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SARS-CoV-2 in wastewater: Occurrence, Detection and Implications

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Abstract

Coronavirus Disease-19 (COVID-19) is presently wreaking havoc on public health and socio-economic development. Besides the upper and lower respiratory tract involvement, gastrointestinal symptoms are also reported in COVID-19 patients through gut-lung axis. Finding its way through the feces of infected individuals and other sources, the genetic material of SARS-CoV-2 (ssRNA) is reported widely in wastewater and is being used as a fingerprint for its detection. With millions of cases arriving every day, there is a need to level up the testing speed efficiency. Due to the restricted sampling potential of testing laboratories, clinical testing is unable to track all the symptomatic and asymptomatic cases. Wastewater-based epidemiology (WBE) bestows an auxiliary monitoring tool that will contribute in community level screening. Sample collection, concentration, RNA extraction, quantification and data analysis are the main steps involved in implementation of WBE that can be relied upon as an alarm call for an upcoming wave, emergence of a new variant or any future pandemic. WBE can be a cheaper and more practical alternative to high end and sophisticated clinical testing for community transmission detection. Worldwide, there are more than 300 reports entailing the occurrence of SARS-CoV-2 in wastewater exhibiting unique temporal trends with five of them in India. This review aims to address the present knowledge on surveillance of SARS-CoV-2 in wastewater and its implications.

Keywords: Wastewater; Surveillance; SARS-CoV-2; Wastewater based epidemiology; COVID-19; Detection; Sewage

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1. Introduction

The COVID-19 (CoronavirusDisease 2019) outbreak began in December 2019 in Wuhan (China) and has since spread throughout the globe. SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus 2), the causative agent for COVID-19 has inflicted nearly 318 million infections and 5.53 million fatalities (as of January 13, 2022). Coronaviruses constitute a huge family of spherical and enveloped viruses possessing spikes on their outer surface and thus resembles a crown, hence the name. The envelope is composed of lipids and spike (S), envelope (E) and membrane (M) proteins. Nucleocapsid (N) protein is associated with its single stranded RNA genome (Kaur et al., 2021) (Fig.1). SARS-CoV-2 represents the most recent addition to the family of beta coronaviruses which are associated with human illnesses. It shares 70% of its genetic makeup with SARS-CoV (Gorbalenya et al.,2020). It is a zoonotic virus with likely origin in bats and has channeled to humans via an unestablished intermediate host. Inhalation via aerosol or droplet transmission as well as fomite and close contact are major routes of transmission for this virus. Cough, breathing problems, fever, and diarrhea are the most often reported symptoms in COVID-19 patients.

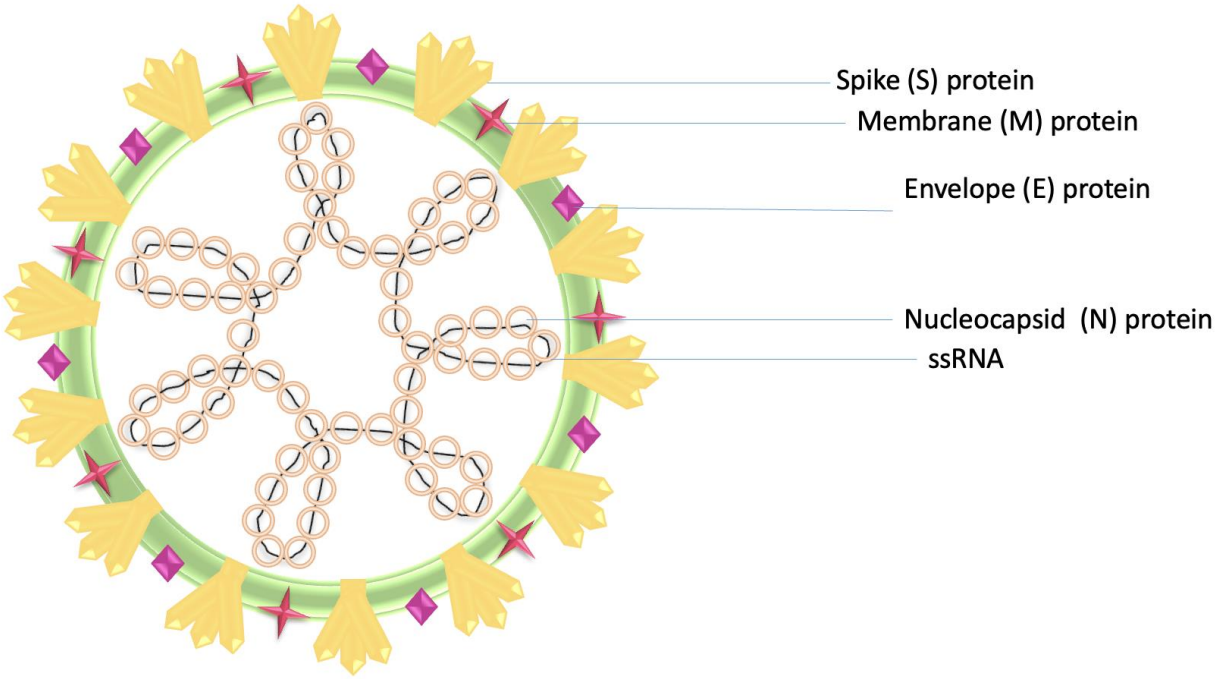

Figure 1: Structure of SARS-CoV-2 showing the 4 structural proteins-S, M, E and N

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The answer to a lot of respiratory manifestations in the pathogenesis of COVID-19 lies in the way the virus enters into the host. SARS-CoV-2 enters via the respiratory system where ACE2 (angiotensin-converting enzyme 2) which serves as an entry gate for the viral spike protein in respiratory and gastrointestinal epithelia, is highly expressed (Arslan et al., 2020). The public outcry about the pathogenicity of this deadly virus has so far been limited to its presence in respiratory secretions but there is now evidence of SARS-CoV-2 RNA in gastrointestinal secretions and in wastewater that has been traced to the presence of viral receptors on gastrointestinal cells (Zhang et al.,2020). Findings suggest that SARS-CoV-2 persists for a longer period of time in GIT (gastrointestinal tract) as compared to the respiratory tract (Grassia et al., 2020). It has been established that even after several weeks following the onset of symptoms, infected patients excrete viral RNA via feces. Hu et al. (2020) described two cases where SARS-CoV-2 was detected in the anal swabs of patients 6 and 14 days after they tested negative. On similar lines, Wu et al. (2020a) reported a patient in China whose fecal sample tested persistently positive even after 33 days of reporting negative results for respiratory samples. Tracking of SARS-CoV-2 RNA in feces (Chen et al., 2020; Young et al., 2020) and urine (Nomoto et al., 2020) of COVID-19 patients reveals pattern of viral propagation through water and raises the likelihood of fecal-oral transmission. From wastewater, this virus may reach sewage treatment plants, households and other areas with high transmissibility (Pandey et al., 2021).

The expeditious spread of SARS-CoV-2 has landed so many people in hospitals forcing the government to implement lockdown and physical distancing measures. However, with easing of the restrictions, new outbreaks are emerging in many countries. Owing to the massive vaccination drive being run in the world, the viral caseload has however decreased. But because of the hyper mutating nature of SARS-CoV-2, this ongoing pandemic has been a roller coaster ride with new variants of the viruses coming up every few months. In November 2021, a new SARS-CoV-2 variant named Omicron was reported. High spike mutations in omicron have enhanced its transmissibility while reducing its sensitivity to antibody neutralization (Dhingra et al., 2022). Therefore, the probability of a new wave of infection is high and hence keeping track of viral distribution and figuring out how viruses disseminate becomes crucial.

Traditionally, pandemic surveillance has relied largely upon clinical testing which is done at existing healthcare facilities or at temporary testing sites. For COVID-19, the classical testing procedure is to use RT-qPCR on nasopharyngeal samples. However, because of the highly contagious nature of the virus and the prevalence of asymptomatic viral carriers, clinical testing capacity has fallen well behind demand. Recognizing the need of bridging the gap and alleviating the stress on testing facilities, recent studies have underlined the potential of wastewater based epidemiology (WBE) as a supplementary alternative. The concept of an early warning

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surveillance system utilizing the WBE approach with relevance to COVID-19 pandemic aims at addressing vital community public health questions that could be answered by investigating and monitoring selected community health indicators which are reflected in the composition of urban wastewater (Kordatou et al.,2020). WBE provides early warnings and insights into the occurrence of COVID-19 in developing or underdeveloped regions where analytical equipment for extensive clinical screening is limited. The predominance of different genetic variants can be uncovered by undertaking sequencing studies (Martin et al., 2020). However, because of the high initial and operating expenses as well as the specialised infrastructure needs, genomic sequencing is restricted and unsustainable (Gwinn et al., 2019). Continuously tracking the prevalence of SARS-CoV-2 and taking necessary steps to prevent and control the disease spread in the population is critical. However, because most people are asymptomatic, tracking the virus is challenging. Also, owing to resource and economic restrictions, active clinical testing of all persons is not practical. Furthermore, COVID-19 may evoke more waves. Under these conditions, the passive yet effective approach of sewage or wastewater monitoring may be utilized to trace and track the prevalence of SARS-CoV-2 RNA and hence aid in screening the entire population.

