparedness and support clinical surveillance in terms of potential early detection in countries with high resources. In low-resource settings, having a functional sewage system in place could be a tool to better control the epidemiological situation.
- Acute phase of pandemic: different aspects need to be considered. In a country with a good testing capacity and which has not reached capacity limits, consideration is required of the possible added value of having an early warning of a few days/weeks in terms of public health actions, assuming that infected people cannot be traced and that the testing, isolation, contact tracing and quarantine strategy could not be applied. In low-resource countries, ES could still be a tool to monitor trends and the general epidemiological situation. In both cases, a cost-effectiveness analysis would be needed.
- Endemic phase: in the case of public health interventions to reduce the burden of the disease, such as implementing a vaccination programme, or less severe virus variants affecting populations, possibly available treatments or reduced large-scale clinical testing, ES gains importance as a complementary testing policy supporting clinical testing for severe and/or hospitalised cases.
- End phase of the pandemic: in a scenario where the virus could disappear or become mild and thus does not represent a public health threat in terms of hospitalisation, patients requiring ICU and deaths, the benefit of having an ES could increase as a tool to monitor the epidemiological situation.

Therefore, contextualising ES could contribute to the discussion of its usefulness as a public health tool, in parallel with the discussion of how to improve its performance. Based on the findings of this review, we believe it is also of considerable importance to highlight when an ES system has added value, regardless of its performance per se. In addition, we strongly suggest assessing the purpose of surveying wastewater, whether the aim is to detect outbreaks, monitor the re-emergence of the virus or keep track of transmission and mutation in the population. Assessment based on both resource settings and stage of the

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pandemic, as well as the aim of the surveillance, could provide reflections on added value and bring ES beyond the research stage in order to focus on improving performance.

### Suggestion for further studies

The assessment of the studies included in this review has identified the need for future studies that take account of long-running surveillance, as opposed to shorter study periods and analysis of signals with defined thresholds, to take public health actions (Agrawal *et al.* 2021a; Kumar *et al.* 2021). Furthermore, correlation modelling that considers people moving to second homes or tourism (Trottier *et al.* 2020) is needed. There is also a lack of research showing environmental factors that might influence the EWS. Most of the studies were carried out in spring or summer, highlighting the need to evaluate the impact of seasons characterised by heavy rain or snow melt (Agrawal *et al.* 2021a). In addition, we did not identify any practical evaluation that included an assessment of the added value of having ES in place (as a tool to manage the crisis and cost–benefit).

### Limitations of the review

A limitation of this review is that the included studies report on results at an early stage of the pandemic, implying that findings need to be interpreted carefully and taking into account the implementation of new assays and monitoring for a new emerging virus. Furthermore, this review needs to be updated to consider studies carried out during the second year of the pandemic that capture the situation after the vaccination programme was implemented, as well as studies with a longer study period, to better evaluate performance in terms of timeliness, sensitivity and specificity and how the information was used to implement public health actions. An update would also capture the numerous publications which had status as 'preprint' at the time the literature search was conducted in this systematic review. Due to the heterogeneity of the included studies, a proper assessment of risk of bias since mimicking a hypothetically RCT and applying PRECEPT for grading the cumulative body of evidence is also challenging, which affect the strength of the outcome in terms of evidence of effectiveness of ES as an EWS for SARS-CoV-2 of the review (Sterne *et al.* 2016; Harder *et al.* 2017). The assessment of bias varies greatly. Bias due to confounding was, in general, high, reflecting the challenge of sampling and analysing wastewater, and low in outcomes of measurement since the clinical data are COVID-19-specific. Although few studies were funded, for example by private companies, the publication bias was assessed as rather low to moderate; however, it is likely that studies with negative findings on the performance of ES for SARS-CoV-2 were not published/reported, affecting the outcome of this review through an overestimation in a positive direction.

## CONCLUSION

In this systematic review, we assessed 29 publications for the effectiveness of ES for the early detection of SARS-CoV-2, six publications for the ability to detect new variants, two for both mentioned purposes and grey literature for potential reporting on practical experience of its added value as a surveillance system. Based on the synthesis of the results, ES showed a potential to be a 1- to 2-week EWS of COVID-19 waves, while there was low evidence for the added early warning value for detecting new variants of concerns in wastewater during the first year of the COVID-19 pandemic. ES could complement clinical surveillance for SARS-CoV-2; however, its cost–benefit value for public health decisions needs to be assessed based on stage of the pandemic and resources available, including the possibility of downscaling/upscaling the environmental surveillance based on needs. Additional studies focusing on filling the methodological knowledge gaps as well as how to use and interpret ES signals and surveillance thresholds for public health actions are needed. Moreover, knowledge should be shared within the international scientific community by countries/areas with long experience of such surveillance. In addition, the review identified a need for harmonised protocols for ES and knowledge on data normalisation as well as cost–benefit evaluations of ES as a public health tool. Considering the rapid increase in the body of knowledge in this field, an update of this systematic review would be useful in re-assessing the effectiveness of ES in light of recent developments. For future assessment of the effectiveness of ES for early warning detection of SARS-CoV-2, studies should include values such as timeliness and, preferably, sensitivity and specificity.

## ACKNOWLEDGEMENT

We thank research librarian Marita Heintz at the Norwegian Institute of Public Health for developing and conducting the literature search.

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## AUTHOR CONTRIBUTIONS

S.H., E.A. and J.A.B.L. screened the publications identified in the literature search for eligibility. M.M. and E.A. assessed the included studies specifically for variants. F.J. and S.K.S. screened the grey literature. E.A. conducted data extraction from the identified studies. S.H. drafted the first version of the manuscript. S.K.S. drafted the graphical abstract. E.A., J.A.B.L., M.M., F.J. and S.K.S. critically reviewed the drafted manuscript and approved its final version.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 5 May 2022; accepted in revised form 7 July 2022. Available online 18 July 2022

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