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Review Article

The prevalence of SARS-CoV-2 in sewage waste water: A review

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ABSTRACT

Since one and half years, the pandemic of SARS-CoV-2 virus (disease caused is Covid-19) has ruined the entire humanity in unimaginable ways, whether it is economy or unemployment or children mental health disorder or large number of deaths. There is no country in the world which is not affected by this virus. In some countries, this pandemic is coming in the form of outbreak such as first wave of pandemic then after some gap period, second wave of pandemic. In country like India, the second wave of corona pandemic has crippled the economy, public health safety and at the same time put a big question on the health infrastructure of entire nation whether it is availability of oxygen cylinders, or testing facility, hospital bed or ventilators. The actual number of patients who can get affected had not been estimated correctly. This poses more problems due to asymptomatic nature of the expression of COVID-19 on individual basis. Tragically, for developing countries like India with high population density, the situation has been more complex. Additionally, more amount of waste from the Covid affected population goes to effluent water, waste water coming out of residential area, hospitals, isolation centres and so on. In this review article, we have focused on presence of corona virus and infection transmission through effluent water in country like India with huge number of population and also provide further scope in research to inform future studies.

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1. Introduction

India is second largest country in terms of population after China where; so pandemic can gain much transmission rate due to more human-human interactions, less social distance behavior and large population density. The only way to counter the spread of Corona virus and reduce the number of infections is lock down and complete curfew. However, restricting people to stay at home or to remain isolated at home, and go out only if it is essential by following Covid appropriate behavior (CAB) protocol like bearing protective mask and use sanitizer and maintaining physical distance, the penetration of virus among population made it minimal. However, with millions of people infected in the cities, the

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waste water releasing from the homes, isolation centers, hospitals, clinics may carry the presence of Corona virus. The viral load in waste water will depend on how much waste is generated from Covid infected families, number of Covid infected patients in the hospitals and testing centers. The virus from mask, sputum droplets, human faeces, saliva, clothes and utensils of infected patients contaminate the drain water which get accumulate into bigger drains and later to sewage waste water. ^{1,2} The asymptomatic nature of corona virus among population leads to uncertainty in the estimation of number of actual patient who are infected. Sewage waste might show the presence of viral RNA of both symptomatic and asymptomatic patient. ^{1,2}

In this review, we have focused on the fact that waste water epidemiological surveillance study can be one of the potential approaches to find out the actual number of patient,

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the extent to which it has had affected the community, disease incidence and transmission rate of the SARS-CoV-2 virus.

2. Sewage Treatment and Waste Water Surveillance Studies

There are literature studies and reports which showed that the SARS-CoV-2 virus has been identified and isolated from municipal waste-water. The presence of SARS-CoV-2 in waste water is possible due to the excretion of the virus in human faeces or through droplets from sputum of infected human being. Moreover, SARS-CoV-2 virus has been isolated from contaminated water and rivers; however, there is insufficient evidence for virus transmission by water and wastewater.² Hatamet al., 2021 also suggested that depending on the nature of waste water and source, Corona virus can be active in such medium for several days. ² Hence, there is an increased risk of its transmission through waste water or contaminated water but more information is needed to validate the data. However, the primary mode of infection is through close contact with the infected person through respiratory droplets when we speak, sing, shout, sneeze or cough.

Waste water sewage monitoring and waste water-based epidemiology surveillance studies have earlier helped in mitigation efforts, such as the Global Polio Eradication Initiative.³ Environmental surveillance has also been used and recommended for other infections, such as typhoid (WHO).⁴

According to 10th May, 2021, Indian Express news report, there were nearly 100 Covid infected dead bodies has been found floating in the river Yamuna and Ganges Ghats at U.P. and Bihar respectively.⁵ Although, experts told that Covid virus does not spread through water. However, disposal of dead bodies of suspected Covid victims in the river Ganga is huge public health concern since the river is already polluted which already has bad impact on the river ecology. It will results in adverse health impact to the people who are directly depending upon river as direct source of livelihood and also impact the aquatic life and river ecosystem.