2. Relationship between SARS-CoV-2 and Gastrointestinal Tract (GIT)

Human gut microbiome comprises a highly complex microbial community that protects the host from pathogens (Vemuri et al., 2017). The gut microbiome is important for maintaining GI equilibrium, and any disruption can cause gastrointestinal complications. Several studies have looked at the pattern of change of the fecal microbiota during the hospitalization of covid patients. In a pilot study, an enrichment of opportunistic microorganisms (such as *Bacteroides nordii, Clostridium hathewayi, Actinomyces viscosus* and *Streptococcus spp*) was discerned in 15 COVID-19 patients, as compared with healthy ones. A decline in beneficial commensals (like *Lachnospiraceae and Ruminococcaceae* families) was also observed (Zuo et al.,2020). Infections with viruses tend to increase the production of cytokines, which significantly influence gut microbiome (Jose and Manual, 2020). The increased cytokines and inflammatory markers in SARS-CoV-2 patients are well established (Ramachandran et al.,2020). Additionally, the use of antibiotics and antivirals can alter gut microflora putting people at risk for GI complications (Wei et al., 2020). Secondary metabolites and antimicrobial peptides generated from beneficial intestinal microbes are important in cellular homeostasis, and the gut microbiome modulates the functioning of immune system (Negi et al., 2019). It has been established that gut microbiota may aid in the development of ARDS (acute respiratory distress syndrome) (Dickson, 2015), implying that the same could impact the onset of SARS-CoV-2-mediated tissue damage resulting from hypercytokinemia (Girija et al., 2020) and SIRS (systemic

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inflammatory response syndrome) (Dhar and Mohanty, 2020). Viral infections raise proinflammatory cytokines in the blood, which causes gut dysbiosis and a breakdown of the protective gut barrier.

Overactive and underactive immunological responses, mediated by gut microbiota result in significant clinical outcomes. Gut microbiota dysbiosis compromises the gut barrier allowing SARS-CoV-2 to travel from lungs to gut through lymphatic and blood circulatory systems (Fig. 2).

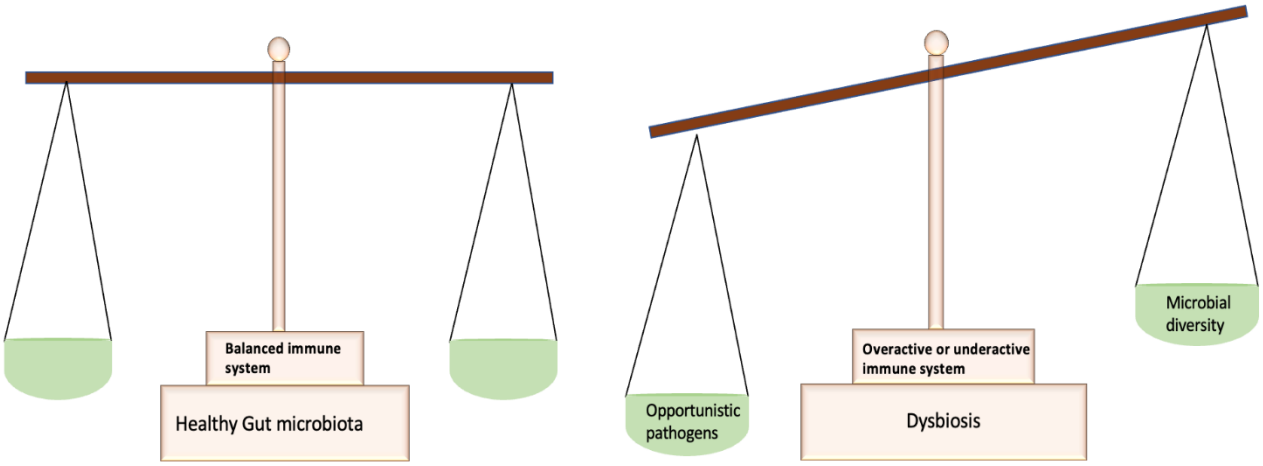

Figure 2: Effect of immune health on gut microbiome

There is a two way lexicon between the gut and lung, which implies that chemicals produced by gut bacteria affect lungs via blood, whereas lung inflammation alters gut microbiota levels. Owing to the respiratory manifestations in COVID-19 patients, a shift in lung microbiota may result in changing the gut microbiome. This gut-lung axis is becoming well recognised as a possible source of gastrointestinal symptoms in patients having respiratory complications (Dhar and Mohanty, 2020).

ACE2 receptor plays a key role during entry of virus into the host cell and lodging subsequent infection. To entertain the possibility of SARS-CoV-2 infection spreading through the oral mucosa, the researchers gathered bulk RNA-seq profiles from two public databases: TCGA (The Cancer Genome Atlas) and FANTOM5 CAGE (Functional Annotation of The Mammalian Genome Cap Analysis of Gene Expression). According to the findings, ACE2 was found to be expressed on the mucosa of the oral cavity (Xu et al., 2020a).

SARS-CoV-2 interacts with ACE2 receptor through S1 domain of its spike protein to penetrate host cells and lodge an infection. TMPRSS2 (Transmembrane serine protease 2) catalyses the breakdown of S protein which allows the virus to release fusion peptide for membrane fusion (Hoffman et al., 2020). Following viral entry, virus-specific proteins are synthesized in the cytoplasm to form new viral particles that are then discharged into the gut. (Batlle et al., 2020) (Fig. 3).

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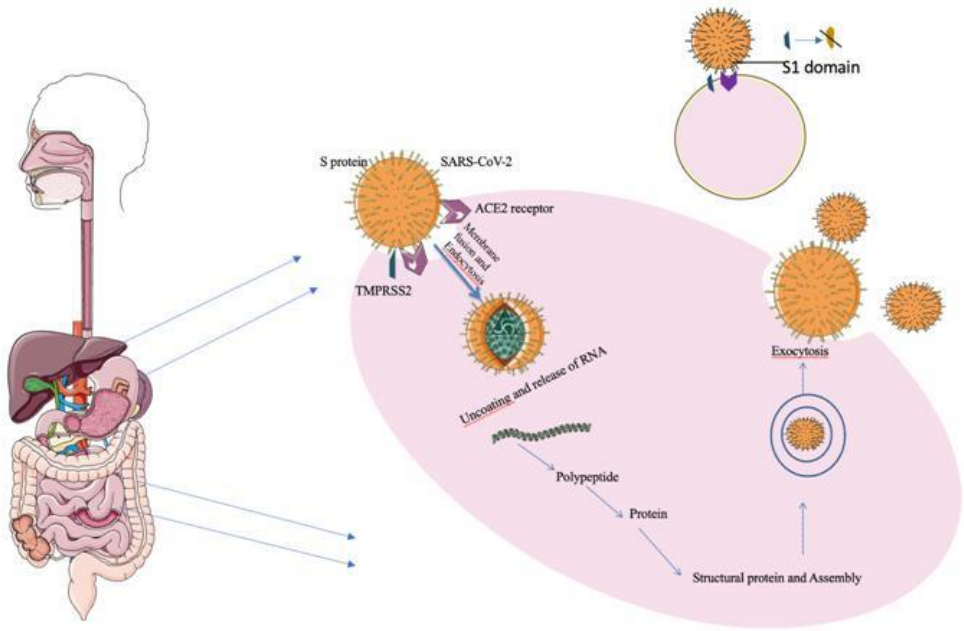

Figure 3: SARS-CoV-2 infecting the cells of GIT mediated by ACE2 and TMPRSS2

It suggests that coexpression of ACE2 and TMPRSS2 makes individuals more susceptible to infection. However, the cells expressing only one of them remain relatively safe (Zhang et al.,2020).

They examined the expression levels of both ACE2 and TMPRSS2 in all cell populations pertaining to gastrointestinal system to ascertain the prevalence of such receptors in the gut.

