Global J. Environ. Sci. Manage. 7(4): 643-658, Autumn 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

REVIEW PAPER

Wastewater-based epidemiology for novel Coronavirus detection in wastewater

G. Saini*, P.S. Deepak

Department of Civil Engineering, Sharda University, Greater Noida, Uttar Pradesh, India

ARTICLE INFO

Article History:
Received 17 January 2021
Revised 28 February 2020
Accepted 31 March 2020

Keywords:
Covid-19 (Coronavirus disease)
Infection
Pandemic
Polymerase chain reaction (PCR)
Polymerase chain reaction (PCR)
Ribonucleic Acid (RNA)
Wastewater-based epidemiology (WBE)

ABSTRACT

The entire world is reeling under the worst pandemic of last 100 years. Over 128 million people have been infected with it and 2.8 million deaths have already taken place, till 30th March 2021. The identification of materials positive cases is the first step towards its containment and treatment. However, testing of individuals is an extensive, expensive and time-consuming exercise. In addition, societal taboos are also associated with infected individuals resulting in very few people volunteering for testing, esp. in the developing and under-developed world. An alternative approach that circumvents individual testing is the wastewater-based epidemiology. A state-of-the-art review of this method is provided in context of its utility for COVID-19 detection. This technique relies on collecting and testing samples from sewers and/or wastewater treatment plants for the presence of pathogens and then using that data to determine and predict the spread of the infection, thereby allowing the provision of appropriate containment and treatment steps. The study covers key aspects of wastewater-based epidemiology application for COVID-19 detection including its need, detailed process of detection and assessment, data analysis, economics and challenges to its application. Findings from a number of case studies are presented to elucidate the utility of this technique. It is clearly seen that WBE-based approach is a much better strategy as compared to individual testing and can be adopted to prevent further spread of Covid-19. The work is expected to further emphasize the application of this method for COVID (and other pandemic) detection and implementing containment strategies. This is clearly a much more economical and non-intrusive approach as compared to the individual testing.

DOI: 10.22034/gjesm.2021.04.10

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
81	1	1

*Corresponding Author:
Email: *gauravsaini@gmail.com*
Phone: +91 828 705 1260
Fax: +91 828 705 1260

Note: Discussion period for this manuscript open until January 1, 2022 on GJESM website at the "Show Article.

INTRODUCTION

Coronaviruses are "nonsegmented, enveloped, positive-sense, single-strand ribonucleic acid viruses", and are part of the *Coronaviridae* family (Weiss and Leibowitz, 2011). There have been seven types of coronaviruses discovered which causes human sickness. The names of known coronaviruses till date are the alpha coronaviruses HCoV-229E and HCoV-NL63, the beta coronaviruses HCoV-OC43 and HCoV-HKU1, MERS-CoV which causes Middle east respiratory syndrome and SARS-CoV which causes Severe acute respiratory syndrome. SARS-CoV-2 is the 7th type and the most recent one to be found out (Lima, 2020). Four of these viruses i.e., alpha coronaviruses and beta coronaviruses induce (mild) respiratory symptoms. The other two viruses (Middle East respiratory syndrome or MERS, and Severe Acute Respiratory Syndrome or SARS) have been a cause for pandemics with a high fatality rate (Kooraki *et al.,* 2020). This is the third time, in last two decades, that a coronavirus infection has taken place (Guarner, 2020). The upsurge of SARS-CoV occurred in 2002 (Zhong *et al.,* 2003), MERS-CoV in 2012 (Nassar *et al.,* 2018), followed by the SARS-CoV-2 in 2019. SARS-Cov-2 was declared a pandemic by the World Health Organization (WHO) on 11th March, 2020 (Cucinotta *et al.,* 2020). As of March 8, 2021 – 117.47 million cases were recorded globally, in which 2.61 million fatalities were reported (Worldometer, 2021a). The SARS-CoV-2 is extremely infectious as compared to SARS-CoV and MERS-CoV. While, the mortality rate associated with the MERS-CoV was higher than that of both SARS-CoV and SARS-CoV-2, the SARS-CoV-2 spread extremely rapidly and infected a very large number of people globally (Meo *et al.,* 2020). Respiratory droplets and direct contact are the major pathways for the spread of SARS-CoV-2 (WHO, 2020). With the median of 2.79 and interquartile range (IQR) of 1.16, the R_0 was calculated to be around 3.28, which implies that an infected person will spread to another 3.28 cases (Liu *et al.,* 2020). The main strategies for control of this pandemic include isolation of positive cases, tracing of contacts (people who were in physical contact with the infected individual), and social/ physical distancing (MacIntyre, 2020). The essence of wastewater-based epidemiology (WBE), known as Wastewater based epidemiology is that infected people shed the virus through excreta and the virus ends up in the wastewater treatment plant where the virus can be detected. This is a community-based method that allows early and reliable detection of infections, and can help in intervention and control of infection spread. Not only viruses, the illegal use of drugs also can be monitored through the sewage. Some countries like China (Cyranoski, 2018), Spain (Huerta-Fontela *et al.,* 2008), France (Karolak *et al.,* 2010), and Canada (Metcalfe *et al.,* 2010) use sewage to monitor the illicit use of drugs. Some research studies (Young *et al.,* 2020; Chen *et al.,* 2020; Xiao *et al.,* 2020) have demonstrated that RNA of SARS-CoV-2 is perceivable in the feces of COVID-19 patients. Chen *et al.* 2020 showed that RNA of SARS-CoV-2 can be diagnosed in human feces a few days to a week ahead of the symptoms onset. This is not the first-time wastewater-based epidemiology has come into rescue to monitor epidemic breakouts. It proved as a promising approach in Brazil and Israel for detecting diseases like polio (Michael-Kordatou *et al.,* 2020) . Sewage surveillance system of Israel, developed in 1989 by their national health authorities to notice poliovirus in wastewater, allowed them to track polio in urban wastewater treatment plants and sewage trunk lines at the time of the re-emergence of polio in 2013, and enabled them to react quickly (Brouwer *et al.,* 2018). It can also be used to access a situation when a new virus enters the community (Sinclair *et al.,* 2011). The biggest advantage of the wastewater-based surveillance for Covid- 19 is the unbiased results. It detects the virus even when the person is asymptomatic or presymptomatic. In addition, testing of sewage ensures that a composite of different human excretions (including, urine, feces, sweat, dead cells, saliva, etc.) is investigated thus providing a broader coverage of infections. Netherlands was one of the first countries to detect the traces of Covid-19 in sewage treatment plants (Hart *et al.,* 2020). Other countries which adopted the wastewater surveillance for routine Covid-19 detection are Australia, New Zealand, Netherlands (Aguiar-Oliveira *et al.,* 2020). Nonetheless, WBE is not without its share of disadvantages, for example, it cannot help in finding specific infected individual in a community. Currently, wastewater treatment facilities serve only 27% of the global population hence decreasing the chances of surveillance of Covid by WBE alone (Mandal *et al.,* 2020). Even with these issues, WBE can be used, esp. in countries/cities with better sewage treatment facilities, as an economic technique for detection and trend analysis. This study provides a state-of-the-art comprehensive review of the application of

WBE-based approach for detection of coronavirus in wastewater. The necessity for this approach is first established, followed by detailed description of the process of detection of Coronavirus in the water. This work was conducted in Greater Noida, Uttar Pradesh, India during November 2020 to March 2021.

Need of WBE for COVID-19 Surveillance

The rapid spread of Covid-19 around the world warranted extensive and rapid testing of the suspected cases. However, the testing rate was very slow as compared to the rate of spread and lagged it by a big margin. This is especially true for developing and underdeveloped countries. Further, the test results are not a current indicator, but rather a lagging indicator of the infection spread. Testing was originally only carried out for the symptomatic individuals. Unfortunately, it may take up to 2 weeks' post-infection for the symptoms to show and there is delay between testing and results. This means that an infected person (whether symptomatic or asymptomatic) may already have infected a large number of individuals even before the detection if carried out. WBE offers a measure of determining the presence and spread trend of the infection, without disturbing anyone. It should be noted that in the initial phases, there was a stigma associated with the Covid infected individuals, which deterred many potentially infected people from getting tested or revealing the test results. On January 30, 2020, India recorded its first case of Covid-19 (Perappadan, 2020). In the last 13 months, 11.2 million cases were recorded, in which 0.15 million fatalities were reported, in India (Worldometer, 2021b). The testing rate should rapidly increase in a country like India where the disease can spread very quickly. There are presently two types of testing methods which are under use: A viral test which shows positive if a person has a current infection, and an antibody test, which shows a positive result if a person had the infection in the past (CDC, 2021a). In general, people only get themselves tested when they start showing symptoms like fever, cough, and headache. So, people who are asymptomatic and presymptomatic can spread the disease unintentionally which is a major concern. This suggests that there is a need of testing method which detects small loads of virus in the body and give results without a need for symptoms to show. Moreover, the surveillance of Covid-19 in wastewater will provide a picture of the community as a whole and help in better hotspot detection. And this type of detection methods does not need the approval of the infected person, as people may be cynical about getting themselves tested due to fear of social stigma.

