



Review article

Wastewater surveillance for viral pathogens: A tool for public health

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ABSTRACT

A focus on water quality has intensified globally, considering its critical role in sustaining life and ecosystems. Wastewater, reflecting societal development, profoundly impacts public health. Wastewater-based epidemiology (WBE) has emerged as a surveillance tool for detecting outbreaks early, monitoring infectious disease trends, and providing real-time insights, particularly in vulnerable communities. WBE aids in tracking pathogens, including viruses, in sewage, offering a comprehensive understanding of community health and lifestyle habits. With the rise in global COVID-19 cases, WBE has gained prominence, aiding in monitoring SARS-CoV-2 levels worldwide. Despite advancements in water treatment, poorly treated wastewater discharge remains a threat, amplifying the spread of water-, sanitation-, and hygiene (WaSH)-related diseases. WBE, serving as complementary surveillance, is pivotal for monitoring community-level viral infections. However, there is untapped potential for WBE to expand its role in public health surveillance. This review emphasizes the importance of WBE in understanding the link between viral surveillance in wastewater and public health, highlighting the need for its further integration into public health management.

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Abbreviations

ACE2	angiotensin-converting enzyme 2
HAV	hepatitis A
HEV	hepatitis E
hAdV	human adenovirus
MCTI	Ministry of Science, Technology, and Innovation
MPXV	monkeypox virus
NWSS	National Wastewater Surveillance System
NPSs	new psychoactive substance
NoV	noroviruses
NSAIDs	nonsteroidal anti-inflammatory drugs
PEG	polyethylene glycol
PHEIC	Public Health Emergency of International Concern
qnrS	quinolone antibiotic resistance gene
SWEEP	Surveillance of Wastewater for Early Epidemic Prediction
WaSH	water-, sanitation-, and hygiene
WBE	Wastewater-based epidemiology
WWTP	wastewater treatment plant (WWTP)

1. Introduction

Public attention and concern for the environment has become increasingly prominent in recent years, particularly in relation to water resources, emerging as a significant societal issue [1]. The proportion of the population with access to safely managed sanitation increased from 47 % to 54 % between 2015 and 2020, despite the global recognition of efforts to improve drinking water facilities [2]. However, current estimates suggest that over 2 billion people lack improved water sources, and more than 3.5 billion lack access to proper sanitation [3]. Socioeconomic and environmental factors exacerbate this crisis, contributing to the degradation of water and sanitation infrastructure, especially in developing regions, exposing billions to water, sanitation, and hygiene (WaSH)-related diseases [4].

The discharge of untreated wastewater in urban areas presents a significant challenge. According to UN-Water, in poor urban regions, a substantial amount of wastewater is released directly into nearby drainage channels or water bodies without any treatment. This unprocessed effluent contains household waste, human excrement, hazardous chemicals, and medical refuse, leading to environmental contamination, particularly in densely populated residential zones [5]. While access to safe drinking water, sanitation, and hygiene is vital for improving living standards, it also plays a crucial role in safeguarding both public health and the environment [6]. As countries strive to enhance their sanitation coverage, it is imperative that they minimize the release of untreated sewage into the environment by harnessing the energy and nutrients contained within it [6].

Water, despite being a recyclable resource, requires effective management to ensure a secure supply and prevent the spread of pathogenic organisms and contaminants [7]. While advancements in water treatment processes have led to a substantial decrease in the occurrence of waterborne diseases [8], the discharge of untreated or poorly treated wastewater into water bodies remains a threat to both water quality and availability, increasing the potential for the spread of pathogens [4,9].

In this context, Wastewater-Based Epidemiology (WBE) serves as a complementary surveillance tool, particularly for monitoring community-level infectious diseases caused by viruses and other microorganisms [10]. WBE serves as a valuable tool for the timely identification of potential outbreaks, monitoring temporal and geographical trends in infectious diseases, gathering comprehensive epidemiological data in specific regions, especially within socially vulnerable areas, and providing real-time insights into the situation [11]. It is distinguished by its heterogeneous composition of biological materials, including wound materials and various bodily fluids, such as respiratory and nasal secretions, saliva, urine, feces, and semen from infected individuals (symptomatic, asymptomatic, presymptomatic, and postsymptomatic) [12].

Conventional public health surveillance strategies rely on the confirmation of suspected cases through clinical, epidemiological, or laboratory outcomes, which limits the identification of asymptomatic and presymptomatic individuals [10]. Conversely, WBE provides a methodology to analyze the composition, detect waste, and quantify pathogens in wastewater [13]. Initially, utilized for poliovirus surveillance [14] and detection of illicit drugs and various chemicals [15], researchers have since applied WBE to screen a wide range of microorganisms, further expanding its scope during the COVID-19 pandemic [16].

WBE facilitates a real-time assessment of community health status, exposure levels to different substances and microorganisms, and tracking of pathogen sources by tracing pipelines and delimiting geographic areas [17]. Additionally [18], it is a valuable tool for issuing early warnings about infectious disease outbreaks through wastewater monitoring, providing epidemiological data for precise interventions by governments and health entities [17].

Despite its advantages, WBE encounters obstacles such as legal constraints [19], technical adjustments [20], and data variation due to multiple factors (e.g., rainfall [21], age [22], infection prevalence [23], and waste discharge [24]). Therefore, further understanding, refinement, and global collaboration are essential to advance the technical processes involved in WBE [18].

Policy and regulatory frameworks are crucial for effective WBE programs for infectious disease surveillance [25,26]. The U.S. National Wastewater Surveillance System (NWSS) exemplifies growing recognition of wastewater monitoring's importance [27]. Collaboration between federal agencies and global initiatives like Surveillance of Wastewater for Early Epidemic Prediction (SWEEP) enhances outbreak resilience [28]. Despite WBE's promise, ethical guidelines are lacking, emphasizing the need for considerations like privacy protection and responsible data use.

This literature review aims to explore the current state of wastewater monitoring for viral pathogens, highlighting its benefits, challenges, and applications as a public health tool.

2. Water quality

Sewer networks (SNs), also known as wastewater networks, constitute a fundamental component of urban infrastructure, serving as conduits for wastewater and stormwater to wastewater treatment plants (WWTPs) for processing or disposal [29]. These networks play a pivotal role in safeguarding public health and safety, preserving the urban water environment, mitigating the risk of waterborne diseases, and averting urban floods [30]. However, the efficacy of SNs is increasingly being challenged by a myriad of factors such as population expansion, climate variations, alterations in pollutant discharge patterns, and evolving human activities [29,31,32].

The ramifications of these challenges on water quality are significant, as underscored by the outbreaks of infectious diseases linked to extreme weather events, often exacerbated by compromised drinking water supplies and deficiencies in water supply and sanitation [33,34]. The World Health Organization (WHO) estimates a substantial toll on human lives due to diarrheal diseases attributable to inadequate water, sanitation, and hygiene (WaSH) practices, particularly in low- and middle-income countries [35,36].

Understanding the intricate interplay between climate change and water quality is paramount. Long-term shifts in temperature, precipitation patterns, and sea levels can exert profound stressors on water resources, thereby affecting availability and quality [34]. Furthermore, warmer ambient temperatures can influence the survival and proliferation of pathogens in water sources, with

Table 1

Examples of potential human biomarkers from public exposure and lifestyle for public health monitoring through WBE.

Biomarkers	Type	Description	Sample	References	
Public exposure biomarkers					
Triazines	Chemicals	Pesticides	Urine	[54–56]	
Pyrethroids				[54–56]	
Organophosphates				[54–56]	
Phosphorous flame retardants and plasticizers				Industrial chemicals commonly used in consumer goods	[57]
Bisphenol A				Industrial chemical commonly used in plastics	[58]
N,N-diethyl- <i>meta</i> -toluamida		Chemical compound present in insect repellent		[60]	
Enteroviruses	Pathogens	Nonenveloped pathogenic virus	Wound materials, respiratory and nasal secretions, saliva, urine, feces, and semen	[73]	
Norovirus				[74,75]	
SARS-CoV-2				Enveloped pathogenic virus	[78]
Monkeypox				[76,77]	
Cryptosporidium		Enteric protozoan parasites	Feces	[79]	
Giardia					
<i>Candida auris</i>		Opportunistic fungal pathogen	Skin, wounds, blood, sputum, urine and feces	[80]	
<i>Campylobacter jejuni</i>		Pathogen causing bacterial gastroenteritis	Feces	[81]	
<i>Campylobacter coli</i>					
Phages of <i>Vibrio cholerae</i> O1 and O139		<i>Vibrio cholerae</i> phages	Feces	[82]	
CrAssphage		Cross-assembly phage		[83,84]	
Lifestyle Biomarkers					
Paracetamol	Medications	Pain and fever medication	Urine	[60]	
Allopurinol				Preventive medication	[61]
Ketoprofen				NSAIDs/Analgesic (nonopioid)	[59,60]
Ibuprofen					[59]
Quinolonas and qnrS		Antibiotic class and antibiotic resistance gene	Urine/feces	[66,67]	
Caffeine	Drugs	Psychoactive drug	Urine	[60,62]	
Alcohol				Recreational substance	[62–65]
Nicotine				Stimulant found in tobacco	[62–65]
Cocaine				Illicit drugs	[53,71,72]
Metametamina					
3,4-metilenodioximetanfetamina (ecstasy)					
Mephedrone		NPSs derived from methcathinone		[68,69]	
Methylone					
1,4-methylimidazoleacetic acid	Metabolites	Metabolite of histamine turnover	Urine	[46]	
8-iso-prostaglandin F2 α				Lipid membrane product	[70]

qnrS: quinolone antibiotic resistance gene; NSAIDs: nonsteroidal anti-inflammatory drugs; NPSs: new psychoactive substance.

implications for public health [37,38].

