

Sewage surveillance for the presence of SARS-CoV-2 genome as a useful wastewater based epidemiology (WBE) tracking tool in India

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ABSTRACT

The infection with SARS-CoV-2 is reported to be accompanied by the shedding of the virus in fecal samples of infected patients. Earlier reports have suggested that COVID-19 agents can be present in the sewage samples and thus it can be a good indication of the pandemic extent in a community. However, no such studies have been reported in the Indian context. Hence, it becomes absolutely necessary to detect the presence of the SARS-CoV-2 in the wastewater samples from wastewater treatment plants (WWTPs) serving different localities of Jaipur city. Samples from different WWTPs and hospital wastewater samples were collected and wastewater based epidemiology (WBE) studies were carried out using the RT-PCR to confirm the presence of different COVID-19 target genes namely S gene, E gene, ORF1ab gene, RdRp gene and N gene. The results revealed that the untreated wastewater samples showed the presence of SARS-CoV-2 viral genome, which was correlated with the increased number of COVID-19 positive patients from the concerned areas, as reported in the publically available health data. This is the first study that investigated the presence of SARS-CoV-2 viral genome in wastewater, at higher ambient temperature (45 °C), further validating WBE as potential tool in predicting and mitigating outbreaks.

Key words | COVID-19, public health data, RT-PCR based detection, SARS-CoV-2, sewage surveillance, wastewater epidemiology

HIGHLIGHTS

- The study reports detection of SARS-CoV-2 in sewage in India.
- The presence of SARS-CoV-2 was confirmed by RT-PCR.
- The presence of viral genome was detected at high ambient temperatures of 45 degrees.
- Corroborates trends in the WWTPs showing viral genome with public health data.
- Treated effluent from WWTPs appears safe for reuse with low public health concern.

INTRODUCTION

Coronavirus can be considered as a paradigm of the phenomenon involving complex dynamics of animals, humans and environments and in the last 18 years, it has caused three new alarming diseases: severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and the current coronavirus disease 2019

(COVID-19) (WHO 2020a). The outbreak of zoonotic coronavirus in humans was first reported in Wuhan, China in 2019 as a pneumonia-like ailment caused by a hitherto uncharacterized etiologic agent (Casanova *et al.* 2020). The coronavirus study group of the international committee of taxonomy of viruses later classified this zoonotic virus as

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SARS-CoV-2 based on the features it exhibited (WHO 2020b). The researchers classified this zoonotic agent into the coronaviridae family on the basis of its genetic structure and crown-like (or halo) structures present on its envelope glycoprotein and characteristic features of chemistry and replication (Tyrrell & Myint 1996). The affliction of this single-stranded positive-sense RNA virus shows varying symptoms in individuals depending upon their immune system. According to the data compiled by World Health Organization (WHO), some of the commonly reported symptoms include fever, dry cough, and tiredness and, in some cases, may also manifest in aches and pains, nasal congestion, sore throat or diarrhea, conjunctivitis, headache, loss of taste or smell, a rash on the skin, or discoloration of fingers or toes. The serious symptoms reported include difficulty in breathing or shortness of breath, chest pain or pressure, loss of speech or movement (WHO 2020a).

The novel strain of coronavirus has evolved after mutations in the previously existing strains. It has been reported that animals played a key role in spreading coronaviruses in humans until recently. But, the mutations, which led to the evolution of SARS-CoV-2, have equipped it with the ability to transmit directly from one human to another (Chan *et al.* 2020). COVID-19 is reported to transmit even through the droplets from an infected person's sneeze or even breath (Chan *et al.* 2020; WHO 2020b). The viable SARS-CoV-2 viral RNA are shed in bodily excreta, including saliva, sputum and feces, which are subsequently found in wastewater (Wu *et al.* 2020a). It is believed that the major transmission route of this virus is inhalation via person-to-person and aerosol/droplet transmission or through fomite, and close contact. At present, COVID-19 is responsible for a rapidly expanding global epidemic with tens of thousands of cases and thousands of deaths (Heymann & Shindo 2020). It is therefore possible that the virus may be released with wastewater and contaminates other water bodies (surface, sea, and groundwater), generating aerosols. Sewage from hospitals, especially infectious disease units, may contain the epidemic virus, thus requiring efficient disinfection before discharge into natural waters. Currently, available evidence indicates the need for a better understanding of the role of wastewater as potential sources of epidemiological data and as a factor in public health risk.

Environmental surveillance is a tool used to monitor the extent and duration of the spread of the virus in specific populations. It gives a measure of contaminants and also provides warning of possible threats emerging in that particular confinement. These monitoring tools have already been successfully implemented for viruses such as poliovirus

and the Aichi virus, in the past. The study of wastewater based epidemiology has been proven successful in other cases as well such as Influenza A (H1N1) epidemic 2009 (Heijnen & Medema 2011), Norovirus, Hepatitis A virus, Hepatitis E virus, Adenovirus, Astrovirus and Rotavirus (Hellmér *et al.* 2014) in determining the viral concentration in the sewage sample both before and after the onset of the symptoms, which have aided in strategies that resulted in their elimination. This technique is in line with the wastewater based epidemiology (WBE) concept. WBE is a reliable surveillance model for identifying global hotspots of COVID-19. Crucially, according to a recent study, SARS-CoV-2 RNA was detected in samples of sewage before any case was reported, suggesting that virus monitoring could be feasible before cases are documented through the health surveillance system (Orive *et al.* 2020).

Human CoVs, including SARS-CoV and MERS-CoV, are known to cause gastrointestinal symptoms as well as respiratory symptoms. In fact, previous studies demonstrated that these viruses replicate in the gastrointestinal tract. The recent reports revealed that 2–10% of COVID-19 patient's had gastrointestinal symptoms, including diarrhea (Chen *et al.* 2020; Wang *et al.* 2020a). Although the exact mechanism of COVID-19-induced gastrointestinal symptoms largely remains unknown, a recent study reported that SARS-CoV-2 infects gastrointestinal glandular epithelial cells (Wu *et al.* 2020b). With diarrhea being reported as a frequent symptom in the patient (Kitajima *et al.* 2020), the shedding of SARS-CoV-2 RNA through feces has been reported in many countries such as the Netherlands (Medema *et al.* 2020) and Wuhan, China (Chen *et al.* 2020; Hindson 2020). A study conducted on the clinical samples collected from different hospitals in China reveals shedding of viral RNA through feces in a significant number of cases, implying the fecal route of transmission (Wang *et al.* 2020a). Some clinical studies reported prolonged fecal shedding of SARS-CoV-2 RNA for up to seven weeks after the first symptom onset (Wu *et al.* 2020a). Another study reported that viral RNA could be detected in the feces of 81.8% cases even with a negative throat swab result (Ling *et al.* 2020). Recent reports implied that significant proportions (17.9–30.8%) of infected individuals are asymptomatic (Tang *et al.* 2020; Wu *et al.* 2020b). It will be easier to survey regions for viral infections, especially in the asymptomatic cases of COVID-19, comprehensively and in real-time, for the reason that the affected individuals start shedding the viral RNA genomes in their feces. This tool will serve as an efficient and sensitive monitoring tool to measure virus levels in the hotspot populations and provide early warning

signs before a potential epidemic in the future. The current approach analyzes wastewater samples to determine the presence of infected individuals and estimate the number of cases. As of 7th May 2020, India counted 52,952 cases out of which 35,902 were active and it showed a tremendous increase by 20.56% and 28.39% in total and active cases, within a month, respectively. As of June 7, 2020, this number was reported to be 257,486 total and 126,431 active cases, respectively (official data from [John Hopkins University 2020](#)). The data from the sewage supports communities with trend analysis that can determine the pandemic hotspots of COVID-19. This can further help researchers to generate early warning and thus help officials to take rapid action regarding the containment or re-emergence of new outbreaks in a population.