It was found that ACE2 was substantially expressed in absorptive enterocytes in the epithelial cells of ileum and colon. The stomach, lung, transverse colon and small intestine all expressed significant levels of TMPRSS2 (Zhang et al., 2020). This suggests a link between classical COVID-19 symptoms and gastrointestinal tract problems. About half the patients with COVID-19 develop GI symptoms. Some of the most common GI symptoms in COVID-19 patients are nausea, diarrhea, anorexia, vomiting and abdominal discomfort. There have also been findings of gastrointestinal bleeding, severe pancreatitis, and colitis. (Perisetti al., 2020). GI symptoms often precede respiratory symptoms (Villapol et al., 2020).

3. SARS-CoV-2 in Wastewater: A potential transmission pathway and wastewater epidemiology (WBE)

Contamination of water systems with human and animal fecal material has been identified as a human health issue due to the fact that water serves as a vehicle for microbes to disseminate and trigger disease outbreaks (La Rosa et al., 2020). The most crucial issue to address is that how the virus enters into the water bodies. The main ways via which SARS-CoV-2 makes its way to the water supply system are (1) wastewater from bathing of patients,

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(2) feces that have been expelled through the toilet (3) contagious waste from infectious items such as contaminated clothing and protective gear being washed away in the laundry, (4) wastewater from health care and funeral homes (Gwenzi., 2021) (5) used masks discarded in the environment, (6) wastewater generated from the public and (7) via toilet flushing and impaired household and municipal plumbing systems (Fig. 4).

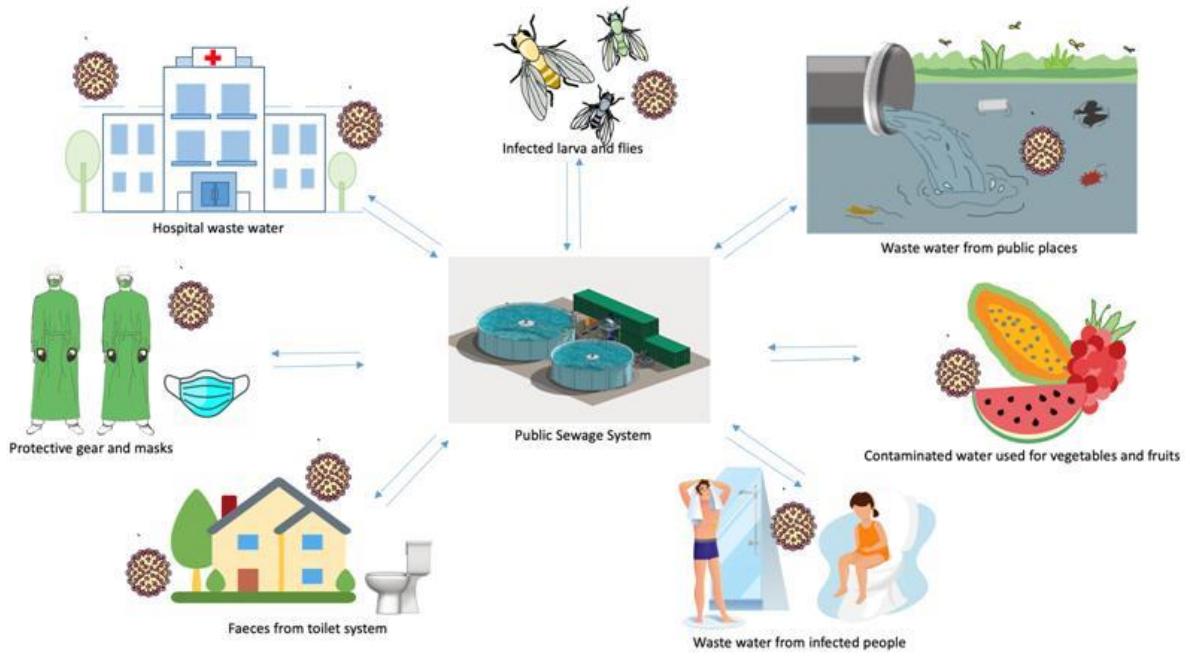

Figure 4: SARS-CoV-2 in wastewater: Routes

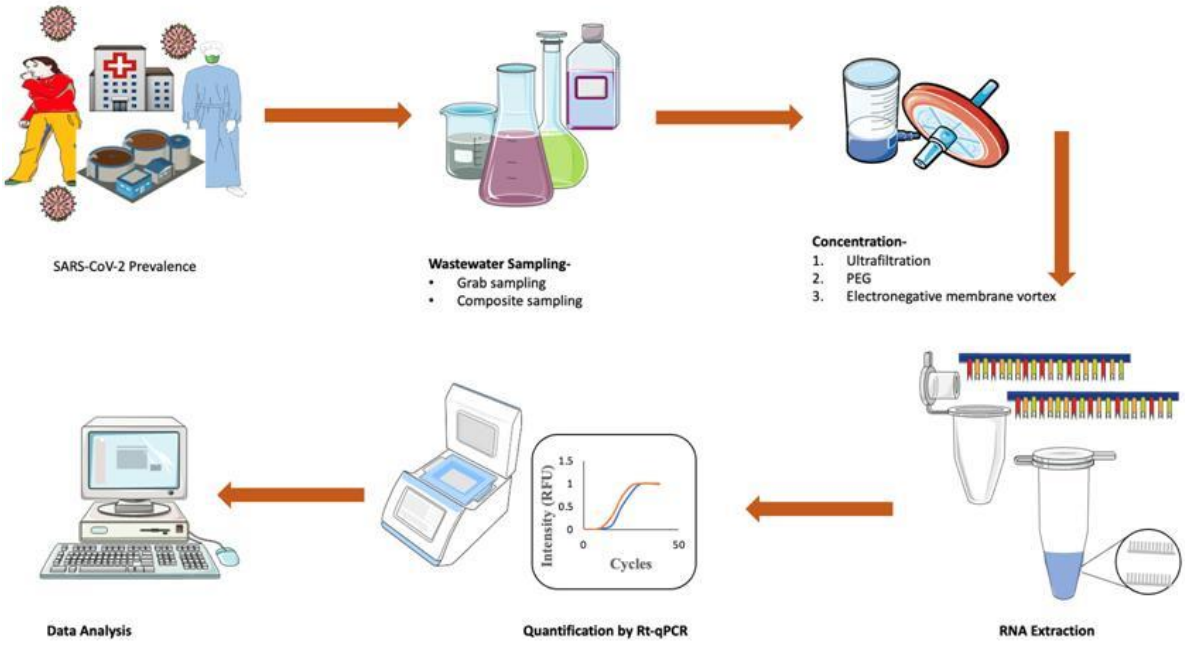

Figure 5: Stepwise illustration of WBE implementation

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According to reports, SARS-CoV-2 is live on the external surface of a surgical face mask for up to 7 days (Chin et al.,2020). Hence discarded face masks that end up in water bodies might provide a high-risk transmission channel for SARS-CoV-2 into water. Viruses reach the sewage system through vomit, urine and feces of infected patients and are transported to the wastewater treatment plant (WWTP) via municipal sewage system. Workers maintaining sewage systems may be exposed to infectious viral particles. The viral load reaching the WWTPs is discharged into surface water as a result of combined sewage overflow incidents. Attributed to the prevalence of viral RNA in stools, there is a profound risk of fecal oral transmission, especially in asymptomatic persons. The virus enters the plumbing system through toilet seats and can then travel to wastewater treatment facilities, where it can infect the personnel. Untreated wastewater, river water, secondary treated wastewater, medical wastewater, municipal sewage, water from cruise ships and aeroplanes, wastewater treatment plant-derived sludges have all been reported to contain traces of SARS-CoV-2 (Ahmed et al., 2020a; Medema et al., 2020).

A study involving 10 pediatric patients provided indications for fecal shedding of SARS-CoV-2. Even after negative nasopharyngeal testing, the rectal samples of eight children remained positive, implying viral shedding by GIT and fecal transmission might occur (Xu et al., 2020b).

Infectious disease-causing microorganisms excreted in feces and urine can be tracked via wastewater analysis. Over the previous year, methods for identifying and quantifying SARS-CoV-2 RNA in untreated sewage have improved with wastewater concentrations corresponding with the reported case trends. In comparison to door-to-door sampling and nasal swab testing, WBE is less costly and more practical. It provides a clearer picture of community distribution of cases than clinical testing does. Table 1 highlights the major difference between these two approaches, stressing the pros and cons of each.

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Table 1: Comparison of clinical testing and WBE

Clinical testing.	**Sewage surveillance**
Pros : • More specific. • Individual specific result. • Easy handling • More accurate	**Pros**: • A quick and efficient technique to check for SARS-CoV-2 infections in small and large populations. • Provides a clearer picture of community distribution of cases than clinical testing does. • Serves as an early warning system for new variants or new waves of disease. • Cost effective and more practical. • Surveillance of the asymptomatic carriers is possible • Can be carried out in remote areas where access to laboratory equipment is shallow.
Cons: • Time consuming. • Sample collection is difficult especially from pediatric patients. • Not feasible for larger population.	**Cons**: • Represent a larger group of people rather than giving individual specific results. • Possibility of false positive and false negative results because of sample dilution, presence of nucleases in sewage etc. • Not feasible in undeveloped areas with improper sewage management.

