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Detecting SARS-CoV-2 in Wastewater An Early Warning Signal for COVID-19

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Summary

Wastewater-based epidemiology (WBE) has emerged as a useful tool to assess the prevalence of the SARS-CoV-2 virus in a given wastewater treatment plant (WWTP) and estimate the spread of infection in the associated catchment population. Research investigations across the globe have detected novel coronavirus in wastewater in Switzerland, France, Australia, Cyprus, Ireland, Italy, Japan, Netherlands, Spain, USA and many other countries.

SRM Institute of Science and Technology, Mu Gamma Consultants Pvt. Ltd (MGC) and The Energy and Resources Institute (TERI), supported by the Swiss Agency for Development and Cooperation (SDC), jointly implemented an intensive wastewater surveillance study during 2020-2021 to detect the presence and load of SARS-CoV-2 virus, the first-of-itskind in Chennai city, India. To develop early signaling of the spread of COVID-19 in communities, wastewaterbased surveillance was conducted in Sewage Treatment Plants (STPs) and Sewage Pumping Stations (SPSs) in Chennai city immediately after the lockdown period in 2020. With support of Chennai Metropolitan Water Supply and Sewerage Board (CMWSSB), raw sewage/ wastewater /sludge samples were collected from four

STPs and five SPSs of Chennai. Surface water, canal water and sewer outlet samples were collected from Adayar and Cooum Riverine belt flowing through the densely populated region of Chennai during the partial and post-lockdown periods in 2020 and Hospital Wastewater (HWW) samples from SRM medical school. All the samples were analysed, and the presence of SARS-CoV-2 was detected for nucleocapsid genes (N1 and N2) in addition, ribonuclease P (RNase P) gene, which serves both as an internal amplification control and indicator of specimen adequacy were detected by using gRT-PCR (quantitative real-time polymerase chain reaction). Further, the number of the ribonucleic acid (RNA) copies was calculated using the results obtained from the Nano Drop. Among caffeine, carbamazepine, and other pharmaceutical and personal care products (PPCPs) detected in wastewater samples, caffeine seems to be potential marker for removal of SARS-Cov-2 from the STPs. The study also confirmed the presence of SARS-CoV-2 genetic material in rivers and sewage outlets in Chennai city. Viral load in a given STP aided in estimation of number of infected person in that particular catchment. Such a WBE approach can provide early signal of future pandemic waves.

Overview of COVID-19 Pandemic situation

The global pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in December 2019 in Wuhan, China. The World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern in January 2020 and a pandemic in March 2020. The confirmed COVID-19 cases have been on the rise globally since February 2020. A year later, in February 2021, the number of positive COVID-19 cases stand at 102 million with more than 2.21 million deaths (Figure 1). Summarily, America (45.6 million confirmed cases) and Europe (34.2 million confirmed cases) have been highly impacted, and South-East Asia (12.8 million) has witnessed a high number of COVID-19 cases, with high recovery rates (WHO, 2021).

For a long time during the pandemic, India had the largest number of confirmed cases in Asia, and the second-highest number of confirmed cases in the world after the United States. On 30 January 2020, India reported its first case of COVID-19 in Kerala, from a student returning from Wuhan, and gradually the transmissions grew after people traveling from affected countries, and their contacts, started testing positive for COVID-19. Since then, there have been a high number of cases and high recoveries, too. As of February 2021, although India has a cumulative 10.7 million number of cases and more than 153,000 deaths (Government of India, 2021), the number of active cases has declined. **(Figure 2)**.

In the southern state of Tamil Nadu, the first case of the COVID-19 was reported on 7 March 2020. Tamil Nadu has the fifth highest number of

confirmed cases in India. All 37 districts of the state are affected by the pandemic, with the state capital of Chennai being the worst. The number of active cases in Tamil Nadu has come down to 4904 and in Chennai, the active cases have reduced to 1693 (Government of Tamil Nadu, 2021). There have been very high recoveries to the tune of 97 to 98 % (Figure 3 A and 3 B).