Addressing these challenges necessitates concerted efforts in wastewater management and treatment. Sustainable Development Goal (SDG) target 6.3 emphasizes the imperative of reducing the proportion of untreated wastewater discharged into the environment by 2030, aiming at improving ambient water quality [39]. Effective wastewater management practices are pivotal in curbing water pollution, given the significant contribution of inadequately treated wastewater to environmental degradation [40].

Wastewater treatment plants (WWTPs) emerge as pivotal players in mitigating water environmental pollution arising from direct wastewater discharge [41]. However, discharges from various sources, including domestic households, municipalities, and industrial activities, underscore the persistent challenge of wastewater pollution, necessitating comprehensive treatment measures [42].

3. Wastewater-based epidemiology (WBE)

Integral components of public health involve interventions aimed at safeguarding societal well-being [43]. Infectious disease surveillance utilizes techniques such as sentinel surveillance, clinical-based surveillance, questionnaires or surveys, hospital admission data and mortality and morbidity rates [44]. However, bias, long execution time, resource insensitivity, ethical issues, and excessive cost hinder these methods [17,45]. Moreover, data collection may be limited to only symptomatic patients seeking clinical care, resulting in underreporting and delayed outbreak identification [16]. In this context, population testing needs to be more strategic and targeted.

Wastewater acts as a collective fingerprint, reflecting population habits and health [46]. Biomarkers from human excretions, including metabolites, endogenous chemical substances, and pathogenic microorganisms, can provide data [17]. Consequently, wastewater has demonstrated its potential as a tool for monitoring human behavior and health [47]. This practice, known as Wastewater-Based Epidemiology (WBE), involves the sampling, concentration, and systematic analysis of wastewater markers, enabling the provision of population-scale information regarding activity and exposure in each sewage system-connected area [48].

WBE's origins date back to the early 20th century, with applications in monitoring typhoid fever in the 1920s [49,50] and poliovirus in the 1930s and 1980s [14,51]. It was formally introduced in 2001 [52] and later used for monitoring illicit drugs [53]. Since then, WBE has been utilized to assess various indicators (Table 1), such as (1) public exposure products such as pesticides [54–56], industrial chemicals [57,58] and pharmaceutical products [59–61] and (2) lifestyle products such as caffeine, alcohol, and tobacco [60,62–65], antibiotics and corresponding resistance genes [66,67], new psychoactive substances [68,69], stress biomarkers [46,70], illicit drugs [71,72], and infectious disease agents, including viruses [73–78] and protozoa [79].

The effectiveness and potential of WBE, particularly for viral infectious agents, stem from the widespread presence and resilience of viruses in both untreated and treated wastewater, as well as in receiving water bodies, reflecting the population's infection pattern [85, 86]. During the global polio eradication program, this method was utilized not only to assess polio circulation in the population but also to evaluate poliovirus immunization [87,88]. Moreover, it facilitated the prediction of hepatitis A and norovirus outbreaks [74].

The unprecedented crisis caused by the COVID-19 pandemic emphasized the necessity for a comprehensive surveillance tool capable of quickly and economically monitoring population dynamics for health professionals [44]. Building on its success in monitoring enteric viruses [74,87,88], WBE was adopted as a supplementary monitoring tool for COVID-19, overcoming logistical and cost challenges associated with traditional surveillance systems [18,89] and revolutionizing the public health landscape.

Currently, more than 4600 sites across 72 countries have implemented WBE for COVID-19 screening (“COVID-19 WBE Collaborative Dashboard,” 2023). This tool has the potential to aid in government policy decision-making before viral transmission or outbreaks, thanks to its predictive capabilities [17,90]. This approach has also demonstrated its value in underdeveloped countries, such as Brazil, which encounter challenges in implementing traditional surveillance systems due to difficulties in conducting widespread testing and subsequent underreporting of cases [91]. Through WBE, viral shedding from asymptomatic patients, who naturally contribute to the spread of COVID-19 [92] and are typically not covered by syndromic surveillance, were also detectable [93]. Moreover, owing to its adaptability, the surveillance infrastructure can be swiftly redirected to new and emerging targets [44], as observed in the California monitoring network (USA), where wastewater underwent routine testing for SARS-CoV-2 and other viruses, demonstrating its potential for Monkeypox surveillance as well [76].

Despite its various applications and benefits, the global adoption of this tool is limited to regions with centralized sewage collection and treatment infrastructure [47]. With approximately 3.5 billion people lacking access to safe sanitation services and 419 million individuals worldwide practicing open defecation [94], the existence of inadequate sanitation systems renders certain populations susceptible to infectious disease transmission and complicates their detection and monitoring through WBE due to the absence of accessible composted human waste [18].

Nonetheless, South Africa, with only 57 % of the population having access to a sewage system [47], has implemented a national wastewater surveillance system for SARS-CoV-2 that is currently operational (<https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-reports/wastewater-based-epidemiology-for-sars-cov-2-in-south-africa/>). However, most WBE studies primarily focus on economically favorable countries or those with adequate or moderate sewage infrastructure [95, 96].

4. Viruses in wastewater

A wastewater treatment plant (WWTP) is a facility that employs a combination of various processes (e.g., physical, chemical, and biological) to treat incoming wastewater, eliminating pollutants [97]. Currently, there are 109,159 identified WWTPs worldwide, located in 129 countries (67 % of the world's countries), receiving substantial amounts of wastewater daily, laden with biomarkers

[47]. These biomarkers include a variety of microorganisms and/or their RNA/DNA residues, particularly enteric pathogens, providing insights into the occurrence of infectious diseases in a local catchment area [13,44]. Among these are various viruses frequently detected in domestic sewage and food industries [13,98]. This diversity of viruses primarily stems from the substantial amount of human excreta received, which may be influenced by factors such as seasonality, characteristics of the covered population area, and the prevalence of circulating viruses in the population [99,100].

Viruses are known to be high-risk pathogens, posing a significant threat due to their propensity for mutation and adaptation to new hosts, particularly RNA viruses [44,101]. According to Murphy (2017), several types of wastewater, such as raw sewage, primary effluent, secondary effluent, and aerated lagoons, have demonstrated that temperature plays a critical role in the viability of viruses. Notably, adenoviruses present in primary and secondary effluent exhibited considerable survival times (T99) of up to 58 and 48 days, respectively, particularly under cold temperatures (4 °C) in the absence of light [102].

Consequently, several viruses have been identified, including pathogenic enteric viruses such as norovirus [74,75], enterovirus [8,73], adenovirus [103,104], astrovirus [105,106], rotavirus [7,107], hepatitis A virus [108], and hepatitis E virus [109,110]. Moreover, researchers have recently applied this approach to SARS-CoV-2 [111,112] and the reemerging Monkeypox virus [76,113]. These viruses have the potential to be transmitted from wastewater to human beings through direct contact as well as through the ingestion or inhalation of aerosols [114]. Table 2 exemplifies some of the viruses found in wastewater.

4.1. Enteric viruses

Enteric viruses are pathogens that infect and replicate within enterocyte cells of the gastrointestinal tract in humans, causing nonbacterial gastrointestinal infections [99,115]. Infected individuals shed millions of viral particles in their feces (10^5 to 10^{11} viral particles per gram of feces), as well as in body fluids, which eventually enter the sewage system [86,99]. These agents possess characteristics that increase their infectious potential, such as stability in acidic environments, a nonenveloped structure, low viral titers needed to infect a host, and high rate of viral particle shedding [115]. Additionally, these viruses are more resistant to standard decontamination processes in drinking water and wastewater treatment [116]. This situation makes viral enteric infections a significant public health concern, contributing to various diseases, particularly diarrhea, which is the second leading cause of death in children under 5 years old, resulting in an estimated 525,000 deaths annually, mainly due to contaminated food and water sources [117].