Process of testing COVID-19 in wastewater

Even though there is no standard testing methodology, different studies have followed different methods to detect Covid-19 in the wastewater treatment plants. A summary of the generic steps involved in the process of COVID-19 testing in wastewater is presented in the Fig. 1.

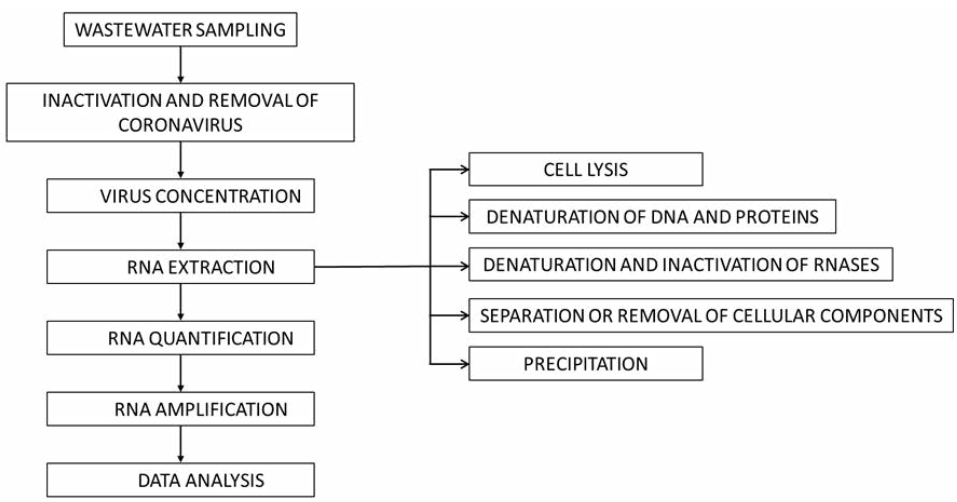


Fig. 1: Process of testing COVID-19 in wastewater

Wastewater sampling

Water sampling is the first step towards detection of Novel Coronavirus in STP. Sampling wastewater for SARS-CoV-2 is used to evaluate trends of infection within the citizenry contributing water to the sewer system (CDC, 2021b). There are two types of wastewater sample collection methods commonly used for this purpose: Grab sampling and Composite sampling. The grab sampling, as the name suggests collects the sample at a time. In comparison, the Composite sampling, involves collecting various individual discrete samples over a specified period of time – usually 24 hours (CDC, 2021b). The composite sampling can be done both manually and automatically. For detection of SARS-CoV-2, both grab and composite sampling methods were used. Sherchan *et al.,* 2020 collected the samples by grab sampling method early in the morning, from 7-11 am. In a study in the Gujrat state of India, Kumar *et al.,* 2020 used the grab sampling method to collect the samples at 11:30am on 8th and 27th May 2020, using sterile bottles. In Netherlands, Medema *et al.* 2020 collected the samples by using composite sampling method in February and March in different selected cities such as Amsterdam, Den Haag, Utrecht, Apeldoorn, Amersfoort, Schiphol. The first Covid-19 case was reported officially after 3 weeks from the first sample round. Nemudryi *et al.,* 2020 used the automatic composite sampling method. The researchers collected the samples with the use of Liquistation CSF34 (Endress+Hauser), an automatic flow proportional sampler located at their facility doorway. In a study in Australia, Ahmed *et al.* 2020 collected samples using the Composite sampling method.

Inactivation and removal of Coronavirus

Getting to know the inactivation or removal process of Coronavirus in the samples can help in establishing better steps to decrease the probability of spread. WHO stated that Covid-19 has a fragile outer membrane as it is known to be an enveloped virus (WHO, 2020). And generally enveloped viruses are environmentally unstable and known to be more vulnerable to oxidants like chlorine. Wang *et al.* 2020 suggested some effective disinfection technologies for deactivating SARS-CoV-2 virus from sewage. The disinfectants included chemicals such as, Chlorine (Cl_2), Sodium hypochlorite (NaOCl), Ozone (O_3), and Chlorine dioxide (ClO_2) and also physical scheme like, UV irradiation. Each disinfection method has its own merits and demerits, so the selection of appropriate disinfection method depends upon various factors like cost, energy consumption, operating parameters, and disinfectants' toxicity. Wang *et al.* 2005 suggested that chlorine is a better disinfectant when compared to chlorine dioxide. It states that SARS-CoV inactivation is completed with 10 mg/L chlorine after 30 minutes of disinfection whereas chlorine requires 40 mg/L in 30 min. Pasteurization is a process where samples are treated with mild heat usually less than 100^{0}C in order to inactivate any live pathogens. Heat pasteurization of wastewater samples was conducted to decrease the biosafety risk from procedures that generate bioaerosols during the processing of wastewater samples (CDC, 2021c). Wu *et al.* 2020 pasteurized the samples at 60^0 C for 90 minutes as a preliminary step before opening the sample containers to increase the protocol safety. Former studies on SARS-CoV-1 suggest that a heat inactivation of 30 minutes at 60°C is adequate for virus inactivation by more than 6 log units (Rabenau *et al*., 2005). Extra precautions were taken by increasing the pasteurization time.

Virus concentration

Virus concentration is the process of enumerating the quantity of virus in a specified sample volume. The methods which were used in various studies are polyethylene glycol (PEG) precipitation (Lewis *et al*., 1988; Bibby *et al*., 2013), skimmed milk flocculation (SMF) (Calgua *et al*., 2013), ferric chloride precipitation – ($FeCl_3$) (Falman *et al*., 2013), ultracentrifugation (Prata *et al*., 2012), ultrafiltration (Hill *et al*., 2007), and glass wool filtration (Cashdollar *et al*., 2007). The effective method of virus concentration should ideally be technically simple, rapid and should have the ability to process large volume of water, repeatable, reproducible and predominantly cost and time effective. As yet, there is no concentration method which meets all these criteria (Michael-Kordatou *et al.,* 2020). Ahmed *et al.* 2020 used two methods for the process of concentration, including direct RNA extraction from electronegative membrane and ultrafiltration. For the first step, the researchers used a RNeasy power microbiome kit®. For ultrafiltration, centrifugation was followed by a QIAcube Connect platform. Westhaus *et al.* 2020 started their process of concentration by centrifugation at 4700g for 30

