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Response of wastewater-based epidemiology predictor for the second wave of COVID-19 in Ahmedabad, India: A long-term data Perspective☆

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ABSTRACT

In this work, we present an eight-month longitudinal study of wastewater-based epidemiology (WBE) in Ahmedabad, India, where wastewater surveillance was introduced in September 2020 after the successful containment of the first wave of COVID-19 to predict the resurge of the infection during the second wave of the pandemic. The study aims to elucidate the weekly resolution of the SARS-CoV-2 RNA data for eight months in wastewater samples to predict the COVID-19 situation and identify hotspots in Ahmedabad. A total of 287 samples were analyzed for SARS-CoV-2 RNA using RT-PCR, and Spearman's rank correlation was applied to depict the early warning potential of WBE. During September 2020 to April 2021, the increasing number of positive wastewater influent samples correlated with the growing number of confirmed clinical cases. It also showed clear evidence of early detection of the second wave of COVID-19 in Ahmedabad (March 2021). 258 out of a total 287 samples were detected positive with at least two out of three SARS-CoV-2 genes (N, ORF- 1 ab, and S). Monthly variation represented a significant decline in all three gene copies in October compared to September 2020, followed by an abrupt increase in November 2020. A similar increment in the gene copies was observed in March and April 2021, which would be an indicator of the second wave of COVID-19. A lead time of 1–2 weeks was observed in the change of gene concentrations compared with clinically confirmed cases. Measured wastewater ORF- 1 ab gene copies ranged from 6.1 x 10^2 (October 2020) to 1.4 x 10^4 (November 2020) copies/mL, and wastewater gene levels typically lead to confirmed cases by one to two weeks. The study highlights the value of WBE as a monitoring tool to predict waves within a pandemic, identify local disease hotspots within a city, and guide rapid management interventions.

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1. Introduction

The global pandemic caused due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected about 43.5 million people across India by July 2nd, 2022 (WHO, 2022). Cases with mild or no symptoms are often overlooked, leading to inaccuracy in epidemiological models and assessment of disease prevalence. A large number of asymptomatic patients employed a never seen challenge over the verified estimation of disease spread based on clinical surveillance (Rimoldi et al., 2020; Medema et al., 2020). According to earlier studies, 18–45% of patients infected with COVID-19 are asymptomatic, but they are capable of spreading the disease, thereby adversely affecting the actual containment of the disease (Lavezzo et al., 2020; Yang et al., 2020; Mizumoto and Chowell, 2020; Nishiura et al., 2020). Wastewater-based epidemiology (WBE) surveillance has gained tremendous recognition as a viable option for COVID-19 surveillance since nearly 67% of infected people showed the presence of SARS-CoV-2 RNA in feces (Chan et al., 2020; Cheung et al., 2020; Wong et al., 2020). COVID-19 patients may shed viruses to wastewater through sputum and saliva (Li et al., 2022a). It can be used to detect the arrival and subsequent decline of pathogen as well as provide an early warning of the forthcoming prevalence of the disease within a community (Aguiar-Oliveira et al., 2020; Hata et al., 2021; Kumar et al., 2021a, b). There are certain advantages of using WBE over clinical testing, which include reduced analytic costs. In addition, wastewater contains viruses shed from a large number of people and thus requires far fewer samples and less labor than clinical testing to know the presence of infected persons in particular location. However, WBE is less sensitive towards detection of SARS-CoV-2 in comparison to norovirus, probably due to its enveloped nature and low SARS-CoV-2 load in the patient's fecal matter and other shedding sources (Hata et al., 2021). Further, it is essential to explore a relationship between the SARS-CoV-2 genetic load in wastewater and the number of cases at the district level in different geographical locations to evaluate WBE's potential as an early prediction tool for COVID-19 pandemic.

Clinical surveillance of COVID-19 is often inadequate to classify the city into specific zones based on the requirement of more tests or attention. This is particularly true for poorly resourced regions where a lower number of confirmed cases may be linked to underreporting. In such cases, *Surveillance of Wastewater for Early Epidemic Prediction* (*SWEEP*)-based information may prove to be critical in the zonation of the city and locating hotspots on a city scale. The concentration of SARS-CoV-2 RNA detected in wastewater would shed light on the true prevalence of COVID-19 infection in the sewer catchment, whereas the numbers reported from the clinically reported cases only account for the diagnosed patients, thereby excluding the undiagnosed or asymptomatic patients from the process. Even though the capability of WBE surveillance to detect RNA of SARS-CoV-2 has been proven, several constraints and bottlenecks exist regarding its practical applicability (Zhu et al., 2021; Tran et al., 2020). It is extremely necessary to match the time-series data of SARS-CoV-2 RNA concentration in the wastewater with the actual clinical survey data in order to confirm the utility and predictability of wastewater surveillance (Wu et al., 2021). This is also essential for the adaptation of the SWEEP on the policy level, which has been suspended for various reasons in major parts of the globe (Tiwari et al., 2021). The effectiveness of WBE has been debated actively on the basis of watersheds, catchment type, complexity of sewer systems, and population (Tiwari et al., 2022). If cases reported from a given city have been substantially high, it is pertinent to check the efficacy of SWEEP on the urban scale. Under this framework, four major directions in the domain of SWEEP may be compiled i) validating the data to unravel the early warning capability of wastewater surveillance for COVID-19 through temporal studies on SARS-CoV-2 RNA detection; ii) the need for an increase of WBE monitoring in various parts of the world to generate data from all the levels of COVID-19 situation; iii) developing the model that can utilize Ct-value acquired through SWEEP into significant predictions for effectual COVID-19 pandemic preparedness; and iv) collectively reaching to the comprehension of crucial issues like removal, discharge, decay, dilution, and infectivity due to the presence of SARS-CoV-2 RNA in wastewater (Kumar et al., 2022; Kumar et al., 2021a; Prevost et al., 2015).

Taking these points into consideration, the present study aims to present the wastewater surveillance results from Ahmedabad, India, and its association with the second wave of the COVID-19 pandemic (starting March 2021) by drawing a comparison between the detected concentration of SARS-CoV-2 RNA in wastewater of various parts of the city and the COVID- 19 confirmed clinical cases. In this study, we analyzed SARS-CoV-2 RNA in the wastewater samples (n = 287) from 9 different locations, including wastewater pumping stations and sewage treatment plant (STP) of Ahmedabad, India, from September 3rd' 2020 to April 12th, 2021 (thirty-two weeks). The main objectives of the study were: **a)** to evaluate the implementation of WBE for the prediction of the second wave of COVID-19 in Ahmedabad; **b)** weekly resolution of the SARS-CoV-2 RNA data for eight months in wastewater samples; and **c)** explicate the potential of WBE for identifying hotspots and public health monitoring at the city level.

2. Materials and methods

2.1. Study area

Ahmedabad is the seventh largest city in India and the second biggest trade centre in the western part of India, with an estimated population of 8.25 million in 2020 (UN world urbanization prospects 2018). It has a sewage network of 2500 km along with 9 sewage treatment plants (STPs) and 45 sewage pumping stations (SPSs). Sampling locations were Motera, Ranip, Paldi, Santivan, Maninagar, Satyam, Vinzole, Odhav, and Vatva, as considered in the previous study by Kumar et al. (2021a), which has been extended for wastewater-based epidemiology prediction for Ahmedabad, India (Fig. 1). The existing treatment capacity of the wastewater treatment plant in the city is 990 MLD (MoHUA, 2021).

