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# Wastewater-based epidemiology approach: The learning lessons from COVID-19 pandemic and the development of novel guidelines for future pandemics

Erica Gagliano<sup>a,b</sup>, Deborah Biondi<sup>a</sup>, Paolo Roccaro<sup>a,\*</sup>

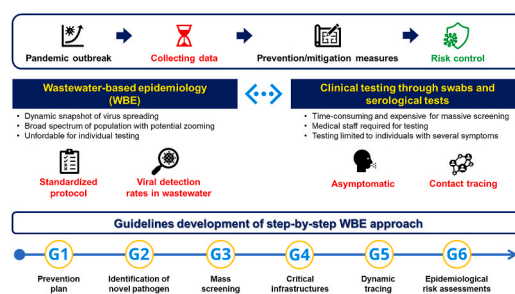
<sup>a</sup> Department of Civil Engineering and Architecture, University of Catania, Catania, Italy

<sup>b</sup> Department of Civil, Chemical and Environmental Engineering, University of Genoa, Genoa, Italy

## HIGHLIGHTS

- Wastewater-based epidemiology (WBE) is fine-tuning as COVID-19 surveillance tool.
- Meant for illicit drug tracing, WBE gives virus spreading snapshot in a community.
- Most cited keywords related to analytical viral method as a main burning topic.
- Data based on clinical testing suffer by contact tracing and asymptomatic cases.
- The trustworthiness of WBE data should be verified through long-term monitoring.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Wastewater-based epidemiology (WBE) provides a comprehensive real-time framework of population attitude and health status. This approach is attracting the interest of medical community and health authorities to monitor the prevalence of a virus (such as the *severe acute respiratory syndrome coronavirus 2*, SARS-CoV-2) among a community. Indeed, WBE is currently fine-tuning as environmental surveillance tool for coronavirus disease 2019 (COVID-19) pandemic. After a bibliometric analysis conducted to discover the research trends in WBE field, this work aimed to side-by-side compare the conventional method based on clinical testing with WBE approach. Furthermore, novel guidelines were developed to apply the WBE approach to a pandemic. The growing interest on WBE approach for COVID-19 pandemic is demonstrated by looking at the sharp increase in scientific papers published in the last years and at the ongoing studies on viral quantification methods and analytical procedures. The side-by-side comparison highlighted the ability of WBE to identify the hot-spot areas faster than the conventional approach, reducing the costs (e.g., rational use of available resources) and the gatherings at medical centers. Contrary to clinical testing, WBE has the surveillance capacity for preventing the virus resurgence, including asymptomatic contribution, and ensuring the preservation of medical staff health by avoiding the exposure to the virus infection during clinical testing. As extensively reported, the time in collecting epidemiological data is crucial for establishing the prevention and mitigation measures that are essential for curbing a pandemic. The developed guidelines can help to build a WBE system useful to control any future pandemic.

\* Corresponding author.

E-mail address: [paolo.roccaro@unict.it](mailto:paolo.roccaro@unict.it) (P. Roccaro).

## 1. Introduction

Municipal wastewaters (MWWs) collected through sewer network and delivered to wastewater treatment plant (WWTP) have been mainly analyzed to assess the removal efficiency of organic matter and nutrients to ensure the protection of receiving water bodies (McCall et al., 2016; van Nuijs et al., 2011). Conversely, the qualitative and/or quantitative analysis of MWW entering the WWTP provides significant information regarding the health status of a community. Deeply, MWW could be considered as a “mirror” of population health.

Based on this statement, wastewater-based epidemiology (WBE) has been developed to obtain population-scale assessments on use/consumption and release of chemical and biological agents within catchment boundaries (González-Mariño et al., 2016; McCall et al., 2016). WBE is a prediction tool based on the analysis of substances excreted by human as metabolites and parent compounds, which were transported through the sewer network to WWTPs (Daughton, 2018; McCall et al., 2016). Moreover, a comparison between areas of different population sizes is possible through the population normalization of data. Lastly, the methodology is non-invasive, and it respects the individual privacy. WBE has been applied to detect different classes of markers/biomarkers such as tetrahydrocannabinol (metabolite from cannabis), tobacco as nicotine, paraxanthine (metabolite from caffeine) (Choi et al., 2018; Gracia-Lor et al., 2017). Over the years, the application of WBE ranges from the evaluation of drug abuse and population health (e.g., exposure to pesticides and heavy metals) to the assessment of lifestyle choice (e.g., consumption of food and chemicals) (Foladori et al., 2020; Mao et al., 2020; van Nuijs et al., 2011). Consequently, the spatial-temporal trends might be monitored through WBE and these trends might provide a comprehensive real-time framework of population attitude and health status (Choi et al., 2018; Daughton, 2018; Mao et al., 2020).

Currently, the entire scientific community is implementing WBE for the detection of *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) RNA in wastewater to screen potential virus carriers and consequently, to assess levels and lineages of coronavirus disease 2019 (COVID-19) in the population providing an early warning tool of pandemic (Bivins et al., 2020; Castiglioni et al., 2022; Foladori et al., 2020; Medema et al., 2020). The keen interest in the monitoring of COVID-19 pandemic through WBE is demonstrated by the global collaborative network recently established and the common platform available online (<http://www.covid19wbec.org/>) for data sharing (Bivins et al., 2020; Castiglioni et al., 2022). However, several challenges and uncertainties related to WBE procedure and related methodological steps (e.g., virus shedding, in-sewer transportation, sampling and storage, analysis of SARS-CoV-2 RNA concentration, back-estimation) are affecting its worldwide application demonstrating the need of a standard procedure implementation (Cervantes-Avilés et al., 2021; Daughton, 2020; Foladori et al., 2020; Kevill et al., 2022; Li et al., 2021). Consequently, further investigations are strongly advised.

As recently experimented during the COVID-19 pandemic, time in collecting epidemiological data is crucial for establishing prevention and mitigation measures and consequently, to promptly identify the virus spreading in a community. To date, most countries including Italy have developed an integrated microbiological and epidemiological surveillance for COVID-19 mainly based on clinical testing (i.e., rhinopharyngeal swabs and serological tests) (World Health Organization, 2022). However, several criticisms regarding time in collecting data and costs arise especially at the peaks of COVID-19 outbreak, highlighting the unfeasibility of traditional method based on clinical testing as early warning tool. Conversely, WBE can provide a comprehensive and timely population exposure portrait although the implementation of WBE as a prevention and early warning tool is still under investigation and some criticisms and limitations should be overcome (Li et al., 2021; Medema et al., 2020; Pandey et al., 2021).

In this study, a bibliometric analysis was conducted to discover the research trends in WBE field also providing a snapshot of the literature

on this research topic by means of VOSviewer software. To fill the existing gap in the scientific literature, this work also aimed to side-by-side compare conventional method based on clinical testing and WBE approach. Indeed, advantages and drawbacks were withdrawn to assess how quickly these approaches could act to control a pandemic. The COVID-19 pandemic has demonstrated that there is an urgent need to establish integrated surveillance systems that are robust, sustainable, and resilient also for potential future pandemics. However, the development of standard protocols and the design of guidelines and consequently their worldwide implementation need more effort and further investigation. Consequently, based on WBE approach and lessons learned from COVID-19 pandemic, novel guidelines have been proposed in this work to improve the existing crisis management plans for future pandemics.

## 2. Bibliometric analysis of research trends in WBE field

In last years, WBE approach has been considered a growing field as evidenced by the number of papers published on this topic from 2014, with a sharp increase in 2020, when the COVID-19 pandemic started (Fig. 1). Indeed, in 2020, and specifically on March 11th, the World Health Organization (WHO) declared a global pandemic related to SARS-CoV-2, which is the causative agent of COVID-19 (World Health Organization, 2020a).