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4. Persistence of SARS-CoV-2 RNA in Wastewater

Environmental factors influencing SARS-CoV-2 transmission (e.g., humidity, particulate material, temperature) have lately been investigated and they reflect that virus spread depends on its ability to persist in a particular environment. The prevalence of various coronaviruses has been documented in both treated and untreated water. SARS-CoV-2 life and sustainability are influenced by the amount of organic matter, initial viral load, temperature, medium type, presence of biological fluids, and chemicals (Carraturo et al., 2020). The envelope of coronaviruses is degraded and generally, the genetic material is found in organic matrices. According to documented data, SARS-CoV remained infectious after 14 days in wastewater at 4 °C, but only 2 days at 20 °C (Wang et al., 2005). Gundy et al (2008), for SARS-CoV, found that coronaviruses survive in wastewater for 2–4 days. They also reported that survival in primary wastewater was only marginally longer than in secondary wastewater, owing to the increased level of suspended particles that protect against inactivation. The half-life mean for SARS-CoV-2 prevalence in wastewater was 0.64 day. Furthermore, 4.3 days were required to reduce infectious virus titre by 99 percent (Bivins et al., 2020).

La Rosa et al. (2020) analysed that coronavirus is susceptible to oxidants such as chlorine and is inactivated in water quickly as compared to non-enveloped human enteric viruses that are known to spread by water. ClO_2 and heat treatments impede the host-cell recognition or binding causing protein damage without impairing genome function. UV_{254}, singlet oxygen, and hypochlorous acid impair genome stability by breaking the capsid protein backbone at specific sites and inhibiting genome replication (Wigginton et al., 2012). Standard disinfection measures are effective against SARS-CoV-2, according to Chin et al. (2020), who found that the virus was undetectable after 5 min of treatment with household bleach (1/49 and 1/99 dilution ratios), chloroxylenol (0.05%), povidoneiodine (7.5 %), ethanol (70%), and chlorhexidine (0.05%) (Chin et al., 2020). Sewage sludge acts as a carrier of SARS-CoV-2 viral particles. Peccia et al. (2020) found that the concentration of SARS-CoV-2 RNA was two to three orders of magnitude greater in primary sludge than in raw wastewater because of the higher solid content.

5. Process workflow

The presence of the SARS-CoV-2 genetic fingerprint can be sensed by sampling and analysing wastewater from a specific location at the treatment facility. Many factors are important to gather useful data for monitoring like collection site, sampling time, sample transport, storage, concentration and quantification method and contextual information regarding the amount of human feces in the sample (Medema et al., 2021).

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5.1 Wastewater sampling

Sampling is a vital step in the deployment of WBE monitoring programme. Based on defecation frequency, sampling technique and sampling frequency, the concentration of SARS-CoV-2 RNA in influent wastewater is projected to alter intermittently. Samples are collected either as grab or composite. Grab samples are taken at a particular place at a specific time and hence provide only a glimpse of wastewater composition. Grab sampling does not take into account WWTP parameters like flow rate, the total volume of wastewater, and temperature. Composite samples (fixed volume of water is taken at fixed time intervals), on the other hand, represent a holistic view of the wastewater composition over a specific period (Gerba et al., 2017). Additionally, the viral detection process depends largely on the time of sample collection. In most cities, fecal shedding is at its peak in the morning or evening which makes these best times to ascertain the viral load during grab sampling. The collection of composite samples will represent the average concentration of viral RNA across the collecting time, without distinguishing between any peak values recorded during the sampling period. As per the reviewed studies (Table 2), the volume of sample varies between 200 mL- 2 L. Sampling is generally done in glass or plastic bottles. The site for sample collection is chosen concerning the population encompassing that particular water body. Samples are often taken at the influent of WWTPs processing household sewage for populations linked to a sewer system to get an overall idea of the viral composition. To determine the most realistic and reliable sampling strategies for detection and quantification of RNA in wastewater, more research and insight is needed in terms of sample type, frequency, duration and sampling site.

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Table 2: Major* case studies providing evidence of SARS-CoV-2 RNA in waste water by employing WBE

Place; Sample collection site and time	**Sample type (Sample treatment); Volume**	**Post sample collection treatment**	**Concentration technique**	**RNA extraction method**	**Genes targeted; PCR assay (PCR kits)**	**Sample positivity and correlation with clinical trends**	**Reference**
Massachusetts (USA); Deer Island Wastewater Treatment Plant and municipal sewer lines, Feb-March, 2020	Grab and composite (Untreated); NR	Pasteurization at 60°C followed by vacuum filtration through 0.2µm membrane.	PEG (8000) precipitation and Ultrafiltration	Trizol reagent	N1, N2; RT-qPCR (TaqMan® Fast Advanced Master Mix; TaqMan™ Fast Virus 1-Step Master Mix; ProtoScriptII Reverse Transcriptase)	NR; Over the studied time period, viral titers in wastewater followed a pattern comparable to new clinical cases. The absence of any case reported before	Wu et al., 2020b

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						march corresponds to no positive detection. A low viral titer is proportional to the lower cases in the population (2 cases in early March) and in late March, the number of titers increased exponentially.	
Connecticut (USA); East Shore Water Pollution Abatement Facility,	NR (Untreated); 40 mL	Stored at −80°C	NR	RNeasey Power Soil Total RNA Kit, Qiagen	N1, N2; RT-qPCR	3632/17661 (20%); No comparison was made	Peccia et al., 2020

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March 19- June1, 2020					(iTaq Universal Probes One-Step Kit)	between clinical positivity and sludge SARS-CoV-2 RNA concentrations.	
Montana (USA); Municipal wastewater treatment plant, March 2020 to June, 2020	Composite (Untreated); 500 mL	Membrane filters (20 mM, 5 mM and 0.45 mM filters)	Ultrafiltration (100kDa molecular weight cut off)	RNeasy Mini Kit (QIAGEN)	N1, N2; RT-qPCR (2019-nCoV CDC EUA Kit)	13/17 (76%); In the first month, SARS-CoV-2 levels were undetected. However, in late May, the wastewater began to test positive, coinciding with	Nemudryi et al., 2020

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						an increase in COVID-19 cases in the population.	
Louisiana (USA); Wastewater treatment plants, January to April, 2020	Composite and grab (Untreated, secondary treated and chlorine disinfected); 1 L	NR	Ultrafiltration and adsorption–elution method using an electronegative membrane	ZR Viral RNA Kit (Zymo Research, Irvine, USA)	N1, N2; RT-qPCR (PerfecTa qPCR Tough Mix Quantabio, Beverly, MA)	2/15 (13%); Clinical and WBE trends were found to be connected. There was no detection in wastewater before to the first COVID-19 case, which was detected on March 9; tests were positive in	Sherchan et al., 2020

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						April, when COVID-19 infections first appeared.	
Virginia (USA); 9 Wastewater treatment plants, March 2020	Composite and grab (Untreated); 1 L	Kept on ice	InnovaPrep Concentrating Pipette Select (InnovaPrep, Drexel, MO, USA) and electronegative filtration	NucliSENS easy-Mag (bioMerieux, Inc., Durham, NC, USA)	N1, N2, N3; RT-ddPCR (One-Step RT-ddPCR Advanced Kit for Probes)	150/198 (75.7%); One WWTP tested positive every time the sample was collected, even when there were just 2 positive cases in the region. The later trends matched	Gonzalez et al., 2020

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						clinical positivity ratio.	
New York (USA); Sewage Network Access Points; 28 April–24 June 2020	Composite (Untreated); 10 mL – 1.9 L	Stored in ice	Ultracentrifugation	AllPrep® PowerViral® DNA/RNA Kit (Qiagen®, Hilden, Ger- many)	RdRP; RT-qPCR [Reliance One-Step Multiplex RT-qPCR Super- mix (Bio-Rad®, California, USA)]	111/169 (65%); Samples containing detectable quantities of SARS-CoV-2 RNA were linked to a greater rate of positive test findings in hospitals.	Wilder, et al., 2021

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Ohio (USA); Hospital, nursing home and city manhole access points, July 2020	Composite (Untreated); 1 L	Stored in ice	PEG (8000) precipitation	TRIzol Reagent and QIAamp Viral RNA Mini Kit	N1; RT-qPCR [TaqMan Fast Viral One-Step Master Mix (ThermoFisher)]	N1: 9/12 (75%) Hospital: 5/6 (83%) Nursing Home: 1/3 (33.3%); There was a slight positive correlation between the number of patients infected and the viral load in the wastewater in hospitals and nursing homes.	Spurbeck et al., 2021