In this context, the present study was planned to achieve the evidence for detection of SARS-CoV-2 RNA samples in municipal WWTPs for untreated and treated wastewater samples, and hospital sewage samples around Jaipur city, and determine the correlations between the positive results for sewage samples from WWTPs with the public health data, as officially reported in the daily newspaper, of positive patients around the area. To date, there have been no reports of detection of SARS-CoV-2 in wastewater in India. The present study provides the probable first reported evidence of the presence of SARS-CoV-2 RNA in sewage samples of Jaipur, Rajasthan (India) and these findings demonstrate the applicability of WBE or sewage surveillance as an early indicator of persistence of the virus in the community and the risk associated with wastewater handling. The work also draws attention to the current wastewater treatment system being used in WWTPs, and the efficacy of sodium hypochlorite or other chlorine compounds being used by hospital authorities as a potent disinfecting agent, to inactivate or attenuate viruses.

EXPERIMENTAL METHODOLOGY

Wastewater sampling

Grab wastewater samples were collected from different units of six municipal wastewater treatment plants (WWTPs) installed at different locations of Jaipur city and wastewater samples from two hospitals, which are the major treatment centers for COVID-19 patients. The locations of eight sites (six municipal WWTPs and two hospitals) are shown in [Figure 1](#). The samples were taken between 3rd May 2020 and 14th June 2020. All the samples



Figure 1 | Map showing locations of sample collection from Jaipur (India) as shown by red pins. Sites 1–6 are Municipal WWTPs, and Sites 7 and 8 are hospitals (SMS Hospital & Mahatma Gandhi Hospital WWTP, Jaipur).

were collected in sterile bottles and transported to the Environmental Biotechnology laboratory at Dr B. Lal Institute of Biotechnology, Jaipur for further investigations. Sample collection was carried out by taking appropriate precautions with the use of standard personal protective equipment (PPE). [Table 1](#) highlights the comprehensive list of all the sample locations along with details on the current secondary & tertiary treatment technologies, average flow rates, and influent and effluent characteristics of WWTPs.

Sample pre-processing

Samples were pre-processed using two different methods based on previously published protocols ([La Rosa et al. 2020](#)). The two methods were tested and standardized for sample pre-processing in the laboratory. In the first method, (Method A), sample processing was carried out in two different steps involving the inactivation of virus particles (which might be present in the wastewater sample), followed by the concentration of the virus by adsorption. Method A began with inactivation of the virus by transferring the samples to 50 mL Tarsons falcon (code 546041) in a biosafety cabinet (BSL2), followed by 70% ethanol spray over the surface of the falcon tubes and UV light exposure for 30 min for surface sterilization. After UV exposure, the samples were transferred to the water bath at 60 °C and kept incubated for 90 min for heat inactivation of the virus. After the inactivation of the coronavirus, the samples were brought to room temperature and filtered through a 0.45µm membrane using a vacuum filter assembly. The

Table 1 | Sampling sites, location, treatment technologies, along with flow rate and characteristics

Site No.	Sampling locations with coordinates	Type of treatment technology (secondary + tertiary treatment)	Flow rate (Avg) (MLD)	BOD ^a mg/L		COD ^a mg/L		NH ₃ -N Mg/L		References
				Influent	Effluent	Influent	Effluent	Influent	Effluent	
Site 1	MNIT J (26.8640°N, 75.8108°E)	MBBR + Cl ₂	1.0	160–200	19.0	512	62.0	NA	NA	Data obtained from STP operator
Site 2	Jawahar Circle, Jaipur (26.8414°N, 75.8°E)	MBBR + UV	1.0	200–250	19.5 ± 4.38	400–450	60 ± 7.53	NA	8.0	Shekhawat <i>et al.</i> (2020)
Site 3	Dravyavati River, Jaipur (26.7980°N, 75.8039°E)	SBR + Cl ₂	65.0	200–250	9–9.5	600–700	40–50	15.0	0.9	Data obtained from STP operator
Site 4	Central Park, Jaipur (26.9048°N, 75.8073°E)	SBR + Cl ₂	1.0	200–250	4.75	400–450	24.32	NA	11	
Site 5	Ramniwas Garden, Jaipur (26.8963°N, 75.8100°E)	MBBR + UV	1.0	363	43	1,055	98.4	NA	NA	Baid (2016)
Site 6	Brahmpuri WWTP, Jaipur (26.9373°N, 75.8250°E)	SBR + No Tertiary	27.0	250–300	9.0	450–500	43	NA	NA	
Site 7	SMS Hospital, Jaipur (26.9054°N, 75.8155°E)	No STP is installed at the site	NA	NA	NA	NA	NA	NA	NA	NA
Site 8	Mahatma Gandhi Hospital, Jaipur (26.7699°N, 75.8546°E)	MBBR + UV	0.600	90.0	11.0	345.0	32.0	NA	Nil	Data obtained from the STP operator

MBBR, Moving Bed Biofilm Reactor; SBR, Sequential Batch Reactor; UV, Ultra Violet disinfection; Cl₂, Chlorine disinfection; BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand; NH₃-N, Ammoniacal nitrogen, JDA, Jaipur Development Authority, NA, Not applicable.

^aThe data is presented as obtained from the secondary sources and references are defined.

filtrate of each respective sample was transferred to a fresh 50 mL falcon containing 0.9 g sodium chloride (NaCl) and 4 g polyethylene glycol (PEG). The contents were dissolved by gentle manual mixing. The samples containing PEG and NaCl were then centrifuged at 4 °C for 30 min at 7,000 rpm. The pellet obtained was further re-suspended in 1X phosphate buffer saline (PBS). In the second method, which is a direct method (Method B), for detection of SARS-CoV-2 in the wastewater sample includes UV treatment of the sample for 30 min, followed by dispensing 1 mL sample in each 1.7 mL centrifuge tube. A spin on 7,000 rpm for 15 min was given to each tube containing the samples. The supernatant was collected in a separate tube and the same process was repeated. The supernatant thus obtained was then processed for RNA extraction.