Considering its worthwhile potentiality, the application of WBE is recently investigated as a valuable tool for COVID-19 surveillance. The ability to monitor the prevalence of SARS-CoV-2 among the population via WBE represents a promising strategy and several studies have been carried out and are still ongoing to validate this approach. The particular focus posed on WBE for COVID-19 pandemic is demonstrated by the number of papers produced only in 2021 (280), which is greater than the sum of papers published between 2014 and 2019 (165) (Fig. 1).

To deepen the bibliometric analysis, VOSviewer (version 1.6.16, developed by Van Eck and Waltman) was employed to construct bibliometric maps displaying the research trends in the field of WBE. VOSviewer (<https://www.vosviewer.com/>) is a freely available software tool for exploring bibliometric networks and then for visualizing such networks in a two-dimensional space based on “visualization of similarities” (VOS) (van Eck and Waltman, 2010). In this study, the co-occurrence keywords maps (Figs. 2 and 3) were created based on bibliographic database files downloaded from Scopus, the largest database of peer-reviewed literature. Firstly, the VOSviewer was performed uploading a Scopus database made of 652 documents (including articles, reviews, book chapters, and conference papers) that matched the

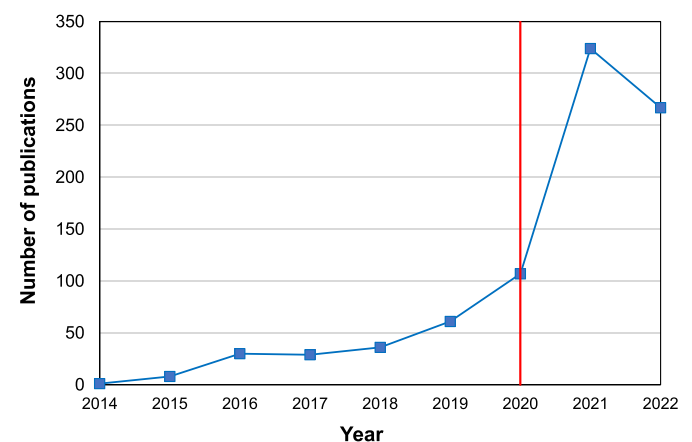


Fig. 1. Temporal distribution of published papers on wastewater-based epidemiology (WBE) (\*data related to 2022 not complete). Red line: start of COVID-19 pandemic. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

followed search query “TITLE-ABS-KEY (wastewater-based epidemiology)”. Consequently, the co-occurrence analysis and the full counting method were chosen in VOSviewer software as type of analysis and counting method, respectively. A threshold of 40 was set as a minimum number of occurrences of a keyword (of the 6099 keywords, 99 met the set threshold). Before visualizing the map, a cleaning step was performed to merge repetitive words (e.g., singular vs. plural forms) and to eliminate unrelated words (i.e., country and organization names).

Fig. 2 is the obtained map from VOSviewer showing the co-occurrence analysis of keywords (depicted as circles) frequently appeared in the publications related to WBE and the nexus between them (depicted as lines) providing a snapshot of the literature on this research topic. Specifically, the circle size represents the frequency of keyword occurrence in the entire Scopus bibliographic dataset, while line thickness stands for the strength of links between two or more keywords indicating the number of times they appeared together in the same document.

Moreover, two main clusters can be distinguished in the map (Fig. 2). Indeed, within the same cluster, the keywords are mutually related, and they were co-studied repeatedly in the same articles. The first cluster, labelled in red, groups keywords related to WBE application for tracing drugs (both illicit and licit) as demonstrated by the specified words such as “amphetamine”, “biomarker”, “drug dependence”, and “unclassified drug”. The second cluster in green encompasses keywords related to SARS-CoV-2 and, consequently, the application of WBE as early warning tool for COVID-19 pandemic. Based on the two clusters, the map clearly discerns the WBE applications between “past” and “present” or either “pre-” and “post-” COVID-19 pandemic. Some keywords related to WBE methodology such as “epidemiology”, “water sampling”, and “sewage” represent the link between the two clusters. Another evidence of the worldwide attention in the WBE application for COVID-19 is provided by checking the occurrences and total link strength of the most frequency keywords. Indeed, among the entire dataset, the following keywords “human” (412 occurrences and total link strength of 5066), “wastewater” (412 occurrences and total link strength of 5031), “wastewater-based epidemiology” (407 occurrences and total link

strength of 4498), “covid-19” (259 occurrences and total link strength of 3213), “sewage” (175 occurrences and total link strength of 2123) belong to the top ten list.

In order to provide more details regarding the correlation between WBE approach and COVID-19 surveillance, a further bibliometric analysis through VOSviewer was conducted. In this case, the bibliographic database files made of 266 documents (including articles, reviews, book chapters, and conference papers) was downloaded from Scopus using with the followed search query “TITLE-ABS-KEY (wastewater-based epidemiology AND Covid-19 OR Sars-CoV-2)” and then uploaded to VOSviewer software. As previously mentioned, the map displayed in Fig. 3 was created by selecting the co-occurrence analysis and the full counting method. A threshold of 30 was set as a minimum number of occurrences of a keyword (of the 1763 keywords, 37 met the set threshold). Then, a cleaning step was performed also in this case for merging repetitive words (e.g., singular vs. plural forms) and for eliminating unrelated words (i.e., country and organization names). Looking at Fig. 3 the frequency of keyword occurrence and the strength of keyword links are depicted through circles and lines, respectively. Specifically, an overlay visualization was selected, and consequently, the colors in Fig. 3 are plotted according to average citations. The highest citations depicted in yellow are related to concentration and quantification viral methods as demonstrated by the related keywords (e.g., “rt qpcr”, “viral RNA”, “quantification”). This evidence matches with the ongoing research on the development of standardized protocols for WBE application as surveillance tool for COVID-19 pandemic and particularly with current studies on analytical viral methods.

### 3. Side-by-side comparison between COVID-19 surveillance based on clinical testing and WBE approach: applicability and limitations

#### 3.1. Clinical testing vs. WBE approach

As recently experienced worldwide, the COVID-19 pandemic caused the over-saturation of the health care systems with serious human health