There are several human enteric viruses responsible for gastrointestinal infections, such as adenovirus, astrovirus, norovirus, hepatitis E virus, enteroviruses, hepatitis A virus, and rotavirus. The most notable among these are the noroviruses (NoV), belonging to the *Caliciviridae* family, which are a group of nonenveloped, single-stranded, positive-sense RNA viruses of approximately 7700 nucleotides in length, with a size of 27–30 nm [115,118,119]. These viruses are responsible for millions of cases of acute nonbacterial gastroenteritis worldwide, with the main genogroups being GI, GII, and GIV [120]. Estimates indicate that noroviruses cause 64,000 hospitalizations due to diarrhea, 900,000 visits to pediatric clinics in industrialized countries, and up to 200,000 deaths of children under 5 years of age in developing countries [121]. In addition, they account for approximately 700 million episodes of diarrhea globally, constituting 20 % of all diarrhea cases annually [119]. Symptoms of NoV infection typically include vomiting and diarrhea of short duration, with an average duration of elimination in feces of up to four weeks but lasting for months in immunocompromised patients [122,123]. Transmission occurs through contaminated food and from person to person via the fecal-oral route [120,123]. Due to its ability to survive at different temperatures and resist disinfection with chlorine or alcohol, NoV easily spreads in the environment [123,124]. Despite its significance, an effective vaccine has not been widely developed, relying primarily on supportive treatment to reverse dehydration and electrolyte abnormalities [122].

Among viral hepatitides, hepatitis A (HAV) and E viruses are the most common agents of acute viral hepatitis globally and are

Table 2
Identified viruses in wastewater samples.

Virus	Location	Methodology	Type of sample	Reference
Norovirus	Sweden	Real-time qPCR	Sewage	[74]
	Spain	Sequencing and phylogenetic Analysis	Sewage	[75]
Enterovirus	Bahrain	Conventional PCR	Influent and effluents	[8]
	United States	Sequencing	Composite wastewater	[73]
Adenovirus	Italy	Real-time qPCR	Inflows and outflows	[103]
	United States	Real-time qPCR	Wastewater, surface water, and combined sewer overflows	[104]
Astrovirus	Uruguay	Sequencing and phylogenetic analysis	Sewage	[105]
	China	Sequencing and phylogenetic analysis	Sewage	[106]
Rotavirus	South Africa	Real-time qPCR	Final effluents	[7]
	Iran	Real-time qPCR	Influent and final effluents	[107]
Hepatitis A	Argentina	Real-time qPCR	Wastewater	[108]
Hepatitis E	France	Real-time qPCR	Influent and effluents	[109]
	Italy	Real-time qPCR	Sewage	[110]
SARS-CoV-2	Netherlands	Real-time qPCR	Sewage	[111]
	Bangladesh	Real-time qPCR	Wastewater	[112]
Monkeypox	Netherlands	Real-time qPCR	Wastewater	[113]
	United States	Droplet Digital PCR	Settled solids and liquid wastewater	[76]

transmitted through the fecal-oral route [125]. In 2015, viral hepatitis accounted for 1.34 million deaths, with 0.8 % and 3.3 % of deaths attributable to HAV and HEV, respectively [126]. HAV belongs to the *Hepatovirus* genus of the *Picornaviridae* family, possessing a single positive-stranded RNA genome of 7.5 kb and causing only acute infections [127]. Infections associated with HAV primarily occur in emerging and low-income countries, and in addition to the fecal-oral route, infections can occur between people, with the latter responsible for most outbreaks in developed countries [127,128]. Despite the availability of a vaccine for HAV, experts estimate that over 100 million infections occur annually worldwide [129]. Like norovirus, HAV has a high potential for dissemination due to its high physical resistance to adverse conditions, which is conferred by its highly cohesive capsid [127]. Hepatitis A can range from mild to severe, with more severe effects in adults, leading to high excretion of viral particles, estimated at 10^{11} genome copies per gram of feces shortly before symptom onset [130]. This, coupled with high physical resistance and the excretion of viral particles, highlights the intrinsic link between oral-fecal transmission and suboptimal sanitation [127].

Hepatitis E virus (HEV), considered the fifth hepatitis virus (after A, B, C, and D), is a nonenveloped virus of approximately 27–40 nm in diameter with a RNA genome with positive single-stranded polarity belonging to the sub-family *Orthohepevirinae* and genus *Paslahepevirus* [131,132]. This virus can be excreted in the feces, coinciding with viremia, appearing a few days before clinical symptoms, and persisting for 14–21 days [131]. Among the species, researchers have identified eight different genotypes, but only four (HEV1, HEV2, HEV3, and HEV4) have been described as infecting humans [133]. Genotypes 1 and 2 exclusively infect humans, with contaminated water as the main route of transmission, leading to large outbreaks of the disease in developing countries; these genotypes only lead to acute viral hepatitis [132,134]. In contrast, genotypes 3 and 4 infect humans through the consumption of meat products or close contact with infected animals, making them zoonotic and using pigs as the main reservoirs; infections from these genotypes may result in extrahepatic manifestations or become chronic in immunocompromised patients [125,134]. Large outbreaks of waterborne hepatitis E viruses have been associated with genotypes 1 and 2 in Africa and Asia, while infections with genotypes 3 and 4 are zoonotic infections of pigs and pork products and cause sporadic cases of hepatitis in industrialized environments [125].

The high levels of excreted nonenveloped enteric infectious agents in stools, along with their insufficient removal from wastewater, pose a risk of contaminating water bodies, which serve as sources of drinking water and crop irrigation [99,116]. Consequently, the provision and adequacy of sanitation services, the quality of wastewater treatment, mismanagement of sewage, and various hygiene practices are inherently linked to infection rates [86,115,116,135]. Viral infections have also been associated with the reuse of partially treated or untreated wastewater for irrigation purposes, particularly in addressing scarce water supplies [136]. This practice of using wastewater-derived water for crop irrigation poses risks to the health of farmers and consumers. Furthermore, groundwater contamination, runoff to surface water, or even exposure to aerosols can affect surrounding communities [136,137].

Several disease outbreaks related to the consumption of vegetables contaminated with enteric viruses have been reported worldwide. For example, eleven outbreaks of lettuce-related gastroenteritis led to 260 notifications in Denmark, caused by human norovirus (NoV) [138]. In addition, positive samples for human adenovirus (hAdV) were obtained in four European countries during the production, processing, and sale phases of fruits [139]. These authors also reported the detection of hAdV in 9.5 % of irrigation water samples. The main challenge with wastewater-derived water is the Wastewater Treatment Plant's (WWTP) inability to completely remove or inactivate the viruses present in sewage [85,137]. Although enteric viruses cannot reproduce in water, they can survive for extended periods (up to 130 days) and cause infections at low viral titers (1–50 viral infectious units from a tissue culture) when ingested [8,99,140]. Typically, conventional wastewater treatment systems can eliminate between 50 % and 90 % of the viruses present in sewage, but the discharged treated water into the aquatic environment still contains a significant viral load [8,99,141].

Microbiological evaluation and monitoring are widely used worldwide to ensure water safety [4]. For this purpose, bacterial indicators such as *Escherichia coli* and total and fecal coliforms are typically used [142]. However, this tool may not demonstrate the occurrence of enteric viruses in water, as WWTPs efficiently remove these bacterial indicators, and their persistence in the environment is shorter than that of the observed enteric viruses [143,144]. Bacteria are more susceptible than certain viruses to disinfection processes, such as UV treatment and chlorination, which are typically used in wastewater treatment [145,146]. Intrinsic factors of virus morphology and biochemistry, such as the virus capsid, size, and the capacity to aggregate with solid waste materials in sewage, can support the survival of the virus during the treatment steps, keeping them potentially infectious [86]. Additionally, they can remain stable in the environment for extended periods, particularly when associated with solids, which act as potential viral reservoirs [147]. Goyal et al. (1984) detected human enteric viruses in sediments from sewage sludge 17 months after sludge disposal ended [148]. Thus, the stability and persistence of enteric viruses in environmental water, even after passing through WWTPs, and their risks to human exposure through the pollution of drinking water sources, recreational waters, and food [99] make the monitoring of these viruses in water sources an essential tool for improving public health.