minutes without a break. Then by using centrifugal ultrafiltration units, the purified wastewater was concentrated. 15 mL of wastewater sample was then added to the filter unit, followed by centrifugation at 3500g for 15 minutes. The concentrated supernatant was then harvested. The step was repeated twice until the quantity of 45 ml of sample was completely concentrated. The pellet was then cleaned to remove any aqueous remains, by deionized water and was centrifuged at 4700g for 5 min. La Rosa *et al.* 2020 used the PEG – polyethylene glycol precipitation method to concentrate the sample. The researchers followed the WHO guidelines for environmental surveillance of poliovirus (WHO, 2003), by modifying the protocol for the enveloped virus. The sample was first centrifuged and, then mixed with dextran and PEG. It is then left in a separation funnel at 4^0 C. The interphase and base layer were collected dropwise, and the concentrate was added from the initial centrifugation to the pellet. WHO protocol intends to follow the chloroform treatment at this stage and was left out to conserve the integrity of the enveloped viruses. Sherchan *et al.* 2020 used two sample concentration methods to enhance the possibility of SARS-CoV-2 occurrence in wastewater. Ultrafiltration, the first process, started with 250 ml sample centrifugation for 30 min. at 3000 g to extract large particles. A centrifugal filter (Centricon® Plus-70) was used to concentrate 70-140 mL of the 250 mL supernatant with a nominal molecular weight limit (NMWL) of about 100 kDa through centrifugation. In order to recover 350 µL of the viral concentrate, the filter unit was inverted and then centrifuged for 2 min at 1000g. Using a pipette, the viral concentrate was then extracted from the sample reservoir. Adsorption-elution was the second method and was carried out with the use of electronegative membrane. 2.5 M $MgCl_2$ was used to attain a final concentration of 25mM $MgCl_2$ in all the samples. Then samples were then passed through an electronegative filter (90 mm diameter and 0.45-µm pore size) attached to a glass filter holder assembly. The viruses were eluted with 10 mL of 1.0 mM sodium hydroxide (pH 10.8). The eluate was recovered for neutralization, in a tube consisting of 50 µL of 100 mM sulfuric acid and 100µL of 100× Tris-EDTA buffer. Later 10 mL of this eluate was centrifuged, containing an ultrafiltration membrane (NMWL = 30 kDa) to obtain approximately 650 µL of final volume. However, the viral recovery yield can be affected by the volume of wastewater to be concentrated. According to Bibby *et al*. 2013, PEG precipitation was proven to be a highly efficient method for virus concentration. Falman *et al*. 2019 suggested that for recovery of poliovirus type 1, SMF was found to be more efficient with a recovery of 106 ± 24.8% while the PEG yielded 59.5 ± 19.4% recovery in wastewater.

RNA extraction

The main aim of method of RNA extraction is to acquire the RNA without any damage from the sample matrix. The most widely used methods for RNA extraction are organic extraction using Phenol-guanidine isothiocyanate, silica-membrane dependent spin column techniques and the use of paramagnetic particles. The fundamental steps in RNA extraction using organic solvents are as follows (Johnson, 2021):

1. *Cell lysis*: Cell lysis is the process which releases the RNA of the cell by breaking down the cell membranes' outer boundary (Michael-Kordatou *et al.,* 2020). It is carried out by using viral, enzymatic, or osmotic mechanisms (Listwan *et al.,* 2010). Through the use of chaotropic agents containing buffers or reagents like guanidinium isothiocyanate, guanidinium chloride, sodium dodecyl sulphate, sarcosyl, urea, phenol or chloroform, the aim of cell lysis is accomplished. And to maintain the integrity of RNA during lysis, solutions such as TRIzol or RNAlater or Qiazol (DiToro *et al.,* 2018) may also be used.
2. *Denaturation of DNA and proteins:* Denaturation refers to the three-dimensional structure loss of the biological molecule viz. DNA or proteins. To break down DNA, DNase can be used whereas proteinase K can be enumerated to digest proteins. Another way to remove the proteins is through repeated organic extraction through the use of phenol and chloroform. This can be achieved by sample dissolution in buffers containing guanidinium salts.
3. *Denaturation and Inactivation of RNases:* By using chaotropic agents such as phenol and chloroform, RNAses can be denatured and inactivated.
4. *Separation or Removal of cellular components:* Addition of chloroform and subsequently centrifuging the solution can be done in order to

isolate RNA from other cellular components. This divides the solution within two phases known as aqueous and organic phases where, RNA is present in aqueous phase.

5. *Precipitation:* Isopropyl alcohol is often used to recover RNA from aqueous phase whereas by using ammonium acetate preferential precipitation of RNA can be done. And Lithium chloride can also be used to for selective precipitation of RNA from DNA and proteins.

RNA extraction can be done through various methods like Magnetic bead technology, Silica technology, Lithium chloride, and urea isolation (Johnson, 2021). But during the process of identification of SARS – CoV – 2, mostly commercial kits have so far been used. Some of the kits used for RNA extraction are RNeasy kits, ZR Viral RNA kit, Combination of RNeasy Power Microbiome Kit and RNeasy Power Water Kit. RNA extraction can also be done by combining two specific kits: RNeasy Power Water Kit and RNeasy Power Microbiome Kit (Ahmed *et al.,* 2020). To accommodate the electronegative membrane, a 5 ML bead tube from RNeasy Power Water Kit was used and a Precellys 24 tissue homogenizer to integrate the samples differing from 3 x 20 s at 8000 rpm at an interval of 10 seconds. From there on the RNeasy Power Microbiome kit was used to extract RNA, as per the manufacturer's instructions. NucleoSpin RNA Virus kit was used to isolate RNA by following the manufacturer's instructions (Westhaus *et al.,* 2020). Nemudryi *et al.* 2020 used RNeasy mini kit to extract RNA. RNA extraction was carried out from samples of concentrated sewage sample using viral RNA kit to get the final volume (Sherchan *et al.,* 2020). Apart from the above kits, the CDC has given a list of viable RNA extraction kits (CDC, 2021d). Some of them are QIAcube® QIAcube, Roche MagNA Pure LC®, Roche MagNA Pure 24, Roche MagNA Pure 96.

RNA quantification

Some detection approaches and quantification of viruses in sewage are epifluorescence microscopy, transmission electron microscopy, throbbing gel electrophoresis, immunofluorescence test, flow cytometry, conventional cell culture and molecular techniques. Molecular methods include conventional polymerase chain reaction (cPCR), Reverse transcription polymerase chain reaction (RT-PCR), Real-time quantitative polymerase chain reaction (RT-qPCR), Multiplex PCR and sequencing (Corpuz *et al.,* 2020).

For SARS-CoV-2 detection in sewage and its quantification, PCR-based methods have been predominantly used. All these methods use the same principle except for RT- qPCR, where the process starts with RNA material in place of DNA, and RT- PCR requires a reverse transcription step before qPCR (Corpuz *et al.,* 2020). The "q" in qPCR stands for quantitative, which means that qPCR tests not only detect the virus's genetic information but also quantify the amount of genetic information (Bustin and Nolan, 2020). Several studies in Australia (Ahmed *et al.,* 2020), France (Wurtzer *et al.,* 2020a), Spain (Randazzo *et al.,* 2020), and Italy (La Rosa *et al.,* 2020) have detected and quantified SARS-CoV-2 by qPCR. With N_Sarbeco and NIID_2019-nCOV_N primers both being nucleocapsid protein gene specific, RT-qPCR was conducted for specific detection and quantification (Ahmed *et al.,* 2020). For reverse transcription and the QPCR, a single-step RT-QPCR was used. Raw sewage and wastewater treatment plant (WWTP) samples were acquired in Israel, and when tested using the RT-qPCR assay, a few of the samples were found positive. The Ct value of less than 40 was obtained in one out of two sewage samples taken from the sewer networks of hospitals treating Covid-19 patients, three out of three isolation facilities (Bar-Or *et al.,* 2020). Wurtzer *et al.* 2020b examined the incidences of SARS-COV-2 in Paris by collecting samples from three different WWTP inlets during the period of 5 March 2020 to 23 April 2020 and tested using RT-qPCR assay. It was found that all the tested samples were positive. The researchers also attempted to associate a relation between quantified number of SARS-CoV-2 genomes in wastewater and carrier numbers. They noticed a significant link between genome units' number of COVID 19 cases reported. They also indicated that monitoring wastewater would serve as a backup and early identification method for SARS-CoV-2 spread. Kumar *et al.* 2020, in India, examined RNAs of SARS-CoV-2 with the use of TaqPath™ Covid-19 RT-PCR Kit for detection of ORF1ab, Ngene and S gene and s gene. Medema *et al.* 2020 from Netherlands quantified by performing qRT-PCR on serial 10 – fold dilutions of quantified synthetic double stranded DNA.