2.2. Sampling approach

The sampling locations were determined by following the approach of Kumar et al. (2021a) for the same study area (Ahmedabad). A total of 287 samples from nine different sites in Ahmedabad were analyzed weekly to detect SARS-CoV-2 RNA in wastewater. Grab sampling method was used to collect the samples in 250 mL sterile bottles (Tarsons, PP Autoclavable, Wide Mouth Bottle, Cat No. 582240, India). In order to detect any contamination during the transport, we examined blanks in the same type of bottle. The samples were transported and maintained at the cooling condition in an icebox until further processing. The samples were processed on the same day after bringing them to the laboratory. All the analyses were conducted in Gujarat Biotechnology Research Centre (GBRC), a laboratory approved by the Indian Council of Medical Research (ICMR), New Delhi.

2.3. SARS-CoV-2 gene detection

2.3.1. Precipitation of viral particles

Firstly, 30 mL of each sample was centrifuged at 4000×g (Model: Sorvall ST 40R, Thermo Scientific) in a 50 mL sterile falcon tube for 40 min, followed by filtration of supernatant using 0.22-μm syringe filter (Mixed cellulose esters syringe filter, Himedia). After filtrating 25 mL of the supernatant, 2 g of PEG 9000 and 0.437 g of NaCl (17.5 g/L) were mixed in the filtrate, which was incubated at 17 °C, 100 rpm overnight (Model: Incu-Shaker™ 10LR, Benchmark). The following day, the mixture was centrifuged at 14000×g (Model: Kubota 6500, Kubota Corporation) for a period of about 90 min. After centrifugation, the supernatant was discarded, and the pellet was resuspended in 300 μL RNase-free water. The concentrated sample was stored in 1.5 mL

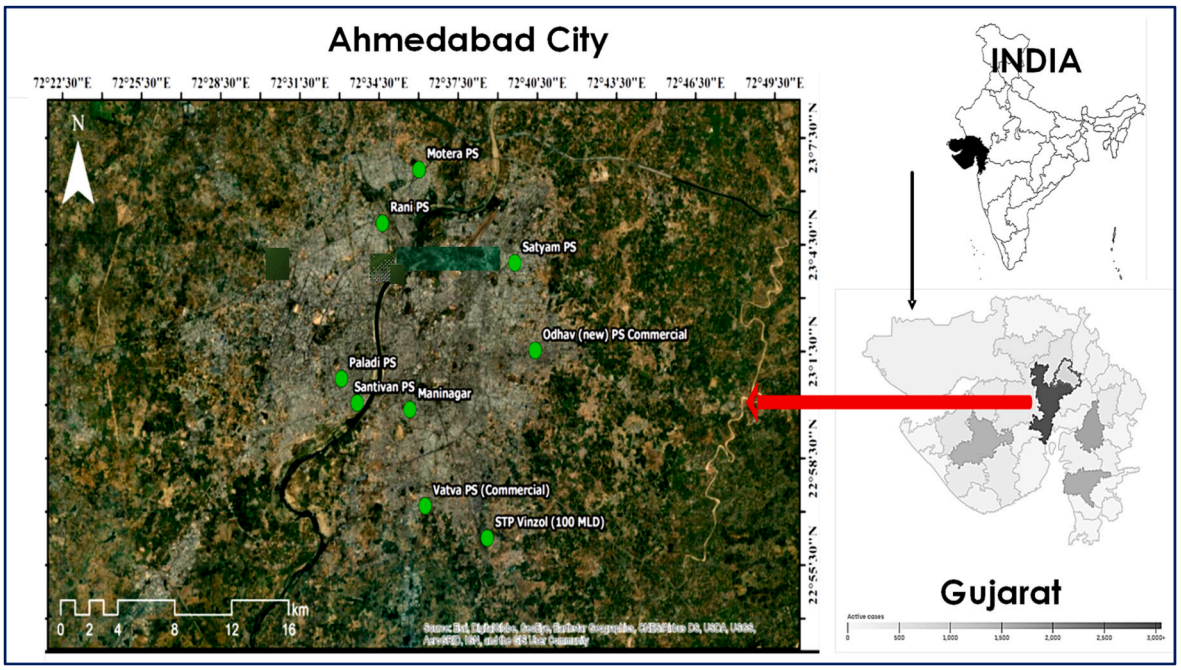

Fig. 1. Location of sampling points in Ahmedabad, Gujarat, India.

eppendorf at −40 °C, and this was subsequently used as a sample for RNA isolation.

2.3.2. RNA isolation, RT-PCR and gene copy estimation

NucleoSpin® RNA Virus (Macherey-Nagel GmbH & Co. KG, Germany) isolation kit was used to perform RNA isolation from the pellet with the concentrated virus. MS2 phage provided by TaqPath™ COVID-19 RT-PCR Kit was utilized as an internal control. Some other particulars include a) the nucleic acid extraction performed by NucleoSpin® RNA Virus Kit (Macherey-Nagel GmbH & Co. KG, Germany) (and Qubit 4 Fluorometer (Invitrogen) was used to estimate RNA concentrations, b) evaluation of molecular process inhibition control (MPC) was done through MS2 phage for QC/QA analyses of nucleic acid extraction and PCR inhibition (Haramoto et al., 2020). The methodology has been described in author's previous works (Kumar et al., 2020; 2021a). The steps were carried out according to the instructions provided in the Macherey-Nagel GmbH & Co. KG product manual, and RNAs were detected using real-time PCR (RT-PCR).

The detection of SARS-CoV-2 was performed by using TaqMan-based chemistry on Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (version 2.19 software). For each run, a template of 7 μL of extracted RNA was used with TaqPath™ 1 Step Multiplex Master Mix (Thermofischer Scientific, USA). The final reaction mixture (25 μL) consisted of nuclease-free water (10.50 μL), Master Mix (6.25 μL) and COVID-19 Real-Time PCR Assay Multiplex (1.25 μL). Positive control (TaqPath™ COVID 19 Control), negative control (from extraction run spiked with MS2), and no template control (NTC) were run with each batch. 40 cycles of amplification were set, and results were explained on the basis of the Ct values for three target genes i.e., ORF1ab, N (Nucleocapsid), and S (Spike) proteins of SARS-CoV-2 along with that of MS2 used as an internal control.

Results were considered conclusive/positive only if two or more genes are detected in the samples. Effective genome concentration was computed semi-qualitatively using the equivalence of 500 copies of SARS-CoV-2 genes as 26 Ct-value (provided with the kit). After this, the RNA amount used as a template and the enrichment factor of wastewater samples during the experimentation were multiplied by the result.

2.3.3. Method of Spearman's rank correlation coefficient

Since the relationship between the SARS-CoV-2 gene concentration in wastewater and the number of new daily confirmed clinical cases (COVID-19, India, 2022) is considered to be non-linear, the relationship was evaluated by Spearman's rank correlation coefficient. Clinical information is based on data from Ahmedabad City, and the number of new daily clinical cases for a total of seven days (the reference date and three days before and after that day) was used to analyze the rank correlation coefficient with the concentration of the SARS-CoV-2 gene in wastewater (to be precise, the virus concentration was substituted by the Ct value). This is because the number of new daily clinical cases is affected by day-of-week variations in the number of tests, including PCR and antigen examination, etc. Specifically, the sampling date was used as the reference, and that reference date was shifted back and forth from the sampling date to analyze the respective rank correlation coefficients. The time lag between the increase or decrease of the SARS-CoV-2 gene concentration (Ct value) in wastewater and that of the number of new daily clinical cases was estimated from the gap between the reference date and sampling date when the rank correlation coefficient was the highest. A negative time lag indicates that wastewater is detecting trends in the viral infection status faster than the clinical tests.