Jayaweera et al., 2020 studies prove that Covid-19 virus only spread through droplets, aerosols, close human contact or surface contact with infected individual. It does not remain in blood of the infected patient and so, it may not infect animals and birds feeding on dead bodies, but when there was a peak of Corona cases, it created panic condition entire nation.

Sewage treatment methods are able to reduce the pollution load caused by this virus in water sources. Water disinfection methods such as chlorination and UV irradiation have an effective role in removing it from water and waste water sources. ⁷ SARS-CoV-2 pandemic is relatively novel type of Corona virus that requires exclusive

epidemiological and other environmental studies such as monitoring of the transmission of this virus through waste water and sewage samples.⁷

Mitali and Mahak, 2021 reported that 70% of urban India sewage is untreated. Although 18% of the world population living in India, but, fresh water resources comprises of only 4% in India which is currently not able to fulfill the water demand of this large population.⁸ Due to increase in population, ground water resources has been depleting at a faster rate. In addition, industrialization and urbanization, land use, creation of dams, building, industries, the numbers of companies has been increased drastically which further reduces fresh water resources. With population explosion, and post pandemic treatment, there is a huge gap between sewage generation and sewage treatment (Table 1). India generates approximately 62,000 million liters per day (MLD) of domestic sewage in urban centers according to Central Pollution Control Board (CPCB), Ministry of Environment, Forest and Climate Change, Govt. of India.⁸ There were 920 sewage treatment plants (STPs) mainly located at urban areas operated mainly by municipal corporations, with a treatment capacity close to 23,000 MLD, merely 37 percent of generation. Only 33 percent of India's urban wastewater is actually treated, and an even smaller portion is reused.⁸ This is the appromixate data of urban areas and there is no data of suburban areas or village wastewater generated. Moreover, these gaps must be reduced to ensure 100% treatment of domestic, commercial and industrial waste water. New sewage treatment plant (STPs) of sufficient capacity must be built with proper maintenance. 9 Table 1 reflects that there is a huge gap between the sewage generated and the actual sewage treated. The probability of the virus remain active in untreated sewage waste remains high since most of the sewage remains untreated which can contaminate the river sources if it mixes with it. Hence, there is a probability that river can be a source of contamination. However, there is no definite evidence about it till now, but the future transmission by this route cannot be overlooked. Therefore, better waste management systems must be prioritized in order to control solid waste pollution and water pollution, which in turn can also add to the better management of air-borne, water borne and animal borne viruses.

Manish Kumar et al., 2021 has suggested that the SARS-CoV-2 virus does not remain alive after disinfection in waste water treatment processes such as chlorination, ozonolysis but data are not sufficient to prove this as these virus has strong envelop of spike proteins unlike polio virus. ¹⁰ However, if the percentage of untreated water is more, then transmission rate of the virus through water must be taken into account as far as villages, districts, small towns, and cities are concerned. Also, the author proposed quantitative microbial risk assessment (QMRA) frameworks that predict the estimated risk of SARS-CoV-2 in natural