An Overview of Wastewater Surveillance in different countries – Research, Policy, and Interventions

Several research groups all over the world including India, have reported detecting novel coronavirus SARS-CoV-2 in wastewater, and such research results are contributing to various evidence-based policy decisions to control the pandemic: Australia (Ahmed et al, 2020), Switzerland (EPFL, 2020), Cyprus (Michael-Kordatou et al, 2020), France (Wurtzer et al, 2020), Ireland (Cahill et al, 2020), Italy (La Rosa et al, 2020), Japan (Haramoto et al, 2020), South Africa (Street et al., 2020), Spain (Chavarria-Miró et al, 2020), USA (Peccia et al and Hart & Halden et al, 2020), and India (Kumar et al 2020, Arora et al., 2020). Inferences of many of these international studies are in agreement with the methods adopted and results obtained from the wastewater surveillance study in Chennai. For example, the number of gene copies found in the SPSs, STP inlets, and sludge in Chennai were comparable to that reported in the untreated wastewaters of Australia, China, and Turkey, and lower than that of the USA, France, and Spain.

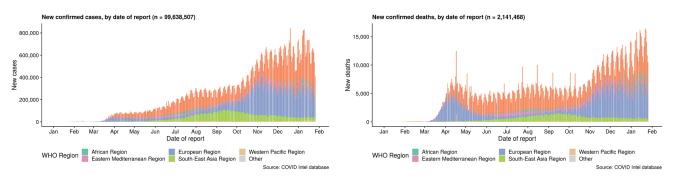


Figure 1: Status of COVID-19 Pandemic Globally Confirmed cases and deaths (January 2020-February 2021 (Source WHO, 2021)

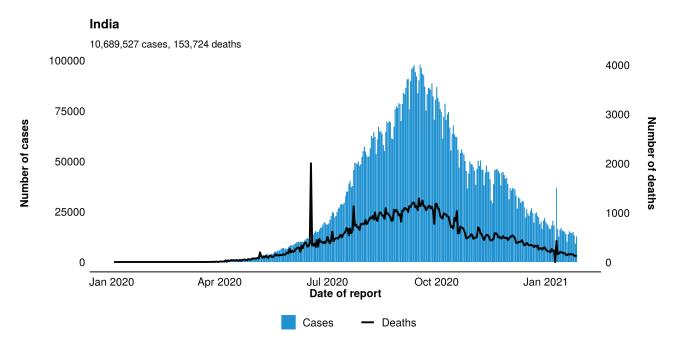


Figure 2: Status of COVID-19 Pandemic in India (January 2020-February 2021 (Source WHO, 2021)

The **United States Environmental Protection Agency (USEPA)** and The Centers for Disease Control and Prevention (CDC) are initiating the National Wastewater Surveillance System (NWSS) as well as developing and applying methods for measuring SARS-CoV-2 levels in wastewater, and standardizing methods to determine infectivity, persistence, and treatment efficacies (CDC, 2021). A study by Hart and Halden, 2020 identified WBE as a potential tool for assessing and managing the pandemic, and reported that about 2.1 billion people could be monitored globally from wastewater samples in 105,600 sewage treatment plants. In terms of policy interventions, in August 2020, the State of New York, announced \$500,000 for Pilot Program to detect SARS-CoV-2 in wastewater systems as early warning signal (State of New York, 2020). The City of Tempe (Arizona) is developing an innovative response to the pandemic from the existing Wastewater Data Analytics - Opioids program supported by the

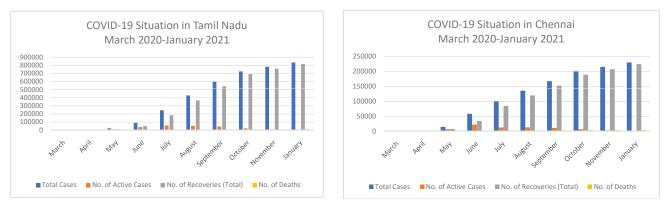


Figure 3: Overview of COVID-19 Situation in Tamil Nadu (A) and Chennai City (B) (Source: Government of Tamil Nadu, 2021)

Tempe City Council's Innovation Fund in 2018 (City of Tempe, 2020).