RNA extraction

RNA extraction and subsequent steps of detection were done at Dr B. Lal Clinical Laboratory Pvt. Ltd, Jaipur (which is authorized by ICMR to conduct COVID-19 testing in humans). For the extraction of viral RNA, FDA and ICMR approved Allplex™ 2019-nCoV Assay kit (cat# RP10244Y RP10243X) was used. 10 µL of proteinase K and 200 µL of lysis buffer were added to 200 µL of the sample into a 1.5 mL centrifuge tube followed by vortex mixing and incubation at 56 °C for 15 min in a heating block. 250 µL of ethanol was added to the sample and mixed by pulse vortexing for 15 seconds. The mixture was then transferred to the spin column and centrifuged at 10,000 g followed by sequential washing with three wash buffers provided in the kit followed by centrifugation at 10,000 g for 1 minute at each washing step. After complete drying of the spin column, the RNA was eluted out using a 50–100 µL elution buffer followed by centrifugation at 12,000 g for 1 minute.

Real time (RT)-polymerase chain reaction (PCR) analysis

RT-PCR assays have been used for the detection of SARS-CoV-2 in wastewater samples (Ahmed et al. 2020; Corman et al. 2020). The assays were performed using FDA approved Allplex™ 2019-nCoV Assay kit (cat# RP10244Y, RP10243X) or TaqPath™ COVID-19 Combo Kit (Cat#A47814) for the qualitative detection of SARS-CoV-2 genomic RNA in the sample on BioRAD CFX96 IVA Real-Time PCR and Applied Biosystems™ Quant Studio™ 5, respectively. The master mix for Allplex™ 2019-nCoV Assay kit was prepared using the kit content which was composed of amplification and

detection reagent, enzyme mix for one-time RT-PCR, buffer containing dNTPs, buffer for one-step PCR and RNase free water. Each PCR tube contained 8 µL RNA sample, 5 µL 2019-nCoV MOM, 5 µL Real-time One-step buffer and 2 µL Real-time One-step enzyme and the final volume of the mixture was adjusted to 25 µL using RNase free water. A list of different genes and fluorophores used for detection is given in Table 2. Thermal cycling reactions were performed at 50 °C for 20 min, 95 °C for 15 min, 44 cycles at 94 °C for 15 seconds, and 45 cycles at 58 °C for 30 seconds, in a thermal cycler. For each run, a set of positive and negative controls were included. For the master mix for TaqPath™ COVID-19 Combo Kit, each PCR tube contained 5 µL RNA sample, 1.25 µL COVID-19 Real-Time PCR Assay Multiplex and 6.25 µL TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X) and the final volume of the mixture was adjusted to 25 µL using RNase free water. Thermal cycling reactions began with UNG incubation at 25 °C for 2 min, followed by reverse transcription at 53 °C for 10 min, activation at 95 °C for 2 min and 40 cycles of denaturation and annealing/extension at 95 °C for 3 seconds and 60 °C for 30 seconds, respectively.

RESULTS AND DISCUSSION

Detection of SARS-CoV-2 genome in wastewater samples

Wastewater epidemiology is a useful study tool in monitoring any infectious disease spread and its community level dynamics in principle (Ahmed et al. 2020). Six municipal WWTPs and two hospitals' WWTPs were selected from Jaipur city (details shown in Table 1). The data on the physico-chemical characteristics of the influent and effluent samples were collected from recent research reports

Table 2 | List of fluorophores used for analyte detection in RT-PCR

Allplex™ 2019-nCoV Assay Kit		TaqPath™ COVID-19 Combo Kit	
Fluorophore	Analyte	Fluorophore	Analyte
FAM	E gene	FAM	ORF1ab
HEX	Internal Control (IC)	VIC	N gene
Cal Red 610	RdRp gene	ABY	S gene
Quasar 670	N gene	JUN	MS2

Note: As instructed by the vendors of the two detection kits.

(Shekhawat *et al.* 2020) and Jaipur Development Authority (JDA) reports and are described in Table 1. The average values of BOD from these WWTPs lie in the range of 160–450 mg/L while that of effluent came out to be 9–30 mg/L, confirming it is well below the standards as prescribed by CPCB (Central Pollution Control Board 2000). The average value of chemical oxygen demand (COD) in the influent lies in the range of 400–1,055 mg/L while that of effluent came out to be 25–90 mg/L. This data shows that the treatment plants are working efficiently. The RT-PCR based detection was done in combinations of the following: one non-structural gene ORF1ab, any two of the structural genes like S (spike) or E (envelope) along with a third structural N (nucleocapsid) gene. COVID-19 RdRp (RNA dependent replicase) was also used as a target to detect the presence of the COVID-19 genome with these structural genes. All the reactions were done with proper internal and positive controls followed by data analysis and result interpretation which is shown in Figure 2. Two separate kits were used for detection of SARS-CoV-2 genome and the efficiency of both the kits was compared. A Ct value of <40 with Allplex kit and Ct values of <35 and <37 (for N

gene and ORF1ab, respectively) with the TaqPath kit was considered to be valid as per manufacturer's instructions and overall, if a sample showed two out of three genes with valid Ct values were considered to be overall positive for the presence of intact viral genome as per manufacturer's instructions. Since both the kits efficiently detected the presence of SARS-CoV-2 genome in the wastewater samples, Allplex kit was used for further analysis.

The wastewater of the entire Jaipur city as well as outskirts area, through a sewerage network, joins the main trunk sewer of 1,800 mm diameter with an average flow of 130 millions of litres per day (MLD) terminating at Delavas sewage treatment plant (STP) based on activated sludge process (centralized treatment facility at 125 MLD capacity). From this main trunk, settled sewage is withdrawn @1 MLD sites 2,4 and 5, connected to three decentralized treatment plants based on moving bed biofilm reactor (MBBR) technology to maintain public parks for more than 10 years. Besides this, site 1 has its own WWTP plant at MNIT Jaipur campus area based on MBBR technology of a capacity of 1 MLD. Site 3 at Dravyavati river WWTP has a capacity of 65 MLD. Site Brahmpuri WWTP (site 6)

Site No.	Sampling location	Date of sampling	Sample type	Target genes					Inference
				Rdrp Gene	Orf1ab Gene	E gene	S gene	N gene	
Site 1	MNIT	04.05.2020	Influent	-	NA	-	NA	-	Negative
Site 2	Jawahar Circle	04.05.2020	Influent	-	NA	-	NA	-	Negative
		12.06.2020		-	NA	-	NA	-	Negative
Site 3	Dravyawati River	04.05.2020	Influent	-	NA	-	NA	-	Negative
		04.05.2020	Effluent	-	NA	-	NA	-	Negative
Site 4	Central Park	04.05.2020	Influent	-	NA	-	NA	-	Negative
		25.05.2020		+	NA	-	NA	-	Negative
		04.05.2020	Effluent	-	NA	-	NA	-	Negative
Site 5	Ramniwas Garden	04.05.2020	Influent	+	NA	+	NA	+	Positive
		26.05.2020		+	NA	+	NA	+	Positive
		12.06.2020		-	NA	+	NA	+	Positive
		04.05.2020	Effluent	-	NA	-	NA	-	Negative
		26.05.2020		-	NA	-	NA	-	Inconclusive
12.06.2020	-	NA	+	NA	-	Negative			
Site 6	Brahmpuri	15.05.2020	Influent	NA	-	NA	-	-	Negative
		20.05.2020		+	NA	+	NA	+	Positive
		12.06.2020		-	NA	+	NA	+	Positive
		12.06.2020	Effluent	-	NA	-	NA	-	Negative
Site 7	SMS Hospital	26.05.2020	Influent	-	NA	-	NA	-	Negative (Method A)
		08.06.2020		NA	+	NA	+	+	Positive (Method B at 4°Celsius)
		12.06.2020		-	NA	+	NA	-	Negative (Method B at Room Temperature)
Site 8	Mahatma Gandhi Hospital	26.05.2020	Influent	-	NA	-	NA	-	Negative
		12.06.2020		-	NA	-	NA	+	Negative
		26.05.2020	Effluent	-	NA	-	NA	-	Negative
		12.06.2020		-	NA	+	NA	-	Negative