In Italy, monitoring revealed trends in hepatitis E virus over nine years (2011–2019), both in terms of virus occurrence and circulating genotypes [110]. These authors described the positivity of 74 out of 1374 (5.4 %) sewage samples for HEV in 13 of the 20 Italian regions, of which 56 (75.6 %) and 18 (24.3 %) belonged to the G3 genotype and G1, respectively. In the Campania Region, La Rosa et al. (2020) detected HAV RNA in 39 out of 463 water samples (20 of them positive in sewage) over four years, with 31 belonging to the IA genotype and 31 to the IB genotype. Thirteen of the 39 samples were collected during the 2015 HAV outbreak, and 10 of them were characterized as IA strains, showing 99–100 % nt identity with the epidemic strain (ISS 1 1 N 2015 Hu) isolated from patients with acute hepatitis in the same region [149]. In France, through wastewater monitoring, a wide range of circulating viral pathogens were detected, including adenovirus, enterovirus, parechovirus, rotavirus, norovirus, and hepatitis A and E [150].

Thus, sewage analysis is a potentially sensitive tool for tracking viral circulation in the area connected to the WWTP and for detecting the cocirculation of several different viral lineages [151]. Furthermore, wastewater surveillance may provide an opportunity for countries that do not have an early warning system for enteric virus outbreaks [152]. In South Africa, researchers detected and characterized NoV GI and GII from wastewater samples [144]. This study detected NoV in 78 out of 108 samples (61.1 %), with 33 out

of 54 in the influent and 45 out of 54 in the effluent, demonstrating the existence of at least 16 NoV genotypes circulating in the Free State and Gauteng.

4.2. SARS-CoV-2

The COVID-19 outbreak, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a profound global impact, leading to significant human and economic losses [153]. Researchers have underscored the zoonotic transmission potential of coronaviruses, highlighting the urgent need for effective surveillance and public health measures [154,155].

The high expression of the angiotensin-converting enzyme 2 (ACE2) receptor, particularly in the lower respiratory tract, has contributed to the severe respiratory manifestations observed in COVID-19 cases [156,157]. SARS-CoV-2, like SARS-CoV, invades cells by utilizing the ACE2 enzyme as a receptor in its pathogenic mechanism, leading to a range of clinical presentations, from mild to severe, often accompanied by a robust immune response [158]. The presence of ACE2 receptors in various organs, including the respiratory tract, lungs, kidneys, blood vessels, heart, and small intestine, has raised questions about the various clinical presentations and extrapulmonary manifestations of the disease [155,159]. Gastrointestinal symptoms associated with COVID-19 have prompted investigations into the detection of SARS-CoV-2 in stool samples, revealing significant viral titers and prolonged shedding of the virus, even in asymptomatic individuals [160–162].

Furthermore, the high prevalence of asymptomatic cases has underscored the need for comprehensive epidemiological surveillance strategies to effectively monitor and control the spread of the virus [17]. WBE has emerged as a valuable noninvasive tool for monitoring the presence of SARS-CoV-2 RNA in sewage, as shown in the examples in Table 3, providing crucial insights into the dynamics of viral transmission [11,15,163]. Studies conducted in Brazil and Germany have demonstrated a temporal correlation between measured concentrations of SARS-CoV-2 RNA in wastewater and the subsequent increase in clinical cases, with lead times ranging from 7 to 14 days [96,164]. Similarly, the relationship between viral titers in wastewater and the corresponding trends in clinical cases has been observed in Mexico, highlighting the predictive capabilities of WBE in tracking disease dynamics [165].

Despite its potential, standardizing WBE methods for SARS-CoV-2 detection remains a significant challenge [90,175]. The complex nature of wastewater, coupled with variations in sewage composition and the unique characteristics of SARS-CoV-2 shedding, demand continued efforts to optimize and refine surveillance strategies [176,177]. Efforts toward standardization are imperative to enhance the reliability and consistency of wastewater-based surveillance, ensuring its continued effectiveness in addressing current and future public health challenges posed by viral outbreaks [111].

4.3. Monkeypox

Monkeypox virus (MPXV) is a double-stranded DNA virus measuring 200–250 nm and enveloped in a lipoprotein membrane [178]. It belongs to the genus *Orthopoxvirus*, *Poxviridae* family, and *Chordopoxvirinae* subfamily [179]. Researchers first discovered MPXV in monkeys in Denmark in 1958 [180]. In 1970, it was first isolated from humans and reported in a 9-year-old child from the Democratic Republic of Congo [181]. Currently, MPXV remains endemic in West and Central Africa, and the infection has been associated with zoonotic transmission from small mammals [76]. However, the global monkeypox outbreak of 2022 presented an unusual scenario. For the first time, the disease emerged in countries that hadn't previously reported cases and lacked established epidemiological links to known monkeypox-endemic regions [182].

On July 23, 2022, the monkeypox virus was designated a worldwide health emergency of international significance, affecting approximately 20,000 people across numerous nations [179,183]. Since then, this zoonotic disease has spread to more than 110 countries beyond Africa, primarily in those not historically considered endemic [113,184].

The World Health Organization (WHO) recognized the global outbreak of monkeypox as a Public Health Emergency of International Concern (PHEIC) on July 23, 2022 [185]. To date, 78,000 cases have been confirmed in 110 locations worldwide (102 with no history of the disease), resulting in 42 deaths [184,186]. Experts have attributed the MPX outbreak in 2022 to mutations in MPXV, climate change, and reduced cross-immunity resulting from the absence of smallpox vaccination in the adult/young population, globalization, and high-risk sexual activity [187].

The infection is a self-limiting condition, with symptoms lasting between 2 and 4 weeks [12]. However, it can occasionally be fatal, with rates of up to 10 % of cases in endemic countries [188] and 3–6% for the 2022 MPX outbreak [189]. MPXV can occur through zoonotic or anthroponotic transmission, with the latter considered the main cause of the current outbreak [190]. Transmission occurs through contact with respiratory droplets, touching characteristic MPX injuries, body fluids, and contaminated objects [191]. Symptomatic cases exhibit main symptoms, including fever, swollen lymph nodes, and localized rashes, which can progress throughout the body [192]. Patient isolation is recommended to prevent transmission [178], and two vaccines are currently available, JYNNEOSTM (live, replication-incompetent vaccinia virus) and ACAM2000® (live, replication-competent vaccinia virus) [193].

In human patients, MPXV genomic DNA has been isolated from skin lesions, the nasopharynx, plasma, semen, urine, and feces samples [179,194]. Among the tested samples, the skin lesions exhibited the highest virus concentration [195]. Therefore, the diagnosis can involve laboratory methods such as viral isolation, tissue immunohistochemistry, molecular diagnosis, electron microscopy, and serology [187]. However, these diagnostic methods are costly, and many are not commercially available, especially in emerging countries [178].

Wastewater surveillance could be a potential approach, given its ability to detect emerging pathogens in small or large communities at a low cost and through noninvasive means [17]. MPXV can reach the sewage system through domestic water discharged into it, which contains human excreta and feces, respiratory secretions, saliva, and runoff from skin lesions released during bathing [12].

Table 3
Wastewater-based epidemiology (WBE) as a surveillance tool for SARS-CoV-2 RNA detection in sewage.

Sampling			Virus detection method		Detection results	Reference
Country	State/City	Sample type	Method	Target gene	Positive rate	
Bangladesh	Dhaka, Sylhet, Chittagong, Mymensingh, Rajshahi, Khulna, Barisal, Rangpur, Habiganj, Kishorgonj, Brahmanbaria, Gaibandha e Cox's Bazar, Cumilla.	Untreated wastewater	RT-qPCR	ORF1ab e N protein	98 % (165/168)	[112]
Brazil	Salvador, Bahia	Untreated and treated wastewater	RT-qPCR	N1 and N2	–	[78]
	Nitério, Rio de Janeiro	Untreated wastewater	RT-qPCR	N2	84,3 % (188/223)	[166]
	ABC Region, São Paulo	Untreated wastewater	RT-qPCR	N1 and N2	N1_53 % (116/220) N2_34 % (74/220)	[164]
Japan	Ishikawa and Toyama prefectures	Untreated wastewater	RT-qPCR	N2, N3 and N protein	47 % (21/45)	[167]
	Yamanashi Prefecture	Untreated wastewater	RT-qPCR	N1 and N2	67 % (88/132)	[168]
The Netherlands	Amsterdam, Den Haag, Utrecht, Apeldoorn, Amersfoort, Schiphol and Tilburg	Untreated wastewater	RT-qPCR	N1, N2, N3 and E protein	14/24 (58 %)	[111]
Mexico	Queretaro State, Santiago de Queretaro	Ativacted sludge, Untreated and treated wastewater	RT-qPCR	RdRP, S and N protein	–	[169]
Australia	Brisbane, Queensland	Untreated wastewater	RT-qPCR	N protein	22 % (2/9)	[170]
Spain	Region of Murcia	Untreated and secondary and tertiary treated wastewater	RT-qPCR	N1–N3	Untreated wastewater_83 % (35/42)/Secondary treated wastewater_11 % (2/18)/Tertiary treated wastewater_0 % (0/12)	[171]
Italy	Castiglione Torinese, Chieri, Cuneo, Govone, Serravalle Sesia, Vercelli, Cerano, Novara, Asti, Domodossola, Omegna, Cossato and Massazza	Untreated wastewater	RT-qPCR	N1 and N2; E protein	–	[172]
Portugal	Amadora, Lisbon, Oeiras, Loures, Cascais, Sintra, Vila Nova de Gaia, Guimaraes, Póvoa do Lanhoso and Vila Nova de Famalicão	Untreated wastewater	RT-qPCR	E protein, RdRP e N protein	48 % (193/404)	[173]
USA	Charlotte, North Carolina	Untreated wastewater	RT-qPCR/RT-ddPCR	N1 and N2	–	[174]

Given the success of WBE in monitoring viral diseases, including during the COVID-19 outbreak, several places worldwide have begun to monitor MPXV in wastewater, using these data as a complementary epidemiological tool (Table 4). The first report of MPXV in wastewater occurred in Spain, with samples collected during the week of the first suspected MPX human infection, detecting the presence of MPXV DNA in 63 out of 312 samples analyzed [196]. MPXV genomic DNA detection was also reported in wastewater samples from the Rome airport in Italy [77].