Table 1: State wise sewage generation and actual quantity treated

S.No.	State	Total sewage generation (MLD)	Actual quantity treated (MLD)	% of Total sewage generated
1	Andaman & Nicobar Islands	23	0	0
2	Andhra Pradesh	2882	309	11
3	Arunachal Pradesh	62	0	0
4	Assam	809	0	0
5	Bihar	2276	0	0
6	Chandigarh	235	188	125
7	Chhatisgarh	1203	6	0
8	Daman Diu	67	7	10
9	Goa	176	25	14
10	Gujarat	5013	2687	54
11	Haryana	1816	1284	71
12	Himachal Pradesh	116	51	44
13	J&K	665	49	7
14	Jharkhand	1510	15	1
15	Karnataka	4458	1786	40
16	Kerala	4256	47	1
17	Lakshadweep	13	0	0
18	Madhya Pradesh	3646	536	15
19	Maharashtra	9107	4242	47
20	Manipur	168	0	0
21	Meghalaya	112	0	0
22	Mizoram	103	0	0
23	Nagaland	135	0	0
24	NCT Delhi	3330	2412	72
25	Odisha	1282	50	4
26	Ponducherry	161	30	19
27	Punjab	1889	1360	72
28	Rajasthan	3185	478	15
29	Sikkim	52	14	27
30	Tamilnadu	6421	995	15
31	Telangana	2660	706	27
32	Tripura	237	1.5	1
33	Uttarpradesh	8263	2510	30
34	Uttarakhand	627	187	30
35	West Bengal	5457	213	4
	Total	72368	20236	28

(Retrieved from Sulabhenvis database, http://www.sulabhenvis.nic.in/Database/STST_wastewater_2090.aspx where; MLD: Million liters per day)

water bodies through different activities of water such as recreation activities like swimming. ¹⁰ They have also shown a statistical analysis based on poisson distribution and dose response model of the virus to monitor possible viral ingestion and chances of getting infection through these water activities. This finding of Manish Kumar et al., 2021 can prove that waste water surveillance studies would be a great tool for the virus monitoring and early warning of pandemic outbreak. Further, detailed comparative research is still needed to explore its future implication and to avoid any future tragedy due to uncontrolled spread of pandemic.

Further, Hindustan times dated 18th June, 2021 reveal the finding of study carried out by Manish Kumar et al., 2021 found out the traces of Covid-19 virus in water samples of Sabarmati river of Gujarat along with two lakes

name Chandola and Kankaria of the city. ¹¹ However, the status of whether those samples contain dead or alive virus is not known.

3. Waste Water Epidemiological Studies as Possible Perspective to Combat Covid-19 Challenges

Wastewater epidemiology study of Covid-19 could have many advantages. It can be a cost-effectual way to investigate the transmission variations of Covid-19 in entire communities. It can be a one of the potential epidemiological indicators from the people who live in sub-urban, peri-urban areas where they lack access to proper healthcare facilities. Also, it might be beneficial in disclosing infection dynamics in advance than diagnostic

testing. Moreover, public health care officials can get near-real-time statistical data on widespread occurrence of the diseases. ¹²

Wide application of waste water monitoring began in the 1990s with efforts to eradicate polio virus. ¹³ However, polio has been eradicated with the help of large scale vaccination. The traditional approach to check polio tracking cases proved incapable of preventing outbreaks. Because polio virus infections often present with non-specific symptoms, severe symptoms of paralysis occurs only in one of 200 cases, the virus was able to spread in areas where it goes unnoticed and was thought to be eliminated. Monitoring sewage waste for polio virus genes is four to five times more effective in detecting outbreaks than monitoring communities for increase in cases of acute paralysis and has allowed entire group to be continuously monitored. When poliovirus was tested in waste water, scale up vaccination campaigns prevented new cases of paralysis. ¹⁴

In contrast to polio, which transmitted mainly by the faecal, oral route, Covid-19 spread by respiratory droplets. 15 Yet, researchers have informed earlier that a SARS-CoV-2 gene is frequently detected in patient stool samples coming in sewage and hospital wastewater. This was not entirely surprising since, earlier epidemic viruses also found in municipal sewage samples. Several studies which were carried out depicted that the faecal and oral route is not likely to be a major cause in the pandemic. 14-17 Concentrations of viral RNA in faeces may change from patient to patient and depending upon the duration and severity of this disease. The faecal-oral transmissions need thoughtful consideration in monitoring Corona infection throughout the population. ¹⁴ In the process of faeces excretion, the viruses are migrated first in sanitary toilet water followed by municipal waste water constituents, which include waste water from showers and washing machines and, in some cases, industrial sewages, rain water and storm waters. When the viruses and their RNA are subjected to complex sewage systems where it might be exposed to different chemicals, pressure, pH, dissolved solids, temperatures and other environmental factors. However, it depends on the variability and stability of Corona viral gene, whether they are stable over different physical conditions and how the viral genome responds to different chemicals which include detergents and soap solutions. 16,18