Figure 2 | Comprehensive data analysis of sampling sites and result interpretation: the samples inferred positive are coded red while those inferred as negative sample for SARS-CoV-2 are coded in green. Samples which showed valid Ct for at least two of the three target genes are shown here in orange (as positive) while the samples with all the three genes having valid Ct values (as positive) are shown here in red. Site 7 (Influent) is color coded yellow, is discussed. Site 5, (effluent) sample, inconclusive (marked white) shows the reaction for which internal control failed in RT-PCR detection. Please note that there is no wastewater treatment plant at site 7, thus there is no effluent sample from site 7. Note: Influent = untreated sample; Effluent = final treated sample.

has a capacity of 27 MLD. All STPs are working on full capacity and hence the same can be considered as their average flow rates. SMS hospital (site 7) does not have its own treatment plant, so the sewage samples were collected from the main drainage, which finally merges with the main trunk sewer without receiving any treatment for the time being. Mahatma Gandhi Hospital (site 8) has a treatment plant of 600 KLD capacity. The results of the study showed the presence of at least two target genes in untreated wastewater samples from site 5 and site 6 (as seen in Figure 3(e) and 3(f)), thus confirming the presence of SARS-CoV-2 genome in these samples. Sites 1–4, however, did not have any valid Ct values and thus indicated that samples were negative for any detectable presence of viral genome. The sewerage system connects all households of the colonies it covers and hence covers almost the entire city population.

The study was conducted on selected eight wastewater treatment plants from the city. Since all the inhabitants are

connected to their respective WWTPs through the sewerage lines, this study has further attempted to correlate the positively tested wastewater samples with the officially published public health data of positive patient cases in the respective area. It was observed that areas served by the WWTPs that showed positive results reported a continuous increase in confirmed positive patients, soon after the first sampling. These observations were in accordance with the previous studies (Ahmed et al. 2020; Medema et al. 2020). A previous study conducted in Paris demonstrated the detection of the viral genome before the exponential phase of the epidemic (Wurtzer et al. 2020), and another study by Randazzo et al. 2020 indicated that the SARS-CoV-2 can be detected weeks before the first confirmed case. The Ramniwas garden WWTP (Site 5) is currently serving the walled city area of Jaipur that includes the major hotspot of the city, the Ramganj area, having maximum reported cases. Our study reveals that a significant increase

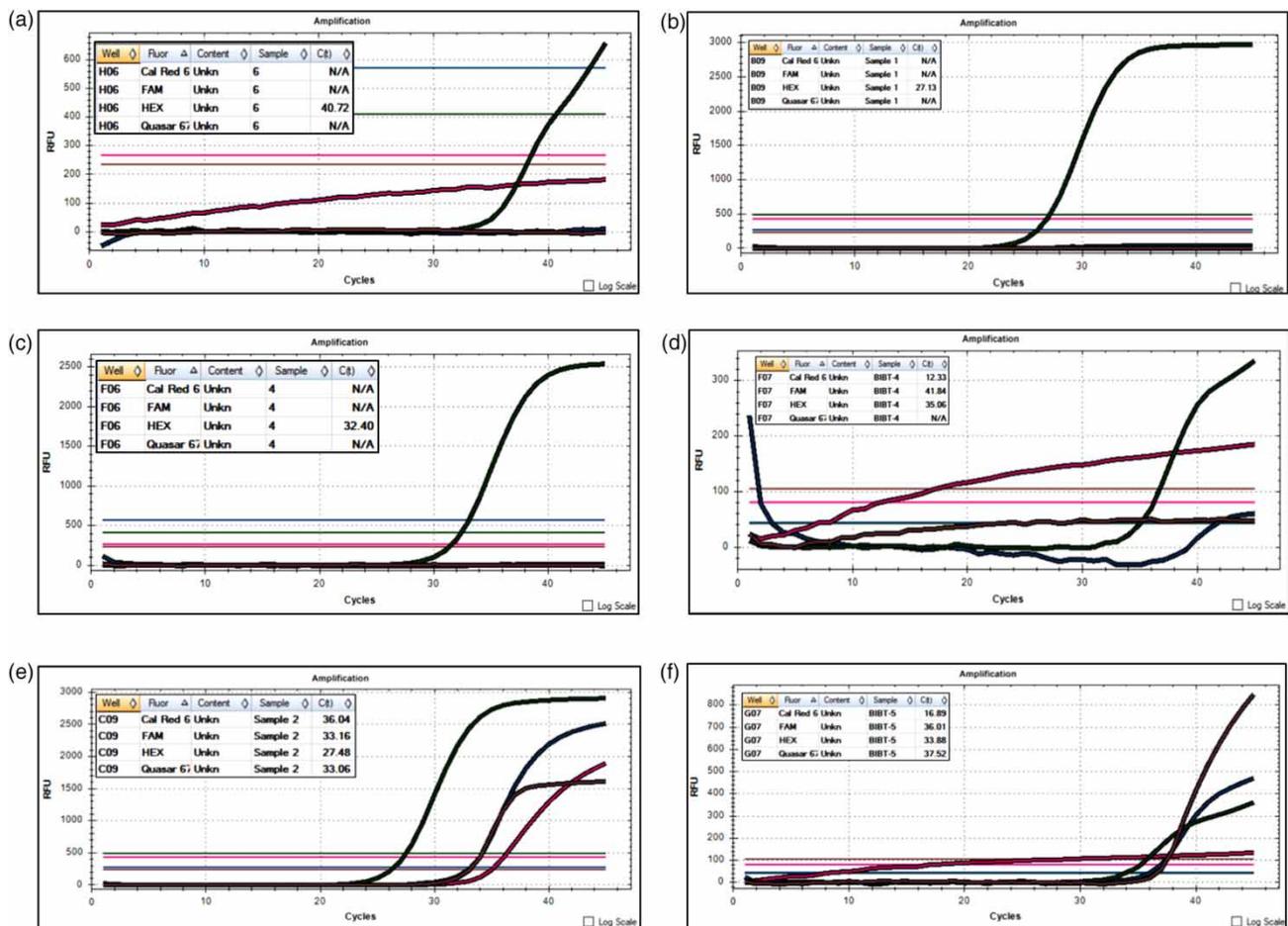


Figure 3 | Results analysis of untreated wastewater samples collected from municipal WWTPs: the graphs of Ct values were analyzed to determine positive and negative samples based on presence of the genome. (a–d) show results from sites 1–4 of untreated wastewater samples and were tested negative. (e and f) show the Ct values of sites 5 and 6 untreated wastewater samples for all the three genes tested were <40, indicating positive presence of the SARS-CoV-2 genome.