RNA amplification

The nucleic acids PCR amplification and detection of the products is concurrently achieved in qPCR method (Cobo *et al.,* 2012). This is a quantitative method because it measures the target sequence unlike conventional PCR methods that provide qualitative data through gel electrophoresis (Kadri *et al.,* 2020). qPCR not only provides quantitative data but also shows high sensitivity and is mostly independent of the amount of reagent concentration (Watzinger *et al.,* 2006). RT-qPCR can also be used for RNA amplification (Ahmed *et al.,* 2020). Kumar *et al.* 2020, applied amplification in a reaction mixture of 25 µL which consists of 7 µL to extract nucleic acids of each sample. 2 µL TaqPath™ COVID-19 Control and purified 5 µL sample were used as the positive and negative controls, respectively. For no template control, nuclear free water has been used. One of the other molecular methods used for amplification is Reverse transcription loop-mediated isothermal amplification (RT-LAMP). The core aspect of the RT-LAMP is a reverse transcription step (RNA to DNA), after which 6 primers attach to the target gene of interest. These 6 primers get attached to the target DNA at an appropriate temperature, and they loop around to create circular constructs that help in extending the DNA. For primers to bind, each loop opens up a new site further amplifying the gene. RT-LAMP allows a rapid and exponential increase in the gene of interest. When probes, dyes, or a fluorophore are added to the mixture, a visible change during the reaction can be detected by eye or with use of special apparatus (Watzinger *et al.,* 2006). LAMP method has an upper hand over RT-qPCR in the point of temperature. LAMP reactions take place at a single isothermal temperature range of 63 ^{0}C to 65 ^{0}C, whereas rapid cycling of multiple temperatures is needed in RT-qPCR method to amplify nucleic acids. This method has also shown substantial potential results by producing them in less than an hour, and on some occasions within 11 minutes (Augustine *et al.,* 2020). Another digital PCR method known as ddPCR - Droplet Digital PCR technology works by using a water– oil emulsion droplet system. In such system, droplets are formed to create the partition that separates the template DNA molecules. In the plate where PCR reaction takes place, the droplets serve the basic purpose as individual test tubes or wells but in smaller space. In the ddPCR technique, the mass sample key partitioning is a critical part. The nucleic acid samples are divided into several thousands of droplets (nanoliter-sized) and PCR amplification is carried out in each of these droplets. The sample requirement is one of the merits for this method. It requires smaller sample size as compared to other digital PCR systems which are commercially available. Reduced cost and preserving precious samples are some of the other merits of this method. This method has shown to have less consumable costs and more accuracy than RT-qPCR (Bio-Rad, 2021).

Data analysis

The results of the studies which were conducted have been reported in one of the following ways (Michael-Kordatou *et al.,* 2020).

- "Absence or presence of the virus in the form of Ct values reported directly by the qPCR instrument"
- "Gene copies/volume of sample, with the use of a quantitative calibration curve of Ct values against known concentrations of the virus for the calculation of the gene copies present in a certain sample volume (relative quantification)".

The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold. Ct levels and the amount of target nucleic acid are inversely proportional to each other (i.e. the lower the value of Ct level, the greater is the amount of target nucleic acid) (Yagci *et al.,* 2020). A patient is considered to be Covid-19 positive if the Ct value is below 40. However, the Indian council of Medical Research (ICMR) does not recommend reliance on Ct values to determine the infectious status of a Covid-19 patient (ICMR, 2020). Some factors which can adversely affect the Ct values are the temperature of transportation, the time between collection and receipt in the lab. Ct values of asymptomatic/mild cases are similar to that of Ct values of severe cases. The number of viral genomes of SARS-CoV-2 per ml of human fecal material are between 6,00,000 to 30,00,000 assuming fecal load of 100-400 g feces/day/person with a density of 1.06 g/mL (Hart *et al.,* 2020). The number of persons infected can be found by using Eq. 1 (Ahmed *et al.,* 2020).

$$\text{Persons Infected} = \frac{\left(\frac{\text{RNA copies}}{\text{liter wastewater}}\right) * \left(\frac{\text{liters wastewater}}{\text{day}}\right)}{\left(\frac{\text{g feces}}{\text{person} - \text{day}}\right) * \left(\frac{\text{RNA copies}}{\text{g feces}}\right)} \quad (1)$$

Eq. 1 has been utilized to calculate the potential number of cases in Brisbane North and Brisbane South primary health networks. The Monte Carlo simulation estimated the median number of infections, which ranged from 1,090 on 27 March 2020 to 171 on 1 April 2020 in the catchment basin. The sensitivity analysis indicated a strong correlation ((−0.977)) between the number of infections and prevalence with $\log_{10}$ SARS-CoV-2 RNA copies in human feces samples.

Accuracy of WBE-based analysis

The accuracy of the results depends upon some of the key variables like seasonal temperature, per person water use, community demographics, average in-sewer travel time and degradation rate of biomarkers as these variables affect the accuracy of data (Hart *et al.,* 2020). These factors influence the precision of the results because the survival of SARS-CoV-2 is influenced by these factors. Chin *et al.* 2020 revealed that when the stability of SARS-CoV-2 was determined at different temperatures, it was found that at 4° C the virus was highly stable and on day 14, there was only ~0.7 -log unit reduction of infectious titer. But when the incubation temperature was increased to 70° C, the time taken for inactivation of virus was brought down to 5 Minutes. Chin *et al.* 2020 also states that SARS-CoV-2 is highly stable over a wide-range of pH values (3-10). Noble *et al*. 1997 which conducted studies on survival of viruses in wastewater discovered that higher molecular weight of dissolved matter has an impact on their survival. Hart *et al.* 2020 also stated that when calibrated carefully, the wastewater surveillance method at worst can detect 1 infected person in 114 individuals and at best can detect one infected case in 2 million uninfected individuals. This shows the advantage of this detection method over the traditional testing methods as they would require significant amount of time and resources. Medema *et al.* 2020 highlighted the ability of wastewater surveillance when spread of the disease was low. An investigation was carried out in Netherlands, for the SARS-CoV-2 presence in wastewater of both domestic and airport, at the start of the pandemic, to find out the effectiveness of sewage surveillance for monitoring. No samples of February 6, 2020 were tested positive i.e. three weeks before the first case was reported. And a N1 fragment was found in the samples of March 5. It indicates that the wastewater can help in finding out the entry of new type of virus or can raise an alarm before the wide spread of virus in the community.

Economics of Covid-19 testing

Per assay costs of both clinical and wastewater-based epidemiology may be different among different countries due to government regulations, labor costs and medical/testing infrastructure. The estimated cost of a test kit which contains key reagents that are essentially identical (qRT PCR buffers, probes, etc.) is available for $10 - $20 USD (Hart *et al.,* 2020). Expenses for reagents required for primary checkup of the community through municipal wastewater was calculated by multiplying the total number of wastewater treatment plants and cost of reagents. The study concludes that countries like Germany can test the people by using 0.014% of the cost required for clinical testing, and in America by 0.00006% of cost required for clinical testing (Hart *et al.,* 2020). These calculations show that WBE costs way less than the cost of clinical test. The price of a RT-PCR test in all states across India ranges from INR 400 to INR 2100, whereas the test was made free in the state of Assam but in order to obtain the report in 24 hours, INR 2200 has to be paid (Timesnow News, 2020). The costs initially ranged between INR 4500 to INR 6000, but once the number of testing labs increased, the cost was brought down significantly. In India approximately 222 million people were tested as of March 8, 2021 (ICMR, 2021), which means that 16.30% people have been tested. Assuming the present cost, it would cost approximately between INR 54,000 Crores and INR 2.83.500 Crores, i.e. USD 7.4 Billion and USD 38.7 Billion to get every person in India tested. WBE-based detection offers a much more economical alternative that will allow the analysis of infection spread without the necessity of testing of all the individuals. In general, the WBE-based approach is to be applied at a broader scale and then subsequently narrowed down to sub-areas of interest (generally locations with higher infection levels). For example, when applied over a city, this approach will indicate the state of spread and its trend (increasing or decreasing). By sub-dividing the city into various sections (or zones), areas with higher occurrence or faster rate of spread can be determined. Targeted individual testing can then be

carried out to isolate the cases and prevent further spread. This is certainly much less expensive than testing all individuals within the city.

Challenges

The foremost challenge to the application of WBE-based approach is the locations from where sampling can be done. These locations are generally the sewage treatment plants and in some cases, the sewer network. Unfortunately, the developing and underdeveloped countries have low prevalence of sewers and STPs. For example, there are only 920 sewage treatment plants in India. Furthermore, out of 61,754 MLD sewage generated, 38,791 MLD remains untreated (International Institute of Health and Hygiene, 2021). Approximately only 37% of the total sewage is passing through the wastewater treatment plants and only those places can be used for community testing through WBE. This leaves a large fraction of the Indian population out of the loop of community-level testing. Another challenge would be the process and test protocol. There are studies being conducted to find out the best and accurate process for detection of virus (Brouwer *et al.,* 2018). Nevertheless, the process is sensitive to key variables like temperature, in-sewer travel time, season, rainfall, sampling time, etc. and data may be affected if not calibrated carefully (Brouwer *et al.,* 2018). For Covid-19 detection through WBE, there are several tests available with varying level of results. A standardized protocol is needed for Covid-19 detection for community based assessment. In addition, the RT-PCR based testing is expensive and requires skilled personnel, both of which are roadblocks for successful implementation in underdeveloped world. Biosensors may be a better approach for real-time monitoring in the sewer networks. Finally, there are still some concerns over the correlation between the results obtained from WBE and actual spread. This may be a function of the population being catered by the sampled WWTP, movement of tourists (esp. for smaller towns), testing method used, etc. (Zahedi *et al.,* 2021). Further studies in these directions are needed.