In the Netherlands, the detection of MPXV in wastewater influent samples collected from five districts of Amsterdam showed the presence of MPXV DNA in 43 out of 108 (42 %) samples [113]. These findings also suggest that the concentration in (bio)solids could

Table 4
Implementation of wastewater-based epidemiology for monitoring MPXV worldwide.

Location	Primers	# of samples	Detection/ Total (%)	Sample source	Concentration method	Detection method	Reference
Netherlands	G2R_WA e G2R_G	108	43/108 (42 %)	solid fraction	–	qPCR	[113]
Italy	N3R, F3L e G2R_G	20	02/20 (10 %)	liquid fraction	Precipitation (PEG/NaCl)	qPCR and nested PCR	[77]
Spain	G2R_WA e G2R_G	312	63/312 (20 %)	–	Aluminum-based adsorption-precipitation	RT-qPCR	[196]
United States	G2R_WA e G2R_G	287	76/287 (26 %)	solid fraction	–	RT-PCR	[76]
	–	18	13/18 (72 %)	raw sewage	Adsorption-elution (AE) and polyethylene glycol (PEG) precipitation	qPCR	[197]
Czech Republic	G2R_WA e G2R_G	24	9/24 (37.5 %)	raw wastewater	–	Sequencing and seminested PCR	[198]

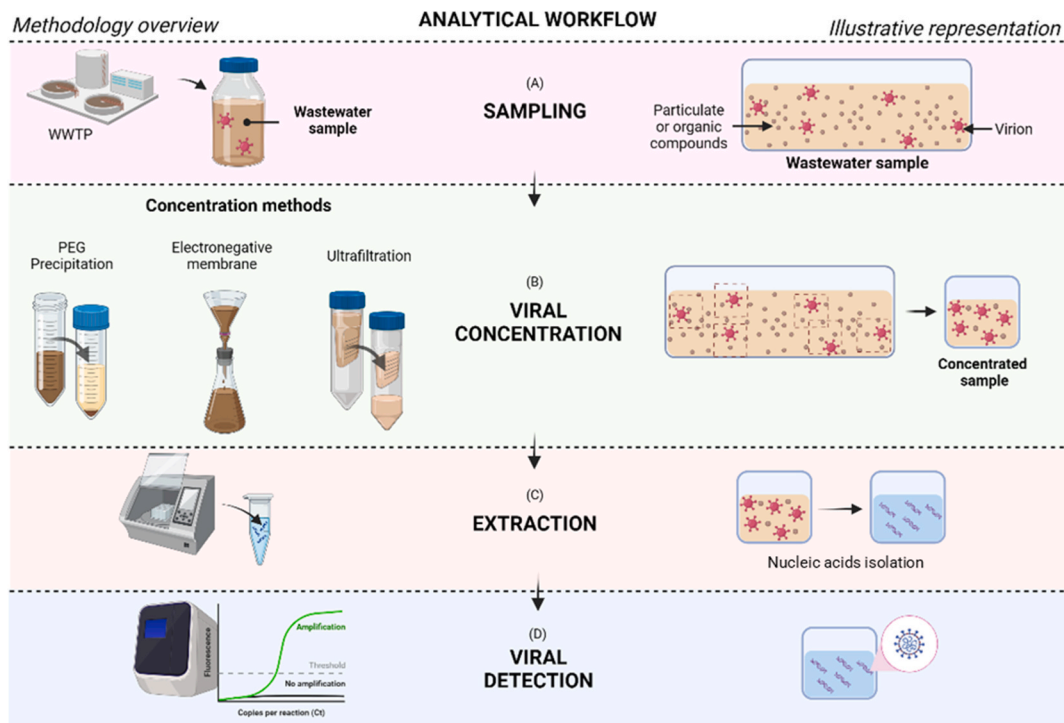


Fig. 1. An illustrative representation of the analytical workflow for viral surveillance in wastewater. (a) The process initiates with targeted sampling from a designated location within the wastewater collection system; (b) subsequently, viral concentration is carried out (showing the three methods most widely used); (c) this is followed by nucleic acids isolation, and (d) the last step involves the detection of viral presence in the samples. Created with [BioRender.com](https://www.biorender.com) (accessed on November 07, 2023).

provide an opportunity for the detection of the virus genetic material. In another study, Wolfe et al. (2022) detected MPXV DNA in settled solid samples from 8 out of 9 monitored WWTPs in San Francisco, California, USA. Notably, the viral concentration was 10^3 higher in the solid fraction of wastewater than in the liquid fraction [76].

Prague's wastewater tested positive for MPXV, with a total of 9 positive samples during periods featuring 1 to 9 new cases per week, aligning with a weekly incidence ranging from 0.07 to 0.64 per 100,000 inhabitants [198]. Scherchan et al. (2023) detected MPXV in untreated wastewater samples from two WWTPs in Baltimore. The findings underscore the potential use of wastewater surveillance as a supplementary early warning tool for monitoring future Monkeypox outbreaks [197].

Recent studies have demonstrated the potential for monitoring MPXV in wastewater. However, the levels of MPXV, in comparison to SARS-CoV-2, are lower in wastewater, potentially contributing to reduced sensitivity in detection [12]. Thus, the choice of the best approach, from sample collection to molecular detection, which could enhance sensitivity and reduce false-negative results, appears to be the current challenge. Another crucial aspect to consider is whether the lower MPXV titers are due to a difference in the number of clinical cases or if MPXV is shed in wastewater less abundantly than other viruses, such as SARS-CoV-2 [199].

5. Viral identification methodology in wastewater

Viral surveillance in wastewater entails a series of systematic procedures, commencing with the sampling of a specific site within a wastewater collection system and subsequently proceeding to the steps of viral concentration, extraction, and detection [200] (Fig. 1). This multifaceted process is imperative owing to the intricate nature of the sample matrix, which encompasses various compounds that could yield inaccurate outcomes, thereby reducing the sensitivity of detecting and quantifying genetic fragments [201].

5.1. Sampling

Wastewater cannot be regarded as a uniformly mixed combination of all materials discharged into the sewer system throughout the day, despite its dilution and blending. Sewers function as dynamic flow systems, leading to constant changes in the composition of wastewater reaching wastewater treatment plants due to fluctuations in flow rates and substances introduced into the system [202]. Therefore, effective wastewater sampling emerges as a crucial tool to address the uncertainties associated with analyzing the spread of the virus [203]. (Fig. 1a). Poorly designed sampling techniques can yield unrepresentative samples, leading to potential false-negative errors [204].

Twenty-four-hour composite capture sampling is a preferred method for monitoring wastewater, as it involves collecting multiple

Table 5
Viral concentration methodologies in wastewater samples.