For the first time from **India**, Kumar et al, 2020 reported the presence of three SARS-CoV-2 genes (ORF1ab, N and S virus) in wastewater. The samples were collected in May 2020 from Old Pirana WWTP in Ahmedabad (Gujarat) to understand the application of WBE surveillance in India. The WWTP has a capacity of 106 MLD receiving effluents of Civil Hospital treating COVID-19 patients. A similar study by Arora et.al, 2020 was conducted by researchers of Dr. B. Lal Institute of Biotechnology (Rajasthan) with samples from hospital wastewater as well as from WWTPs of Jaipur, which has been a pandemic hotspot (red zone) since April 2020. At a higher ambient temperature of above 40°C, WBE studies for the presence of SARS-CoV-2 were conducted using the RT-PCR technique to confirm the presence of COVID-19 target genes (S, E, ORF1ab, RdRp, and N genes) in the samples that correlated with the increased number of reported COVID-19 positive patients.

the above-mentioned WBE Although studies were conducted in India earlier in 2020, the relevance and expected outcomes of the wastewater surveillance study in **Chennai** are quite significant. This is the first study to be conducted in any metropolitan city in India. The sudden outbreak of COVID-19 spread in Chennai during April-May 2020 ultimately led to it being the most affected district in Tamil Nadu as well as one of the major COVID-19 hotspots of India. Chennai is one of the largest and most populated Indian cities. The government agencies and supporting institutions have been doing their best to conduct clinical testing and impose necessary restrictive measures. However, in communities/zones where COVID-19 clinical testing is underutilized or unavailable, the results of wastewater monitoring can complement the existing COVID-19 surveillance systems by providing an efficient pooled community sample and useful data. There exist other local challenges in Chennai that also make the present study relevant. For example, in the northern part of the city along the Cooum riverine belt, the city houses low-income groups practicing open defecation. Rivers are stagnant and several sewer outlets have direct discharge points along the rivers. Being the

first-ever study in an Indian metropolitan city, it will initiate other cities to develop a road map for wastewater monitoring initiative.

In terms of knowledge management of the current research efforts and to empower collaboration on a global scale for WBE of SARS-CoV-2, there are various ongoing initiatives like the European Union's NORMAN-SCORE Joint Initiative (Sewage Analysis CORe group Europe), COVID-19 WBE Collaborative, Biobot Analytics, NSF Research Coordination Network (RCN) and Global Water Pathogen Project (GWPP). COVIDPoops19 that can be accessed through the COVID-19 WBE Collaborative Platform is a global dashboard on tweeting/retweeting on all matters related to wastewater and SARS-CoV-2/#COVID19, and brings together 39 dashboards, 182 universities, 45 countries and 900 sites under a single platform. COVIDPoops19 platform includes the summary of our study of SARS-CoV-2 Wastewater Monitoring in Chennai, and recognizes it as a global effort.

Research on SARS-CoV-2 monitoring in Chennai City and suburban areas

More than 70% of the wastewater generated in India is directly discharged into water bodies (CPCB, 2015). Given the uncertainties of the continuing pandemic situation, it is imperative to predict and prevent the subsequent waves in the catchment areas. Hence, an intensive wastewater surveillance was conducted with support of CMWSSB, to detect the presence of SARS-CoV-2 from the Sewage Treatment Plants (STPs) and sewage pumping stations (SPSs) of Chennai city during the post lockdown period. Simultaneously, weekly monitoring of wastewater from a hospital located about 30 km away from the city was also conducted during partial lockdown and post lockdown periods at different time intervals of the day.

In terms of sampling design for SARS-CoV-2 monitoring, analysis of 156 samples (using four concentration methods) was carried out from the STPs of Chennai City (during post lockdown period), and SRM hospital wastewater (during partial and post lockdown period). SARS-CoV-2 was detected in about 48% (75 nos.) of the tested samples. The sampling sites included 5 SPSs, 4 STPs (inlet, outlet,

and primary sludge) of Chennai city and SRM Hospital wastewater. The study site map of Chennai city is presented in **Figure 4**. The overall sampling design for the study in Chennai city entailing the SPSs, STPs, SRM Hospital wastewater, ground water and surface water sampling sites of Chennai city is given in **Table 1**.