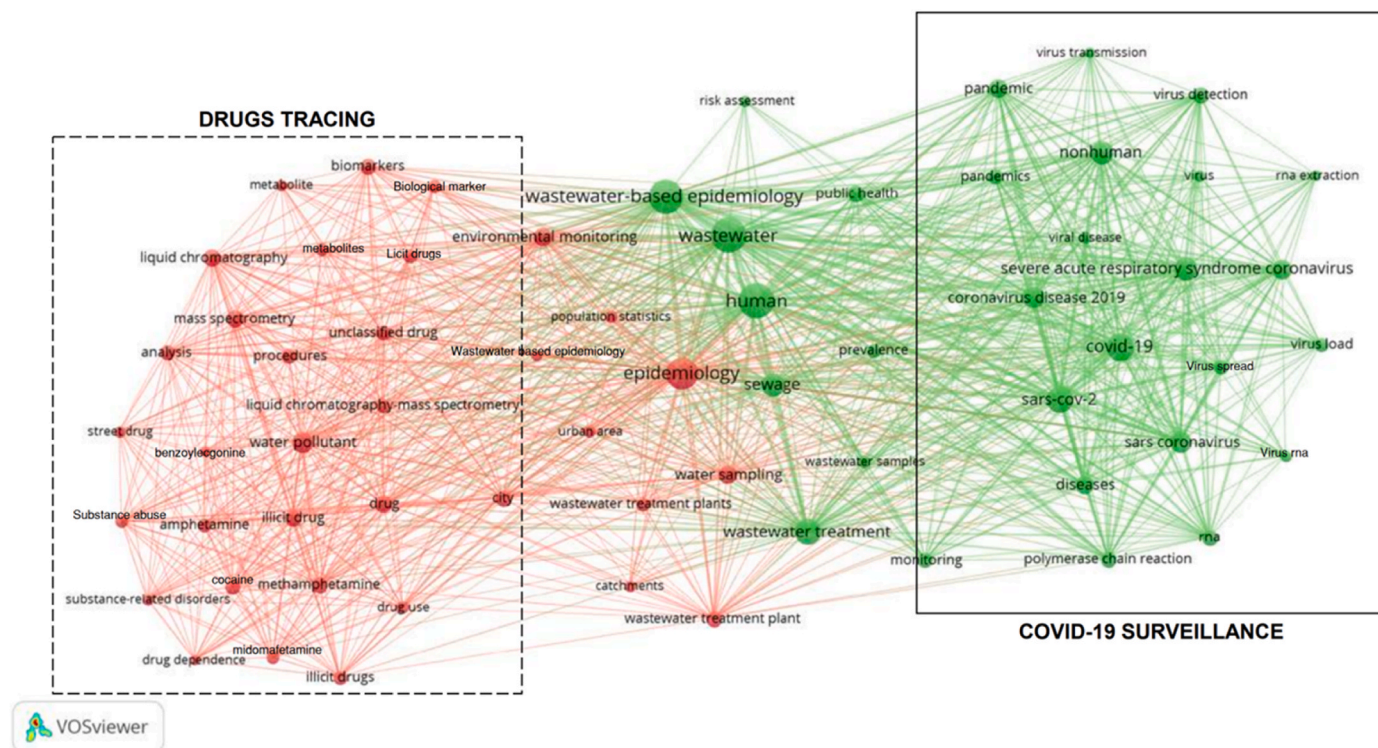


Fig. 2. Co-occurrence keywords map by cluster (search query: “TITLE-ABS-KEY (wastewater-based epidemiology)”).

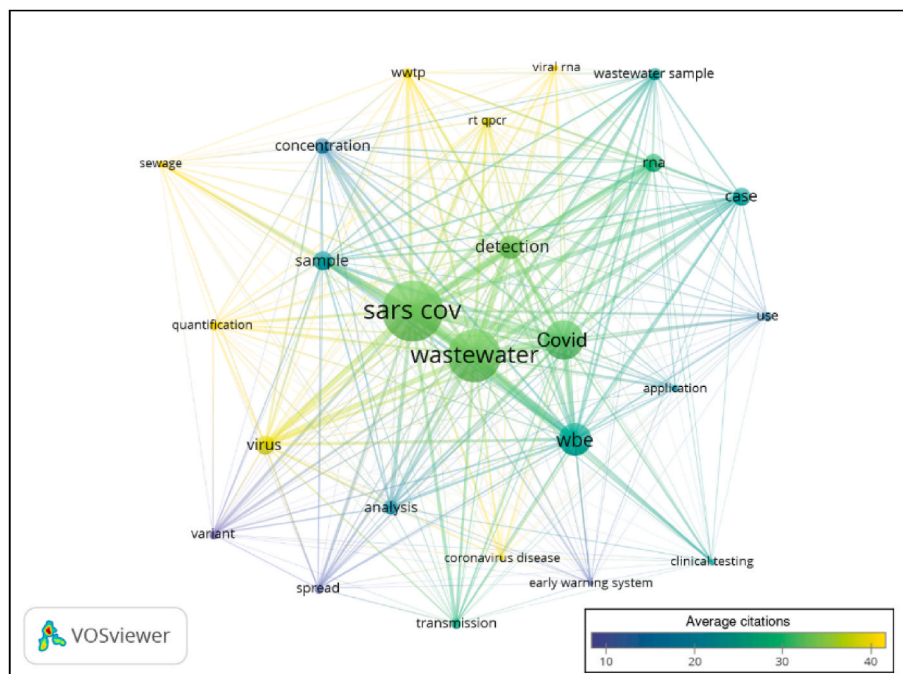


Fig. 3. Co-occurrence keywords map by average citations (search query: “TITLE-ABS-KEY (wastewater-based epidemiology AND Covid-19 OR Sars-CoV-2)”).

complications and a high mortality rate especially during the 1st wave due to the absence of specific antiviral drugs/vaccines and the delay in the establishment of virus containment measures (Sohrabi et al., 2020; World Health Organization, 2022). The entire world has experienced that health and safety of humans, financial and economic stability of each nation, and proper functioning of health care facilities are strongly related to the availability of fast and effective analytical test methods (Daughton, 2020).

In a pandemic scenario, time in collecting epidemiological data and consequently the time in responding to virus spreading through the establishment of prevention/mitigation measures is paramount. Indeed, lower time in recognizing hot-spot areas and infected clusters results in reducing virus transmission and in saving human lives (World Health Organization, 2022, 2021).

To identify the extent of the COVID-19 pandemic especially the distribution and magnitude of infected population, many efforts were made by governments to set up control, mitigation, and containment measures to minimize the domino effect (World Health Organization, 2021). However, some measures of control such as lockdown, “stay at home” and curfew orders should be introduced as the only change to counteract the virus spreading in a community due to their severity and harshness (Aguar-Oliveira et al., 2020; Manica et al., 2021). For instance, the first lockdown nationally emitted in several countries (including Italy) to reduce COVID-19 transmission had serious impacts on both human daily life and economic system whose negative implications have been enormous and still evident (Organization for Economic Cooperation and Development, 2020). However, it was proven that the transmission of COVID-19 in certain areas was not so acute, certainly not required those severe restrictions (Manica et al., 2021; Randazzo et al., 2020).

Most countries, including Italy, have developed an integrated microbiological and epidemiological surveillance for COVID-19 based on clinical diagnostic testing (i.e., rhino-pharyngeal swabs and serological tests). However, scaling diagnostic tests for mass surveillance of populations is characterized by numerous challenges and concerns such as time lag in collected data, high costs, and medical staff being greatly involved (Daughton, 2020; Hart and Halden, 2020a).

In this framework, the epidemiological approach based on WBE

could provide reliable and accurate data for health authorities to act appropriately and effectively. Undoubtedly, WBE cannot replace clinical testing for identifying infected individuals, it might thus provide complementary information to clinical reporting. WBE might serve to monitor health population status acting as sentinels for public health authorities in case of fast outbreak (Daughton, 2020; Kumar et al., 2022; Mao et al., 2020; Thompson et al., 2020). Moreover, WBE approach could be also employed as decision-making support to guide the health authorities’ decisions related to restraint and mitigation measurements (Aguar-Oliveira et al., 2020; Medema et al., 2020; Randazzo et al., 2020).

A side-by-side comparison between COVID-19 surveillance based on clinical testing (i.e., rhino-pharyngeal swabs and serological tests) and WBE approach was accurately performed by reviewing previously published papers, and the main findings are reported on Table 1. Particularly, several factors such as time lag in collecting data, costs, massive screening, source of data bias, and surveillance capacity have been analyzed and compared to identify the potentiality and limitations of each approach.

Especially in large urban areas characterized by faster virus spreading, the time in collecting epidemiological data is crucial for avoiding the domino effect. A time lag between the infection and the inclusion of the new case in the COVID-19 integrated surveillance system based on clinical testing is extensively documented (Daughton, 2020; Kumar et al., 2022). This delay varies locally due to the available medical staff and local/regional health services and the analytical capacity of laboratories in providing a quick diagnosis (negative or positive result) as well as other social-economic factors such as the individual access to health care facilities (distance and economic welfare) (Daughton, 2020; Hart and Halden, 2020a; Pandey et al., 2021). For example, considering the microbiological and epidemiological surveillance for COVID-19 established in Italy in January 2020 (<https://www.epicentro.iss.it/en>), an average time lag of 2–3 weeks (i.e., 13–23 days) occurs between the moment a person becomes infected with SARS-CoV-2 (“day zero”, when the infection occurs) and the notification of the individual disease to The Italian National Institute of Health (ISS) by lastly including the new case in the COVID-19 integrated surveillance system. Deeply, Fig. 4 shows the timeline of the notification

**Table 1**  
Side-by-side comparison between Covid-19 surveillance based on clinical testing (i.e., rhino-pharyngeal swabs and serological tests) and WBE approach.