3. Results and discussion

Variations in SARS-CoV-2 RNA were detected and quantified from influent wastewater samples for eight months (September 2020 to April 2021) to understand the pandemic situation in Ahmedabad, Gujarat, India. Out of 287 samples analyzed in the study, 258 were found positive, comprising two out of three target genes. Whereas, 253 samples displayed positive RT-PCR results for all of N, ORF 1b, and S genes. The average Ct values for S, N, and ORF 1 ab genes were 32.89, 31.84, and 32.48, respectively. The average Ct value of internal control (MS2 bacteriophage was 27.42. Also, no SARS-CoV-2 gene was detected in the negative control samples.

3.1. Monthly variation

Monthly variation portrayed a notable decline of 89.7%, 63.7%, and 90.8% in N, ORF-1ab, and S gene concentration (copies/L), respectively,

in October 2020 compared to September. This was followed by a sharp increase in November 2020, i.e., about 25, 22, and 26 folds in the N, ORF 1 ab, and S genes, respectively. The concentration of all three genes started decreasing in the month of December and continued till February 2021. After this, there was a pronounced increase in the concentration of all three genes in March 2021 as compared to February 2021, i.e., about 19, 10, and 6 folds in the S, ORF1ab, and N genes, respectively. In April, the average gene concentrations for N, ORF-1ab, and S genes were $10.5x10^3$, $8.3x10^3$, and $3.6x10^3$ per liter, respectively. The highly infectious and fatal Delta variation (B.1.617.2), which caused the second wave in India, and is responsible for the dramatic increase in gene concentration in March 2021 and April 2021.

The descending order of monthly variation in ORF1ab gene concentration in wastewater samples was: November 2020> April 2021> March2021> September2020> December2020> January2021> October2020> February2021. Likewise, the decreasing order of N gene in wastewater samples followed a similar pattern and was found in the order of November 2020> April 2021> March 2021> September 2020> December 2020> January 2021> February 2021> October 2020 and that of S gene was found to be November 2020> March 2021> April 2021> September 2020> January 2021> December 2020> February 2021> October 2020 (Fig. 2 a-d). The genome concentration of SARS-CoV-2 RNA was maximum in the month of November 2020 ($1.1x10^4$ copies/L), followed by April 2021 ($7.5x10^3$ copies/L), March 2021 ($4.5x10^3$ 524 copies/L), September 2020 ($3.0x10^3$ copies/L), December 2020 ($1.8x10^3$ copies/L), January 2021 ($1.6x10^3$ copies/L), February 2021 ($4.7x10^2$ copies/L) and October 2020 ($4.4x10^2$ copies/L).

There had been a decrease of 20.47% in active cases in October 2020 with respect to September 2020, followed by a rise of 1.82% in November 2020 compared to the preceding month, October 2020. The rise of active cases of November 2020 with respect to October 2020 is analogous to a change of 59 cases (3234 cases on November 1, 2020–3293 on November 26, 2020). Though the percentage change in active cases appears to be insignificant, the sharp increase in SARS-CoV-2 gene concentration can be attributed to a huge 37.93% increase in average daily confirmed cases in November 2020 (i.e., 252 cases) as compared to October 2020 (i.e., 183 cases). The casual and reluctant attitude of people during the festive season in India (mid-Oct 2020 to mid-Nov 2020) might be the reason for the surge in COVID-19 cases. This was followed by a decline of 2.27% in active cases in December 2020 with respect to November 2020 (2998 cases on December 14, 2020–2930 cases on 28th December 2020). In January 2021, there was a further decrease of 49% in the number of active cases with respect to December 2020 (2894 cases on January 4, 2021–1478 cases on January 29, 2021). Likewise, in February 2021, there was a reduction of 36% in active cases with respect to January 2021 (855 cases on February 8, 2021–548 cases on February 22, 2021). Finally, a 3-fold increase in the number of active cases was noticed in the month of March 2021 with respect to February 2021. This corresponds to a change of 1455 cases (642 cases on 1st March 2022–2097 cases on 30th March 2022). Lockdown was imposed upon the city in April 2021, when the number of active cases had already reached 7165 (as on 12th April 2021). A whopping rise in the average daily confirmed cases in March 2021 (i.e., 317 cases) and April 2021 (i.e., 1017 cases till 12th April) was noticed due to the second wave of COVID-19. Like WBE surveillance in Ahmedabad, other countries including New Zealand (Medema et al., 2020), Spain (Vallejo et al., 2020), Italy (La Rosa et al., 2020), Australia (Ahmed et al., 2021), England (Martin et al., 2020), Japan (Hata et al., 2021), and Brazil (Fongaro et al., 2021) have also confirmed presence of SARS-Cov-2 RNA in wastewater, first reported cases, and national lockdown in respective countries (Table 1). These results showed that outbreak for COVID-19 can be predicted via trends of viral loads in wastewater through WBE surveillance.

One of the main purposes behind WBE for COVID-19 detection is its capability to make an early warning of the disease outbreak or emergence of new trends within communities. The distinctive increase in the number of active cases, viz. 7165 appeared on April 12, 2021, which is 2 weeks post the significant increase in the viral genome concentration in wastewater samples (on March 30, 2021) (Fig. 3). Therefore, this time period of 2 weeks could be adequately utilized to control the pandemic situation in the city. Some other studies around the globe reported early detection of SARS-CoV-2 RNA in wastewater even before the first report of clinical diagnosis. For example, in Netherlands, SARS-CoV-2 genetic material was detected in wastewater in early February 2020, even before the official declaration of the first case in late February 2020 (Medema et al., 2020). Similarly, La Rosa et al. (2020) reported SARS-CoV-2 genetic material in wastewater samples from two different cities in Italy before the first official documented report. Likewise, Randazzo et al. (2020) identified SARS-CoV-2 RNA in wastewater samples from Spain. Thereafter, various studies have been carried out where the presence of SARS-CoV-2 RNA in wastewater samples were detected and reported successfully (D'Aoust et al., 2021; Ahmed et al., 2021, Kumar et al. 2020). The researchers from Gujarat found the genomic traces of the B.1.617.2 in wastewater samples before a month of a clinically confirmed case of the same variant in Ahmedabad (Joshi et al., 2022). A few studies, however, focused on evaluating the potential of WBE on the temporal scale with respect to the changes in COVID cases. Conversely, in USA, Nemudryi et al. (2020) have reported that SARS-CoV-2 RNA concentration in municipal wastewater lagged behind the laboratory test results by 8-days (Pearson's $r = 0.989$) in mid-March 2020, whereas preceded clinical results by 2 days ($r = 0.92$) in May–June 2020. Li et al. (2022b) have reported that genome concentrations in longitudinal monitoring of SARS-Cov-2 in wastewater lagged 7-days behind the clinical test reported. Recently, Swift et al. (2023) have reported that RNA genome counts in multiple WWTPs were lagged 2-days were strongly correlated 6 out of 7 WWTPs than clinical tests, due to SARS-Cov-2 signals being diluted at one WWTP, which needs to be taken care of while performing lag-optimization.