in the numbers of positive tested cases was reported within 6–14 days of our first date of sampling (4th May 2020). This increase was even more significant owing to a particular hot-spot area of Jaipur city (Central jail), which showed a surge in the number of positive cases within six days of testing of the sample from site 5 (WWTP) that confirmed the presence of the viral genome. Additionally, the untreated wastewater sample from Brahmपुरi WWTP (site 6), the first sample (collected on 15th May 2020) was interpreted negatively as it did not show the presence of virus, which correlated with the corresponding low number of positive cases around the area. However, during sampling in the subsequent week, the Brahmपुरi WWTP showed positive detection of the viral genome, and this was well correlated by a sudden surge in COVID-19 cases up to five fold within the next six days of the detection. Previous reports have mentioned that the presence of the viral genome can be as early as 14 days of actual positive testing (Bernard Stoecklin *et al.* 2020). This difference in the gap of detection can be due to the following reasons: firstly, in contrast to the previous studies, our window of testing started much later, when a complete lockdown was underway and the corona spread had already reached the third phase. Secondly, we also noticed that after 15th May 2020, the numbers of COVID-19 positive cases increased and the gap between our detection and surge of positive tested cases of an area decreased from 14 to 6 days. We acknowledge that in each of these areas where samples were tested positive, the number of officially reported positive cases by the city was only in double digits. This low number of cases reported by the city can be attributed to a very low number of COVID-19 tests done per area per day by the city. This data has reinforced our confidence in proposing that WWTPs can indeed serve as good checkpoints in predicting the surge in COVID-19 cases well ahead of the individual testing. This is critical as even a delay of three days in COVID-19 detection can lead to a potential spread to thousands of people (Mallapaty 2020). Out of the municipal WWTPs that were selected for the current study, wastewater samples from two WWTPs (sites 5 and 6) showed positive viral detection. We wondered if this pattern held any significance and if it could be related to the status of the positive tested cases in the respective area. We thus surveyed the officially published daily public health data of the total and new COVID-19 positive cases from the areas linked with our test WWTPs. The results of our study predicted that samples collected early from 4th to 14th May 2020, were tested positive, 10–14 days preceding a large jump in the number of reported corona positive cases in

the respective area. However, during the late sampling (15th May to 12th June 2020), this gap decreased from 14 days to 6 days, between our detection and the surge of numbers in positive cases. One of the reasons for this contrasting trend might be the window of our observation. While the previous studies were done during the initial phases of disease spread along with the increasing imposition of restricted interactions in the respective countries, we have investigated the samples along with the progressive relaxation in the lockdown of the city. This is an interesting observation, and is reported for the first time. We thus hypothesize that this reduction in the gap could be because of a rapid spread of disease along with the accompanying relaxations in the lockdown in the city by the local government. Because, during a rapidly spreading pandemic, everyday can potentially account for spread to tens of thousands of more people, we infer that this study highlights that a warning obtained even 6 days ahead, could be crucial in taking relevant steps against the outbreak. The early detection of SARS-CoV-2 RNA in wastewater could have alerted about the imminent danger, giving a valuable time to the managers to coordinate and implement actions to curb the spread of the disease. Therefore, our outcomes support the proposition that WBE could be used as an early warning tool to monitor the status of COVID-19 infection within a community. Additionally, we believe that the environmental surveillance could be used as an instrument to drive the right decisions to reduce the risk of lifting restrictions too early. For instance, a key question is how to reduce the risk of a ‘second wave’ and/or recurring local outbreaks. Massive population tests are the first choice, but in their absence, wastewater monitoring of SARS-CoV-2 RNA can give a reliable picture of the current situation. Our study also highlighted the importance of the WBE tool by monitoring viral RNA in wastewater to assess disease prevalence and spread in defined populations, which may prove beneficial for predicting COVID-19 related public health policy.

Validation of SARS-CoV-2 genome in wastewater samples by two different protocols

There has been growing evidence of the presence of the SARS-CoV-2 genome in sewage samples, but the major challenge in the detection is the lack of an optimized and standardized protocol (Bivins *et al.* 2020). As there are several reports for viral RNA isolation to check for the genomic presence of the COVID-19 causing virus in human fecal samples and sewage samples (Chen *et al.*

2020; Hindson 2020; Wang *et al.* 2020a), the present study tried to establish a protocol, which would be robust under local conditions. As mentioned earlier, both methods (A and B) were standardized to achieve the detection of viral RNA in the sample. Firstly, Method A uses filtration and PEG adsorption method to increase the concentration of the SARS-CoV-2 virus before RNA extraction. This process was developed with the purpose of investigating probable samples with diluted loads and for the samples with low expected viral load. In this protocol, the samples were subjected to heat inactivation of virus particles. After the inactivation, the COVID-19 viral particles present in the sample were concentrated by filtration and adsorption onto PEG into 1 mL of final volume. This ensured an increased concentration of viral particles present per mL. Secondly, Method B directly processes the sample for RNA extraction after removal of large suspended solids by a simple centrifugation. This method was standardized for the untreated wastewater samples and fecal samples where the probability of striking a heavy load of viral particles existed. In the direct method, the samples were subjected to a centrifugation step to remove larger solid particles and debris and directly processed for the RNA extraction and RT-PCR detection assay. The results of the study showed that the samples could be similarly tested for the detection of viral genomic RNA regardless of the pre-processing and concentration differences due to the two protocols. Thus, the samples from a particular site (processed by method A or B) tested either positive or negative regardless of the methods used and that the testing results were linked to the area, rather than the sample processing method. As we observed that both methods were effective uniformly in the case of municipal wastewater samples (sites 1 to 6), and we could detect the presence of SARS-CoV-2 in a sample regardless of the method employed. Thus, each method acted as an internal control of another for each sample tested by them. As many research groups across the globe are mobilizing to monitor wastewater for SARS-CoV-2 RNA for this purpose, it is necessary to validate methodologies, and data sharing to maximize the yield of WBE. Efficient harmonization of sampling, quality control, analysis methods and publications will help to ensure a high-quality evaluation of WBE (Bivins *et al.* 2020).