Items	Clinical testing (i.e., rhino-pharyngeal swabs and serological tests)	WBE approach
<b>Time lag in collecting data</b>	Well documented and varying due to several factors, for example supply of swabs and chemicals, possibility of individual access to health care facilities (distance from the nearest and economic welfare), analytical capability of laboratory facilities and their widely occurrence/availability. Time lag between the infection and the inclusion of the new case in the COVID-19 integrated surveillance system is 2–3 weeks on average.	Almost real-time monitoring, lead time depends on existing standardized procedure and facilities. Lead time is ascribed to the time needed for collecting samples, performing analysis, and transmitting data.
<b>Analytical detection</b>	High-sensitive and accurate.	Sensitive and accurate only once the establishment of a protocol validation and standardization of the entire process (from sampling to viral detection).
<b>Tests effect on human</b>	Potential human-to-human transmission at medical center. Invasive and debilitating in the long run.	Non-invasive and anonymous. Understanding the virus prevalence keeping individuals isolated.
<b>Number of analyses</b>	High, especially in presence of fast virus spread.	Limited and localized at specific sampling points (WWTP, sewage).
<b>Size population</b>	Individual level.	Community at any level with the ability to discern among sub-areas. Potential zooming on sensitive and high-density infrastructures (e.g., hospitals, schools).
<b>Massive screening</b>	Time-consuming, expensive, high mobility of potential infected people and overwhelm medical care systems.	Covering a broad spectrum of population with a limited number of chemical analyses. Also including individuals that are asymptomatic or mildly symptomatic, people that are reluctant to get tested, and/or who have less access to testing.
<b>Risk control</b>	Low due to the time needed for performing clinical tests and consequently collecting data especially during fast virus spreading.	Prompt acting. Fast capacity in assessing the magnitude of sanitary emergency and its re-emergence.
<b>Virus spreading</b>	Difficult to control due to the high number of individual tests required.	Dynamic and fast tracing. Countering the rapid rise of infections.
<b>Contact tracing</b>	Paramount especially at fast virus transmission.	Not required to determine the extent of population infection.
<b>Costs</b>	Expensive and unaffordable especially during pandemic and when resources are limited.	Affordable.
<b>Reagents</b>	The required amount depends on the number of individuals tested.	Moderate usage. At emergency, the rational use of those available (such as swabs).
<b>Medical and health-care staff</b>	Needed for testing individuals. High risk of virus infection during testing due to aerosols from patients. Worst scenario: overwhelm	Not required for testing.

**Table 1 (continued)**

Items	Clinical testing (i.e., rhino-pharyngeal swabs and serological tests)	WBE approach
	and/or collapse of the medical care system.	
<b>Restrictions</b>	Widely applied. Their application depends on number of individuals tested and the promptness of collecting data.	Site-specific and strictly functional, minimizing the long stay-at-home periods.
<b>Strategic planning</b>	Due to time needed to individually test and data collection, a delay in acting is observed.	Ad-hoc measures where and when are necessary and their short-term implementation.
<b>Social distancing</b>	Not ensured during massive screening due to potential gatherings.	Always guarantee particularly at stages with high contagious rate.
<b>Asymptomatic Stigma and discrimination</b>	Not accounted for. Stigma and discrimination are typical in little communities.	Included. Respectful of the individual privacy.
<b>Source of data bias</b>	False positives, asymptomatic cases, close contacts to positive cases not identified by contact tracing.	Variability of wastewater parameters (e.g., seasonality temperature variations) and the occurrence of extreme weather events.
<b>Surveillance capacity</b>	Feasible although cost-prohibitive and massive testing needed.	Feasible for preventing the re-emergence of virus, almost in real-time. Investigation of the temporal trend and application of backward approach.
<b>Risk of virus resurgence</b>	High, it is infeasible to monitor sporadic outbreaks.	Continuously controlled through the monitoring of virus prevalence in communities.
<b>Criticisms</b>	<ul style="list-style-type: none"> <li>- Time lag.</li> <li>- Testing is often limited to individuals with severe symptoms.</li> <li>- Time-consuming and expensive for massive screening.</li> <li>- Unfordable when resources (e.g., reagents and swabs) are limited.</li> <li>- Medical and health-care staff is required for testing.</li> <li>- Weakness of contact tracing to identify the potential individuals to being tested.</li> <li>- The need of access to health centers (distance and capacity of analytical laboratory).</li> <li>- The contribution of infected but asymptomatic individuals is not accounted for.</li> </ul>	<ul style="list-style-type: none"> <li>- Development of a standardized protocols for WBE procedure dealing with MWW sampling, virus recovery/concentration and its quantification.</li> <li>- Feasible in regions with existing infrastructures (e.g., sewage and WWTP).</li> <li>- Unfordable for individual testing.</li> <li>- Knowledge gaps in the dynamics of viral shedding in the feces influencing viral loads in wastewater.</li> <li>- Complexity of wastewater matrix and effect of MWW seasonal variability on viral detection rates.</li> <li>- Long-term monitoring needed to obtain trustworthy data and to conduct the correlation with clinical testing.</li> </ul>

process according to the earliest Italian surveillance for COVID-19 and the potential virus transmission pathways during the notification process to ISS. The promptness in sending the updated number of positive cases and other relevant information (such as the recognition of clusters and new hot spots of concern) to the ISS and consequently, to include them in COVID-19 integrated surveillance system strongly depends on the local management procedures and on the effectiveness of regional coordination (<https://www.epicentro.iss.it/en>).

It should be highlighted that Fig. 4 is mainly referred to the initial stages of the pandemic where some delays might be caused by limited resources (such as swabs and available laboratories). However, the steps within the notification process and consequently the days needed to count a new case will not vary significantly due to the hierarchical

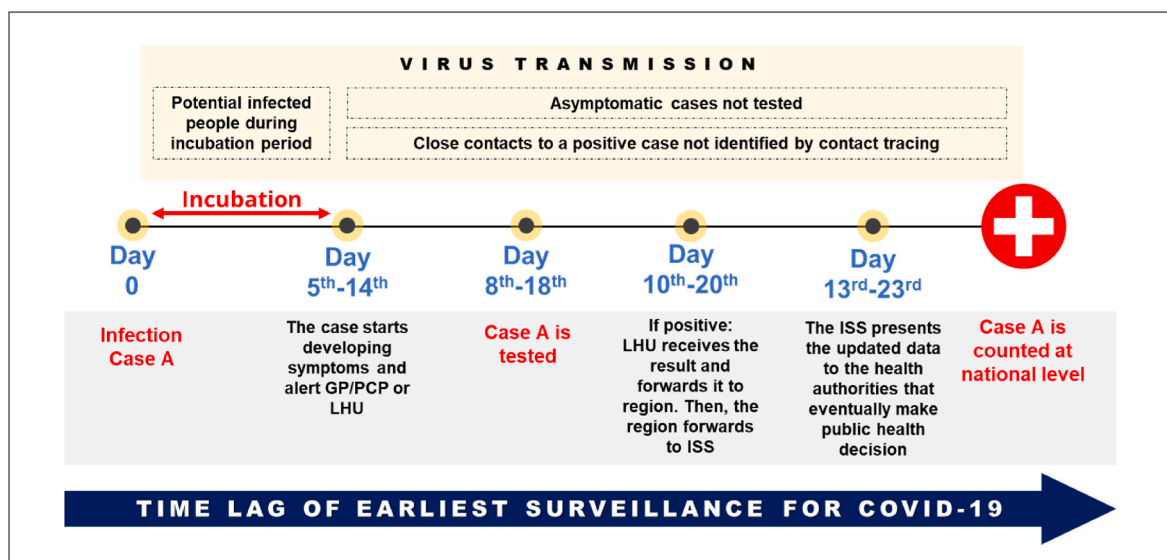


Fig. 4. Time lag of earliest microbiological and epidemiological surveillance for COVID-19 and potential virus transmission during the notification process to The Italian Institute of Health (ISS) (LHU: local health unit; GP: general practitioners; PCP: primary care pediatricians) (source: Epicentro, ISS, <https://www.epicentro.iss.it/en>).

structure of national public health systems despite the improvement of clinical testing capacity (Kumar et al., 2022).