3.2. Early warning capability

The current investigation is based on our first proof of concept study, in which we discovered SARS-CoV-2 genetic material in wastewater and asserted that it may be used for community COVID-19 surveillance (Kumar et al., 2020). The percentage change in genome concentration level on a particular date maintained a positive correlation with the confirmed cases registered 1–2 weeks later by the regulatory authority based on clinical tests (Fig. 3). This authenticated the early warning

Table 1
Country-wise detection of SARS-Cov-2 viral RNA in wastewater before the confirmed COVID-19 cases and related casualty data.

Name of the country	Confirmed presence in wastewater	First reported cases	Date of National Lockdown Measures	National casualties	Reference
Amsterdam (New Zeeland)	Feb 6, 2020	Feb 27, 2020	Mar 15, 2020	3134	Medema et al. (2020)
Barcelona (Spain)	Jan 25, 2019	Feb 25, 2020	Mar 14, 2020	18276	Vallejo et al. (2020)
Milan (Italy)	Dec 18, 2019	Feb 21, 2020	Mar 9, 2020	31106	La Rosa et al. (2020)
Brisbane South (Australia)	Feb 27, 2020	Mar 13, 2020	Apr 3, 2020	2121	Ahmed et al., 2021
Southeast England	Feb 11, 2020	Feb 13, 2020	Mar 23, 2020	1800	Martin et al. (2020)
Ishikawa (Japan)	Feb 19, 2020	Feb 21, 2020	July 29, 2020	316	Hata et al., 2021
Toyama (Japan)	Mar 30, 2020	Apr 4, 2020	July 29, 2020	236	Hata et al., 2021
Santa Catarina, (Brazil)	Nov 27, 2019	Mar 03, 2020	Mar 21, 2020	51	Fongaro et al., 2021

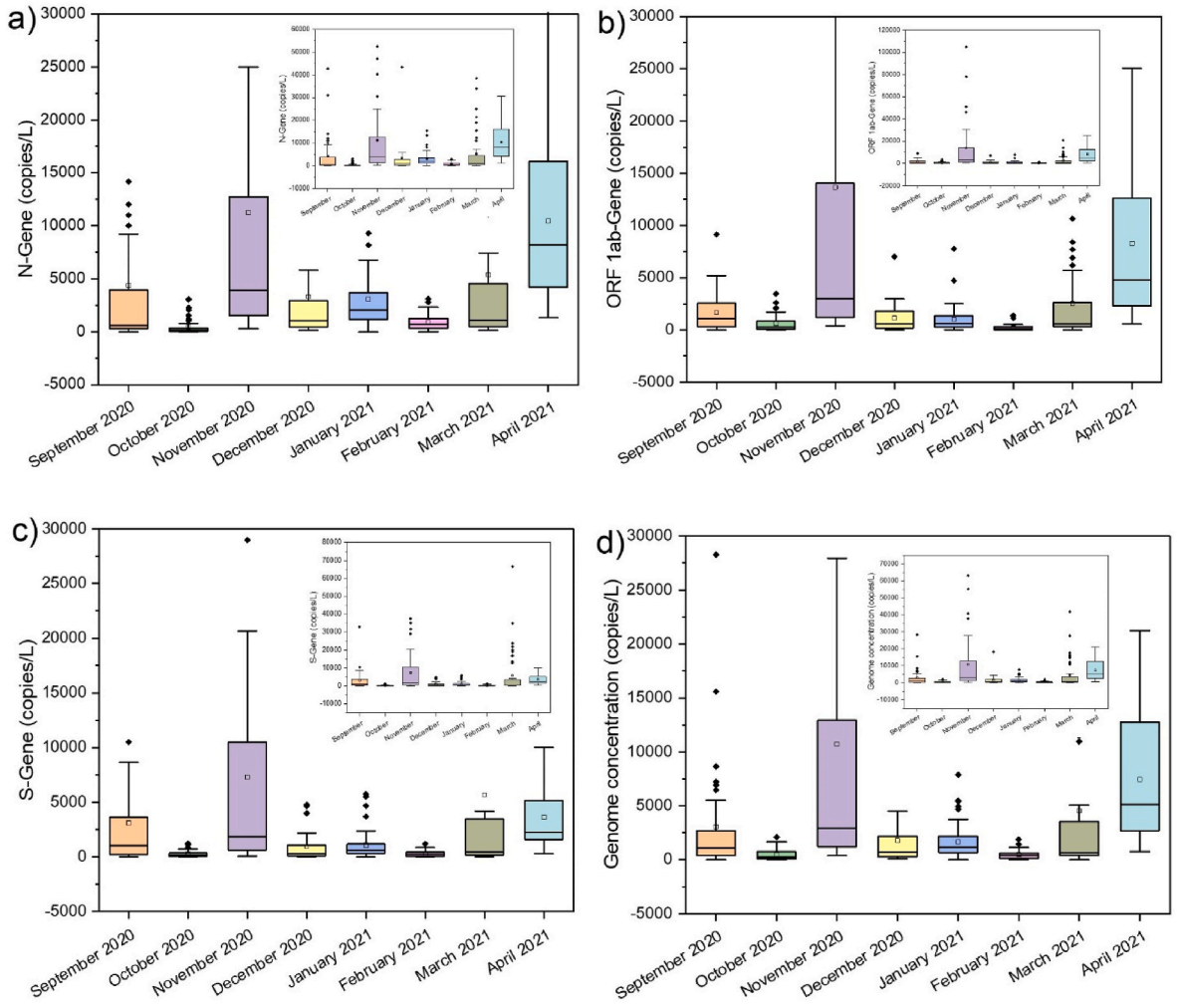

Fig. 2. Monthly variations in average SARS-CoV-2 gene copies collected from different STPs in Ahmedabad, a) N-Gene, b) ORF1 ab Gene, c) S-Gene, and d) Genome concentration.

capability of WBE for COVID-19 surveillance on a temporal scale. A temporal variation in SARS-CoV-2 genetic material loading in effluent samples from different treatment plants and active cases for eight months is shown in Table 2. However, no linear relationship exists between the SARS-CoV-2 gene concentration and epidemiological data. Therefore, we demonstrated the relationship between percentage changes in SARS-CoV-2 genome concentration and daily confirmed cases (Fig. 3), which can be used as a pre-alarming tool, as it offers a lead of around 2 weeks for the upcoming scenario. From the present study, we can see that on 8th October 2020, a sharp decline of 134% was noticed in the percentage change in the genome concentration, followed by 4.8% decline in the percentage change in daily confirmed COVID-19 cases on 22nd October 2020. Similarly, on 5th November 2020, a significant increase of >22-folds was noticed in the in the genome concentration compared to the earlier sampling date, which was followed by 11.16 and 45.58% increment in the percentage change in confirmed COVID-19 cases on 19th November and 26th November 2020, respectively. In contrast, more than >1000% and 500% increase were observed in percentage change in SARS-CoV-2 genome concentration in wastewater in early September and mid- October, respectively. However, no notable increase in the number of confirmed cases was detected 1–2 weeks later. In spite of this, the aforementioned technique exhibited positive prediction in most of the cases during the study period.

The variations in SARS-CoV-2 RNA in wastewater were further detected and quantified to understand the pandemic situation during the second wave in Ahmedabad. On March 30th, 2021, a steep hike of >1800% in the percentage change in the genome concentration was noticed compared to the earlier sampling date, which was followed by 120% increment in the percentage change in confirmed COVID-19 cases on April 12th, 2021 (Fig. 3). Therefore, the severity of the pandemic situation can be predicted 1–2 weeks prior to the official reports based on clinical tests. The results from the study highlighted the potential of WBE surveillance as an early warning tool for COVID-19 in the presence of adequate SARS-CoV-2 genetic material in wastewater samples. The findings of the study further supported those of Ahmed et al. (2021), who detected a longitudinal decline in the presence of SARS-CoV-2 RNA with the subsidence of the first epidemic wave.