Case Studies_of WBE application for Coronavirus detection

In different case studies and research conducted around the world, the observations of viral load were made and, in some instances, WBE helped in detecting Covid-19 infected cases in real time (Table 1). In a study conducted in Paris, the concentration of vRNA in raw sewage was found to be 5.104 genome units /L during an early stage when total confirmed cases in Paris were 91 (Wurtzer *et al.,* 2020b). Wu *et al.,* 2020 suggested that the concentrations indicated a much higher COVID-19 prevalence (0.1%−5%) than confirmed clinical cases (0.026%). The study implies that WBE can help in finding the hotspots of spread in a much better way than the clinical testing. In another study in India, the estimated maximum concentration was 3.5 x 10^2 copies/L in the month of May 2020 (Kumar *et al.* 2020). According to Foladori *et al.,* 2020, the analytical data does not give the correct information of the viral load as it is variable to number of cases. It is also suggesting that the load can be up to 4 orders of magnitude, from 5·103 to 107.6 copies/mL and there is a need of further research to get the exact values. There are cases when the testing of wastewater for detection of Covid-19 helped prevent the outbreak of the disease. In University of Arizona, daily screening of sewage was conducted (Pineda and Leingang, 2020). On 25 August 2020, the official found the traces of coronavirus. They immediately got the 311 people in the dorm tested on 26 August 2020 and found two students who were infected with the virus (although they were asymptomatic). They sent the infected students into isolation and the next day the samples were clear of the traces. It helped the authorities to stop the outbreak of virus to other students living in the same dorm. Two recent developments have taken place: the availability of the vaccines and the detection of new strains. Multiple vaccines have been approved, in different countries, and mass-vaccination programs have also been started to protect the frontline workers and people at-risk as a priority. On the other hand, new strains have emerged and have led to an upsurge in the new cases. Globally, around half million new cases are being reported daily. This shows that while human beings have come up ways to tackle the virus, its mutations have allowed it to continue the infection. This calls for a concerted effort in detection, containment, treatment and prevention. WBE-based testing is a cornerstone of such efforts.

Table 1: A summary of case studies of WBE application for Covid-19 detection in wastewater

Place of study	Location of sample collection	Process of Sample collection and Dates of collection	Virus concentration method	RNA extraction method	RNA quantification & amplification method	Genome value / C_t value	Reference
Ahmedabad, Gujarat India	Wastewater treatment plant	Composite and Grab; 8th May 2020 – 27th May 2020	Use of PEG	Commercial available kit (NucleoSpin® RNA Virus)	RT-PCR using TaqPath™ Covid-19 RT-PCR Kit	Maximum concentration of 3.5×10^2 copies/L	Kumar *et al.*, (2020)
Australia	One suburban pumping station and two WWTPs	Conventional refrigerated autosampler and Submersible in-situ high frequency autosampler as well as grab sampling; 24th February 2020 – 5th April 2020	Direct RNA extraction from electronegative membranes And Ultrafiltration	Combination of two kits (RNeasy power water Kit and RNeasy power microbiome Kit.	RT-qPCR	Maximum of 12 copies / 100ml	Ahmed *et al.*, (2020)
Brazil	Sewage treatment plants, hospital wastewater and sewers network	Ten-hour composite samples; 15th April 2020	Ultracentrifugation Method	Using QIAamp® Viral RNA Mini kit and a QIAcube® automated system.	RT-qPCR	SARS-CoV-2 RNA was detected in 5 of 12 samples, Ct values: 36.3 to 39.8	Prado *et al.*, (2020)
Italy	Three WWTPs	Composite; 3rd February 2020 – 2nd April 2020	PEG-dextran method	NucliSENS miniMAG semi-automated extraction system.	PCR using Kit Platinum™ SuperFi™ Green PCR Master Mix	6 of 12 WWTP'S tested were positive.	La Rosa *et al.*, (2020)
Paris, France	Three WWTPs	Composite; 5th March 2020 – 23rd April 2020	Ultra centrifugation and extraction	Using PowerFecal Pro kit, QIAsymphony automated extractor	Using Fast virus 1-step Master mix 4x	$3x10^6$ GU/L on peak	Wurtzer *et al.*, (2020b)
Israel	Hospital treating SARS-COV-2 patients, Isolation facility, Sewer network, Wastewater treatment plant	Using Automatic sampler; 10th March 2020 – 21st April 2020	Centrifugation, and using polyethylene glycol (PEG)	Using viral RNA extraction kit (RNeasy mini kit- QIAGEN and EasyMAG)	Reverse transcribed and qPCR and Step One Plus real-time PCR system	10 out of 26 collected samples tested positive with Ct values: 32.76 to 38.5	Bar Or *et al.*, (2020)
Japan	Five times from a wastewater treatment plant and Three times from a river	Grab sampling; 17th March 2020 – 7th May 2020	Electronegative membrane-vortex (EMV) method and adsorption-direct RNA extraction method	Using QIAamp Viral RNA Mini Kit in a QIAcube automated platform	Using Thermal Cycler Dice Real Time System TP800	Ct value: 39.96, 2.4×10^3 copies/L in the water sample	Haramoto *et al.*, *(2020)*
Istanbul, Turkey	WWTPs and Man holes	Composite and Grab sampling; 21st April 2020 – 25th April 2020	Centrifugation	Using Pathogen Mini Kit	RT-qPCR	Five out of Seven samples collected from WWTP were positive	Kocamemi *et al.*, (2020)

Table 1: A summary of case studies of WBE application for Covid-19 detection in wastewater

Place of study	Location of sample collection	Process of Sample collection and Dates of collection	Virus concentration method	RNA extraction method	RNA quantification & amplification method	Genome value / C_t value	Reference
Louisiana, USA	Two WWTPs	Composite and Grab; 13th January 2020 – 8th April 2020	Ultrafiltration and Adsorption–elution method using an electronegative membrane	Use of *Pseudomonas* bacteriophage	RT-qPCR using a CFX96 RealTime PCR Instrument	3.1×10^3 - 7.5×10^3 copies/L	Sherchan *et al.* (2020)
England	WWTP	Composite; 14th January 2020 – 12th May 2020	Centrifugation	RNA extracted from the National Institute for Biological Standards and Control	RT-qPCR	3.50 - 4.20 Log10 gc/L	Martin *et al.* (2020)
Southeastern Virginia, USA	Nine WWTPs	Composite and Grab; 9th March 2020 – 26th July 2020	n InnovaPrep Concentrating Pipette Select and electronegative filtration	Using NucliSENS easyMag	RT- digital droplet PCR	98 out of 198 water samples were found positive.	Gonzalez *et al.* (2020)
North Rhine-Westphalia Germany	Nine WWTPs	Composite; 8th April 2020	Amicon® Ultra-15 Centrifugal Filter Unit	NucleoSpin RNA Virus kit	OneStep RT-qPCR using Luna Universal Probe One-Step RT-qPCR Kit or LightCycler® Multiplex RNA Virus Master and the CFX96 Real-Time System, with a C1000 Touch Thermal Cycler.	3 - 20 gene equivalents per mL in the inflow, and 2.7 - 37 gene equivalents in the WWTP effluent	Westhaus *et al.* (2020)
Santiago, Chile	Two WWTPs	Composite; March 2020 – June 2020	Ultracentrifugation	Using the QIAampÒ Viral RNA Mini kit	Using the TaqMan 2019-nCoV Assay Kit v1	Ct values: 28.1- 37.7	Ampuero *et al.* (2020)
Netherlands	WWTPs	Composite; 7th February 2020 – 25th March 2020	Ultrafiltration	Using the RNeasy PowerMicrobiome Kit and Biomerieux Nuclisens kit	RT-qPCR	14–30 gene copies (gc)/mL	Medema *et al.* (2020)
Southern Nevada, USA	WWTP and from lake	Grab and Composite; March 2020 – May 2020	Primary concentration with hollow fiber ultrafiltration (HFUF) and secondary concentration with Centricon ultrafiltration	Purelink Viral RNA/DNA Mini Kit	q-PCR	4.5×10^9 gc/L	Gerrity *et al.* (2020)

RECOMMENDATION

Given the unique advantages offered by the WBE-based approach, it should be the preferred choice for Covid-19 detection and spread analysis, globally. This pandemic has severely affected the entire world and hence a global approach is required to its containment and remediation. It is expected that countries with more resources will come forward to help the lesser-endowed nations in terms of scientific and technical know-how, testing kits and other accessories needed for testing and control. Formation of a collaborative group (Covid19WBEC) is a welcome step in this direction. WBE-based detection, followed by human testing in areas with high concentration of cases and mass-scale vaccination is likely to be the key to overcome this pandemic.