Method principle	Concentration method	Advantages	Drawbacks	References
Size-exclusion based on particle	Ultrafiltration (dead-end and tangential flow)	<ul style="list-style-type: none"> - Simultaneous concentration of viruses and other pathogens; - Inexpensive filters. 	<ul style="list-style-type: none"> - Filter clogging in samples with high turbidity (except tangential flow); - It can require filter conditioning; - Slow filtration rate 	[219–222]
Centrifugation-based	Ultracentrifugation	<ul style="list-style-type: none"> - No preconditioning; - One step method; - Concentrate solid and liquid phase viruses 	<ul style="list-style-type: none"> - Requires expensive equipment (ultracentrifugation); - Can't be used for large volume sample 	[170,222]
Precipitation	Precipitation with PEG	<ul style="list-style-type: none"> - Greater efficiency in concentrating RNA viruses; - Large volume water processing; - Concentrate viruses from both solid and liquid phases; - Relatively cheap 	<ul style="list-style-type: none"> - Coprecipitation of PCR inhibitors; - Time consuming; - Require centrifugation 	[85,222]
Flocculation	Skim milk flocculation	<ul style="list-style-type: none"> - One-step method; - Simultaneous concentration of several samples; - Field deployable; - Large volume water processing (Up to 40 L samples) 	Time consuming	[85,222]
Membrane Adsorption	Negative filters adsorption	<ul style="list-style-type: none"> - Extraction can be done directly from the membrane; - Low cost; 	<ul style="list-style-type: none"> - No useful for turbid samples; - Requires conditioning; - No useful for large volume samples 	[219,220, 222]
	Positive filters adsorption	<ul style="list-style-type: none"> - No require preconditioning; - Capable of concentrating viruses over a wider pH range; - Useful for large volumes; - Field deployable 	No useful for turbid samples	
Size-exclusion based on particle/Centrifugation-based	Centrifugal ultrafiltration	<ul style="list-style-type: none"> - Simple and quick method; - Reduces the amount of PCR inhibitors; - Multiple sample processing 	<ul style="list-style-type: none"> - Filter clogging; - Requires preconditioning at high turbidity (prefiltration) 	[85,224]

samples throughout the day and consolidating them into a single volume [205]. This strategy is believed to offer a more comprehensive representation of viral content and is less susceptible to the inherent variations of seasonal and diurnal changes [204]. However, the implementation of this approach can be costly and challenging. It requires the installation of automated equipment (\$2300 to \$7500 USD per unit) and personnel to conduct sampling at fixed locations, which can be difficult to access (e.g., sewer manholes, rivers), along with ongoing maintenance and security costs [205–208].

Alternatively, some studies have utilized the individual capture method for monitoring purposes [170,209–212]. This approach involves collecting a single sample at a specific point in time [204]. However, the representativeness of the results may be compromised, as it overlooks the influence of factors such as flow rate, total wastewater volume, temperature, and variations in viral excretion profiles [200]. Additionally, small sample volumes combined with low recovery rates could lead to false-negative outcomes [213]. Nevertheless, random samples can be employed to monitor various locations within a region, including pumping stations or hospitals, which can serve as potential infection sentinels, along with the option of sampling small communities or communities without a sanitary sewage system [208,214].

Another cost-effective option is passive sampling (trap sampling), which entails the deployment of an adsorbent device (e.g., gauze, pads, and macroporous glass) for a predetermined duration that is capable of interacting with significant wastewater volumes [207, 208]. This method is advantageous, particularly for poor countries, due to its simplicity and safety, and has proven to be effective in monitoring enteric viruses and enveloped viruses [215,216]. However, unlike random sampling, trap sampling limits the ability to quantify the amount of virus present in wastewater, hindering a comprehensive understanding of method sensitivity (the ability to detect low levels of virus) and efficiency (the ability to recover the virus from the sample) [208].

5.2. Viral concentration

Viral pathogens are typically diluted in wastewater, often leading to low or undetectable concentrations in small volume samples (i.e., <1 L) [175]. To address this issue, the concentration step, preceding the extraction of genetic material (DNA/RNA), is utilized to increase method's sensitivity and yield detectable levels of viral nucleic acid [217]. In this step, intrinsic wastewater factors such as turbidity, volume, and quality must be considered, as they can significantly affect virus concentration efficiency and downstream detection techniques [218]. However, no method can uniformly concentrate all viruses, given the significant structural differences between enveloped and nonenveloped viruses, even when using the same sample type [170].

Several techniques have been employed for the concentration of viral particles in wastewater samples. Among these, three primary concentration methods (Fig. 1b) have emerged as the most widely used: vacuum filtration utilizing an electronegative membrane; a

precipitation method employing PEG 8000; and ultrafiltration [90].

In response to the need for a reliable concentration methodology for viral markers, various concentration procedures are currently employed across different wastewater volumes (15 mL–40 L). These methods rely on techniques such as size exclusion based on particles, centrifugation, precipitation, flocculation, membrane adsorption, or a combination of these principles [170,219–224] (Table 5). While most of these techniques were originally developed for enteric virus surveillance [202], several have proven applicable for concentrating other viruses in wastewater, including SARS-CoV-2 and Monkeypox virus [76,112,113,225].

The variability in inactivation rates among different viruses, coupled with the differential stability exhibited by infectious viruses and their nucleic acids, underscores the intricate dynamics at play within wastewater systems [226]. These differences are likely influenced by variations in the microbiological composition of different water sources and the distinct mechanisms governing virus inactivation [227]. Viral nucleic acids, unlike viral capsids, can persist and remain detectable even after the virus loses infectivity, which means they can be found in the environment for longer durations [228]. [44,229,229,230].

5.3. Nucleic acid isolation

In PCR (polymerase chain reaction)-based detection methods, the viral genome's target sequence is amplified. However, this necessitates the isolation of genetic material (DNA/RNA), typically involving stages of lysis, purification, and recovery [231], a process commonly performed on human specimens for diagnostic purposes. In the investigation of viruses in wastewater samples (Fig. 1c), commercially available kits have found wide application in concentrated isolates from wastewater samples [200]. Nonetheless, caution is warranted regarding the indiscriminate use of these kits, given the variability in efficiency and consistency in isolating the viral genome and removing PCR inhibitors, even when sourced from the same supplier [232,233]. Consequently, automated extractors have been employed to isolate virus nucleic acids from influent and effluent wastewater samples [78,234,235], as they can bind nucleic acids, while minimizing the co-extraction of various other materials commonly found in wastewater, such as organic matter, humic substances, and microbial debris, which reduces variability in analytical results and improves sample purity [85].

5.4. Viral detection

For many years, cell culture-based methods have served as the gold standard for detecting infectious viruses in environmental samples [236]. However, these techniques are limited to the ability to cultivate viruses of interest *in vitro*. Consequently, PCR and qPCR (Fig. 1d) methods have become crucial for identifying viruses in water samples due to their rapid detection, high sensitivity, and specificity, enabling test adaptation based on the desired specificity, whether to cover broad viral groups such as enteroviruses or target more specific viral strains based on selected target DNA/RNA sequences [218].

Genome sequencing has emerged as a pivotal tool for the discovery and characterization of viral variants in wastewater-based epidemiology [237]. Studies have underscored the significance of employing virome sequencing and next-generation sequencing approaches to unveil insights into viral evolution, dynamics between different viral species or variants, and the prevalence of emerging variants within communities [238]. WBE, leveraging virome sequencing, offers a unique perspective on aggregate community loads of specific pathogens and their genomic diversity, complementing clinical reporting biases [239]. Monitoring viral genome sequences in wastewater improves estimates of community prevalence and makes it easier to identify new variations early on [240]. Recent advancements in genomic sequencing technologies enable comprehensive scanning of the entire viral genome, enabling the detection of unique and functionally important mutations and VOC-defining clusters [241,242]. Innovative computer techniques improve the parsing of several viral lineages in wastewater samples even further [243]. In general, viral pathogens in wastewater may be sequenced using whole genome sequencing, which is a versatile and effective method for determining the frequency of circulating viral variations and assisting in the prompt identification of newly emerging variants in populations [244,245].

Urban wastewater is a complex mixture that extends beyond fecal waste, encompassing pharmaceutical products, personal hygiene items, rainwater, sediments, household detergents, industrial effluents, metals, and other substances [202]. However, some of these compounds can partially or completely prevent PCR amplification, resulting in false negatives. These compounds, known as PCR inhibitors, comprise a variety of substances, such as humic acids, polysaccharides, fats, proteins, metal ions, RNases, and others that are recognized to interfere with PCR-based methods [219,220,246]. Given this, processing controls, such as viruses with similar structures, have been recommended in the analysis of environmental samples, particularly in wastewater, since controls serve as an internal verification of the extraction and detection process [218,247]. If the control virus exhibits a significant decrease in recovery compared to expected yields, it may indicate the presence of inhibitors in the sample.

WBE holds promise for tracking the spread of viral lineages (variants) within communities, yet it presents inherent challenges due to the vast diversity of genetic material present in wastewater samples [44,229]. The presence of genetic material from viruses with diverse evolutionary backgrounds can complicate the precise identification and phasing of specific circulating lineages [230]. To address these challenges, the field of WBE is actively developing and refining bioinformatic tools. These tools employ various approaches, such as identifying mutations specific to variant lineages within sequencing data, utilizing sample deconvolution techniques, and comparing lineage-specific mutations [229]. While advancements are being made, the application of standard metagenomic methods, like ordination analysis, remains relatively new in WBE research.