These findings were further supported by Spearman's rank correlation for the early warning potential of WBE. The results showed the highest rank correlation coefficient was observed in the ORF1 ab followed by S and N genes for all pumping stations (PS) and sewage treatment plants (STP). In the three PS, Santivan PS, Paldi PS and Ranip PS, the time lag was negative for all viral genes, i.e., the gene detection preceded clinical test results. Furthermore, for the ORF-1ab gene, having the highest rank correlation coefficient, the time lag was −8 days for Santivan PS ($r = 0.45$, $p < 0.01$), −10 days for Paldi PS ($r = 0.54$, $p < 0.01$), and −11 days for Ranip PS ($r = 0.60$, $p < 0.01$) (Fig. 4). These PS results showed that we could detect trends in viral infection status of the community from the wastewater analysis, approximately 10 days earlier than the clinical examination. Although the rank correlation coefficients were smaller than those in the ORF-1 ab gene, the same three PS also showed negative time lags in the N and S genes at about the same time as

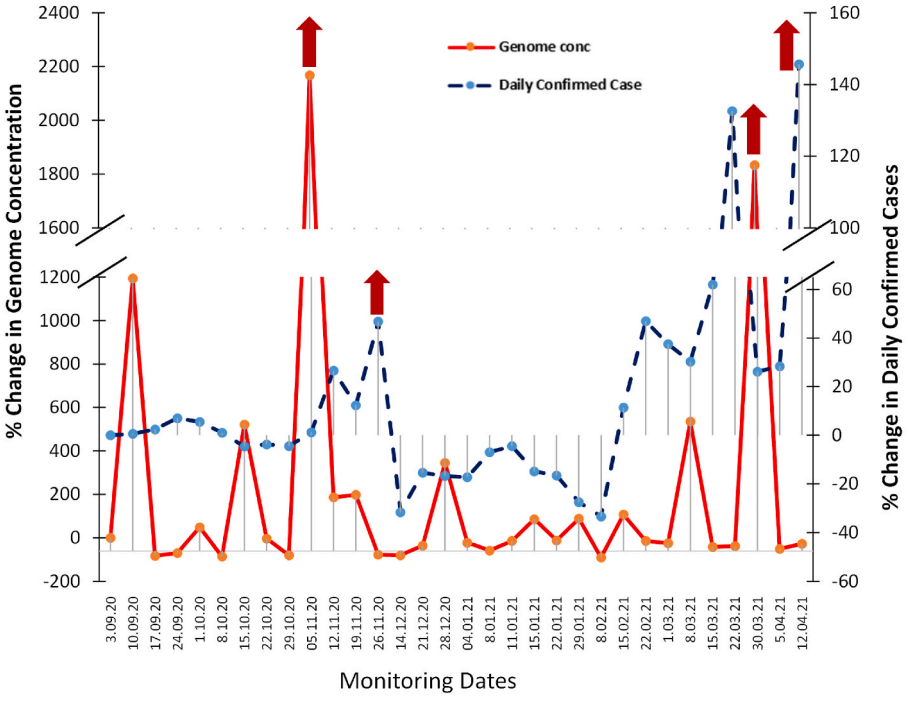

Fig. 3. Potential and evidence of wastewater-based epidemiology surveillance of Covid-19 pandemic as an early warning tool in Ahmedabad.

Table 2
Temporal variation in SARS-CoV-2 genetic material loading found in the influent and effluent samples collected from different wastewater treatment plants.