During August-September 2020, with the guidance and support from CMWSSB, wastewater monitoring during post lockdown

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Table 1: Sampling Design for the wastewater surveillance study in Chennai City								
SN	Sampling Sites		Lockdow	/n		Post Lockdown		Total Samples
		No. of Samples	Methods (n=4)	Total RT-PCR Sample in duplicates	No. of Samples	Methods (n=4)	Total RT-PCR Sample in duplicates	
1	R K Nagar north SPS	NC	-	-	1	1x4=4	4x(3*2)=24	24
2	K P Park SPS	NC	-	-	1	1x4=4	4x(3*2)=24	24
3	Padmanabha Nagar SPS	NC	-	-	1	1x4=4	4x(3*2)=24	24
4	Mandavelipakkam SPS	NC	-	-	1	1x4=4	4x(3*2)=24	24
5	Jai Balaji Nagar SPS	NC	-	-	1	1x4=4	4x(3*2)=24	24
6	Kodungaiyur STP (Inlet, Outlet, Primary Sludge)	NC	-	-	3	3x4=12	12x(3*2)=24	72
7	Koyambedu STP (Inlet, Outlet, Primary Sludge)	NC	-	-	3	3x4=12	8x(3*2)=48	48
8	Nesapakkam STP (Inlet, Outlet, Primary Sludge)	NC	-	-	3	3x4=12	8x(3*2)=48	48
9	Perungudi STP (Inlet, Outlet, Primary Sludge)	NC	-	-	3	3x4=12	8x(3*2)=48	48
10	Adayar groundwater	NC	-	-	1	1x4=4	4x(3*2)=24	24
11	Adayar Surface water	3	3x4=12	12x(3*2)=72	3	3x4=12	12x(3*2)=72	144
12	Potheri lake water & Groundwater	NC	-	-	2	2x4=8	8x(3*2)=48	48
13	Cooum stored water	01	-	2x(3*2)=12	2	2x2=4	4x(3*2)=24	36
14	Cooum Surface Water	03	3x4=12	12x(3*2)=72	5	5x4=20	20x(3*2)=120	192
15	Buckingham Canal	02	2x4=8	8x(3*2)=48	2	2x4=8	8x(3*2)=48	96
16	Kodungaiyur STP-Groundwater	NC	-	-	1	1x4=4	4x(3*2)=24	24
17	Otteri Nala	NC	-	-	2	2x4=8	8x(3*2)=48	48
18	SRM Hospital wastewater	18	18x4=72	72x(3*2)=432	5	5x4=20	20x(3*2)=120	552
		Total Sample		636	Total Sample		1500	

NC – Not Collected

Policy Brief

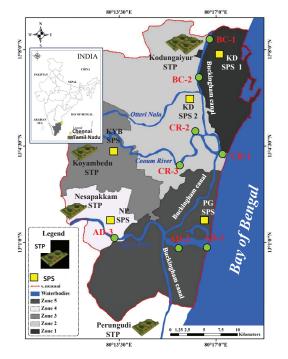


Figure 4 : Study Site Mapof Chennai City

phases was conducted to detect the presence of SARS-CoV-2 in the four major STPs and SPSs of Chennai city (Figure 4). Wastewater surveillance was conducted to map the spread of SARS-CoV-2 from the STPs and SPS of Chennai city during the first two weeks of September 2020. Simultaneously, wastewater of SRM University Hospital were monitored on a weekly basis during partial and post lockdown periods. The hospital wastewater monitoring was carried out at different time intervals (7am, 10am, 1pm, 4pm and 7pm).

Human wastewater markers such as caffeine, carbamazepine and other organic tracers were monitored to track the load of these contaminants along with SARS-CoV-2, which also mirrored the functional efficacy of STPs. This was done by using RT-PCR technique.