Detection of SARS-CoV-2 genome at high ambient temperatures during summers

As mentioned previously, wastewater treatment plants cater to tens of thousands of people in a given community. It has

been previously reported that the virus can stay for very long periods in water and wastewater but at the same time, there are other studies that have reported that high temperatures can negatively affect the viral persistence outside the human body (La Rosa *et al.* 2020). In India, especially in Rajasthan, however, where the ambient temperatures can go as high as 50 °C in May–June, the study aimed to check if the WWTP samples could still have the presence of SARS-CoV-2 genome at a higher temperature. The idea was to check if WWTPs in India could act as a checkpoint and alert against the COVID-19 outbreaks. Therefore, the samples were collected in the months of May and June 2020, where the ambient temperatures in the city were high, up to 45 °C. The presence of the viral genome was detected at a high ambient temperature of 45 °C, which showed that WWTP samples can serve as a checkpoint where the presence of the COVID-19 genome can be checked. These results correlated with a recent study that predicted the linear upward (increasing) trend of positive patients found in most states of India with increasing temperature and humidity (Goswami *et al.* 2020). The present study reports the first evidence of SARS-CoV-2 genome in wastewater samples from India, under higher ambient temperature conditions. The present study reports the evident presence of viral genome in untreated wastewater even during high ambient temperatures of 45 °C. There have been epidemiology based studies outside India on SARS-CoV-2 virus which were done at colder seasons and quite low ambient temperatures (Bernard Stoecklin *et al.* 2020; Medema *et al.* 2020). The detection of the COVID-19 genome in wastewater by these studies can be easily explained as the low range of temperature is reported to increase the duration of viral persistence (La Rosa *et al.* 2020). We could not trace any studies that report the viral behaviour at high temperatures. This is perhaps the first study to check untreated and treated wastewater samples during the months of May–June 2020 which are the hottest months in the summer, especially in Jaipur. Thus, it is proposed that the testing of wastewater appears to be a useful approach for early detection and studying the infection spread dynamics of the community population throughout the year. Although the study was able to establish the temperature independence of the viral genome detection in case of SARS-CoV-2 by successful detection in the wastewater samples at high temperatures, there are still other factors, which are yet not investigated. The RT-PCR based detection of SARS-CoV-2 RNA genome could be affected by individual or combinatorial effects of multiple factors, including the temperature. These factors can be the duration between

shedding and testing, collection and testing and the composition of the wastewater itself. It is possible that the samples have certain non-permissive conditions for the stability and detection of viral particles, thus impacting the results. It is also possible that certain parameters like pH range, total suspended solid, COD, BOD might interfere with the RT-PCR detection itself. These questions are still widely open and need to be pursued by the scientific community in order to improve the WBE based surveillance.

Efficacy of current treatment systems at WWTPs against SARS-CoV-2

In India and other developing countries, the effluents from wastewater treatment plants find their way to the nearby gardens and agricultural areas for irrigation reuse. In this context, it becomes necessary to validate the presence of the viral genome in the treated effluent samples from WWTPs. It is highly likely that this wastewater may be originating from areas under coronavirus spread. It has been reported previously that the virus can persist with its active state (La Rosa et al. 2020) and this could pose a risk to the safety of people involved in the irrigation and public health in general. The contamination due to this wastewater may also lead to a new chain of viral spread. So, the present study also tried to investigate the efficacy of the current wastewater treatment technologies against SARS-CoV-2.

Interestingly, the results of the study (as shown in Figure 4) revealed that all the treated wastewater samples showed negative results for the presence of viral RNA from all the WWTPs. From the present study, it was inferred that even in those WWTPs which tested positive for untreated wastewater; the treated wastewater had no detectable viral genome. This is important to mention that the positive site 5 (Ramniwas garden) currently uses MBBR technology, and site 6 (Brahmpuri) used sequencing batch reactor (SBR) technology, and the results conclude that the different wastewater treatments technologies being used are currently effective and able to decrease the viral particles below the detection limit, thus confirming the efficacy of treatment technology in attenuation of the virus, concluding that there is little risk to public health with the reuse of treated water for irrigation. Our results showed that the probability of the presence of any detectable viral genome load was very less and that the water could be considered safe for public consumption by the current standards.

Efficacy of current sanitization practices (hypochlorite and other chlorine agents as disinfecting agent) in hospitals

The present study also investigated decontamination and sanitization practices used in the hospitals. This is a critical aspect as, although the suspected patients are quarantined

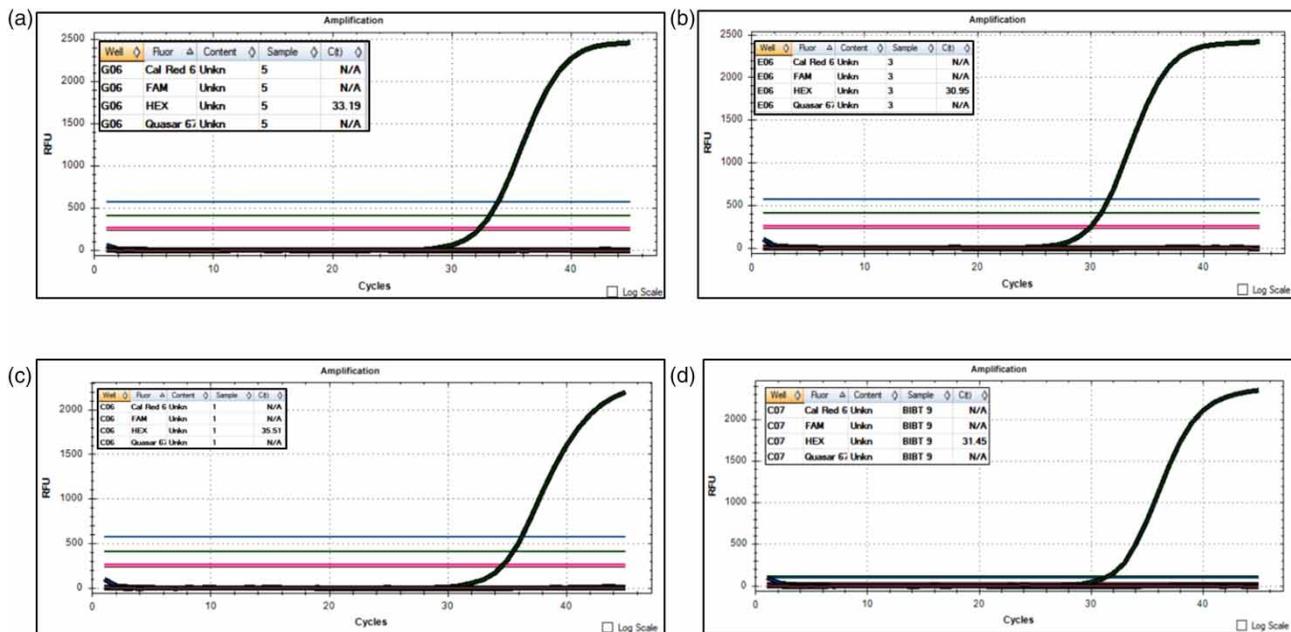


Figure 4 | Results analysis of treated wastewater samples collected from the municipal WWTPs (a) Site 3 (b) Site 4 (c) Site 5 (d) Site 6; shown here are graphs for Ct values for the three target genes tested. The graphs shown here have valid Ct value for the internal control but not for the target genes; hence all the samples are negative.