Station	Sampling date	September, 2020				October, 2020					November, 2020				December, 2020			January, 2021						February, 2021			March, 2021				April, 2021		
		3.09	10.1	17.1	24.1	0.1.10	8.1	15.1	22.1	29.1	5.11	12.1	19.1	26.1	14.1	21.12	28.12	4.01	8.01	11.01	15.01	22.01	29.01	8.02	15.02	22.02	1.03	8.03	15.03	22.03	30.03	5.04	12.04
	Active Cases	3671	4168	4038	4252	4122	3614	3472	3451	3372	3283	3280	3362	3293	2998	2994	2930	2894	2848	2796	2552	1991	1478	855	544	548	642	729	909	1594	2097	2586	7165
	SARS-CoV-2 Genes	Gene Copies (copies/ L) x 10^2																															
Motera PS	N	19.9	120	0.36	1.56	7.99	2.84	1.23	30.8	ND	28.7	70.8	522.7	57.8	7.29	5.85	50.81	34.39	8.48	31.48	11.79	11.27	19.38	6.55	5.91	15.24	4.08	21.48	8.04	55.55	384.68	68.52	15.90
	ORF	5.84	16	1.43	5.73	1.7	1.16	10	20.8	ND	3.86	104	783.2	44.4	6.06	2.31	24.44	9.75	6.59	3.69	6.51	12.91	3.12	ND	1.01	4.08	ND	17.73	2.84	26.43	208.25	56.77	37.53
	S	4.4	71.1	0.78	4.6	1.27	1.32	3.17	11.3	0.34	3.65	63.9	350.8	18.2	7.40	2.30	8.06	22.30	5.02	4.31	3.13	6.08	3.77	0.26	3.53	7.58	0.73	34.73	4.51	17.13	667.28	19.37	14.36
	Genome	10.1	69.1	0.86	3.96	3.65	1.77	4.8	21	ND	12.1	79.5	552.2	40.1	6.92	3.49	27.77	22.15	6.69	13.16	7.14	10.09	8.76	2.27	3.48	8.97	1.60	24.64	5.13	33.04	420.07	48.22	22.60
Ranip PS	N	3.18	310.4	9.8	5.4	6.61	3.73	2.17	0.68	0.17	64.2	33.1	471	124.7	22.50	4.34	433.41	13.64	27.00	24.21	12.03	65.62	15.55	4.09	5.07	1.97	3.82	250.46	14.36	8.42	123.57	95.99	112.15
	ORF	ND	51.9	41.7	14.8	0.86	ND	13.3	3.59	0.95	29.9	30.5	463.2	101.9	28.27	0.27	70.13	5.67	17.14	15.33	0.00	5.92	ND	ND	2.07	1.18	1.20	84.07	13.24	2.93	49.88	49.88	95.99
	S	0.46	105	39.2	15.2	1.67	0.51	5.91	0.18	0.78	15.8	24.8	289.9	37.04	39.97	0.42	47.54	7.49	14.81	8.92	2.94	15.83	1.74	ND	1.19	1.68	3.26	136.20	8.48	0.48	190.00	15.79	35.72
	Genome	1.22	155.8	30.2	11.8	3.05	1.41	7.14	1.48	0.63	36.6	29.4	408.1	87.9	30.25	1.68	183.69	8.93	19.65	16.15	4.99	29.13	5.76	ND	2.78	1.61	2.76	156.91	12.03	3.95	121.15	53.89	81.29
Paldi PS	N	5	40.5	3.26	ND	12.1	0.27	0.23	0.55	0.3	8.69	12.6	99.8	39.1	2.21	8.90	25.69	26.03	21.90	8.57	23.51	56.48	32.00	ND	31.03	8.41	2.64	15.79	40.86	10.14	339.23	306.46	42.63
	ORF	1.73	11.7	11.1	0.28	3.31	0.19	0.69	2.17	0.27	5.77	24.4	140.5	21.9	1.29	0.96	7.88	22.00	8.95	ND	5.10	8.85	ND	ND	13.78	3.46	ND	5.23	40.57	5.88	141.42	250.46	23.01
	S	0.79	29.6	9.8	0.75	1.76	ND	0.66	2.78	0.69	3.52	27.2	118.9	9.93	1.74	0.58	0.84	10.72	6.32	3.97	6.44	22.38	ND	0.56	11.80	5.28	ND	28.14	37.79	0.62	350.04	79.87	14.96
	Genome	2.51	27.2	8.07	0.34	5.74	0.15	0.53	1.83	0.42	5.99	21.4	119.7	23.6	1.75	3.48	11.47	19.58	12.39	4.18	11.68	29.24	ND	0.00	18.87	5.72	ND	16.39	39.74	5.55	276.90	212.26	26.87
Santivan PS	N	12.4	100	3.07	1.37	2.15	2.37	0.87	0.96	ND	15.1	2.74	116.3	12	9.72	9.82	4.30	66.61	36.93	6.54	11.34	22.72	ND	9.20	11.00	23.27	1.73	13.79	74.24	14.76	214.73	13.33	41.74
	ORF	4	30.4	9.74	4.13	0.65	0.24	3.9	5.17	ND	12.2	3.89	129.6	12.9	7.36	2.42	3.41	24.28	24.30	10.91	3.25	4.70	0.72	0.82	ND	11.41	3.66	5.80	57.18	5.84	77.00	5.80	20.33
	S	3.14	86.6	10.4	4.57	1.2	ND	1.87	1.55	0.15	6.03	4.24	141.9	3.67	10.78	1.48	ND	57.41	14.26	2.74	6.03	19.12	ND	1.21	4.36	8.44	5.37	1.79	20.33	3.05	200.43	3.03	18.22
	Genome	6.51	72.3	7.74	3.36	1.33	0.87	2.21	2.56	ND	11.1	3.63	129.3	9.5	9.29	4.57	2.57	49.43	25.16	6.73	6.88	15.51	ND	3.74	5.12	14.37	3.59	7.13	50.58	7.88	164.05	7.39	26.76
Maninagar	N	5.8	48.5	6.15	0.62	15.4	3.5	2.78	ND	0.15	8.3	NA	168.6	34.5	3.49	7.01	16.26	67.67	3.51	18.52	63.21	27.96	48.70	12.60	3.26	2.00	10.91	2.40	7.43	18.34	45.46	230.10	98.87
	ORF	1.05	10.3	26.3	2.62	3.95	0.26	26.1	2.54	1.68	5.93	NA	172.7	28.3	1.38	5.68	5.13	2.18	2.54	6.17	25.12	13.69	1.30	ND	1.96	0.82	5.47	ND	5.17	8.65	18.85	151.34	44.49
	S	1.18	20.6	35.2	2.08	2.84	0.73	12.1	ND	0.47	2.17	NA	105.2	10.4	2.96	4.73	2.21	36.99	2.75	5.44	23.56	14.31	2.46	0.48	3.17	1.31	1.73	ND	4.34	9.00	41.74	51.70	43.87
	Genome	2.68	26.4	22.5	1.78	7.39	1.5	13.7	ND	0.77	5.47	NA	148.8	24.4	2.61	5.80	7.87	35.62	2.93	10.04	37.30	18.65	17.49	4.36	2.80	1.38	6.04	ND	5.65	11.99	35.35	144.38	62.41
Satyam PS	N	14.1	141.7	4.91	6.48	30.4	4.88	2.23	0.21	0.28	10.2	8.21	23.2	29.5	1.89	2.54	14.25	133.93	12.03	0.43	15.70	36.81	81.68	2.16	19.05	7.06	2.34	4.02	3.05	7.53	110.50	63.27	36.48
	ORF	1.4	39.9	23	24.8	10.8	1.05	17.2	3.34	2.15	7.52	5.82	13.3	27.6	ND	1.04	3.24	47.25	6.37	1.05	11.76	19.64	ND	1.18	3.69	3.79	2.70	0.88	4.02	0.10	49.53	42.03	13.24
	S	2	78	23.3	24.8	7.01	0.36	7.09	1.69	0.51	0.68	3.03	12.1	10.3	0.92	0.81	0.74	55.06	3.21	0.89	4.80	11.25	ND	0.52	3.23	5.49	0.54	ND	2.27	ND	168.24	17.49	16.67
	Genome	5.82	86.5	17.1	18.7	16.1	2.1	8.85	1.75	0.98	6.15	5.69	16.2	22.5	0.93	1.46	6.08	78.75	7.20	0.79	10.75	22.56	ND	1.28	8.66	5.45	1.86	1.63	3.11	2.54	109.42	40.93	22.13
STP Vinzol	N	11	92.2	2.57	ND	20.6	ND	1.68	3.16	0.92	111.5	127.4	470.9	56.4	36.71	29.42	18.59	25.81	14.53	9.37	62.92	6.93	154.19	27.95	13.52	8.72	5.44	3.80	12.98	13.42	154.80	133.17	208.25
	ORF	3.97	22.3	34.7	ND	6.44	ND	16.8	34.7	6.8	43.9	187.1	1049	17.1	19.94	15.57	18.03	6.22	1.67	6.31	77.78	5.20	2.07	2.85	5.21	2.24	10.62	3.52	2.86	6.27	61.91	126.37	155.98
	S	6.23	51.1	37.3	ND	4.97	ND	5.98	11.6	2.52	18.5	105	374.5	11.7	12.11	4.87	17.30	4.18	0.30	4.02	23.70	8.78	5.37	3.61	2.56	4.06	8.53	1.48	2.74	2.92	127.32	36.74	87.22
	Genome	7.06	55.2	24.9	ND	10.7	ND	8.15	16.5	3.41	57.9	139.8	631.5	28.4	22.92	16.62	17.97	12.07	5.50	6.57	54.80	6.97	53.88	11.47	7.10	5.01	8.20	2.93	6.19	7.54	114.68	98.76	150.48
Odhav PS	N	38.1	427.5	5.84	1.69	22.7	0.29	2.2	ND	1.61	155.2	305.2	249.9	401.7	45.53	40.30	58.17	11.84	11.64	12.65	19.17	4.06	51.22	ND	4.02	ND	4.26	14.07	1.42	4.81	190.00	18.47	170.81
	ORF	17.9	91.4	31	3.46	9.05	ND	21.3	40.5	8.38	132.5	512.1	277.8	305.5	24.79	15.90	29.69	15.43	5.61	2.94	12.41	5.76	3.11	ND	ND	ND	9.87	7.53	3.59	2.19	106.47	14.36	111.32
	S	6.87	329.2	31.5	7.43	6.96	ND	8.57	0.15	3.36	69.3	316.5	206.4	131.7	21.61	8.32	46.51	7.44	2.45	3.71	11.55	1.95	10.33	0.30	ND	ND	0.51	0.86	0.62	1.81	237.30	4.72	100.34
	Genome	20.9	282.7	22.8	4.19	12.9	5.74	10.7	0.2	4.45	119	377.9	244.7	279.6	30.64	21.51	44.79	11.57	6.56	6.44	14.38	3.92	21.55	ND	ND	ND	4.88	7.49	1.88	2.94	177.92	12.52	127.49
Vatva PS	N	13.2	110	3.12	0.87	10.3	0.8	0.22	2.02	0.46	23.9	28.5	34.7	15.2	1.39	26.20	10.31	6.05	24.35	38.47	15.01	8.67	92.96	7.35	6.82	4.79	10.00	5.30	9.55	9.36	155.98	160.77	64.66
	ORF	7.94	35.4	15.5	0.13	4.63	0.14	7.7	17.2	2.14	6.54	23.2	31.8	10.8	1.63	5.99	2.05	ND	15.88	16.81	15.51	11.45	0.73	0.94	2.54	3.34	5.27	4.18	4.91	3.12	69.02	244.73	45.78
	S	1.33	48.7	20.2	1.01	3.01	ND	1.09	8.56	0.76	7.93	17.5	25.3	5.62	2.22	0.88	6.08	1.66	6.64	9.12	8.27	3.63	46.65	ND	5.24	3.12	3.12	6.96	2.62	4.54	218.05	68.52	25.53
	Genome	7.51	64.7	12.9	0.67	5.97	0.47	3	9.27	1.12	12.8	23	30.6	10.5	1.75	11.02	6.15	2.57	15.62	21.47	12.93	7.92	46.78	2.76	4.87	3.75	6.13	5.48	5.69	5.68	147.68	158.01	45.32