RT-PCR was used to detect the presence of SARS-CoV-2 in samples by using nucleocapsid (N1 and N2) specific primers and probes to monitor the genetic fragments of the virus present in the wastewater samples. Altogether 17 grab samples (Figures 4 and 5) from the WWTPs and SPSs of Chennai city and 43 samples from SRM Hospital STP, were collected and analysed. The experiment



Figure 5: Wastewater samples being collected from Hospital at SRMIST

was conducted in a Bio-safety level 2 Cabinet at SRMIST laboratory (Figure 6); a biosafety cabinet being an enclosed, ventilated laboratory workspace for safely working with materials contaminated with pathogens requiring a defined biosafety level. Wastewater was extracted for SARS-CoV-2 detection from the samples using composite, supernatant, sediment, and syringe



Figure 6: Wastewater concentration experiment



Figure 7: Level-II Biosafety Cabinet

filtration methods. The extracted RNA (Figure 7) from each method was concentrated and subjected to RT-PCR analysis (Figure 8).

Several positive and negative samples were detected (Figures 9). Out of the 17 STP samples collected and analysed using all the four above mentioned methods, all the inlets were found to **be positive** for both N1 and N2 nucleocapsid genes (Chakraborty et. al., 2020). The semi-quantitative estimation of RNA was done by counting the number of RNA copies in the positive samples using NANODROP. Chemical markers, such as caffeine and carbamazepine have been preferred as potential markers for untreated wastewater or nonfunctional STPs (Chen et al., 2014; O'Brien et al., 2017, Chakraborty et al., 2019 and 2020).

In this study, higher dynamic range and concurrence of caffeine and carbamazepine with the detected RNA copies highlight the potential



Figure 8: RT-PCR

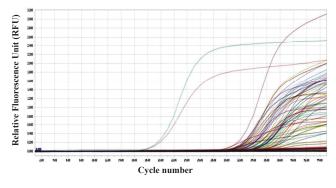


Figure 9: RT-PCR Signal

application of these compounds as chemical markers for SARS-CoV-2 in wastewater surveillance. Caffeine is found to be an effective chemical marker and its high level was observed in Koyambedu (population of 14 Lakhs, 90 Million Litres per day (MLD) wastewater), as compared to Kodangaiyur (population 30 Lakhs, 202 MLD wastewater) and Perungudi (population 30 Lakhs, 95 MLD wastewater) reflecting the direct release of higher volume of wastewater. Unlike Koyambedu, Perungudi catchment houses a population of 30 lakhs, yet generates almost similar amount of wastewater as Koyambedu (95 MLD) and the estimated positive cases of SARS-CoV-2 was one-fourth of both Koyambedu and Kodangaiyur.

River water monitoring for SARS-CoV-2 surveillance in Chennai

SARS-CoV-2 surveillance was also conducted in the **river water**, **sewage**, **ground water and tap water of Chennai city** (Figure 10). Sewage cleaning systems to the waterways in Chennai city are discharged to Adyar (49 locations), Buckingham canal (183 locations) and Cooum (105 points) rivers. Except during the rainy season, these rivers are almost stagnant. Untreated sewage water outlets are present along the important rivers-Adayar, Cooum and canals (Buckingham Canal) (Lakshmi, 2011). Literature suggests evidence of fecal shedding of the SARS-CoV-2 virus and the presence of viral RNA in wastewater and river water (Xu et al., 2020), (Lodder and de Roda Husman, 2020) & (Rimoldi et al., 2020).

During the partial and post lockdown periods in 2020, surface water samples were collected from Cooum River, Adayar River, and Buckingham canal. Further, groundwater samples and tap water were collected. 500 mL of the samples were collected



Figure 10: Sample collection from STP in Chennai city

in sterile plastic containers and transferred to the laboratory in ice boxes.

Throughout the sample collection and processing, strict safety protocols and compliances (Figure 11) were followed. The safety protocols were exercised as per WHO 2020 and ICMR 2020 guidelines, which were mandatory for conducting the study at the SRM IST Laboratory. These include, Personal Protective Equipment, Biosafety Level 2 Cabinet, and WHO Guidelines and Standard Operating Procedures (SOPs) related to safe collection and transportation of wastewater samples and laboratory safety. The compliances which was mandatory for conducting this study at the SRM IST Laboratory included Approval from SRM Institute's Biosafety Committee, Clearance from SRM Research Institute's Biosafety Committee (that all protocols are met as per WHO standard including Biosafety Level Cabinet), and Preliminary as well as Final Approval from CMWSSB, Government of Tamil Nadu.