Even at high laboratory testing capacity, a comprehensive massive screening through clinical testing characterized by individual testing size is time-consuming, prohibitive in terms of extreme high costs and it may lead to overwhelm medical care system (Daughton, 2020; Hart and Halden, 2020a). Conversely, after the isolation of target virus and the standardization of WBE procedure, the virus spreading in a community may be continuously monitored by analyzing limited and localized sampling points tracking any potential outbreak (or resurgence) with a potential zooming on sensitive and high-density infrastructures (e.g., hospitals, schools) (Thompson et al., 2020; World Health Organization, 2020b).

Moreover, WBE is able to cover a broad spectrum of population including also the contribution of asymptomatic and mildly symptomatic individuals. Based on the current routine of diagnostic process, a diagnostic swab test is usually carried only after symptoms development and consequently, some asymptomatic cases may not be tested providing a fragmented and incomplete epidemiological situation at local/regional and even at national level. However, people that are reluctant to get tested or have less access to clinical testing are not accounted for (Shrestha et al., 2021; Thompson et al., 2020). Indeed, it was estimated that the unreported cases represent about 40–50% of all infections and this percentage could be higher in small communities (Hart and Halden, 2020a; Qiu, 2020; Schmitz et al., 2021). Several studies have been recently performed to systematically compare the number of infected cases reported by clinical testing with SARS-CoV-2 RNA concentration in wastewater samples highlighting the contribution of asymptomatic cases (Cluzel et al., 2022; Petala et al., 2022; Schmitz et al., 2021). It was demonstrated by Petala et al. (2022) that the ratio of unreported cases (e.g., asymptomatic and mildly symptomatic individuals, people being reluctant to seek medical care) to reported infected people by clinical testing varies proportionally with number of reported cases. According to the model developed, a rising of unreported cases could lead to an earlier wastewater signal than medical surveillance based on clinical testing (Petala et al., 2022). Moreover, Schmitz et al. (2021) pointed out the role of wastewater monitoring to successfully reduce the SARS-CoV-2 transmission events at the student dormitories of the University of Arizona. Indeed, the positive wastewater samples have triggered the clinical testing inside the community, which revealed a plentiful of asymptomatic cases (~80% of all

infections) otherwise not accounted for. These findings displayed the capacity of WBE as early detection tool also in the presence of an increased number of unreported cases.

Contrary to clinic tests that are invasive and debilitating in the long run, WBE approach is non-invasive and anonymous, and it allows the quick identification of infection clusters located in a distinct area, preventing the domino effect caused by a fast virus infection rate (Daughton, 2020; El-Baz et al., 2020; Randazzo et al., 2020).

In case of severe and acute virus transmission and when the number of infected people increases daily, the mass screening through diagnostic tests will certainly cause an over-saturation of local health services (e.g., drive-through test centers) leading also to an expected risk of human-to-human transmission since people potential or even already infected with symptoms are allowed to move for getting tests (Daughton, 2020). Moreover, the contact tracing has been a key strategy for breaking the chains of transmission and consequently disease-associated mortality (World Health Organization, 2020c). However, the process of identifying and assessing people who have been exposed to infected people is neither fast nor often feasible due to issues related to backward tracing (i.e., infection history such as the identification of infection source and potential contacts) and forward tracing (i.e., monitoring the identified contacts such as daily follow-up of signs and symptoms) (Daughton, 2020; World Health Organization, 2020c). Indeed, in most infection scenarios due to the complexity of backward tracing, prioritization for follow-up is given to contacts with higher risk of infection based on exposure degree (mainly relatives and work/study colleagues) and to contacts with some pre-existing pathology considered vulnerable people.

Conversely, the surveillance capacity is feasible and dynamic with WBE approach also allowing the investigation of temporal trends without resorting to contact tracing, which is sometimes an unreliable source. Indeed, some sources of data bias in the clinical testing are the close contacts to positive cases not identified by the contact tracing, false positives and asymptomatic (or mildly symptomatic) cases. Whereas data collected through WBE approach could be affected by the seasonal variability of MWW parameters (e.g., temperature, flow rate, turbidity) and by some extreme weather conditions (e.g., storms) (Hart and Halden, 2020a; Kevill et al., 2022; Kumar et al., 2022).

The cost of both clinical and WBE screening vary worldwide due to different labor costs, availability of laboratory equipment, and existing infrastructures (Hart and Halden, 2020a; Kitajima et al., 2020; Shrestha

et al., 2021). Moreover, it is noteworthy that during a sanitary emergency the resources are limited and consequently, the rational use of those available such as reagents, swabs, and medical staff is paramount. Indeed, the economic advantage of WBE is significant. Overall, the cost for WBE surveillance reagents would constitute only 0.0014% of those required for clinical testing of individuals and the per capita cost of WBE is lower than clinical testing even in rural areas with an estimated cost of \$0.10 per person per week (Hart and Halden, 2020a; Weidhaas et al., 2021). Stigma and discrimination on infected individuals typically reported in little communities are avoided through WBE guaranteeing the respect of individual privacy (El-Baz et al., 2020; World Health Organization, 2020c). Furthermore, the massive screening through clinical testing is not only time-consuming and expensive but also involves a high number of medical staff. Conversely, through the WBE approach, the medical staff could exclusively focus on sick patients, particularly during the pandemic peaks (Ahmed et al., 2022). Social distancing is always guaranteed by applying the WBE approach, while it may be not ensured during clinical massive screening due to potential gatherings at medical centers. Based on the updated local map of virus circulation, WBE allows the minimization of unnecessary long stay-at-home policies (such as lockdown) that contribute to increase human stress and to negatively impact the economy of local area (D'Aoust et al., 2021; El-Baz et al., 2020). Data from WBE approach could usefully support the strategic planning through the short-term implementation of *ad hoc* measures (Medema et al., 2020).

Furthermore, WBE capability has been recently investigated as an early warning system also for variants of concern (VOCs) occurrence inside a community. Indeed, VOCs are commonly characterized by high transmissibility and virulence/clinical disease presentation interfering with the implemented diagnostic assays and public health measures, as well as the effectiveness of vaccines and therapeutics (Oloye et al., 2022; World Health Organization, 2022). When the sequencing capacity of all clinical cases is limited, the monitoring of SARS-CoV-2 infections through WBE could enhance the prompt identification and rapid estimation of virus load also in presence of VOCs complementing the national testing efforts. Monitoring studies have pointed out the detection of a specific VOC (e.g., Omicron and Delta) in MWW prior to its recognition through clinical testing (Chassalevris et al., 2022; Oloye et al., 2022; Reynolds et al., 2022). Specifically, WBE was able to identify the emergence of a variant (Omicron) inside a community (Thessaloniki, Greek city) earlier than its identification via routine surveillance based on clinical samples preceding the first declared Omicron case by approximately 7 days and highlighting the Delta-to-Omicron BA.1 transition patterns (Chassalevris et al., 2022). Similarly, other findings have shown the occurrence of the Alpha variant in Dublin MWW before the sequencing of the first genome in clinical sampling, while the Delta variant was recorded almost simultaneously on both clinical and wastewater samples (Reynolds et al., 2022).