CONCLUSION

A global pandemic has engulfed the humanity and has infected 128 million people (so far), killing millions in a very brief span of 15 months. Unfortunately, despite the best efforts, it is yet to be controlled. The first step in the fight against Covid-19 is its detection. Unfortunately, the targeted human testing is expensive, intrusive and a lagging indicator of the infection. An alternate approach such as, wastewater based epidemiology may act as a potential solution. Here, a comprehensive literature review focusing on the application of wastewater based epidemiological approach to the detection of Covid-19 is presented. This method obviates the need for extensive, non-targeted individual testing and helps in determining the spread and its trend at a much lower cost, in a non-intrusive and timely manner. It has been established, through various studies, that WBE-based Covid-19 detection can provide an early indication of the spread as compared to direct-human testing, thus providing significant lead time. These results can then be used to focus, in a targeted manner, on areas with high level of viral spread leading to human testing, and containment and treatment efforts. This approach has been applied in different geographies (Table 1) and has proven to be an asset in containing Covid-19 and preventing further spread of the infection, saving lives and resources in the process. Different variations of PCR-based assays have been utilized in these studies to a varying degree of success. With the availability of multiple vaccines globally, this approach can be coupled with the targeted vaccination program to prevail over this pandemic. The biggest challenge to this approach is the non-availability of representative sampling locations, especially in developing and underdeveloped countries. This brings the focus back on the lack of extensive sanitation programs in such countries. Standardized protocols and knowledge/resource sharing, through concerted global efforts, is expected to enhance the application of this technique in different parts of the world and is likely to aid humanity's fight against Covid-19.

AUTHOR CONTRIBUTIONS

P.S. Deepak wrote the first draft of the manuscript and helped with updates (as needed), including the post-review activities. G. Saini developed the concept, supervised the manuscript preparation and edited it.

ACKNOWLEDGMENTS

The authors acknowledge the anonymous colleagues and reviewers for their valuable comments and suggestions that helped improve this manuscript.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
Cl_2	Chlorine
ClO_2	Chlorine dioxide
cPCR	Conventional polymerase chain reaction
Ct	Threshold Cycle
ddPCR	Droplet Digital polymerase chain reaction
DNA	Deoxyribonucleic acid
$FeCl_3$	Ferric chloride
ICMR	Indian council of Medical Research
IQR	Interquartile range

kDa	Kilodaltons
MERS	Middle East respiratory syndrome
MLD	millions of liter per day
NaOCl	Sodium hypochlorite
NMWL	Nominal molecular weight limit
O_3	Ozone
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Real-time quantitative polymerase chain reaction
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SMF	Skimmed milk flocculation
vRNA	Viral Ribonucleic Acid
WBE	Wastewater based epidemiology
WHO	World health organization
WWTP	Waste water treatment plant

REFERENCES

Aguiar-Oliveira, M. L.; Campos, A.; R Matos, A.; Rigotto, C.; Sotero-Martins, A.; Teixeira, P.; Siqueira, M. M., (2020). Wastewater-Based Epidemiology (WBE) and Viral Detection in Polluted Surface Water: A Valuable Tool for COVID-19 Surveillance-A Brief Review. Int. J. Environ. Res. Public Health, 17(24): 9251 **(19 pages)**.

Ahmed, W.; Angel, N.; Edson, J.; Bibby, K.; Bivins, A.; O'Brien, J.W.; Choi, P.M.; Kitajima, M.; Simpson, S. L.; Li, J.; Tscharke, B.; Verhagen, R.; Smith, W.; Zaugg, J.; Dierens, L.; Hugenholtz, P.; Thomas, K.V.; Mueller, J.F., (2020). First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ., 728: 138764 **(9 pages)**.

Ampuero, M.; Valenzuela, S.; Valiente-Echeverría, F.; Soto-Rifo, R.; Barriga, G.P.; Chnaiderman, J.; Rojas, C.; Guajardo-Leiva, S.; Díez, B.; Gaggero, A., (2020). SARS-CoV-2 detection in sewage in Santiago. Chile. MedRxiv **(10 pages).**

Augustine, R.; Hasan, A.; Das, S.; Ahmed, R.; Mori, Y.; Notomi, T.; Kevadiya, B.D.; Thakor, A.S., (2020). Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic. Biology, 9(8):182 **(17 pages)**.

Bar Or, I.; Yaniv, K.; Shagan, M.; Ozer, E.; Erster, O.; Mendelson, E., (2020). Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. MedRxiv **(11 pages)**.

Bibby, K.; Peccia, J., (2013). Identification of viral pathogen diversity in sewage sludge by metagenome analysis. Environ. Sci. Technol., 47(4): 1945–1951 **(7 pages)**.

Bio-Rad, (2021). Droplet Digital™ PCR (ddPCR™) Technology.

Brouwer, A.F.; Eisenberg, J.N.; Pomeroy, C.D.; Shulman, L.M.; Hindiyeh, M.; Manor, Y.; Grotto, I.; Koopman, J.S.; Eisenberg, M.C., (2018). Epidemiology of the silent polio outbreak in Rahat, Israel, based on modeling of environmental surveillance data. Proc. Natl. Acad. Sci. U.S.A., 115(45):E10625–E10633 **(9 pages).**

Bustin, S.A.; Nolan, T., (2020). RT-qPCR Testing of SARS-CoV-2: A Primer. Int. J. Mol. Sci., 21(8): 3004. **(9 pages).**

Calgua, B.; Rodriguez-Manzano, J.; Hundesa, A.; Suñen, E.; Calvo, M.; Bofill-Mas, S.; Girones, R., (2013). New methods for the concentration of viruses from urban sewage using quantitative PCR. J. Virol. Methods, 187(2): 215–221 **(7 pages)**.

Cashdollar, J.L.; Wymer, L., (2013). Methods for primary concentration of viruses from water samples: a review and meta-analysis of recent studies. J. Appl. Microbiol., *115*(1): 1–11 **(11 pages)**.

CDC, (2021a). COVID-19 Testing Overview. Centers for Disease Control and Prevention.

CDC, (2021b). Developing a Wastewater Surveillance Sampling Strategy. Centers for Disease Control and Prevention.

CDC, (2021c). Wastewater Surveillance Testing Methods. Centers for Disease Control and Prevention.

CDC, (2021d). CDC 2019-Novel Coronavirus (2019-nCoV). Centers for Disease Control and Prevention. Real-Time RT-PCR Diagnostic Panel, CDC-006-00019, revision: 06 CDC/DDID/NCIRD/ Division of Viral Diseases Effective: 12/01/2020.

Chen, Y.; Chen, L.; Deng, Q.; Zhang, G.; Wu, K.; Ni, L.; Yang, Y.; Liu, B.; Wang, W.; Wei, C.; Yang, J.; Ye, G.; Cheng, Z., (2020). The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J. Med. Virol., 92(7): 833–840 **(8 pages)**.

Chin, A.; Chu, J.; Perera, M.; Hui, K.; Yen, H. L.; Chan, M.; Peiris, M.; Poon, L., (2020). Stability of SARS-CoV-2 in different environmental conditions. Lancet. Microbe, 1(1): e10 **(2 pages)**.

Cobo, F., (2012). Application of molecular diagnostic techniques for viral testing. Open Virol. J., 6: 104–114 **(11 pages)**.