This study confirmed the presence of SARS-CoV-2 genetic material in rivers, sewage from rivers, groundwater and tap water in Chennai city. The presence of caffeine, carbamazepine, and other pharmaceutical and personal care products (PPCPs) in these samples suggest that the samples were from direct discharge from the surrounding population. In this study, it was assumed that **quantification of SARS-CoV-2 viral genome in sewage discharge points, ground water and tap water** provides an indication of the number of symptomatic and asymptomatic COVID-19 cases.

Prospectively, studies detecting the presence of SARS-CoV-2 genetic material are urgently needed in other diverse water sources. Altogether, the results obtained from this study could underline the monitoring of SARS-CoV-2 in water sources, to support necessary public health interventions



Figure 11: Safety Protocols and Compliances

in the control and prevention of subsequent viral infection.

Issues and Challenges in Wastewater Monitoring

- Inadequate data: Sporadic studies on wastewater surveillance for detection of SARS-CoV 2 are being conducted in specific locations across the world. The existing data are inadequate for developing epidemiological models that can map the concentrations of SARS-CoV 2 in the sewer water linked to the probable number of local positive cases in an area.
- Fear of stigmatization: Despite the public health benefits, local authorities and municipalities may be reluctant and may object to the idea of monitoring the sewage for the presence of SARS-CoV-2 in their respective area, for fear of stigmatization of their locality being marked as a 'virus hotspot'.
- Privacy and ethical considerations: Wastewater surveillance may be linked to privacy considerations and ethical concerns of the concerned area, city, state, or country. Whether it is a city, a catchment area, a factory premise, a hospital, a senior care center, a residential community, an individual house, or a neighborhood — as the data collected from wastewater sampling becomes more individualized, there might be objections from people or institutions, and such privacy considerations must be dealt with at the policy level.
- Lack of uniform coverage: The current wastewater surveillance studies do not cover all geographically and demographically diverse populations, and mostly does not always include regions that are disconnected to the municipal sewerage system.
- Necessity to cover areas with limited access to clinical surveillance: Wastewater surveillance studies are not always conducted in areas with limited clinical surveillance access or facilities, which is critical for the detection of SARS-CoV-2 in such locations.

Discussion

WBE could trace the actual infected persons when the detection of SARS-CoV-2 in asymptomatic infections are not effective during clinical surveillance. Evidently, SARS-CoV-2 can be shed in the feces of individuals with symptomatic or asymptomatic infection; therefore, wastewater surveillance can **capture data on spread of infection in a given catchment**. SARS-CoV-2 is reported to be discharged in the feces of people five weeks after their respiratory samples had tested negative for the virus. Studies also record persistent shedding of virus genetic material RNA in human excretions like stool, saliva, sputum, and urine, which find their way into the sewers (WHO Scientific Brief, 2020).

The wastewater surveillance study in Chennai has gathered evidence from analysis of samples collected from 12 STPs, 5 SPSs, a Hospital Wastewater Plant, rivers, surface water and groundwater, for detection of SARS-CoV-2 viral particles during partial lockdown and post-lockdown periods in the city. Clinical results of community wastewater were collected from the COVID-19 treatment/ care centre to perform simulation studies. With the estimated RNA copies/L of wastewater samples, the number of infected individuals that can be expected from a population of a given catchment area was predicted. Interestingly, negative RT-PCR results for the outlet samples and more than 90% removal of caffeine showed the functional efficacy of the STPs in Chennai city.