Overall, several criticisms related to clinical testing and the WBE approach could be highlighted, and they were briefly summarized in the last row of Table 1. The limitations of clinical testing are well known and extensively documented. Moreover, those drawbacks are derived from clear, objective, and foreseeable issues, for example, the testing scale and costs. Consequently, overcoming those limitations is unfeasible as well as improving the effectiveness of clinical testing in massive screening by reducing the turnaround times and cost. In this context, antigen rapid test kits (RTKs) through saliva sampling have been proposed as alternative assays to rhino-pharyngeal swabs making them ideal for massive screening (Ahmed et al., 2022; Canete et al., 2021). However, studies are still ongoing to assess the accuracy and performance efficiency of RTKs since the key aspects of saliva sample collection, storage and handling should be carefully considered to obtain reliable and comparable results to rhino-pharyngeal swabs (Ahmed et al., 2022).

### 3.2. Main challenges in WBE design and implementation

Despite its promising application, worldwide application of the WBE is still limited due to the numerous shortcomings and flaws, which affect the accuracy and trustworthiness of WBE results. To date, the main challenge in introducing WBE as environmental surveillance tool is the need for the standardization of procedure, which includes the development of protocols for several steps from MWW sampling to virus quantification. Indeed, the analytical method of viral quantification, the long-term monitoring, the frequency of wastewater sampling, and the variability of MWW are currently the most investigated topics (Cervantes-Avilés et al., 2021; Foladori et al., 2020; Weidhaas et al., 2021). For instance, most studies have employed RT-qPCR for SARS-CoV-2 quantification in wastewater although the detection efficiency of RT-ddPCR was also demonstrated at low viral load. Which analytical method employed for SARS-CoV-2 quantification is currently a topic very debated (Alygizakis et al., 2021; Cervantes-Avilés et al., 2021; Kevill et al., 2022). A reasonable long-term monitoring during WBE study is necessary to test the accuracy of obtained data and to comprehensively identify the relationship between concentrations of SARS-CoV-2 RNA in MWW and rates of COVID-19 cases in the corresponding communities (Li et al., 2021; Weidhaas et al., 2021). Moreover, in the WBE design and subsequent implementation, the frequency of MWW sampling should be accounted for, as pointed out by several studies (Cluzel et al., 2022; Graham et al., 2021; Huisman et al., 2022). Overall, the obtained findings regarding the impact of sampling frequency on the representativeness of the results show that the frequency of sampling up to 3 days per week provides results comparable to a daily sampling. Below this value (e.g., 1 day per week) the robustness of findings decreases and the results might depend on which days were selected for the MWW sampling (Cluzel et al., 2022; Graham et al., 2021; Huisman et al., 2022).

Lastly, the effect of seasonal variability of MWW on WBE data is currently under investigation (Foladori et al., 2020; Hart and Halden, 2020a, 2020b). For instance, it was demonstrated that MWW temperatures lead to under-/over-estimation of infected cases making the WBE data unreliable (Hart and Halden, 2020a, 2020b). The MWW temperatures mainly affect the in-sewer travel time of the target virus. A study conducted in Detroit (USA) pointed out that the tolerable duration of the virus was about 100 h and 20 h in winter and summer, respectively (Hart and Halden, 2020a). Generally, the time required for the virus titer to decrease by 90% (T90) is high at low MWW temperatures (Foladori et al., 2020; Hart and Halden, 2020b). Other factors affecting the virus concentration/extraction such as the MWW turbidity and the presence of surfactants are still under investigation due to the potential increase in inhibitors factors (Kevill et al., 2022).

## 4. Development of guidelines based on WBE approach for monitoring future pandemics

In the framework of innovative and multidisciplinary approach, novel guidelines of WBE approach for future pandemic have been developed and presented in this section. Specifically, the learned lesson from COVID-19 pandemic as well as the fundamental principles of WBE were involved to develop a step-by-step methodology for preventing and tracking future pandemic. The proposed guidelines can be categorized into six steps and related actions as summarized in Table 2.

### 4.1. G1) Prevention plan

The aim of the prevention plan is to continuously monitor the health status of a community within catchment boundaries. In this framework, the prevention plan should be implemented based on the available resources and testing capacity. Moreover, it should need to be regularly updated to comply with public health needs. The prevention plan also requires the engagement of WWTPs in order to develop an accurate monitoring strategy accomplishing all the procedures needed for



**Table 2**  
Proposed guidelines of the step-by-step WBE approach for pandemic control.

Phase	Action
<b>G1) Prevention plan</b>	<ul style="list-style-type: none"> <li>- Wastewater monitoring strategy (including selection of biomarkers and sampling campaign).</li> <li>- Development of protocols for testing methods.</li> <li>- Creation of analytical laboratories network.</li> <li>- Introduction of local and national experts specialized in WBE.</li> </ul>
<b>G2) Identification of novel pathogen</b>	<ul style="list-style-type: none"> <li>- Alert public health authority.</li> <li>- Collaboration inside the analytical laboratories network to develop analytical protocol.</li> <li>- Coordination with local/national surveillance and health authorities (multidisciplinary task force).</li> <li>- Collaboration between selected laboratories and health authorities.</li> <li>- Indicators for the capacity to conduct testing at scale</li> </ul>
<b>G3) Implementation of WBE approach for mass screening</b>	<ul style="list-style-type: none"> <li>- Data collection from WWTPs</li> <li>- Comparison of results obtained from wastewater analysis at different sites (from local to worldwide labs).</li> <li>- Perform clinical testing based on spreading rate</li> </ul>
<b>G4) Application of WBE in selected areas and critical infrastructures</b>	<ul style="list-style-type: none"> <li>- Zoom on sensitive and high-density infrastructures (e.g., schools, prisons, hospitals, health care facilities, and airports).</li> <li>- Attention to areas with poor sanitary conditions and “high-risk” populations</li> <li>- Identify the hot-spot areas.</li> <li>- Zoom on hot-spot areas (selected sewer sampling points) to find the cluster and perform clinical testing.</li> </ul>
<b>G5) Data elaboration for the dynamic tracing of a disease</b>	<ul style="list-style-type: none"> <li>- Data from WBE are complemented with clinical testing results.</li> <li>- Normalization to compare viral WW concentration over time and across locations.</li> <li>- Statistical analysis for trend calculation.</li> <li>- Modeling for short- and long-term prediction through prediction models.</li> <li>- Promote the collaborative learning.</li> </ul>
<b>G6) Implementation of <i>ad hoc</i> mitigation and prevention strategies tailored to WBE and epidemiological risk assessments</b>	<ul style="list-style-type: none"> <li>- Introduction of control measures that are site-specific and tailored to WBE results and real-time epidemiological risk assessment.</li> <li>- Preservation and rational use of available resources.</li> <li>- Evaluation the effectiveness of introduced measures at different action radius and temporal stages.</li> <li>- Monitoring any re-emergence of virus and countering the rapid rise of infections.</li> </ul>

sampling campaign. Background information related to the characteristics of sewer system, the population served, the WWTPs operating factors and data (e.g., chemical/physical water quality measurements, flow rate measurements) should be accounted for monitoring strategy.

In the prevention plan, a particular focus should be posed on the selection of biomarkers to track in WW. Indeed, it is paramount that biomarkers are stable also during the sampling and storage phases, they must be at proper concentration for accurate detection in WW, and they should come from human metabolism whose process is known (Choi et al., 2018). The latter is essential to discern them from exogenous ones mainly derived from external contamination in the sewage system and from other microbial communities that characterize biological treatment at WWTPs.

Considering the several criteria to be satisfied (e.g., stable and detectable in MWW, low variance in the daily excretion, not influenced by seasonal and geographic variations) and the extent of existing biomarkers, their selection could be very challenging (Gracia-Lor et al., 2017). Consequently, a benchmarking analysis is a useful preliminary step in the development of the prevention plan, ensuring the scientific and clinical meaningfulness of candidate biomarkers. Based on the obtained results, a panel of biomarkers is selected to provide objective insights on community health status, complementing it with traditional water quality parameters. Among human health biomarkers, antibiotics and the related metabolites could be included in the list since their use is related to bacterial infections. Promising findings on virological surveillance could be achieved through DNA-based health biomarkers (Choi et al., 2018).