6. Epidemiological insights through wastewater surveillance

WBE has found extensive applications beyond infectious disease surveillance, extending its utility to diverse realms of public

health. It has been instrumental in the evaluation of food safety measures [48], monitoring drug misuse trends [248], evaluating the effects of temperature on health [249], assessing population exposure to various environmental toxins such as pesticides and heavy metals [250], and contributing to overall environmental health management [98]. By providing insights into the prevalence and distribution of various contaminants and substances within communities, WBE has emerged as a versatile and robust tool in the broader context of public health management and policy formulation [251].

The unpredictability of infectious outbreaks, regional epidemics, and pandemics poses a formidable challenge for public health preparedness. This vulnerability is highlighted by the difficulty in timely responses, which stems from the limitations of traditional epidemiological approaches [236,252]. These methods rely primarily on specific clinical symptoms to identify the onset of a disease epidemic, leaving a critical gap in the ability to foresee and proactively address emerging health threats [236].

Epidemiological insights derived from wastewater monitoring have become increasingly instrumental in tracking the emergence and spread of infectious diseases, particularly in the context of the ongoing COVID-19 pandemic [253,254]. Numerous studies and scientific reports have emphasized the efficacy and cost-effectiveness of wastewater surveillance in providing early warnings of disease burden to policymakers and health officials, thereby facilitating evidence-based decision-making for disease control and preparedness [254–256]. Moreover, the diverse applications of wastewater monitoring extend beyond tracking SARS-CoV-2, encompassing the potential for monitoring other emerging pathogens and disease prevalence within communities [257–259]. WBE demonstrates its utility in detecting infection and transmission in the general community, regardless of the presence of clinical symptoms or socio-economic biases related to case reporting [260]. This emphasizes the inclusive nature of wastewater surveillance, capturing a more comprehensive picture of disease prevalence, including cases that might otherwise go undetected through traditional clinical surveillance alone.

The pivotal role of wastewater monitoring as an essential component of global disease surveillance systems has gained recognition, emphasizing the need for comprehensive frameworks to unify monitoring programs worldwide [256,261,262]. While the current wastewater research landscape remains fragmented due to its grassroots origins, there is a pressing need for global and country-level leadership, financial investments, and adaptable implementation frameworks to maximize the potential of wastewater surveillance [256]. Establishing wastewater-based surveillance (WBS) initiatives and integrating collected data would be extremely beneficial to public health experts, local authorities, and decision makers, enabling a more integrated approach to disease monitoring and response [260]. This comprehensive integration would facilitate informed decision-making, policy development, and targeted interventions, contributing to improved public health outcomes.

Environmental surveillance can play an essential role in supplementing clinical surveillance efforts or prompting more extensive monitoring in regions with limited or underdeveloped epidemiological data [263]. People who live in these areas, which often encompass densely populated regions, resource-limited informal settlements, or marginalized communities facing socioenvironmental vulnerabilities, may encounter restricted access to healthcare facilities, decreased care-seeking tendencies, insufficient testing capabilities, and overwhelmed clinical surveillance capacities [11].

Wastewater surveillance's utility for infectious diseases is evident through its historical applications in tracking water-borne pathogens and its recent proactive role in monitoring SARS-CoV-2 during the COVID-19 pandemic [44,254,262]. Wastewater monitoring serves as a valuable complementary surveillance technique, offering insights into the presence of various pathogens and aiding in disease outbreak monitoring [44]. WBE has the potential to demonstrate its efficacy in COVID-19 surveillance, enabling the prediction of the broader pandemic landscape [264]. By pinpointing the hotspots and refining public health interventions, WBE can offer regulatory bodies the necessary lead time to effectively manage challenging circumstances [265]. Moreover, WBE could serve as an early indicator of potential outbreaks and seasonal upsurges in the times ahead [17,265]. Implementing this technique enables the nearly real-time detection of specific targets across the community [266]. In the case of infectious diseases such as COVID-19, real-time monitoring is crucial for effectively addressing their transmission and minimizing their impact [267].

The undeniable benefits of wastewater surveillance for infectious disease monitoring are tempered by challenges in equity, timeliness, and data utilization, which highlight the need for its integration and mainstreaming [259,261]. Promoting responsible and ethical monitoring practices, ensuring data representativeness, and enhancing the spatial resolution of surveillance systems emerge as crucial factors in advancing the efficacy of wastewater monitoring for public health [257,261,262].

7. Unveiling the impacts of wastewater-based epidemiology on public health

The exploration of WBE has unveiled significant implications for public health, particularly in the context of disease surveillance, outbreak detection, and monitoring of population health indicators [10]. Historical milestones, including John Snow's seminal work in 1854, highlighted the association between contaminated water sources and disease transmission, laying the foundation for modern wastewater analysis [268]. The subsequent use of wastewater surveillance for tracking the spread of poliomyelitis [269] and monitoring cocaine usage [53] in specific populations further underscored the versatility of this approach in understanding diverse health phenomena [268].

Professor Masaaki Kitajima emphasized that WBE holds significant potential as a valuable tool in healthcare research, supported by various researchers [270]. First, through wastewater analysis, early warning signs of disease outbreaks can be detected, aiding in timely interventions, particularly in the case of infectious diseases such as COVID-19 [271]. Second, WBE enables a comprehensive understanding of infectious disease prevalence by examining various pathogens in wastewater, offering insights crucial for public health planning and policymaking [272]. Finally, WBE complements traditional healthcare surveillance methods, providing a population-level perspective that covers a broader segment of the population, including asymptomatic individuals, thereby enhancing the overall understanding of public health [273].

In Brazil, wastewater-based epidemiology (WBE) projects conducted throughout the COVID-19 pandemic were primarily spearheaded by universities, research institutes, and regulatory agencies [89]. These initiatives benefitted from collaborative efforts involving water and sanitation companies as well as health authorities at municipal or state levels [15]. By leveraging the collective expertise and resources of these institutions, comprehensive WBE data on SARS-CoV-2 were systematically collected and disseminated to the public. This information was made accessible through various channels, including publicly available online dashboards, weekly or monthly bulletins, and warning alerts issued during periods of heightened transmission [274]. Such proactive measures not only facilitated real-time monitoring of viral prevalence in communities but also served to inform public health interventions and mitigation strategies in response to evolving pandemic dynamics.

[271,275] However, it is crucial to acknowledge that WBE cannot be used in isolation in epidemiology studies. It requires a comprehensive approach that integrates multiple surveillance methods to enable proactive interventions and effective disease control [275].

Since 2018, China has implemented wastewater monitoring programs at local, provincial, and national levels, serving as a cornerstone for evaluating the efficacy of drug control efforts [276]. The utilization of WBE data has been instrumental in aiding law enforcement agencies in cracking down on drug-related crimes [277]. According to Li et al. (2023), routine monitoring at sewage plants has enabled the identification of suspect manufacturing activities, prompting further investigations at upstream pump stations and sewer networks to pinpoint specific areas of interest. Police raids that followed, directed by monitoring data, were effective in taking down many covert labs [15]. Concurrently, in Europe, the Sewage Analysis CORE group Europe (SCORE) collaboration has been pivotal in providing annual data on the population's use of psychoactive drugs to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations Office of Drugs and Crime (UNODC) [278]. Moreover, in Australia, the Australian Criminal Intelligence Commission (ACIC) established the Australian National Wastewater Drug Monitoring Program in 2016 [279]. This approach has since been commercialized by Biobot Analytics in the United States, showcasing the global adoption and commercialization of wastewater-based drug monitoring initiatives [15].

The utilization of human biomarkers present in sewage offers a valuable early warning system for disease outbreaks, allowing for timely policy interventions and governance strategies [98]. However, careful consideration of community practices and beliefs is essential in selecting appropriate monitoring sites, ensuring accurate representation and the effective utilization of resources without compromising WASH initiatives aimed at improving overall public health [280].

Furthermore, the significance of wastewater treatment facilities as critical venues for epidemiological investigations of emerging diseases has been underscored, particularly in vulnerable populations that are disproportionately affected by regional epidemics and pandemics [275]. Studies conducted in various regions, such as Israel and Brazil, have further emphasized the utility of WBE in detecting community-level disease prevalence, highlighting its role in supporting targeted public health interventions and informing municipal policies [166,281].

Looking ahead, experts recognize the potential of WBE to contribute to the attainment of various United Nations (UN) Sustainable Development Goals. These goals include ensuring healthy lives, promoting sustainable economic growth, and building resilient infrastructure [47]. Its role in monitoring infectious diseases and assessing the efficacy of vaccination campaigns has positioned WBE as a complementary method for evaluating the spread of infections, particularly in areas with limited diagnostic resources [172].

A recent study illustrates that WBE could effectively monitor progress toward achieving more than half of these goals, set for 2030. These encompass a broad spectrum, including ending hunger, promoting healthy living, sustainable economic growth, resilient infrastructure development, and fostering inclusive and sustainable societies [47].