Since sediment (SED) and supernatant (SUP) were the most effective wastewater concentration methods for SARS-CoV-2 detection in wastewater samples, the values obtained from these methods were further used to predict the number of infected people in each of the catchment areas. It was noted that during post lockdown, the estimated persons infected (3757) was in line with the number of actual number of active COVID-19 cases (3389). Furthermore, the predicted experimental data of Chennai city was in line with the simulated results of the study. It was found that **Kodungaiyur (KD)** and **Koyambedu (KYB) regions had the maximum number of infected individuals**. It is noteworthy that KYB-STP caters to half the population (14

lakhs) and receives half the amount of wastewater (90 MLD) in comparison with KD catchment (30 lakhs, 202 MLD). Yet the numbers of predicted infected cases (6,386) in both the catchments are similar. The high population density in KYB can be an important factor for the spread of infection. Maximum number of RNA copies/L (1,63,341 copies/L) and very high level of caffeine have been observed in this catchment. Also, the percentage of predicted infected individuals in the **Perungudi** (**PG**) catchment (a high-income locality with better sanitation and hygiene conditions) was four folds lesser than the KYB catchment.

Evidently, the incidence of COVID-19 cases in Tamil Nadu with a high number of cases from Chennai was attributed to the delay in closing the **Koyambedu market**, one of the largest perishable goods market complexes in Asia. Since people from diverse socio-economic backgrounds, belonging to varying levels of access to hygiene and population sizes in different communities were universally infected with SARS CoV-2, it can be conclusively stated that this marketplace was a **possible trigger point for the community spread** of COVID-19 in the city.

Therefore, the study uncovers practical insights to the local government and public health experts in understanding the occurrence of SARS-CoV-2 through viral loading as an important tool for public health decision making including containment and relaxation measures in the catchment community. The expected number of cases of infected persons could provide the details of the severity of the infection in each catchment area, and identify the zone that needs immediate attention to combat the spread of this pandemic. It also supports the need for monitoring of community wastewater as a **cost-effective** mode of obtaining the early signaling for the spread of COVID-19 in other urban and rural areas of India. Such studies have the potential to map the SARS-CoV-2 infection in the region thereby aiding major policy decisions like intensifying testing and imposing/lifting restrictions related to social distancing or quarantine orders.

Recommendations and Way Forward

Wastewater surveillance is a comprehensive assessment of community health. It provides a range of information on the health of the community, existence/prediction of diseases, monitoring disease outbreaks and spread among the population, track new emerging viruses, provide health and wellness of a community, drug surveillance, track lifestyle and drug abuse, and provide information on environmental exposure of community (Chakraborty et. al, 2020).

The key biological markers from bacteria, viruses, and fungi have the potential to indicate diseases within a population. Large number of wastewater treatment facilities globally can be used to effectively monitor the health profile of billions of people worldwide, as it can track information of both asymptomatic virus through viral RNA shedding, and even where no clinical cases have been reported. It thus fills in important gaps in data required for containment of the region and control of the epidemic/pandemic.

WBE provides an overall status of the disease in a particular watershed, especially considering the nonviability of 100 percent clinical testing in most regions. It is also a rapid and reliable technique for gathering information at the community level. It has also proven to provide unbiased data and beneficial in regions where testing is delayed.

The **recommendations** for overall management of wastewater surveillance system and better use (application) of the data collected from monitoring are given below:

- In terms of **data management**, there needs to be a concrete plan for dealing with limitations related to data generation, analysis, access, sharing, and use of information. Centralized data access systems should be developed for wider dissemination, application, and further enrichment of key research results. As discussed earlier, many such online platforms are already being developed that needs to be strengthened further.
- There is a need to identify the key issues and challenges related to the development of validated methodological protocols for the quantitative analysis of SARS-CoV-2 in

wastewater. This will enable the methodological optimization of SARS-CoV-2 wastewater analyses, transforming the wastewater infrastructure into a source of vital information for making important health policy decisions and necessary interventions.