from the general public, the waste they generate can potentially cause contamination to the external water resources after being discharged outside. The present study tried to investigate if there is any presence of the viral genomic RNA in the wastewater samples of two such local hospitals where patients with positive COVID-19 were being treated on campus. The results showed a discrepancy between the samples processed under the two different conditions, as highlighted in Figure 2 (coded yellow). The untreated wastewater samples of site 7, when processed by method A (using heat-inactivation and concentration methods) tested negative for the presence of viral genome. These results suggested that the heavy use of hypochlorite and detergent solutions used in current treatment systems in hospitals for sanitizing are effective in inactivating the viral genome and hence showed negative results (Wang et al. 2020b). The sanitization treatment seems to be successful in the destabilization of the viral coat in the case of enveloped viruses like SARS-CoV-2 that leads to faster degradation of the viral genomic RNA to below detection limits. Additionally, to further confirm our hypothesis, samples from one of the hospitals (site 7) was collected on 8th June at 4 °C and processed by direct protocol (Method B) and showed positive results (Figure 5) for the viral RNA. This confirms that

this degradation was stalled by using lower temperatures for stabilizing coronavirus, as reported by La Rosa et al. 2020. The sample from site 7 was again collected on 12th June, at ambient temperature and processed by Method B, and, interestingly, gave negative results. As we know, the viral genome is possibly detected by the RT-PCR based tests, being sensitive enough to pick up even a single copy of the genome, this further confirmed our hypothesis. Thus, it is highly likely to report that under the normal physiological conditions, the sanitization practices being followed by hospitals are able to remove the viral particles of SARS-CoV-2, and are safe to be reused or discharged in the public domain. The wastewater samples from the second hospital which we tested (site 8), showed consistently negative results. Since the hospitals in this study were following the sanitization protocol guidelines given by the ministry of health, we could conclude that the sanitization guidelines are sufficient to curb the viral presence and prevent the spread of this virus to a great extent in the present scenario. These kinds of studies are equally important to keep in check the sanitization practices being followed by hospitals and in generating the new standard and guidelines, if required. The future implications of the work may include understanding the WBE tool as an alert system for preventing outbreaks.

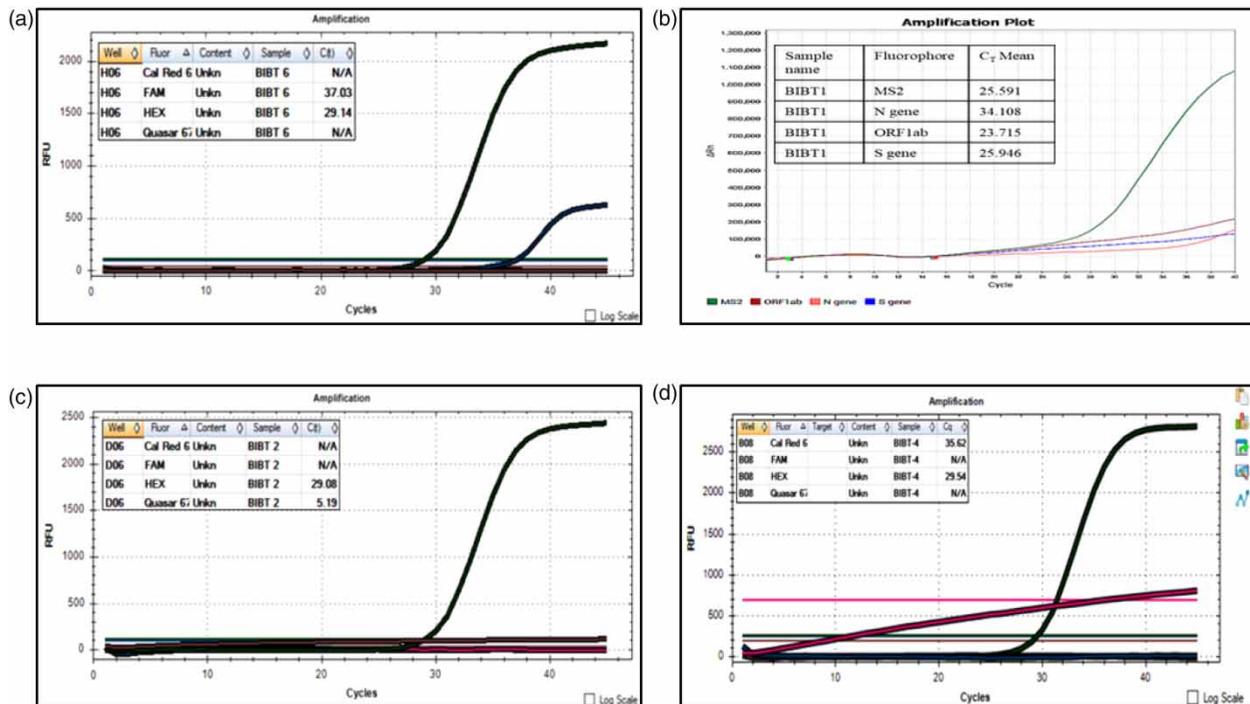


Figure 5 | Results analysis of wastewater samples collected from the hospitals: valid C_t values were detected for Site 7, untreated wastewater sample, (a) processed at room temperature, (b) processed at 4 °C, (c) Site 8, untreated wastewater samples, (d) treated wastewater samples both showed negative results.

CONCLUSIONS

This is the first study that reports the detection of SARS-CoV-2 in wastewater in India using RT-PCR assay for the detection of viral genome in wastewater. The study also highlights the need for WBE tools and surveillance as an alert system that provides population-level estimates of the burden of SARS-CoV-2 against future outbreaks. This approach can become the basis for further developing a useful warning system for the cities of India, where in-person testing may not be available. The findings of the present study confirmed the detection of COVID-19 genome at ambient temperature (45 °C) and can prove to be a useful tracking tool in understanding the dynamic behavior of this pandemic's spread. The study further highlights the efficacy of sodium hypochlorite or other chlorine compounds being used by hospital authorities as an effective disinfecting agent, to inactivate or attenuate viruses. However, future studies would be required to further validate the virulence or infectivity of these viruses in wastewater samples.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Sudipti Arora: Conceptualization, investigation, resources, sample collection, manuscript writing and editing, corresponding author

Aditi Nag: Experimental design, protocol standardization, experimental conduction, data analysis, writing original draft of manuscript, investigation

Jasmine Sethi: Experimentation, pre-processing, manuscript preparation

Jayana Rajvanshi: Sampling, experimentation, RT-PCR testing

Sonika Saxena: Resources providing, sample collection protocol standardization and supervision

Sandeep K. Shrivastava: Experimental design in COVID-19 detection

A. B. Gupta: Conceptualization, motivation, supervision, data analysis, resource providing and manuscript review

DECLARATION OF COMPETING INTEREST

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

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

- Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J. W., Choi, P. M., Kitajima, M., Simpson, S. L., Li, J. & Tschärke, B. 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](#). *Science of the Total Environment* **728**, 138764. doi: 10.1016/j.scitotenv.2020.138764.
- Baid, N. 2016 *Microbiological Analysis of MBBRs with Special Emphasis on the Assessment of Nosocomial Pathogens – A Case Study*. M. Tech thesis, Malaviya National Institute of Technology, Jaipur, India.
- Bernard Stoecklin, S., Rolland, P., Silue, Y., Mailles, A., Campese, C., Simondon, A., Mechain, M., Meurice, L., Nguyen, M., Bassi, C. & Yamani, E. 2020 [First cases of coronavirus disease 2019 \(COVID-19\) in France: surveillance, investigations and control measures, January 2020](#). *Euro Surveillance* **25** (6). doi:10.2807/1560-7917.es.2020.25.6.2000094.
- Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Been, F., Bhattacharya, P., Bijlsma, L., Boehm, A. B., Brown, J. & Buttiglieri, G. 2020 [Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19](#).