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Wisconsin (USA); Wastewater treatment plants, October 2020 mid to beginning of Jan, 2021	Composite (Untreated); 500 mL-1 L	Stored at 4°C	PEG/NaCl precipitation	Wizard® Enviro Wastewater TNA kit ; Maxwell® Enviro Wastewater TNA kit	N1, N2 , E; RT-qPCR [SARS-CoV-2 RT-qPCR Detection Kit for Wastewater (Promega Corp.)]	N1:36/36 N2: 36/36 E: 36/36 (100%) ; The peak of positive SARS-CoV-2 cases which were reported in mid-November 2020 conforms to the peak of the SARS-CoV-2 genetic evidence in the wastewater.	Mondal et al., 2021
Spain; Hospital treating COVID-19 patients and	Composite (Untreated); 100 mL	Samples were centrifuged at 4000g for 30	Ultrafiltration (30 kDa molecular weight cut off)	QIAmp viral RNA mini kit	N; RT-qPCR	NR; There was a strong	Vallejo et al., 2020

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wastewater treatment plants, 15 April 2020 - 4 June 2020		min and were filtered through 0.22 µm membranes			qCOVID-19 kit (GENOMICA, SPAIN)	correlation between active cases and logarithm of daily mean viral load.	
Valencia (Spain); Wastewater treatment plants, February 12 2020 to April 14 2020	Grab (Treated and untreated); 200 mL	Stored at 4°C	Aluminum driven flocculation	Nucleospin RNA virus Kit (Macherey-Nagel)	N1, N2; RT-qPCR (Prime- Script One Step RT-PCR Kit)	N1: 22/73 (30%) N2: 26/73 (35.6%) N3: 28/73 (38.3%) ; On February 24, first wastewater sample tested positive	Randazzo et al., 2020a

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						coinciding with the first positive case confirmed on 25 Feb, 2020. Similar trends followed thereafter.	
Murcia (Spain); Wastewater treatment plants, March 12 2020- April 14 2020	Grab (Untreated, secondary and tertiary treated); 500 mL-1 L	Stored at 4°C	Aluminum hydroxide adsorption-precipitation	Nucleo- Spin RNA virus kit (Macherey-Nagel GmbH & Co., Düren, Germany)	N1, N2, N3; RT-qPCR [One Step PrimeScript RT-PCR Kit (Perfect Real Time)]	13/24 (54.1%) When cases were identified inside the municipality, amplification signs were observed in wastewater.	Randazzo et al., 2020b

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Barcelona (Spain); Wastewater treatment plants and sewer maintenance holes, April 13 2020 - 7 July 2020	Grab (Untreated); 800 mL	NR	PEG6000 precipitation	NucliSENSminiMAG extraction system (bioMérieux)	N1, N2, E, IP2, IP4, RdRp; RT-qPCR (RNA UltraSense one-step quantitative RT-PCR system; Invitrogen, Life Technologies)	NR; Based on the actual number of reported symptomatic cases, the fall in genome copy numbers corresponded to a decrease in the predicted cumulative number of shedders.	Chavarria-Miró et al., 2021
Catalonia (Spain); Wastewater treatment plants,	Composite (Untreated); 250 mL	Stored at −80°C	Ultrafiltration (30 kDa molecular weight cut off)	QIAamp Viral RNA Mini Kit using the QIAcube automatic system (Qiagen)	N1, N2; RT-qPCR (RNA UltraSense™ One- Step RT-	N1: 118/184 (64%) N2: 102/184 (56.5%)	Rusinol et al., 2021

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Mid-march to early November 2020					qPCR System (Invitrogen)	Overall: 128/184 (69.5%); During the first wave of the pandemic, the infection peak in Barcelona coincided with the highest proportion of SARS-CoV-2 in waste water. While in the second wave, the proportion of N1 in wastewater was less than during	

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						the first wave. Larger WWTPs have higher proportion of N1 and N2 than smaller ones.	
Gujarat (India); Waste water treatment plants, 8th and 27th May 2020	Grab (Untreated and treated); 500 mL	Stored at 4°C	Centrifugation and filtration followed by PEG 9000 precipitation	NucleoSpin® RNA Virus, Macherey-Nagel GmbH & Co. KG, Germany	ORF1ab, S, N; RT-PCR (TaqPath Covid-19 RT-PCR Kit)	NR; Positive samples were detected with more than tenfold increase on May 27 than on May 8, which corresponded the frequency of	Kumar et al., 2020

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						infections in the area.	
Gujarat, Gandhinagar (India); Wastewater treatment plants, 7 August 2020 to 30 September 2020	Grab (Untreated); 250 mL	Kept in ice	Centrifugation and filtration followed by PEG 9000 precipitation	NucleoSpin® RNA Virus (Macherey-Nagel GmbH & Co. KG, Germany)	ORF1ab, S, N; Real time-PCR [TaqPath™ 1 Step Multiplex Master Mix (Thermofischer Scientific, USA)]	Overall: 39/43 (90%) Wards: 32/33 (96.9%) Academic institute: 7/10 (70%); The percentage change in genomic concentration level on a certain day was positively	Kumar et al., 2021a

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						associated to confirm cases reported 1–2 weeks later. In September, the SARS-CoV-2 RNA concentration was greater than in August.	
Gujarat, Ahmedabad (India); WWTP, pumping station, lakes, river, 3 September 2020 and 26 November 2020	Grab (Untreated); 250 mL	Kept in ice	Centrifugation and filtration followed by PEG 9000 precipitation	NucleoSpin® RNA Virus isolation kit (Macherey-Nagel GmbH & Co. KG, Germany)	ORF1ab, S, N; RT-qPCR [TaqPath^{TM} 1 Step Multiplex Master Mix (Thermofischer	111/116 (95.6%) Positive correlation of clinically confirmed cases with WBE trends.	Kumar et al., 2021b

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					Scientific, USA)]		
Chennai (India); STPs , SPSs, hospital, 5 September 2020 to 11 September 2020	Composite and grab (Treated and untreated); NR	Kept in ice	Composite (COM), supernatant (SUP), sediment (SED) and syringe filtration (SYR)	QIAamp Viral RNA mini kit, Qiagen, Germany	N1, N2 RNase P; RT-qPCR (IDT 2019-nCoV CDC-EUA kit)	12/17 (70.5%) Wastewater surveillance revealed a larger proportion of affected persons in places with high population density	Chakraborty et al., 2021

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Jaipur (India); Wastewater treatment plants, hospitals, February 202021-June 82021	Grab (Untreated, secondary treated, tertiary treated); 1 L	Surface sterilized using UV treatment for 30 min followed by centrifugation.	NR	MagMAX Viral/Pathogen NucleicAcid Isolation Kit (Applied Biosystems	N, E, RdRp; RT-PCR (Allplex^{TM} 2019- nCoV Assay RT-PCR)	WWTP:5/18 (27.7%) Hospitals: 1/7 (14.2%); Areas covered by WWTPs with positive detections reported a significant rise in confirmed positive cases soon after the first sampling.	Arora et al., 2021

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Pune (India); Wastewater drains, 23 December, 2020- 22 February 2021	Grab (Untreated); 1L	Heat inactivation by placing in water bath (60°C for 60 min)	Vacuum filtration, Ultrafiltration	RNeasy Power Water Kit (Qiagen; 14700-50-NF)	NR; RT-qPCR (Blunt/TA Ligase Master Mix (New England Biolabs; M0367L), NEB Next Ultra II End Repair/dA-Tailing Module (New England Biolabs; E7546L) and Native Barcoding Expansion 1–12 (PCR-free) (Oxford Nanopore	NR; Before clinical detection, novel mutations were discovered in wastewater samples. In wastewater, the presence of SARS-CoV-2 Delta variant lineage related mutations (B.1.617) corresponded with clinical suspicion.	Dharmadhikari et al., 2022

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					Technologies; EXP-NBD104)		
Netherlands; Wastewater treatment plants and airport, 5 February, 2020 – 25 March 2020	Composite (Untreated); 250 mL	Stored at 4°C until further processing.	Ultrafiltration (100kDa molecular weight cut off)	RNeasy Power Microbiome Kit (Qiagen, Hilden, Germany)	N1, N2,N3,E; RT-qPCR [Taqman Fast Virus 1-Step Master Mix (Applied Biosystems, Fisher Scientific, Landsmeer, The Netherlands)]	WWTP:12/25 (48%) Airport: 3/4 (75%); As the pandemic progressed, the clinically confirmed cases correlated with wastewater sample	Medema et al., 2020