In order to ensure that the monitoring studies performed worldwide provide comparable results, protocols for testing methods (i.e., sample processing, preparation and concentration, and the RNA extraction and measurement) should be also implemented. In this regard, the creation of an analytical laboratories network is necessary to guarantee the robustness of the analytical procedure for monitoring MWW and to attain the maximum reliability of analytical results. According to the precaution recommendation, Biosafety Level 3 (BSL-3) laboratories owing virus isolation, inactivation and cultures should be involved in this network. Moreover, the introduction of experts specialized in WBE is crucial to develop a national surveillance database constantly updated and to build an effective interlocution with surveillance and health authorities. WBE experts would play a key role to bridge the gap between analytical laboratories network nationwide and the health authorities. Consequently, the communication channels between WBE experts (at national, subnational, and local levels) and the health authorities should be strengthened.

Moreover, to validate the flexibility of prevention plan and to test the strategic and operational response, pandemic simulation exercises need to be periodically conducted.

Several preparedness measures are paramount to the correct implementation of the prevention plan. For instance, they may include the designation of both WBE experts at both local and national levels, the stockpile of products and equipment essential to deal with a public health emergency at multiple sites distributed across the country, and the training of multi-purpose health personnel.

All the measures included in the prevention plan should be defined in protocols to enable timely follow-up.

#### 4.2. Identification of a new pathogen

After an emergency such as the detection of a new pathogen, the analytical laboratories network would act for the prompt and quick identification of the new pathogen and, consequently, for the tuning of viral RNA detection and quantification. Indeed, a meticulous approach is needed for the assessment of viral RNA sequences, the potential transformation and/or its ability to retain viability. In this regard, the newly identified pathogen is promptly included in the prevention plan (G1) updating the panel of biomarkers. The analytical protocol must also include information regarding the sampling procedure due to the strong fluctuations in terms of flow rate and pollutants concentration in the influent MWW during the day. Advises on samples handling (temperature and light conditions) and RNA isolation should be also included since they could affect the quantification of viral load and its viability. Moreover, a process control is strongly recommended to fully understand viral recovery and consequently, the amount of virus lost during sample processing. The intra- and inter-laboratory assessment on sampling regimes and molecular methods, the evaluation of WW pre-treatment effect on genetic signal and the understanding of potential dilution and persistence of the genetic signal in the sewer collection system could be mentioned in the checklist of prioritized tasks.

After its development, the analytical protocol for the detection and

quantification of the newly identified pathogen in MWW will be disseminated to other accredited national laboratories. The attained result of this step is the inclusion of the newly identified pathogen in the list of target compounds in order to quickly monitor its occurrence and eventually, its spread in different communities and/or sensitive infrastructures dislocated nationwide. Consequently, indicators should be properly developed for evaluating the capacity of each laboratory belonging to the network to conduct testing at scale. Moreover, the cooperation with health authorities is strongly recommended to promptly alert the public health regarding the potential outbreak of the newly identified pathogen. Indeed, created with the scope of enhancing coordination, the multidisciplinary task forces would play a key role in communication efforts across government units.

#### 4.3. Application of WBE for mass screening

After the development of the analytical protocol and the coordination of accredited national laboratories with the official analytical lab network, the next step is represented by the mass screening of a community through WBE approach. Indeed, in the case of newly detected pathogen, the mass screening should be quick and with a broad spectrum. Consequently, the occurrence of new pathogen and its spread could be feasible by analyzing the influent of WWTPs. At this stage the standardization of sampling analysis, data collection, and statistical interpretation is paramount and fundamental to successfully apply the WBE approach and to track the epidemiological “picture” of the community. Moreover, the investigation of the temporal trend and level of infection in a target community could be also estimated through the backward approach. Indeed, based on evidence of pathogen circulation, the prediction of re-emergence viral transmission could be feasible. The overall health status of a community might be obtained by integrating the results of WBE approach, able to include the contribution of infected but asymptomatic individuals, with clinical observations, mostly focused on patients with symptoms. Epidemiological data obtained from mass screening through WBE approach should be collected and health authorities will be updated regularly with weekly reports. Communication channels and tools that are mainly computer-based and already implemented should be operated to enhance timely and effective coordination.

Meanwhile clinical testing should be performed if the collected data revealed a fast virus spreading inside the community. Consequently, correlations to clinical data for the assessment of community prevalence should be identified.

#### 4.4. Application of WBE in selected areas and critical infrastructures

When the detection of newly identified pathogen is extensively documented, a targeted WBE is needed in order to identify hotspot areas. Therefore, a sampling campaign at specific locations in the WW network (e.g., manholes outside buildings) is required to focus on sensitive and high-density infrastructures (e.g., schools, prisons, airports, hospitals, and healthcare facilities). Consequently, the application of WBE approach in selected areas is crucial to develop an epidemiological connection with a confirmed outbreak providing complementary data to the mass screening (G3). Indeed, the crowded infrastructures could be defined as high risk since the virus spreading and regrettably the transmission is faster and more dangerous due to the presence of frail people. Moreover, a zooming of the infected population can be obtained by collecting wastewater samples at sub-catchment of the sewerage system in order to identify potential clusters. Finally, it should give particular attention to areas with poor sanitary conditions (high density/low resource setting) and vulnerable/“high-risk” populations (e.g., health workers, elderly people, people with comorbidities that increase the risk of severe disease), and all other places where conditions might favor fast disease transmission.

#### 4.5. Data elaboration for the dynamic tracing of a disease

The application of G3 and G4 will allow the data collection through a cost- and time-effective screening approach (few samples comprise a pool of many individuals), which should be complemented with the results from clinical testing.

Based on the dynamic nature of WBE, the trend of the new pathogen spreading in a selected area and/or in a whole community is constantly monitored, and any space-time variation is tracked. This information will anticipate the clinical testing results and will be essential to depict an updated epidemiological map of the disease, which will be under constant review by WBE specialists.

A normalization step should be performed to compare the viral concentrations over time. Particularly, this normalization should account for the changes in WW contributions providing data in units of viral gene copies per day. Moreover, in order to compare the viral levels in different areas (such as considering different sampling locations) the viral concentration is normalized by the number of people served by the sewer system, obtaining data in units of viral gene copies per person per day. Furthermore, the forecasting capacity of WBE could be enhanced by normalizing the measured viral concentration with human waste indicators (e.g., fecal coliform bacteria concentration). As recently investigated, the use of fecal coliforms as normalization target for SARS-CoV-2 concentrations improved the correlation with COVID-19 cases at campus scale, consequently further investigations on the development of normalization targets was advised (Zhan et al., 2022).

The virus trends can be calculated through a statistical analysis assessing the changes in the normalized concentrations. As a result, data from different WWTPs and related to different locations are analyzed despite differences in population size and WW volume. For better evaluation of WW data, the variability in each measurement is also incorporated using weighted least squares regressions in order to encompass the variability in the sampling, processing, and quantification phases.

The static validation of the collected data is strongly required to corroborate the feasibility of proposed guidelines. In this regard, in order to strengthen the applicability of WBE approach for tracing the pathogen circulation, some variabilities should be included such as the knowledge of community attitude and behavior including the timeline of travels (especially daily commuting for business and education purposes). Other items to consider are for example the presence of recreational activities which may attract tourists and other non-resident people in the investigated area. Consequently, the population behavior and other information inferred from updated census data should be well known and accurately included in the prevention plan (G1).

Indeed, the deep knowledge of the target community (G1), the protocol validation of the entire analytical protocols from sampling to viral detection (G2), and the statistical interpretation of the findings through mathematical models (G5) are essential tasks to guarantee accuracy, robustness, and comparability of collected results along time and across different communities.