- Casanova, J. L., Su, H. C. & Effort, C. H. G. 2020 A global effort to define the human genetics of protective immunity to SARS-CoV-2 infection. *Cell* **181** (6), 1194–1199.
- Central Pollution Control Board report 2000 Available from: <http://www.cpcbenvi.nic.in/scanned%20reports/PCL%204%20Environmental%20Standards.pdf>
- Chan, J. F., Yuan, S., Kok, K. H., To, K. K., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C. C. Y., Poon, R. W. S. & Tsoi, H. W. 2020 A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* **395** (10223), 514–523. doi: 10.1016/s0140-6736(20)30154-9.
- Chen, Y., Chen, L., Deng, Q., Zhang, G., Wu, K., Ni, L., Yang, Y., Liu, B., Wang, W., Wei, C. & Yang, J. 2020 The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *Journal of Medical Virology* **92** (7), 833–840. doi: 10.1002/jmv.25825.
- Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L. & Mulders, D. G. 2020 Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance* **25** (3). doi: 10.2807/1560-7917.es.2020.25.3.2000045.
- Goswami, K., Bharali, S. & Hazarika, J. 2020 Projections for COVID-19 pandemic in India and effect of temperature and humidity. *Diabetes & Metabolic Syndrome* **14** (5), 801–805. doi: 10.1016/j.dsx.2020.05.045.
- Heijnen, L. & Medema, G. 2011 Surveillance of influenza A and the pandemic influenza A (h1n1) 2009 in sewage and surface water in the Netherlands. *Journal of Water and Health* **9** (3), 434–442. doi: 10.2166/wh.2011.019.
- Hellmér, M., Paxéus, N., Magnusius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T. & Norder, H. 2014 Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. *Applied and Environmental Microbiology* **80** (21), 6771–6781. doi: 10.1128/aem.01981-14.
- Heymann, D. L. & Shindo, N. 2020 COVID-19: what is next for public health? *Lancet* **395** (10224), 542–545. [https://doi.org/10.1016/S0140-6736\(20\)30374-3](https://doi.org/10.1016/S0140-6736(20)30374-3).
- Hindson, J. 2020 COVID-19: faecal–oral transmission? *Nature Reviews Gastroenterology & Hepatology* **17** (5), 259–259. doi: 10.1038/s41575-020-0295-7.
- Johns Hopkins University 2020 COVID-19 Dashboard. Last accessed: 15.06.2020. Available from: <https://www.arcgis.com/apps/opsdashboard/index.html#/85320e2ea5424d4faa75ae62e5c06e61>
- Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C. P., Hamilton, K. A., Haramoto, E. & Rose, J. B. 2020 SARS-CoV-2 in wastewater: state of the knowledge and research needs. *Science of The Total Environment*. 139076. <https://doi.org/10.1016/j.scitotenv.2020.139076>.
- La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S. & Suffredini, E. 2020 Coronavirus in water environments: occurrence, persistence and concentration methods – A scoping review. *Water Research* **179**, 115899. doi: 10.1016/j.watres.2020.115899.
- Ling, Y., Xu, S. B., Lin, Y. X., Tian, D., Zhu, Z. Q., Dai, F. H., Wu, F., Song, Z. G., Huang, W., Chen, J. & Hu, B. J. 2020 Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chinese Medical Journal* **133** (9), 1039–1043. doi: 10.1097/CM9.0000000000000774.
- Mallapaty, S. 2020 How sewage could reveal true scale of coronavirus outbreak. *Nature* **580**, 176–177. doi:10.1038/d41586-020-00973-x. Available from: <https://www.nature.com/articles/d41586-020-00973-x>.
- Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. & Brouwer, A. 2020 Presence of SARS-Coronavirus-2 in sewage. *medRxiv*. 2020.2003.2029.20045880. doi: 10.1101/2020.03.29.20045880.
- Orive, G., Lertxundi, U. & Barcelo, D. 2020 Early SARS-CoV-2 outbreak detection by sewage-based epidemiology. *Science of The Total Environment* **732**, 139298. <https://doi.org/10.1016/j.scitotenv.2020.139298>.
- 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 Research* **181**, 115942. <https://doi.org/10.1016/j.watres.2020.115942>.
- Shekhawat, S. S., Kulshreshtha, N. M. & Gupta, A. B. 2020 Investigation of chlorine tolerance profile of dominant gram negative bacteria recovered from secondary treated wastewater in Jaipur, India. *Journal of Environmental Management* **255**, 109827.
- Tang, A., Tong, Z., Wang, H., Dai, Y., Li, K., Liu, J., Wu, W., Yuan, C., Yu, M., Li, P. & Yan, J. 2020 Detection of novel coronavirus by RT-PCR in stool specimen from asymptomatic child, China. *Emerging Infectious Diseases* **26** (6), 1337–1339. <https://dx.doi.org/10.3201/eid2606.200301>.
- Tyrrell, D. A. J. & Myint, S. H. 1996 Coronaviruses. In: *Medical Microbiology*, 4th edn (S. Baron ed.). University of Texas Medical Branch at Galveston, Galveston, TX, USA.
- Wang, J., Shen, J., Ye, D., Yan, X., Zhang, Y., Yang, W. & Pan, L. 2020a Disinfection technology of hospital wastes and wastewater: suggestions for disinfection strategy during coronavirus disease 2019 (COVID-19) pandemic in China. *Environmental Pollution* **262**, 114665. <https://doi.org/10.1016/j.envpol.2020.114665>.
- Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G. & Tan, W. 2020b Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* **323** (18), 1843–1844. doi: 10.1001/jama.2020.3786.
- World Health Organization 2020a Coronavirus. Last accessed: 15.06.2020. Available from: <https://www.who.int/health-topics/coronavirus>
- World Health Organization 2020b Last accessed: 15.06.2020. Available from: <https://www.who.int/news-room/detail/27-04-2020-who-timeline-covid19>
- Wu, F., Xiao, A., Zhang, J., Gu, X., Lee, W. L., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N., Endo, N. & Duvall, C. 2020a SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *medRxiv* <https://doi.org/10.1101/2020.04.05.20051540>

Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X. & Kuang, L. 2020b [Prolonged presence of SARS-CoV-2 viral RNA in faecal samples](#). *The Lancet Gastroenterology & Hepatology* **5** (5), 434–435. [https://doi.org/10.1016/S2468-1253\(20\)30083-2](https://doi.org/10.1016/S2468-1253(20)30083-2).

Wurtzer, S., Marechal, V., Mouchel, J. M., Maday, Y., Teyssou, R., Richard, E., Almayrac, J. L. & Moulin, L. 2020 [Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters](#). *medRxiv* <https://doi.org/10.1101/2020.04.12.20062679>.

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