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						positivity. The proportion of SARS-CoV-2 RNA increased as the number of COVID-19 patients reported increased.	
North Rhine-Westphalia (Germany); Wastewater treatment plant, 8-9 April, 2020	Composite (Untreated, tertiary treated); NR	Transported to laboratory on melting ice.	Ultracentrifugation	NucleoSpin RNA Virus kit (Macherey Nagel)	M, N, E, RdRp; RT-qPCR (Luna Universal Probe One-Step RT-qPCR Kit, LightCycler® Multiplex RNA virus Master)	13/13 (100%); No conclusive correlation between clinical cases and SARS-CoV-2 concentration in waste water was established.	Westhaus et al., 2021

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Yamanashi Perfecture River (Japan); River and wastewater treatment plant, 17 March, 2020 –7 May 2020	Grab [Untreated, river water, secondary treated water (activated sludge)]; 1 L	Transported to laboratory on ice and processed within 6h.	Electronegative membrane-vortex (EMV), Adsorption-direct RNA extraction method	RNeasy Power Water kit (Qiagen) and β-Mercaptoethanol	N, N1, N2, ORF1a, S; RT-qPCR, Nested RT-qPCR (NR)	WWTP:1/10 (10%) River: 0/3 (0%); In Yamanshi Prefecture, there was a correlation between RNA detection and the maximum peak in daily cases. When confirmed cases were high, RNA concentration was also high.	Haramoto et al., 2020

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Greater Doha (Qatar); Wastewater treatment plant, 21 June 2020 to 30 August 2020	Composite (Untreated); 1 L	On site heat treatment at 56°C for 30 min.	PEG8000 Precipitation	Quick-RNA Viral Kits (Zymo Research, Irvine CA, USA)	N1, N2; RT-qPCR (SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay Research Use Only (RUO) kit)	43/43 (100%) During the research period, daily reported SARS-CoV-2 positive cases declined by almost 66 percent, which was paralleled by lowering CRNA trends in all WWTPs. In the initial two weeks of August 2020, there was a brief increase in	Saththasivam et al., 2021

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						CRNA, which correlated with an increase in daily reported cases.	
Hong Kong (China); Manholes of isolation ward hospital, public housing estate, Waste water treatment plant , sewer network, 8 June, 2020 to 29 September, 2020	Composite (Untreated); NR	Inactivation at 60°C for 30 min prior to subse- quent processing followed by centrifugation	Ultrafiltration (10kDa molecular weight cut off)	Trizol plus RNA Purification Kit (Thermofisher)	N; RT-qPCR (TaqMan Fast Virus 1-Step Master Mix)	23/107 (21.49%); SARS CoV-2 was detected in sewage samples two days before COVID-19 was discovered in two housing estate buildings. Relationship	Xu et al., 2021

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						between WWTP RNA concentration and clinically confirmed cases was not seen.	
Rio De Janeiro (Brazil); Sewer network, hospital waste water and sewage treatment plant, 15 April, 2020 - 25 August 2020	Composite (Untreated); NR	Pasteurisation at 60°C for 90 min	Ultracentrifugation	QIAamp® Viral RNA Mini kit (QIAGEN, CA, USA) ,QIAcube® automated system (QIAGEN)	N2; RT-qPCR (NR)	WWTP:5/10 (50%) Hospital :0/2 (0%); SARS-CoV-2 RNA titers of sewage samples were higher at the time when the city had the	Prado et al., 2021

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						largest number of COVID-19 patient cases.	
Pakistan (38 Districts); Quarantine center drainage, open drains, pumping station, 20 March–28 April 2020	Grab (Untreated); 1 L	Transported in cold chain.	PEG precipitation	Spin star viral nucleic acid kit 1.0	ORF1ab, E, N; RT-qPCR, RT-PCR [Real-Time Fluorescent RT-PCR Kit, 2019-nCoV Nucleic Acid Diagnostic, Kit (PCR	21/78 (26.9%); SARS-COV-2 RNA was found in wastewater samples collected from sites where COVID-19 patients had	Sharif et al., 2020

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					Fluorescence Probing)]	recently been confirmed.	
Milan (Italy); Waste water treatment plant, River, Canal, 14 and 22 April 2020	Grab (Treated wastewater, river water); 1 L	Transported in dark glass bottles under refrigeration conditions.	Not undertaken	QIAMP Viral RNA mini kit (Qiagen, Hilden, Germany)	N, ORF1ab, E; RT-qPCR (2019-nCoV real-time RT-PCR kit panel)	WWTP:4/12 (33.3%) River: 4/6 (66.6%); The study was conducted during declining phase of peak and lower viral load was observed on 22 April than on14 April which corresponds to	Rimoldi et al., 2020

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						the overall declining case trend.	
Queensland (Australia); Pumping station and wastewater treatment plant, 24 February 2020–1 April 2020	Composite and grab (Untreated); NR	Stored at 4°C.	Electronegative membrane and ultrafiltration (10kDa)	RNeasy Power Microbiome Kit (Qiagen).	N; RT-qPCR (iTaq™ Universal Probes One-Step Reaction Mix)	2/9 (22.2%); Wastewater samples were generally positive during the period with highest caseload data.	Ahmed et al., 2020a

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Melbourne (Australia); Waste water treatment plant and main sewer pipes, 25 Aug 2020 – 27 Oct 2020	Composite and grab (Untreated); NR	Stored at 2-8°C until further processing.	NR	MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific)	N, ORF1ab; RT-qPCR (PerkinElmer® SARS-CoV-2 Nucleic Acid Detection Kit)	71/346 (20%); Even when a single case was present, the WWTP gave a positive RNA signal, providing some evidence that even a single infected individual in a community on a given day could trigger virus detection at the sampling site on	Black et al., 2021

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						that same day.	
Helsinki (Finland); Waste water treatment plant, 9–20 April and 24–25 May 2020	Composite (Untreated); 2 L and 500 mL	Stored at 4°C, −20°C, and −75°C for later analysis after dividing into aliquots.	Ultrafltration	Chemagic Viral300 DNA/RNA extrac-tion kit	E, N2; RT-qPCR (TaqMan Fast Virus 1-step Master Mix and a QuantStudio 6 Flex real-time PCR system)	NR; SARS-CoV-2 was found in wastewater influent samples, which matched verified COVID-19 cases.	Hokajärvi et al., 2021

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12 North eastern cities (France); Wastewater treatment plant, 2 April 2020 –28 May 2020	NR (Untreated); NR	NR	Ultrafiltration, PEG6000 precipitation	NR	RdRp, E ; RT-ddPCR, RT-PCR (RNA UltraSens™ One-Step Quantitative RT-PCR system)	NR; A drop in cases in patients was detected in tandem with a decrease in genome concentration in wastewater, establishing the relationship between the virus circulation in the human population and its presence in wastewater.	Bertrand et al., 2021

*Among 350+ studies only major studies have been included in the table

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5.2 Storage and treatment after collection

In most cases, samples obtained for viral determination in environmental matrices are processed and analysed promptly, however, sometimes the samples are needed to be stored before further processing. The samples are stored at 4°C, transferred to a transport container, and then to the laboratory at 4°C (Table 2). Too low temperatures (-20°C /-80°C) are not recommended because thawing can degrade viral genetic material (Brisebois et al., 2018). RNA viruses are vulnerable to degradation, owing to the fragility of their envelope and the instability of RNA due to the ubiquity of RNases in environment. This could result in a lesser viral load giving false analytical results. According to previous data, SARS-CoV isolated from a patient's sputum could be heat-inactivated and incubation at 60°C for 30 min gave 100% SARS-CoV eradication. (Rabenau et al., 2005). In some studies, samples were given heat treatment (56°C or 60°C for 30 min) before viral concentration to enhance the laboratory staff's safety during sample handling (Table 2). Thermal treatment of the sample reduces SARS-CoV-2 infectivity by more than 5 logs without distrupting RNA structure (Pastorino et al., 2020). In some cases, UV disinfection was also employed (Arora et al., 2021). UV impairs genome stability by breaking the capsid protein backbone at specific sites, inhibiting genome replication (Wigginton et al., 2012). Some detection and quantification analyses began with centrifugation and/or filtering to eliminate bacterial debris and coarse particles from wastewater, as shown in Table 2.