- The wastewater samples and data collected should be **representative** (Kitajima et al, 2020) for all age groups, gender, ethnicity, and for all types of people, to understand how the disease is unfolding or prevalent in different individuals. In this regard, specific studies may be taken up to develop a technique that may result in impositions on individual behavior.
- The legal use of wastewater data should be well defined. A legally binding policy might enable the use of wastewater data for deciding on screening programs in areas with SARS-CoV-2 have been detected. In such places, people might be requested to voluntarily participate in clinical screening programs. However, in cases where people refuse testing, the person(s) might be subjected to a quarantine (for a specific period) and be ordered to be tested prior to being able to leave their home.
- The wastewater surveillance study should be monitored by a Coordination Committee consisting of representatives from research institutions, public utilities, public health authorities, etc. to facilitate the quick translation of research results into policy decisions like clinical testing and follow-up orders like area lockdown and quarantine orders for affected population groups.
- Countries must introduce national policies on wastewater surveillance for detection of SARS-CoV-2, to facilitate efforts by state or local authorities to screen wastewater for the virus. This recognition will permit state and local governments to have broader authority for immediate action and regulation.
- Existing national wastewater testing programs can be used as a **cost-effective way** to track the spread of COVID-19 across the population and provide early warnings of resurgence. For

example, in India, the existing polio environmental surveillance network can be used to monitor SARS-CoV-2 at the wastewater treatment plants across the country.

- For better understanding of the best uses or benefits of wastewater surveillance, it is important to conduct intense **research studies** on biological, epidemiologic, technical, economic, and other aspects of SARS-CoV-2 wastewater monitoring that will enable providing recommendations on necessary future actions.
- Wastewater surveillance studies have not detected any infectious SARS-CoV-2 virus in untreated or treated sewage. Therefore, SARS-CoV-2 sewage sampling is not expected to endanger any infection risk to workers. However, it is important to establish and follow **biosafety standards** (like Bio-Safety Level 2 that covers laboratories working with agents associated with human diseases) while processing wastewater samples for detection of SARS-CoV-2 in laboratories.
- In many countries (including India), the COVID-19 situation seems to be improving, yet there is unpredictability especially considering second and third waves in a few countries and the occurrence of **new strains of the virus**. Monitoring and surveillance on a regular basis could alert in such situations.
- Once a reliable SARS-CoV-2 wastewater monitoring system is established, wastewater data might be used to indicate and show a roadmap for tracking disease, intensifying testing, re-introducing public orders related to social distancing or quarantines, and even lifting restrictions once a cessation of infection is confirmed.
- According to the Sustainable Development Goals (SDG) Report 2020, the world had been making progress to meet the global goals. However, since early 2020, within a short period of time, the COVID-19 pandemic has created a huge crisis, causing disruption to SDG progress reversing years of progress on poverty, healthcare, and other key sectors. If WBE-induced policy decisions (related to clinical testing and preventive measures) are adopted in a timely manner, the COVID-19

pandemic might be controlled globally that could enable the SDG progress pathway.

About 70% of wastewater in India is discharged untreated into waterbodies or in the open (CPCB, 2015). Vast majority of rural areas and several urban areas do not have access to centralized sewerage systems. Such areas that are not covered by the municipal sewerage system must also be **included in the wastewater surveillance** for detection of SARS-CoV-2. For this purpose, the wastewater samples can be obtained from sewer system manholes to find the sources of the virus, and a robust monitoring system must be devised for the same.

In the past, wastewater surveillance has been successfully used as a method for early detection of other viral diseases such as poliovirus and other enteroviruses. In India, surveillance methods including screening of wastewater was successfully used to demonstrate wild poliovirus transmission and to understand the effectiveness of polio immunization campaigns in the country. India continues wastewater surveillance at 52 wastewater treatment plants (Next Trends Asia, 2020) and unregulated catchment areas where sewage is drained in nine states and one Union territory. Therefore, in line with the polio surveillance studies, the presence of SARS-CoV-2 can also be monitored using the alreadyexisting wastewater monitoring system of the country. Countries like the United States (CDC, 2021), Finland (Mehrotra et al, 2020), Germany (Cortizo et al, 2020), the Netherlands (Dutch Water Sector, 2020), and Pakistan (Sharif et al., 2020) are either considering or have already launched national wastewater surveillance programs to track any resurgence of COVID-19. This kind of real-time monitoring of sewage in India can help detect and contain COVID-19 infections early on. Regardless of the intensity, frequency, and efficacy of clinical testing in an area, sewage surveillance can be a leading indicator of changes in COVID-19 burden within a community leading to timely decisions to protect public health of the region.

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