in the ORF1 ab gene. In Motera PS, the time lag was negative for all the genetic regions except ORF-1 ab. Among N, S, and average genome regions, S showed the highest rank correlation coefficient ($r = 0.48$, $p < 0.01$) with a time lag of −8 days (Fig. 4). Likewise, In Vatva PS, negative time lag was noticed for all genes except N. The highest rank correlation coefficient was observed for ORF1 ab gene ($r = 0.38$, $p < 0.02$) with a lag time of −19 days. However, in the case of STP Vinzol, Odhav, Satyam, and Maninagar PS, no negative lead time was noticed for all the genes. The latter can be ascribed to **i)** grab sampling approach in the present study rather than composite sampling; **ii)** the clinical cases used for the

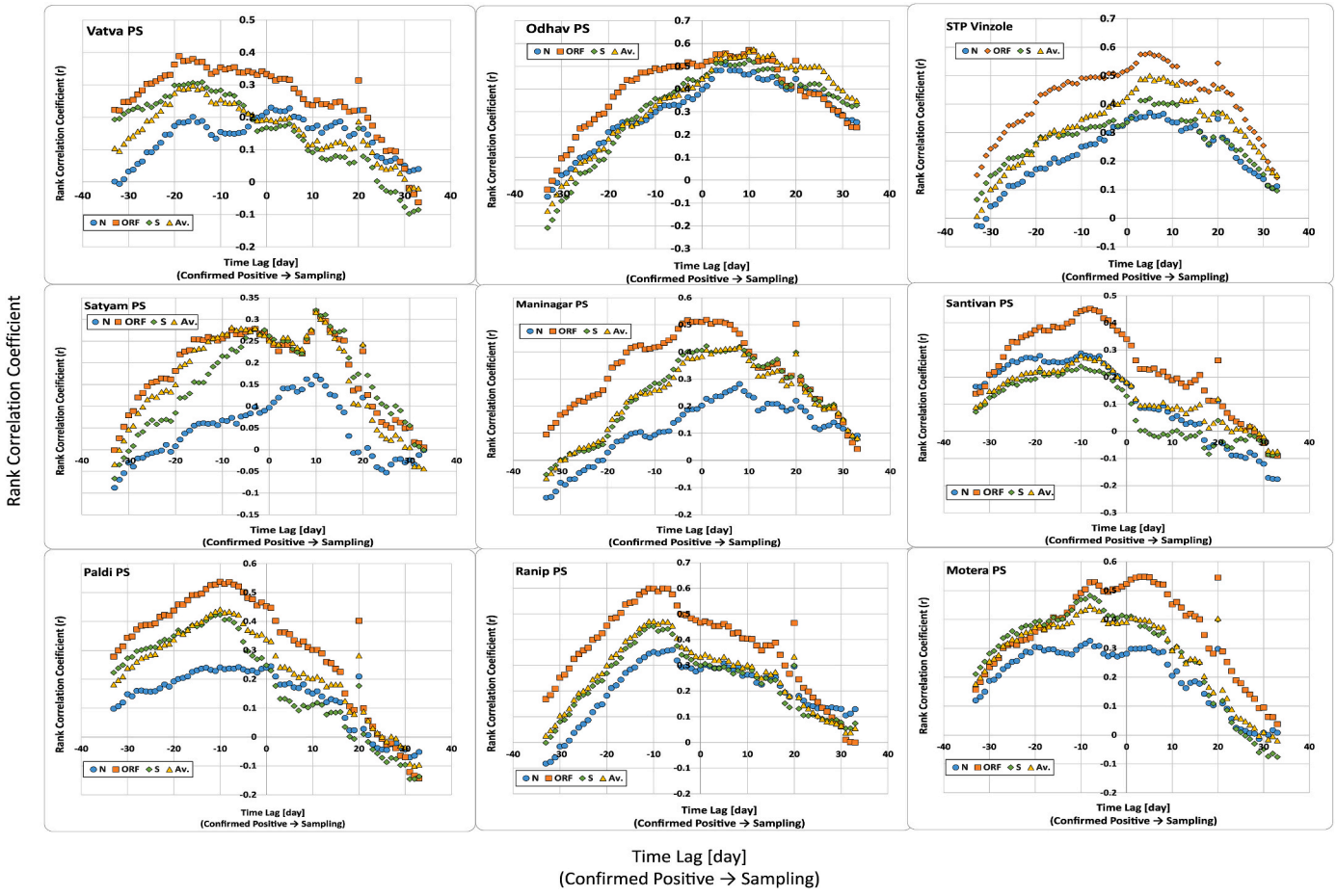

Fig. 4. Rank correlation coefficient with the concentration of the SARS-CoV-2 gene in wastewater during September 03, 2020 to April 12, 2021.

rank correlation analysis were the numbers for the whole area, not for the specific catchments of PS/STPs. The different time lag implies that the center of infection moves around the city, from catchments such as Santivan, Paldi, Ranip, which had negative lags, to Odhav, Vinzole, Satyam, which had positive lags, can possibly due to different sampling characteristics, i.e., sampling from boundaries of the wastewater sample (the relevant PS) and the clinical cases (the relevant residential region); **iii)** two peaks in Satyam PS may mean that center of infection visited twice in this catchment; **iv)** disparity in the actual infected individuals and clinical data. Nevertheless, the following observations were critical in Spearman's rank correlation between new daily positive cases and Ct values of different RT-PCR detection genes of different sites: **i)** ORF1 ab gene can be used as a marker gene for the early detection or changes in the spike of COVID-19 cases, and **ii)** WBE can be used for the early detection of COVID-19 at sub-city levels reflected by a clear-cut lead time. Therefore, it is suggested that we would be able to predictably capture the signs of the trend of increase or decrease in the number of infected people from wastewater.