5.3 Concentration

After entering the sewer systems via human excretions, the SARS-CoV-2 genome gets diluted below the detection limit. For the enrichment of SARS-CoV-2 in wastewater, multiple concentration approaches are applied which include PEG precipitation, ultrafiltration, adsorption elution, electronegative membrane filtration, electronegative membrane vortex, aluminium driven flocculation, aluminium hydroxide adsorption precipitation, filtration through the mixed cellulose-ester membrane, ultracentrifugation (Table 2). Ahmed et al. (2020b) compared seven concentration methods of SARS-CoV-2 recovery from untreated wastewater using murine hepatitis virus (MHV) as a surrogate. MHV is an enveloped virus and has a positive sense single-stranded RNA genome. Because of their structural and physical similarities, MHV and other murine viruses (e.g., murine norovirus) have been effectively employed as surrogates for a variety of viruses. (Casanova et al., 2009; Patel et al., 2017; Ye et al., 2016). The performance of seven techniques was determined for CoV recovery and compared by seeding MHV in untreated wastewater samples and RT-qPCR assays were used to analyse MHV concentrations in the seeded untreated domestic wastewater samples. Three out of seven methods were based on electronegative membrane

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adsorption with varying pH, two were ultrafiltration methods with centrifugal devices where one of them used the Amicon ultra-15 centrifugal filter and the other one used Centricon-70 plus. The last two methods were based on PEG precipitation and centrifugation. Their results demonstrated that adsorption extraction methods with neutral pH and added $MgCl_2$ gave the maximum MHV recovery. The concentration method to be considered effective and applicable should be able to process large volumes of water, be easy and rapid, provide a high viral recovery yield, be applicable for a variety of viruses, be repeatable, reproducible and cost and time-effective. Lu et al. (2020) did highlight that PEG-based separation approach is most commonly utilised for the COVID-19 in WBE. The authors indicated that the electronegative membrane filtration approach may have issues with the organic matter being preferential adsorbed on the charged membrane surface and the possibility of blockage when dealing with turbid wastewater samples.

5.4 RNA extraction

Following virus concentration, another critical step is RNA extraction from the sample matrix without destroying it. Isolation of RNA is usually performed by commercially available kits (Table 2). Organic extraction with silica-membrane based spin column techniques, phenol-guanidine isothiocyanate, and the use of paramagnetic particles are the three most often used RNA extraction procedures. There have been no studies that have compared various RNA extraction methods to see how effective they are at extracting RNA from influent wastewater.

5.5 Detection and quantification of RNA

5.5.1 RT-qPCR

Once the RNA has been isolated, the next process is to detect and quantify it. As detailed in Table 2, majority of the studies have employed RT-qPCR which are based on the presence of TaqMan probe assay, for the quantification of SARS-CoV-2 RNA in wastewater. RT-qPCR targets RdRp, N2, N3, N1, E and Orf1ab genes. The sensitivity of this assay is hampered by non-specific primer annealing and the presence of PCR inhibitors. PCR inhibitor compounds include bile salts, phenol, urea, calcium ions, ethanol, SDS, proteins like haemoglobin, collagen, myoglobin, polysaccharides and proteinases (Schrader et al., 2012). Column chromatography, solvent extraction, silica columns, cation exchange resins and magnetic silica beads are all suggested strategies to combat the presence of inhibitors. In Table 2, it can be seen that every study uses different PCR reaction mixes, having different reaction efficiency with specific primers. The threshold of C_t values, and hence of related viral quantities, is influenced by the makeup of each PCR reaction mix. To evaluate the performance of primer-probe sets, Vogels et al. (2020) examined the analytical efficiency and sensitivity of four RT-qPCR tests. Although all primer-probe sets were shown to be capable of detecting SARS-CoV-2, significant differences in analytical sensitivity were

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found in instances where the viral load was very low. A lot of discrepancies have been seen in the PCR reaction reagents which have been used. The wide range of reagents and methodologies used in testing laboratories underscores the need for technique and reagent optimization among laboratories to ensure that analyses are carried out consistently and correctly.

It's critical to utilise process controls to keep track of goal recovery levels and measurement efficiency. The use of quality control measures prevents the appearance of false-negative results and it also assures that even very low concentrations of SARS-CoV-2 in complex matrices like influent wastewater can be detected. The use of process controls, include three types: (I)molecular process controls inoculated into the viral concentrate, ii) whole process surrogate controls to be inoculated in a water sample before virus concentration and iii) inoculation of RT-qPCR controls before running the reaction (Haramoto et al., 2018) makes potential measurement of inhibition from each step of the sample treatment/analysis process more obvious. Use of positive, negative and non-template controls prior to RT-qPCR provides significant evidence of sample and reagent contamination, low reaction efficiency, and the need for further process optimization.

Besides RT-qPCR, other more sensitive methods like nested PCR, Droplet Digital PCR (ddPCR) are also being used (Table 2). Nested PCR improves sensitivity by decreasing non-specific binding by using two sets of primers, allowing it to detect very low levels of virus load in wastewater. ddPCR also improves the LOD (limit of detection) of SARS-CoV-2 (Gonzalez et al., 2020). Serial dilutions of a positive control linear DNA standard of SARS-CoV-2 were examined using primers/probe sets targeting ORF1ab and N gene of SARS-CoV-2 with both ddPCR and RT–PCR to compare their dynamic range (Suo et al., 2020). The results revealed that ddPCR has a much shorter minimum detection range than RT-PCR. LAMP (Loop-mediated isothermal amplification) is another fast and sensitive DNA amplification technique which uses four or six primers to bind six sections of a target DNA (Huang et al., 2020).

5.5.2 **Other methods**

Biosensors are the devices that use biological materials such as nucleic acids or peptides as input signals to generate information using physical means such as optical or electrical signals. (Demeke et al., 2020). Biosensors could be used to detect and provide a qualitative response in areas where RT-qPCR is not feasible due to resource and cost constraints (Sharma et al., 2021). Nucleic acids, proteins, tiny molecular antibodies, and viruses have all been detected using **electrochemical biosensors** (Bhalla et al., 2020). EBs (Electrochemical biosensors) are a type of biosensors that detects biological molecules using an electrochemical transducer (Osman et al., 2019). Seo et al. (2020) introduced a FET biosensor for detecting SARS-CoV-2 in clinical samples which was developed by

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covering the gate of a graphene-based transistor with a SARS-CoV-2 spike protein-specific antibody. Tests with the cultured virus, SARS-CoV-2 S- protein antigen, and nasal swab specimens from COVID-19 patients reveal the intended functionality of the sensor. This biosensor was also successfully utilised to identify viral strains in culture media (Seo et al., 2020). Another type of biosensor which could be used to detect the presence of distinct RNA amplicons in wastewater samples are the printed circuit board (PCB) biosensors. PCB biosensors were recently uncovered to be able to detect SARS-CoV-2 N1 RNA in control samples (Kumar et al., 2021c). Concentration of certain small molecular biomarkers in blood is found to be useful for the prognostics of the COVID-19. Elevated levels of polymorphonuclear lymphocytes, high production of ROS, increased levels of CRP, CysC, creatinine, urea, lymphocytes are all the potential biomarkers for designing a biosensor for COVID-19 detection (Xu et al., 2020c; Xiang et al., 2020). The specificity and sensitivity of assays geared at early detection of COVID-19 disease should be the primary focus of current biosensing advancements. Because pandemic viral strains are highly contagious, such as SARS-CoV-2, which has a reproductive number of 1.5 to 2, single-use (disposable) sensors are essential for preventing contamination from detection devices. For SARS-CoV-2 pandemic, **Optical biosensors** also offer an alternative way for virus detection since they are safe, cost-effective, and do not require an amplification step, unlike RT-qPCR.

Paper analytical devices, or PADs, are considerably easier, less costly, and more convenient to use. The process entails filtering the pathogen nucleic acid from collected wastewater samples using a paper based set up. Paper based detection of SARS-CoV-2 counts as LFT (lateral flow tests). A standard biochemical experiment utilising particular reagents can detect the presence of SARS-CoV-2 RNA. As a result of this method, a green circle (indicating positive) and a blue circle (indicating negative) can be observed macroscopically (Yang et al., 2020). A broad range of diagnostic devices is included in the developing area of **microfluidics**, also known as (LOC) **lab-on-a-chip** or micro complete analysis system. LOC technologies have progressed from single-task devices to integrated systems which are capable of executing complicated tasks. An LOC platform usually consists of several microfluidic components dedicated to specific activities like fluid transport and mixing, reagent storage, detection, and maybe collection.

As SARS-CoV-2 becomes endemic, it is mutating perpetually, resulting in a variety of novel lineages. SARS-CoV-2 Variants of concern and variants of interest show enhanced disease severity, transmissibility, and/or immune evasion in emerging variants. Effective public health strategies need a timely and precise measurement of local proportion of SARS-CoV-2 variants. Employing clinical genomic surveillance, Karthikeyan et al. (2021) were able to identify VOCs up to two weeks before they were detected. Genetic viral surveillance and molecular

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diagnostic techniques are required for virus and variant monitoring for quick differentiation of variations in laboratory or point-of-care (POC) testing setups. However, wastewater genomic monitoring is difficult. Low virus loads, highly fragmented RNA, and PCR inhibitors result in poor sequencing coverage and quality in heterogeneous environmental samples.