A set of indicators and related scoring system or scale are created to allow effective data visualization. In this regard, the maps with color coded symbols showing the recent trend of target virus could be useful.

Models for short- and long-term prediction will be developed allowing a further WBE data elaboration. A time series analysis can be applied to predict future re-emergence of the virus, while the machine learning (ML) can be accurately developed to the prediction of medical risks. For instance, ML could predict the risk for human to be infected, in developing severe health complications and in pinpointing the species acted as hosts for the disease. Moreover, ML can help to predict the interactions between viral proteins and drugs already tested for human safety but currently used to treat other diseases.

Undoubtedly, the periodic meetings promote the collaborative learning among health departments, WW utilities, laboratory networks could help shape best practices, share practical suggestions, and

troubleshoot challenges.

#### 4.6. Implementation of *ad hoc* prevention and mitigation strategies sized on WBE and epidemiological risk assessments

The continuously updated “portrait” of the health situation of a community provided by WBE represents a key finding to prevent a potential pandemic. Consequently, the obtained information might be paramount to guide the health authorities in adopting measures of control of virus spreading and transmission. Some of them are currently included in the pandemic plans, which are suggested by WHO (World Health Organization, 2020c), although their application at national, and local level are in an early stage.

Based on the updated information provided by WBE, the health and surveillance authorities supported by WBE specialists can establish *ad hoc* and stepwise prevention and mitigation strategies, which might act locally and/or nationwide. To ensure the flexibility, these *ad hoc* strategies should be based on epidemiological risk-assessment allowing the implementation of site-specific measures at different temporal stages of a pandemic and the progressive introduction of restrictions aimed at promoting physical distancing.

The most effective prevention and mitigation measures/strategies could be summarized as follow:

- Introduction of national guidance on personal protective practices (e.g., hygiene measures, personal protective equipment).
- Mass screening through individual testing of districts and sensitive infrastructures, which have been recognized as hot-spot areas.
- Introduction of public health orders to limit person-to-person transmission such as social distancing measures (e.g., closure of recreational activities, banning of mass gatherings) and other restriction measurements (e.g., stay-at-home orders, curfew, lockdown).

The most severe actions to limit the virus transmission such as the restrictions of stay-at-home orders, nonessential business closure, and lockdown should be established for the highest scenario risk (i.e., domino effect, which causes the over-saturation of health systems) due to the adverse socio-economic impacts on society and daily life disruption.

The implementation of site-specific mitigation and prevention strategies allows the preservation and the rational use of available resources such as reagent and consumables for individual testing. Meanwhile, the effectiveness of the introduced measures of control can be evaluated based on the time-space monitoring of virus through WBE approach. The latest also enables to monitor any re-emergence of the virus inside a community and, eventually, to counter the rapid rise in infections.

## 5. Conclusion and final remarks

Initially developed for tracing the illicit drug occurrence, WBE could be successfully applied as early warning tool for infectious virus presence and to prevent virus outbreak by promptly applying strategic plans with prevention actions. Indeed, WBE can be used for several purposes such as an early and quick indicator of population infection (i.e., environmental surveillance tool) and a decision-making support to guide the health authorities’ decisions related to restraint and mitigation measurements. It is evident that WBE approach could provide site-specific and timely data to quickly assess the magnitude of sanitary emergency avoiding the exponential increase of infected people, the collapse of medical health facilities and consequently the related mortality rate. Indeed, as recently experimented during COVID-19 pandemic, the time in collecting data (including clinical testing and contact tracing system) regarding virus spreading in a community is crucial for deciding the prevention and mitigation measures.

The scientific papers published on this research topic increased

sharply suggesting the growing interest on WBE approach for COVID-19 pandemic. Deeply, the keywords frequently utilized in the publications and their nexus were analyzed through VOSviewer software and the co-occurrence analysis map discerned the WBE applications between “past” and “present” since one cluster gathered the keywords related to WBE application for tracing both illicit and licit drugs, while the other encompasses keywords related to SARS-CoV-2 and COVID-19. Moreover, the most cited keywords related to quantification/concentration viral methods revealed the main burning issue, which negatively affected the accuracy of WBE and consequently its worldwide application as early warning tool in the COVID-19 pandemic.

The side-by-side comparison between clinical testing and WBE approach emphasized the following aspects:

- With more affordable costs comparing to clinical testing, WBE could provide a real-time monitoring of virus spreading in a community with a potential zooming on sensitive and high-density infrastructures (e.g., hospitals, schools) quickly identifying cluster and virus re-emergence.
- WBE covers a broad spectrum of population also including the contribution of asymptomatic (and mildly symptomatic) individuals and who are reluctant to get tested or have less access to medical services.
- Massive screening through clinical testing is not only time-consuming and expensive but also requires a high number of medical staffs involved with a potential risk to be infected during testing.
- At fast virus spreading, the weakness of contact tracing in identifying the close contacts to infected people should be mentioned as one of main sources of data bias on clinical testing contributing to the increase of time lag in collecting data.

The advantages of WBE during a pandemic spreading are extensively documented and they were recently confirmed by the COVID-19 pandemic. Indeed, WBE can be used to test large communities (cities) at minimal cost and controlled risk as well as to control the pandemic spread at critical infrastructures, such as hospitals, and at neighborhood level. WBE will anticipate data on infected population that can lead to the identification of population to be tested individually and to the adoption of measures at cluster level that can minimize the pandemic spread. The latest represents an important improvement compared to the current approach based on clinical testing.

The WBE approach is rapidly evolving for environmental surveillance of COVID-19 pandemic and numerous studies are ongoing to address several questions in different fields. Specifically, the following research needs are arising:

- **Biologic**, understanding of (i) the persistence of SARS-CoV-2 RNA fragments in sewage, (ii) the viral shedding across all infection stages, (iii) the correlation between faecal excretion and detection through environmental surveillance.
- **Technical**, developing of standardized protocols dealing with collection timing, time integration, sample conservation, pre-treatment, identification of appropriate virus surrogates for process control, concentration and extraction methods, quality assurance. Efforts are needed (i) to quantify the limits of detection and quantification, false-positive and false-negative rates, (ii) to develop validate analytical procedures for testing areas without sewage systems, (iii) to understand the effect of MWW physical/chemical characteristics (e.g., turbidity, temperature), (iv) to identify an appropriate SARS-CoV-2 surrogate marker.
- **Epidemiologic**, the development of standardized protocols is strongly recommended also to identify the best location for sampling points and to define the epidemiological data management in view of the integration between WBE and clinical testing. Separate observations should be carried out for tourists and passers-by by keeping track of their contribution with specific measures.

- **Economic**, detailed evaluation of costs and benefits ascribed to the environmental surveillance through WBE also including limitations and shortcomings.
- **Other**, training WWTP and sanitation workers to adequately adopt prevention measures during sampling campaigns.

To sum up, the proposed guidelines developed on the lessons learned from COVID-19 pandemic are helpful to develop a step-by-step WBE approach for pandemic control. They suggest a checklist of activities to be undertaken from the continuous monitoring of health status inside a community (G1, prevention plan) to the implementation of *ad hoc* mitigation and prevention strategies, passing through the WBE data collection (G3 and G4), the integration with clinical testing and the development of prediction models (G5).

The main findings of the present study are useful to guide the development of an integrated surveillance tool that is dynamic and promptly implemented, enhancing effective prevention efforts, timely preparedness, and careful resilience against future pandemics.

Shared knowledge between the medical community and wastewater surveillance practitioners as well as the inter-disciplinary collaborations with analytical chemists will provide fruitful information to develop an integrated approach based on WBE and clinical testing that undoubtedly will support the health authorities.

#### Credit author statement

Erica Gagliano: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. Deborah Biondi: Investigation, Writing – review & editing. Paolo Roccaro: Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision, Funding acquisition.

#### 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.

#### Data availability

Data will be made available on request.

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