At least, wastewater surveillance provides a real-time situation of the pandemic, nearly on the day of wastewater sample collection. An infected individual (symptomatic, asymptomatic, pre-symptomatic, post-symptomatic) immediately starts to contribute virus particles to a sewage network (through feces, nasal mucus, and sputum), and if the WW sample collection event, particularly with grab sampling as in this study, matches with the time of arrival of such infected wastewater, then SARS-CoV-2 can be detected (Grijalva et al., 2022). However, regarding clinical cases, many factors such as symptoms of the infected individual, his/her willingness to be tested, availability of the testing facility, waiting in the queue for testing, collection of nasal swab samples, sample processing, and analysis (RNA extraction from clinical samples, and RT-PCR), may delay the reporting at least three to four days. Therefore, WBE information could be available in advance than clinical reporting. Further, an infected individual is mostly reported once as a clinical case, but biologically she/he continuously contributes virus particles to the sewage system for an average of 17.0 days (Cevik et al., 2021).

On comparing the SARS-CoV-2 genome concentration in wastewater of Ahmedabad, we found high genome concentration during the first wave (November 2020) compared to the second wave (April 2021), while daily new confirmed cases were much higher in the second wave in comparison to the first wave. The latter can be ascribed to i) the greater infectivity and transmissibility of the Delta variant (B.1.617.2) compared to the Alpha variant (B.1.1.7), which might lead to increased clinical testing during the second wave; ii) more asymptomatic patients and less clinical testing during the first wave. The mass vaccination campaigns could have a role in a surge of infection, mainly in heavily populated countries like India, as such campaigns concentrate crowds at a single facility. But, later, due to vaccination, the host immune could be improved and infectivity and load of virus particles per infected individual could be lowered. Therefore, a huge number of infected individuals could need to have a similar detection rate during the second wave of infection (Tiwari et al., 2022). Also, the virus particles in the sewage system are continuously degrading. Mass, vaccination in communities could affect not only the virus load in infected individuals but also the fate and decay in sewage networks. Therefore, the detection trend of virus RNA targeted with three different genes was varied (Fig. 4). However, for clinical sampling, it could not be the case; virus particles are always more intact and healthier for molecular processing.

3.3. SWEEP-based city zonation and identification of hotspots

On the basis of SARS-CoV-2 genome concentration in wastewater samples, we successfully recognized locations that are highly susceptible for COVD-19 infection and its transmission among the community. Despite not having explicit epidemiological data at the ward level/ sampling locations, the variations of SARS-CoV-2 gene concentration in wastewater samples were sufficient to classify the city. In September 2020, maximum effective gene concentration was displayed by the wastewater samples collected from the east zone ($5.7x10^3$ copies/L), followed by the north zone ($3.5x10^3$ copies/L) (Fig. 5). Likewise, in November 2020 the north (Motera and Ranip) and east (Odhav and Satyam) zones were particularly affected, with an average genome concentration of $1.6x10^4$ and $1.3x10^4$ copies/L, respectively (Fig. 5). Even though areas present in the north and east zones revealed high virus genetic load, a sharp rise in SARS-CoV-2 RNA could be seen in all the zones in November 2020. On 28th December 2020 the north zone showed higher SARS-CoV-2 genome concentration ($1.1x\ 10^4$ copies/L) as compared to the other zones. On 8th March 2021 an ascent in virus genetic load ($9.1x10^3$ copies/L) could be seen from the north zone. At the end of March 2021 (30th March 2021), the wastewater samples collected from the north zone showed maximum genome concentration ($2.7x10^4$ copies/L), followed by the west zone ($2.2x10^4$ copies/L), even though sharp rise in SARS-CoV-2 RNA was noticed in all the zones.

This implies the capability of SWEEP technology to distinguish the study area at the sub-city or zone level based on SARS-CoV-2 gene concentration. SWEEP data can offer insight into the actual extent of the infection due to the SARS-CoV-2 since it covers both asymptomatic and symptomatic patients. It is, thus, possible to identify hot spots within the city, which can assist in increasing preparedness in advance. Contrarily, clinical surveillance usually falls short while classifying the city into distinct zones as it is primarily dependent upon the location of test centres. Also, it does not take the number of asymptomatic patients into account.

4. Conclusions

Wastewater-based epidemiology holds a lot of promise as a favorable tool that could be used to detect real-time and early disease signals. It could also be utilized to determine emerging hot spots in the surveillance of COVID-19 prevalence at the community level. WBE could prove to be extremely essential in an Indian context, where there is a scarcity of resources both in terms of disease management and diagnosis. In the present study, the relation between the percentage change in SARS-CoV-2 genome concentration and confirmed cases for a period of eight months more or less followed a similar trend on the temporal scale,. Additionally, the findings of the study successfully detected the resurge of viral RNA load in wastewater, approximately 2 weeks prior to the second wave of COVID-19 in Ahmedabad, India. This gap of 2 weeks between the change in genome concentration in wastewater samples and the number of confirmed cases of COVID-19 unveils the potential of WBE surveillance as an early warning. Likewise, Spearman's rank correlation suggested the highest rank correlation coefficient for ORF1 ab gene followed by S and N genes. Also, the results showed that we could detect trends in viral infection status from the wastewater approximately 10 days earlier than the clinical examination. This time gap may prove to be sufficient to take effective management interventions to stop the spread of the disease and further assist the authorities in identifying the hotspots within a city. However, additional research should be promoted to develop a predictive model that can interpret SWEEP data to policymakers to boost the awareness and management of pandemics. Besides, advancements in detection of RNA copies, prompt analysis, daily-to-daily lead/lag-time analysis, and effective sampling technique need to be followed in order to avoid dilution of the wastewater samples to predict and monitor COVID-19 outbreak prior to utilizing resources effectively in a short time span to save humanity.

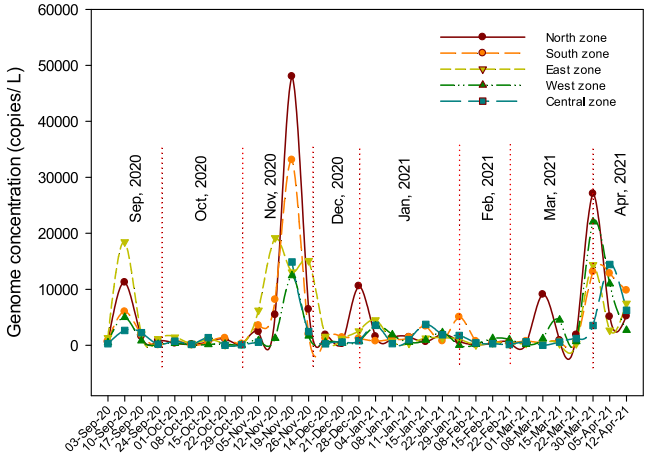

Fig. 5. Zone-wise COVID-19 pandemic status in Ahmedabad city.

Credit author statement

Manish Kumar: Conceptualization, Visualization, Writing- Reviewing and Editing. Madhvi Joshi: Data curation, Methodology, Investigation, Writing – original draft preparation. Guangming Jiang: Data curation, Visualization. Rintaro Yamada: Data curation, Visualization. Vaibhav Srivastava: Data curation, Investigation, Visualization, Writing-original draft preparation, Reviewing and Editing. Ryo Honda: Data curation, Visualization. Jürgen Mahlknecht: Writing- Reviewing and Editing. Damia Barcelo: Writing- Reviewing and Editing. Sabarathinam Chidambram: Writing- Reviewing and Editing. Anwar Khursheed: Writing- Reviewing and Editing. David W Graham: Writing- Reviewing and Editing. Ritusmita Goswami: Writing- Reviewing and Editing. Keisuke Kuroda: Writing- Reviewing and Editing. Anand Tiwari: Writing-Reviewing and Editing. Chaitanya Joshi: Project Supervision, Writing-Reviewing and Editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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