Through the analysis of various markers, including dietary patterns, pharmaceutical consumption, and stress hormones, WBE enables the identification of health disparities across different communities, facilitating targeted interventions and policy planning [282]. [10,15,44].

8. Policy and regulatory aspects

Policy and regulatory aspects play an essential role in the successful implementation and management of WBE programs for infectious disease surveillance. The establishment of the National Wastewater Surveillance System (NWSS) by the U.S. Centers for Disease Control and Prevention (CDC) is a significant milestone, signifying the growing recognition of the importance of wastewater monitoring as a surveillance tool [258,283]. However, challenges persist, particularly concerning the coordination of protocols and policies, as highlighted by Wolfe (2022). To ensure the effective deployment of these programs, it is crucial to consider ethical guidelines, especially in smaller community sites [284].

Furthermore, the legal frameworks governing wastewater governance and monitoring vary across states and territories, with historical regulations often stemming from public health acts [284]. The roles of key stakeholders in WBE programs, including research laboratories, utilities, health authorities, and nongovernmental organizations (NGOs), need to be well defined and coordinated [280].

The application of WBE in the pandemic context, as seen through the NWSS initiative in the U.S., emphasizes the importance of collaboration between federal agencies and their role in establishing a formalized approach to WBE [283]. Moreover, global collaborations such as the Surveillance of Wastewater for Early Epidemic Prediction (SWEEP), proposed by Tiwari et al. (2021), hold promise in enhancing the resilience of future generations to viral outbreaks [28].

Diverse countries, including Finland, Hungary, Luxembourg, the Netherlands, Spain, and Turkey, have integrated WBE as a comprehensive approach to monitor the transmission of SARS-CoV-2 nationwide [89]. In contrast, the United States, Canada, Australia, France, Switzerland, and the United Kingdom have adopted regional monitoring strategies [89].

Between 2021 and 2022, the MCTI (Ministry of Science, Technology, and Innovation) established a COVID-19 wastewater-

monitoring network in several Brazilian states. The monitoring initiative, documented at <http://redevirus.mcti.gov.br/novidades/monitoramento-de-covid-19-em-aguas-residuais-2022/>, has been active since the early months of 2021 in key cities, including Belo Horizonte, Curitiba, Distrito Federal, Fortaleza, Recife, and Rio de Janeiro. Epidemiological bulletins released up to March 2022 displayed a notable correlation between fluctuations in the levels of SARS-CoV-2 genetic material detected in sewage and the incidences of suspected and confirmed COVID-19 cases in each evaluated city [78].

While the applications of wastewater research hold promise, there is currently a lack of well-developed ethical guidelines governing its use under specific conditions [285]. Various subfields of applied ethics offer perspectives to address the ethical dilemmas posed by wastewater research, as highlighted by Doorn et al. (2022):

- Collected data remain untraceable to individuals, requiring a balance between disease surveillance and privacy protection;
- The specificity of wastewater sources impacts their effectiveness and privacy. It is crucial to consider the data in conjunction with other sources and handle it with care to avoid excessive datafication of society;
- Wastewater surveillance offers a valuable complement to conventional diagnostic testing methods for infectious diseases; WBE can influence the choice of treatment methods to enhance the ecological health of water systems.

9. Challenges and future directions

Wastewater-based epidemiology has gained acceptance in several countries as an effective method for preventing, intervening in, and controlling new and reemerging infectious diseases [44,166]. Its potential for understanding population behavior and its consequences for public health has also been emphasized [286].

This approach has the advantage of being a practical model with minimal resource requirements, capable of gathering comprehensive data with broad geographic and population coverage [287]. Moreover, data collection can occur in real time, independent of clinical data, thereby reducing the potential for bias and under detection [45]. However, despite the potential benefits, researchers need to address the remaining limitations and challenges through additional studies, research, and development.

The complexity of the wastewater matrix, which includes challenges such as the dilute nature of biomarker concentrations compared to urine and the intricate composition of the wastewater substrate, hinders the analysis and extraction of biomarkers and pathogens, and accurately determining the population size covered by WWTPs poses a significant challenge [17]. According to Mao et al. (2020), to address this, a thorough understanding of the metabolic process associated with biomarkers is crucial, ensuring that the biomarkers identified in wastewater originate exclusively from human sources and not external sources.

A national wastewater monitoring study in Australia linked sampling with the Australian Census, demonstrating the effectiveness of synchronizing wastewater sampling with a national census. This enabled an estimation of the population size and the acquisition of more reliable data on per capita consumption, exposure, and chemical release [286].

Despite its undeniable potential as a public health tool for detecting and effectively combating prevalent infectious diseases at a lower cost, further studies and research are necessary to solidify WBE as an internationally accepted tool.

Notably, numerous microorganisms, including viruses, bacteria, and protozoa, have been identified in water matrices, especially in wastewater, some of which are pathogenic to humans. Although they hold significant potential, the universal establishment of monitoring for certain viruses, such as enteric viruses, SARS-CoV-2, and the reemerging Monkeypox virus, has not yet occurred. This lack of universal monitoring is compounded by the need for major changes in water management and the design of water infrastructure [47]. These changes are crucial to effectively address challenges arising from climate change, alterations in the water cycle, the evolution of digitalization, infrastructure improvements, and the protection of privacy [288].

Enteric viruses represent a notable example of microorganisms with the potential for infectious spread through water, leading to food-related outbreaks, particularly prevalent in developed countries and significantly challenging in emerging nations due to inadequate sanitation, notably affecting children. Although monitoring initiatives for these viruses in wastewater have yet to be globally established, their significant potential has been recognized. For instance, Bisseux et al. (2018) in France developed a method for real-time monitoring of the circulation of a wide range of human enteric viruses in urban wastewater during environmental surveillance, indicating pollution derived from wastewater and assessing global water quality risk [150].

The COVID-19 pandemic underscored the necessity of effective strategies to monitor the behavior of the SARS-CoV-2 virus. Infectious disease surveillance involves techniques such as sentinel surveillance, clinical-based surveillance, surveys, hospital admission data, and mortality rates [289,290], yet it faces obstacles such as biases, long execution time, insensitivity to resource limitations, ethical concerns, and high costs [291]. Consequently, wastewater-based epidemiological surveillance proved valuable in addressing the pandemic by facilitating the identification of SARS-CoV-2 and enabling the implementation of monitoring initiatives that issue predictive alerts in a noninvasive and cost-effective manner, making it advantageous even for poor regions [112,292].

Apart from the benefits, obstacles continue to arise in the broad implementation of WBE, mostly because of the complex structure of the wastewater matrix. Efficient sample processing and viral DNA and RNA extraction techniques are necessary to ensure the dependability of WBE by removing matrix interferences and improving detection sensitivity. To scale up and increase the accuracy of WBE, further research is needed to produce a “gold standard” analytical approach for the complex wastewater matrix [293].

The surveillance of the reemerging Monkeypox virus in the USA and Europe now efficiently utilizes the established frameworks initially designed for testing SARS-CoV-2 [76]. Thus, wastewater monitoring programs can swiftly adapt to the screening of new targets, establishing them as flexible tools in epidemiological surveillance.

10. Conclusion

In conclusion, we highlight the promise and effectiveness of Wastewater-Based Epidemiology (WBE) as a crucial tool for monitoring and predicting the spread of infectious diseases, especially during the COVID-19 pandemic. This study emphasizes that despite the challenges associated with the collection, concentration, and detection of viruses in wastewater samples, PCR and qPCR techniques emerge as essential methods for viral pathogen detection, offering robust sensitivity and specificity. With the continued development and implementation of wastewater surveillance strategies worldwide, these innovative approaches are expected to continue playing a crucial role in protecting public health and preventing infectious disease outbreaks.

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Data availability

Data are contained within the article.

CRediT authorship contribution statement

Matheus Carmo dos Santos: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Ana Clara Cerqueira Silva:** Writing – original draft, Conceptualization. **Carine dos Reis Teixeira:** Writing – original draft, Conceptualization. **Filipe Pinheiro Macedo Prazeres:** Writing – original draft, Conceptualization. **Rosângela Fernandes dos Santos:** Writing – original draft, Conceptualization. **Carolina de Araújo Rolo:** Writing – review & editing. **Emanuelle de Souza Santos:** Conceptualization. **Maisa Santos da Fonseca:** Writing – review & editing. **Camila Oliveira Valente:** Writing – review & editing. **Katharine Valéria Saraiva Hodel:** Writing – review & editing. **Larissa Moraes dos Santos Fonseca:** Writing – review & editing. **Bianca Sampaio Dotto Fiuza:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Rodrigo de Freitas Bueno:** Writing – review & editing, Project administration. **Jailson Bittencourt de Andrade:** Writing – review & editing, Project administration. **Bruna Aparcida Souza Machado:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

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

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