5.6 Statistical analysis

After the quantification of viral RNA has been done, the next step is to analyse the data and compare it with clinical studies. To establish the usefulness and predictability of wastewater monitoring, time-series data of SARS-CoV-2 RNA concentration in wastewater that can be linked with real clinical survey data, is urgently needed. This is also necessary for the policy level adaption SWEEP (Surveillance of Wastewater for Early Epidemic Prediction) (Tiwari et al., 2021). Among the reviewed case studies only few had undergone statistical analysis. Most studies geared at comparing the SARS-CoV-2 RNA removal efficiency using different wastewater treatment techniques rather than prediction of a new wave. Table 3 highlights the path of different countries in this line.

The mingling of science and mathematics has always brought beautiful results. A mathematical model is presented by Petala et al (2022) where it is argued that early warning capacity varies along the days of an outbreak. It depends on the number of days between the day of maximum shedding rate of infected individuals in their disease cycle and the day of their medical testing. F (T) is the total number of cases that have been reported at time t. f (t) however is the number of positive cases at a particular time t. It is important to note that F(t) comprises those who at the time t either had a positive test or are in the early stages of infection and hence have no symptoms, but who nevertheless shed virus and will be tested positive later following the onset of symptoms. Here, τ represents the time in number of days. Infection begins at τ=1 and it is detected at $\tau = \tau_d$. τ_e marks the end of viral shedding by the infected individual. The number of days between discovery and the end of shedding is not consistent among cases, but there is a dispersion that may be explained by the probability density functions $P_d(\tau_d)$ and $P_e(\tau_e)$. av is the average value of the corresponding variable.

Considering the preceding, F(t) and f(t) can be related to each other as:

$$F(t) = \int_{\tau d1}^{\tau d2} \int_{\tau e1}^{\tau e2} P_d(\tau_d) P_e(\tau_e) \int_{t+\tau d-\tau e}^{t+\tau d} f(X)\, dX d\tau_e d\tau_d$$

This relationship is the mathematical representation of the assertion that infected people at time t include those who have already been detected as well as those who will be detected in the coming days. The required global shedding rate evolution function R may theoretically be obtained by multiplying this amount by the average

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shedding rate per person R(t). Such an average model is sufficient only when shedding pace of people during their illness cycle had a steady worth which, however is not the case. Hence for this, S(τ) marks the function of daily shedding rate per person.

6. Case studies highlighting the detection of SARS-CoV-2 in wastewater

Most of the studies pinpointed the existence, survival and infectivity of SARS-CoV-2 in stools. Such studies reported the practical viability of routine SARS-CoV-2 RNA monitoring in wastewater. The virus can be excreted in the fecal matter before the onset of symptoms, even during illness, and after recovery (Gwenzi, 2021). Xiao et al. (2020) collected fecal samples of 28 COVID-19 patients throughout the months of January and February and 12 among them were tested positive for SARS-CoV-2 virus. Among 12 patients, 2 patients were positive for SARS-CoV-2 virus suggesting the prevalence of infectious virus in stool. Infectious SARS-CoV-2 was found in fecal samples by Yeo et al. (2020), who also hypothesized fecal oral transmission. In March and April of 2020, researchers in Australia used RT-qPCR to identify SARS-CoV-2 in wastewater (Ahmed et al., 2020a). To concentrate viral samples, ultrafiltration and direct RNA extraction using electronegative membranes were utilized. SARS-CoV-2 RNA load ranged from 19-120 copies/L. Sherchan et al. (2020) were the first one to detect SARS-CoV-2 RNA in North America. In case of untreated water, two of fifteen tests were positive. In March and April 2020, research conducted in Spain employed an aluminium hydroxide adsorption precipitation method to concentrate different samples and RT-qPCR to identify viral RNA (Randazzo et al., 2020b). Among them, 83% of influent samples and 11% of effluent samples were positive for at least one SARS-CoV-2 RT-qPCR target out of total 42 influent samples, 18 secondary samples, and 12 tertiary treated effluent samples. A study in Netherlands was carried out by Medema et al. (2020), which offered a preliminary estimate of COVID-19's incremental prevalence. SARS-CoV-2 RNA was tracked down in city sewage of Netherlands six days before the first clinical cases were actually recorded. These findings emphasize that WBE might be a sensitive and useful tool for tracking down SARS-CoV-2 infection levels and changes in communities. Many other such stories from the same line project towards the presence of SARS-CoV-2 RNA in wastewater and sewage. Table 2 summarizes some of the major studies which provided evidence for the same. More than half of the studies have been done by using RT-qPCR as the desired molecular tool to detect the presence of viral RNA.

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Table 3: Statistical analysis of results of WBE

Place	Method	Reference
Finland	Kruskal-Wallis test followed by the Dunn post hoc test was used to analyse the differences in copy numbers in samples held at different temperatures. IBM SPSS statistics were used for statistical analysis. The degradation characteristics at the storage temperatures were investigated using the GInaFiT Version 1.7 (Geeraerd and Van Impe Inactivation Model Fitting Tool) freeware add-in for Microsoft® Office 365® Excel.	Hokajärvi et al., 2021
India (Gujarat)	SARS-CoV-2 genome removal effectiveness was compared by using paired t-tests on the total effective genome concentrations collected.	Kumar et al., 2021d
India (Jaipur)	R was used to visualise the co-detection of genes using various kits, as well as the eradication effectiveness resulting from various treatment modalities. To analyse the temporal effect, viral concentration data was merged with a 7-day average of new cases for Jaipur and India.	Arora et al., 2021
USA (Virginia)	To investigate variations in the total number of SARS-CoV-2 test detections, Kruskall-Wallis analysis was utilised. The pair-wise comparisons between separate assays were then examined using Dunn's testing. R Statistical Computing Software version 3.6.3 was used to make the figures.	Gonzalez et al., 2020

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USA (Connecticut)	The association between SARS-CoV-2 RNA copies/ml results for replicated RNA extractions of each day sample was estimated using linear regressions. Two-tailed t-tests (= 0.05) were carried out in PCR inhibition studies to check if spiked sludge RNA extracts yielded the same C_t values as spiked water samples at various dilutions.	Peccia et al., 2020
Qatar	GraphPad Prism and Origin Pro were used for statistical analysis, and Matlab was used to write mathematical modelling routines.	Saththasivam et al., 2021

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Limitations

Although waste water epidemiology is a very promising tool for analysing COVID-19 spread scenario, yet there are many issues associated with the same. The degradation of target viral RNA during its transit through the sewage system is a hot topic in wastewater-based epidemiology. PCR inhibitors are another big hurdle in the quantification of viral RNA. Added to that, the whole technique, from sampling to final RNA quantification has not been optimised. It is necessary to explore the development of accepted methodological tools for viral quantitative analysis. RT-PCR has several flaws that may limit its application globally. For example, expert technicians, expensive equipment, and time-consuming procedures are required for RT-PCR. With COVID-19 cases increasing worldwide, this approach has been unable to satisfy the requirements of detecting a large number of suspicious and asymptomatic cases in a short period of time despite the fact that PCR has proven excellent sensitivity and specificity in some cases.

7. Conclusion

Along with respiratory symptoms, GI problems are becoming increasingly prevalent in COVID-19 patients, resulting in viral release into sewage. Several studies have confirmed the ability of sewage surveillance to detect a rise in viral circulation before the health surveillance system reports the cases. WBE can help emergency responders detect sick people in towns, cities, and particular drainage sections of huge areas. Remarkable efforts to investigate SARS-CoV-2 in wastewater have been made all over the world; however, a gold standard approach for the concentration, extraction and detection of the virus in key environmental matrices such as sewage is yet to be created. The fate of SARS-CoV-2 in WWTPs is predicted using comparable CoVs that are highly influenced by environmental variables (e.g., solids, pH, temperature, pollutants) due to a paucity of analytical data. The WWTPs assisted in demonstrating the feasibility of regular SARS-CoV-2 RNA monitoring in wastewater. The virus can be excreted in the feces before the beginning of symptoms, throughout the illness, and after recovery. WBE provides a cheap, real-time, and unbiased picture of the entire population in the area under study. It has proven to be effective in identifying SARS-CoV-2 circulation patterns across countries during the COVID-19 pandemic. Wastewater surveillance can therefore be a very promising tool for analysis of any future COVID -19 case peak at the earliest, especially for asymptomatic carriers.

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Conflict of interest

The authors declare no conflict of interests

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