For the first time, Medema et al., 2020 conducted the study in March 2020, at the Netherland to identify SARS-CoV-2 presence in sewage waste water system. ¹⁹ It was the first reported study that was carried out as far as SARS-CoV-2 is concerned in sewage samples that is transmitted to the drainage facilities of waste water. Although, sufficient information is not there to show the extent to which this virus live in fresh ground water or wastewater. However, extensive studies are required to monitor the active or

inactive state of this virus in waste water samples as if it is found in contagious state, then it can turn out to be a more drastic community spreader. More research and statistical analysis of waste water surveillance study can indicate that there might be a relationship between detection of viral gene in waste water and diseases outbreak in the community. Also, in the study of Medema et al., 2020, Reverse transcription polymerase chain reaction (RT-PCR) test has been carried out with sewage samples of seven cities and the airports against three fragments of the nucleocapsid protein gene (N1-3) and one fragment of the envelope protein gene (E). ¹⁹ First case has been reported on February 27, 2020 but, no SARS-CoV-2 was detected in samples of February 6, which is three weeks until the first case was reported. On March 5, the N1 fragment was detected in sewage of five sites. On March 15 and 16, the N1 fragment was detected in sewage of six places, and the N3 and E fragment were detected at 5 and 4 places correspondingly. The identification of the virus in sewage, even when the Covid-19 widespread presence was low, suggested that sewage epidemiological surveillance could be an important tool to monitor the movement of the virus in the population.

There is a likelihood that SARS-CoV-2 viruses inside infected body are inactivated in the gastrointestinal-tract fluid and might be excreted primarily as in a non-infective way. In order to support the statement, Darnell et al., 2004 found that under highly alkaline pH 12 & 14 and extremely acidic pH 1 & 3 cause inactivation of SARS-CoV-2 virus, whereas virus may remain stable around neutral pH. 20 Therefore, in the stomach area where highly acidic environment prevails in the presence of gastric acid, SARS-CoV-2 shall not be capable of infecting other cells. However, if any person who is under proton-pump inhibitor (PPI) medication for any diseases such as gastric ulcer, gastroesophageal reflux diseases, the acid secretion in the person's stomach get reduces that can make gastrointestinal tract area susceptible to Corona virus infection. 21 Almario et al., 2020 conducted a cohort study in which they found that individuals taking PPIs were significantly showing positive Covid-19 test than those not taking PPIs. ²²

In contrast to India, where sewage generation load is extremely high (Table 1); waste water epidemiological study in the sewage sample (released from each state) can exhibit an important role to control the pandemic outburst in nation as a whole. The correlation between concentration of viral RNA in municipal waste water and disease prevalence in community might be helpful in determining the early warning of transmission of disease and so this waste water surveillance data can be fruitful weapon to control the pandemic in those areas where sewage load is relatively higher. ^{18,23} This is another advantage of virus detection study on effluent water samples to combat its penetration to other localised area. Another aspect to India is that, around 450 million people do not have access to toilets

(UNICEF-India, Water, sanitation and hygiene data 2019) and practiced open defecation. ²⁴ As a result of that, consequently transfer the human faecal material goes to waste water and provide a great risk of contamination of surface water and ground water, which reveals definitive risk of virus exposure to the human beings.