Corpuz, M.; Buonerba, A.; Vigliotta, G.; Zarra, T.; Ballesteros, F.; Jr, Campiglia, P.; Belgiorno, V.; Korshin, G.; Naddeo, V., (2020). Viruses in wastewater: occurrence, abundance and detection methods. Sci. Total Environ., 745: 140910 **(27 pages).**

Cucinotta, D.; Vanelli, M., (2020). WHO declares COVID-19 a pandemic. Acta Biomed: Atenei Parmensis, 91(1): 157–160 **(4 pages)**.

Cyranoski, D., (2018). China expands surveillance of sewage to police illegal drug use. Nature 559: 310-311 **(2 pages).**

DiToro, D.; Winstead, C. J.; Pham, D.; Witte, S.; Andargachew, R.; Singer, J.R.; Wilson, C.G.; Zindl, C.L.; Luther, R.J.; Silberger, D.J.; Weaver, B.T.; Kolawole, E.M.; Martinez, R.J.; Turner, H.; Hatton, R.D.; Moon, J.J.; Way, S.S.; Evavold, B.D.; Weaver, C.T., (2018). Differential IL-2 expression defines developmental fates of follicular versus nonfollicular helper T cells. Science, 361(6407):

eaao2933 **(29 pages)**.

Falman, J.C.; Fagnant-Sperati, C.S.; Kossik, A.L.; Boyle, D.S.; Meschke, J.S., (2019). Evaluation of secondary concentration methods for Poliovirus detection in Wastewater. Food Environ. Virol., 11(1): 20–31. **(12 pages).**

Foladori, P.; Cutrupi, F.; Segata, N.; Manara, S.; Pinto, F.; Malpei, F.; Bruni, L.; La Rosa, G., (2020). SARS-CoV-2 from feces to wastewater treatment: What do we know? A review. Sci. Total Environ., 743: 140444. **(13 pages).**

Gerrity, D.; Papp, K.; Stoker, M.; Sims, A.; Frehnera, W., (2020). Early-pandemic wastewater surveillance of SARS-CoV-2 in Southern Nevada: Methodology, occurrence, and incidence/prevalence considerations. Water Res X. 10: 100086 **(11 pages)**.

Gonzalez, R.; Curtis, K.; Bivins, A.; Bibby, K.; Weir, M.H.; Yetka, K.; Thompson, H.; Keeling, D.; Mitchell, J.; Gonzalez, D., (2020). COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Res, 186: 116296. **(32 pages).**

Guarner, J., (2020). Three emerging Coronaviruses in two decades. Am. J. Clin. Pathol., 153(4): 420–421 **(2 pages)**.

Haramoto, E.; Malla, B.; Thakali, O.; Kitajima, M., (2020). First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci. Total Environ., 737: 140405. **(9 pages).**

Hart, O.E.; Halden, R.U., (2020). Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities andchallenges. Sci. Total Environ., 730: 138875 **(9 pages)**.

Hill, V.R.; Kahler, A.M.; Jothikumar, N.; Johnson, T.B.; Hahn, D.; Cromeans, T.L., (2007). Multistate evaluation of an ultrafiltration-based procedure for simultaneous recovery of enteric microbes in 100-liter tap water samples. Appl. Environ. Microbiol., 73(13): 4218–4225 **(8 pages)**.

Huerta-Fontela, M.; Galceran, M.T.; Martin-Alonso, J.; Ventura, F., (2008). Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. Sci. Total Environ., 397(1-3): 31-40 **(10 pages)**.

ICMR, (2020). Evidence based advisory on correlation of COVID-19 disease severity with Ct values of the real time RT-PCR Test. Indian Council of Medical Research.

ICMR, (2021). SARS-CoV-2 (COVID-19) testing status.

International Institute of Health and Hygiene, (2021). ENVIS Centre on hygiene, sanitation, sewage treatment systems and technology, National status of waste water generation and treatment.

Johnson, M., (2012). RNA Extraction., Mater. Meth., 2: 201.

Kadri, K., (2020). Polymerase chain reaction (PCR): principle and applications. Synthetic Biology - New Interdisciplinary Science **(18 pages)**.

Karolak, S.; Nefau, T.; Bailly, E.; Solgadi, A.; Levi, Y., (2010). Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). Forensic Sci. Int., 200(1-3): 153–160 **(8 pages)**.

Kocamemi, A.B.; Kurt. H.; Hacıoglu. S.; Yaralı. C.; Saatci. A.M.; Pakdemirli, B.; Yaykıran, S., (2020). First data-set on SARS-CoV-2 detection for Istanbul wastewaters in Turkey. Medrxiv **(10 pages).**

Kooraki, S.; Hosseiny, M.; Myers, L.; Gholamrezanezhad, A., (2020). Coronavirus (COVID-19) outbreak: What the Department of Radiology should know. J. Am. Coll. Radiol.: JACR, 17(4): 447–451 **(5 pages)**.

Kumar, M.; Patel, A.K.; Shah, A.V.; Raval, J.; Rajpara, N.; Joshi, M.; Joshi, C.G., (2020). First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Sci. Total Environ., 746: 141326 **(8 pages)**.

La Rosa, G.; Iaconelli, M.; Mancini, P.; Bonanno Ferraro, G.; Veneri, C.; Bonadonna, L.; Lucentini, L.; Suffredini, E., (2020). First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci. Total Environ., 736: 139652 **(6 pages)**.

Lewis, G.D.; Metcalf, T.G., (1988). Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Appl. Environ. Microbiol., 54(8): 1983–1988 **(6 pages)**.

Listwan, P.; Pédelacq, J.D.; Lockard, M.; Bell, C.; Terwilliger, T.C.; Geoffrey S.W., (2010). The optimization of in vitro high-throughput chemical lysis of *Escherichia coli*. Application to ACP domain of the polyketide synthase ppsC from *Mycobacterium tuberculosis*. J. Struct. Funct. Genomics, 11**:** 41–49 **(9 pages)**.

Liu, Y.; Gayle, A.A.; Wilder-Smith, A.; Rocklöv, J., (2020). The reproductive number of COVID-19 is higher compared to SARS coronavirus. J. Travel Med., 27(2): taaa021 **(4 pages)**.

MacIntyre, C.R., (2020). Case isolation, contact tracing, and physical distancing are pillars of COVID-19 pandemic control, not optional choices. Lancet Infect. Dis., 20(10): 1105–1106 **(2 pages)**.

Mandal, P.; Gupta, A. K.; Dubey, B.K., (2020). A review on presence, survival, disinfection/removal methods of coronavirus in wastewater and progress of wastewater-based epidemiology. J. Environ. Chem. Eng., 8(5): 104317 **(11 pages)**.

Lima, C.M.A.O., (2020). Information about the new coronavirus disease (COVID-19). Radiol. Bras., 53(2): V-VI. **(2 pages).**

Martin, J.; Klapsa, D.; Wilton, T.; Zambon, M.; Bentley, E.; Bujaki, E.; Fritzsche, M.; Mate, R.; Majumdar, M. (2020). Tracking SARS-CoV-2 in sewage: evidence of changes in virus variant predominance during COVID-19 pandemic. Viruses, 12(10): 1144 **(17 pages).**

Medema, G.; Heijnen, L.; Elsinga, G.; Italiaander, R.; Brouwer, A., (2020). Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett., 7 (7): 511-516 **(6 pages).**

Meo, S.A.; Alhowikan, A.M.; Al-Khlaiwi, T.; Meo, I.M.; Halepoto, D.M.; Iqbal, M.; Usmani, A.M.; Hajjar, W.; Ahmed, N., (2020). Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. Eur. Rev. Med. Pharmacol. Sci., 24(4): 2012–2019 **(8 pages)**.

Metcalfe, C.; Tindale, K.; Li, H.; Rodayan, A.; Yargeau, V., (2010). Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environ. Pollut., 158(10): 3179–3185 **(7 pages)**.

Michael-Kordatou, I.; Karaolia, P.; Fatta-Kassinos, D., (2020). Sewage analysis as a tool for the COVID-19 pandemic response and management: the urgent need for optimised protocols for

SARS-CoV-2 detection and quantification. J. Environ. Chem. Eng., 8(5): 104306 **(24 pages)**.

Nassar, M.S.; Bakhrebah, M.A.; Meo, S.A.; Alsuabeyl, M.S.; Zaher, W.A., (2018). Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection: epidemiology, pathogenesis and clinical characteristics. Eur. Rev. Med. Pharmacol. Sci., 22(15): 4956–4961 **(6 pages)**.

Nemudryi, A.; Nemudraia, A.; Wiegand, T.; Surya, K.; Buyukyoruk, M.; Cicha, C.; Vanderwood, K.K.; Wilkinson, R.; Wiedenheft, B., (2020). Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. Cell Rep. Med., 1(6): 100098 **(11 pages).**

Noble, R.T.; Fuhrman, J.A., (1997). Virus decay and its causes in coastal waters. Appl. Environ. Microbiol., 63(1) : 77–83 **(7 pages)**.

Perappadan, B.S., (2020). India's first coronavirus infection confirmed in Kerala.

Pineda, P.; Leingang, R., (2020). University of Arizona wastewater testing finds virus at dorm, prevents outbreak, Arizona Republic.

Prado, T.; Fumian, T.M.; Mannarino, C.F.; Maranhão, A.G.; Siqueira, M.M.; Miagostovich, M.P., (2020). Preliminary results of SARS-CoV-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz, 115: e200196 **(3 pages).**

Prata, C.; Ribeiro, A.; Cunha, Â.; Gomes, N.C.; Almeida, A., (2012). Ultracentrifugation as a direct method to concentrate viruses in environmental waters: virus-like particle enumeration as a new approach to determine the efficiency of recovery. Environ. Monit. Assess. JEM, 14(1): 64–70 **(7 pages)**.

Rabenau, H.F.; Cinatl, J.; Morgenstern, B.; Bauer, G.; Preiser, W.; Doerr, H.W., (2005). Stability and inactivation of SARS coronavirus. Med. Microbiol. Immun., 194(1-2): 1–6 **(6 pages)**.

Randazzo, W.; Truchado, P.; Cuevas-Ferrando, E.; Simón, P.; Allende, A.; Sánchez, G., (2020). SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res., 181: 115942 **(9 pages)**.

Sherchan; S.P.; Shahin, S.; Ward, L.M.; Tandukar, S.; Aw, T.G.; Schmitz, B.; Ahmed, W.; Kitajima, M., (2020). First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA. Sci. Total Environ., 743: 140621 **(7 pages)**.

Sinclair, R.G.; Choi, C.Y.; Riley, M.R.; Gerba, C.P., (2008). Pathogen surveillance through monitoring of sewer systems. Adv. Appl. Microbiol., 65: 249–269 **(21 pages)**.

Timesnow News, (2020). COVID-19: How much does RT-PCR test cost? Find state-wise price here.

Wang, J.; Shen, J.; Ye, D.; Yan, X.; Zhang, Y.; Yang, W.; Li, X.; Wang, J.; Zhang, L.; Pan, L., (2020). Disinfection technology of hospital wastes and wastewater: Suggestions for disinfection strategy during coronavirus Disease 2019 (COVID-19) pandemic in China. Environ. Pollut., 262: 114665. **(11 pages).**

Wang, X.W.; Li, J.S.; Guo, T.K.; Zhen, B.; Kong, Q.X.; Yi, B.; Li, Z.; Song, N.; Jin, M.; Xiao, W.J.; Zhu, X.M.; Gu, C.Q.; Yin, J.; Wei, W.; Yao, W.; Liu, C.; Li, J.F.; Ou, G.R.; Wang, M.N.; Fang, T.Y.; Li, J.W., (2005). Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital. J. Virol. Methods, 128(1-2): 156–161 **(6 pages)**.

Watzinger, F.; Ebner, K.; Lion, T., (2006). Detection and monitoring of virus infections by real-time PCR. Mol. Aspects Med., 27(2-3): 254–298 **(45 pages).**

Weiss, S.R.; Leibowitz, J.L., (2011). Coronavirus pathogenesis. Adv. Virus Res., 81: 85–164 **(80 pages).**

Westhaus, S.; Weber, F.A.; Schiwy, S.; Linnemann, V.; Brinkmann, M.; Widera, M.; Greve, C.; Janke, A.; Hollert, H.; Wintgens, T.; Cilesek, S., (2021). Detection of SARS-CoV-2 in raw and treated wastewater in Germany - Suitability for COVID-19 surveillance and potential transmission risks. Sci. Total Environ., 751: 141750 **(13 pages)**.

WHO, (2003). Guidelines for environmental surveillance of poliovirus circulation. World Health Organization Publication.

WHO, (2020). Water, sanitation, hygiene, and waste management for SARS-CoV-2, the virus that causes COVID-19.

Worldometer, (2021a). Coronavirus cases of World.

Worldometer, (2021b). Coronavirus cases of India.

Wu, F.; Zhang, J.; Xiao, A.; Gu, X.; Lee, WL.; Armas, F.; Kauffman, K.; Hanage, W.; Matus, M.; Ghaeli, N.; Endo, N.; Duvallet, C.; Poyet, M.; Moniz, K.; Washburne, A.D.; Erickson, T.B.; Chai, P.R.; Thompson, J.; Alm, E. J., (2020). SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. mSystems. 5(4):e00614-20 **(9 pages)**.

Wurtzer, S.; Marechal, V.; Mouchel, J. M.; Moulin, L. (2020a). Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. MedRxiv. **(4 pages).**

Wurtzer, S.; Marechal, V.; Mouchel, J.M.; Maday, Y.; Teyssou, R.; Richard, E.; Almayrac, J.L.; Moulin, L., (2020b). Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in waste water, Greater Paris, France, 5 March to 23 April 2020. Euro Surveill., 25(50): 2000776. **(7 pages).**

Xiao, F.; Tang, M.; Zheng, X.; Liu, Y.; Li, X.; Shan, H., (2020). Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterology, 158(6): 1831–1833.e3 **(7 pages)**.

Yagci, A.K.; Sarinoglu, R.C.; Bilgin, H.; Yanılmaz, Ö.; Sayın, E.; Deniz, G.; Guncu, M.M.; Doyuk, Z.; Barıs, C.; Kuzan, B.N.; Aslan, B.; Korten, V.; Cimsit, C., (2020). Relationship of the cycle threshold values of SARS-CoV-2 polymerase chain reaction and total severity score of computerized tomography in patients with COVID 19. Int. J. Infect. Dis., 101: 160–166 **(7 pages)**.

Young, B.E.; Ong, S.; Kalimuddin, S.; Low, J.G.; Tan, S.Y.; Loh, J.; Ng, O.T.; Marimuthu, K.; Ang, L.W.; Mak, T.M.; Lau, S.K.; Anderson, D.E.; Chan, K.S.; Tan, T.Y.; Ng, T.Y.; Cui, L.; Said, Z.; Kurupatham, L.; Chen, M.I.; Chan, M., (2020). Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. Singapore 2019 Novel Coronavirus Outbreak Research Team, J. Am. Med. Assoc., 323(15): 1488–1494 **(7 pages)**.

Zahedi, A.; Monis, P.; Deere, D.; Ryan, U., (2021). Wastewater-based epidemiology- surveillance and early detection of waterborne pathogens with a focus on SARS-Cov-2, *Cryptosporidium* and *Giardia*. Parasitol. Res. **(22 pages)**.

Zhong, N.S.; Zheng, B.J.; Li, Y.M.; Poon.; Xie, Z.H.; Chan, K.H.; Li, P.H.; Tan, S.Y.; Chang, Q.; Xie, J.P.; Liu, X.Q.; Xu, J.; Li, D.X.; Yuen, K.Y.; Peiris.; Guan, Y., (2003). Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet, 362(9393): 1353–1358 **(6 pages)**.

AUTHOR (S) BIOSKETCHES

Saini, G., Ph.D., Associate Professor, Department of Civil Engineering, Sharda University, Greater Noida, Uttar Pradesh, India.

Email: *gauravsaini@gmail.com*

Deepak, P.S., B.Tech., Department of Civil Engineering, Sharda University, Greater Noida, Uttar Pradesh, India.

Email: *2018016302.pinakana@ug.sharda.ac.in*

COPYRIGHTS

©2021 The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

HOW TO CITE THIS ARTICLE

Saini, G.; Deepak, P.S., (2021). Wastewater-based epidemiology for novel Coronavirus detection in wastewater. Global J. Environ. Sci. Manage., 7(4): 643-658.

DOI: 10.22034/gjesm.2021.04.10

url: https://www.gjesm.net/article_243222.html