Prosun Bhattacharya et al., 2021 also focused on persistent shedding of viral RNA of SARS-CoV-2 in the human faeces that have created a possibility to track the prevalence of Covid-19 in communities through wastewater surveillance epidemiology. 1 Presence of viral gene in human faeces of infected individual, both symptomatic and asymptomatic can open the way forward to track the disease penetration in the community where there is a lack of diagnostic facilities for the detection of disease. Presently, there are some challenges for using sewage sample as a tool to detect the viral gene but more extensive research will help to synchronize the process of isolation, purification, identification, and diagnostic. Standard Operating Procedure (SOP) for sample collection, location details, and sewage data should be developed for more consistency in result analysis. 15 Different Government Institutes of India such as Central Pollution Control Board (CPCB), Jal Board, Waste management officials, National Centre for Disease Control need to work in collaboration to bring waste water surveillance study to benefit the entire community.

In general, the main indicators for Covid-19 prevalence are the number of positive cases and number of deaths in a particular area. However, with advancement of technology and detailed epidemiological study of Corona virus data, many other key indicators are emerged like number of testing, tracking, positivity rate and treating the cases, number of beds occupied and number of deaths etc, to control the surge of pandemic in different states. These indicators proved useful in combat the Covid-19. However, these information are approximate and absolute which broadly depends on people willingness to give sample for testing, easy access to diagnostic, false testing reports, time lag between actual death happening and reported death and fear associated with Covid-19 influenced social stigma. Since the diverse population of India have diverse mind-set to represent or hide the case but, nevertheless, waste water surveillance epidemiology studies may become an important tools to address the liveability of virus in a particular geographical location; free from all above mentioned bias. 7,18,23,25,26

As vaccination is the only way to control this pandemic and bringing back the economy and livelihood to normalcy, waste water epidemiological surveillance study coming from the different places like hospitals, nursing homes, diagnostic clinics, pathological centres, residential areas, prison and other areas can also be of added advantage in overall controlling of the pandemic and new surge in positive cases. ^{13,14,16} Also, necessary research studies need to be carried out to understand the kinetics of virus presence, its concentration, rate of decay and the factors which influence its transmission and mitigation through waste water. ^{25,26} Comparative analysis among treated, untreated and fresh water sources is also important to study the persistence of SARS-CoV-2 in waste water. On a different scale, it will help country to come back to normal in terms of socio economic aspects too.

4. Conclusions

New viruses, like SARS-CoV-2, have been detected in human populations and in the other host that might give a possibility to a emergence of new strain or virus. Some viral strains might have the potential to recombine with human and other animal viruses and undergo the process of mutation to produce new viral strains and has a possibility to find the new host through different route including waste water or sewage waste.

Not only Corona virus but different kinds of viruses, representing many taxonomic groups, are likely to be present in municipal sewage wastes involving human and animal faecal wastes, combined faecal and urine wastes, respiratory secretions entering sewage and other domestic waste streams. The persistence of enteric and respiratory emerging viruses in various contaminated faecal and sewage samples has been studied, but in respective of that, inevitable quantitative information is not sufficient for these emerging viruses, especially under certain environmental conditions such as large population density, pollution and other factors that represent potential reservoirs and super spreader of transmission. Therefore, there is substantial unpredictability about the extent at which some human animal viruses survive in the waste water, their kinetics of inactivation and the rate at which they released into the environment where it may be transported for further human or animal exposure, contact and transmission.

5. Future Implications

Further studies are recommended to better characterize virus survival times and its inactivation rates, particularly for the newly emerging viruses of like SARS-CoV-2 which has crippled the human habitat and economy of the world. In addition, it is necessary to identify environmental condition, parameters of waste water, different soil-water interfaces, air-water, surface water interfaces that can facilitate virus persistence and its transmission over the period of time. The factors governing the transmission rate, prevalence in different species, virus gene survival rate in waste water, the medium at which virus present, ambient conditions and influential parameters to understand its virology and virulence properties.

Furthermore, reliable methods to quantify viral survival rate in waste water when subjected to chemical treatment, various other waste treatment processes in sewage treatment plants and its co-existence with another waste is also be recommended to understand the extent to which they survive, activated and inactivated so that we can completely win over this tragic pandemic throughout the world.

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None.

7. Conflict of Interest

The authors declare no conflict of interest.

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