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Genetic sequencing detected the SARS-CoV-2 delta variant in wastewater a month prior to the first COVID-19 case in Ahmedabad (India)☆

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ABSTRACT

Wastewater-based genomic surveillance can identify a huge majority of variants shed by the infected individuals within a population, which goes beyond genomic surveillance based on clinical samples (i.e., symptomatic patients only). We analyzed four samples to detect key mutations in the SARS-CoV-2 genome and track circulating variants in Ahmedabad during the first wave (Sep/Nov 2020) and before the second wave (in Feb 2021) of COVID-19 in India. The analysis identified a total of 34 mutations in the spike protein across samples categorized into 23 types. The spike protein mutations were linked to the VOC-21APR-02; B.1.617.2 lineage (Delta variant) with 57% frequency in wastewater samples of Feb 2021. The key spike protein mutations were T19R, L452R, T478K, D614G, & P681R and deletions at 22029 (6 bp), 28248 (6 bp), & 28271 (1 bp). Interestingly, these mutations were not seen in the samples from Sep/Nov 2020 but did appear before the massive second wave of COVID-19 cases, which in India started in early April 2021. In fact, genetic traces of the Delta variant were found in samples of early Feb 2021, more than a month before the first clinically confirmed case of this in March 2021 in Ahmedabad, Gujarat. The present work describes the circulating of SARS-CoV-2 variants in Ahmedabad and confirms the consequential value of wastewater surveillance for the early detection of variants of concerns (VOCs). Such monitoring must be included as a major component of future health protection systems.

1. Introduction

The Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) has had a disastrous impact on human life (Hu et al., 2021). It continues to disrupt healthcare systems worldwide since the first declaration of the COVID-19 pandemic by the World Health Organization (WHO) on Mar 11, 2020. SARS-CoV-2 has infected over 37 million people and caused ~0.48 million deaths in India alone by Jun 18, 2022. Governments are taking considerable steps to expedite the vaccination drive to control the pandemic everywhere in the world, which has had great successes (Oliu-Barton et al., 2022). However, a public health challenge still exists due to continuing mutation of SARS-CoV-2 owing to its positive-sense single-stranded RNA genetic core and high circulation.

Mutations in the SARS-CoV-2 genome has led to the emergence of different highly infectious variants of concern (VOCs). For example, the B.1.1.7 lineage of SARS-CoV-2 (VOC-20-DEC-01), which was detected in the United Kingdom (UK) in Nov 2020, is supposed to be 40–80% more contagious compared to the original strain (Davies et al., 2020; Volz et al., 2021). Likewise, other SARS-CoV-2 lineages from Brazil (P.1; VOC-21JAN-02), Southern African countries (B.1.351; VOC-20DEC-02), India (B.1.617.2; VOC-21APR-02) are more transmissible than the variants reported in early 2020. Recently, a new SARS-CoV-2 variant viz., Omicron (B.1.1.529), was reported from South Africa on Nov 24, 2021. The Omicron variant is highly transmissible compared to the earlier lineages, having significantly lower neutralization titers by post-vaccination sera (Dejnirattisai et al., 2022). The variants of concern (VOCs) are important in terms of viral pathogenicity, virulence, and transmission. The variants of concern (VOCs) can be more transmissible,

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resulting in likely greater disease severity outcomes, and are also known for reduced sensitivity to antibody neutralization (Davies et al., 2020; Wang et al., 2020).

Multiple mutations in the spike protein and other important genomic areas are common in these variants, leading to attenuated efficacy of SARS-CoV-2 therapeutic interventions. For example, E484K mutation is found in the receptor binding ridge of the spike protein, which has been identified in many lineages, including B.1.351 (VOC-20DEC-02), P.1 (VOC-21JAN-02), A.23.1 (VUI-21FEB-01), B.1.525, B.1.1.318, P.2 (VUI-21JAN-01), B.1.324.1, a subclade of B.1.526, and P.3 (VUI-21MAR-02). This mutation reduces virus binding to polyclonal sera (Greaney et al., 2021a, 2021b) and evades virus from the treatment with monoclonal antibody REGN10933, which is one of the antibodies in the REGN-COV2 cocktail (Starr et al., 2021). Mutation E484K also leads to avoidance from class 2 antibodies and results in a 5-fold (approx.) reduction in neutralization by COV47 plasma (Greaney et al., 2021a, 2021b). Similarly, P681H and P681R mutations are present in the proximity of the furin cleavage site in the viral spike glycoprotein. P681H mutation has been reported in B.1.1.7 (VOC-20DEC-01), B.1.1.318, and P.3 (VUI-21MAR-02) lineages, while P681R mutation has been witnessed in A23.1 and all B.1.617 lineages. Both P681H and P681R mutations are supposed to enhance the spike protein cleavage and augment viral fusion to the host cell (Brown et al., 2021; Saito et al., 2021). Though the latter implication of P681H mutation is not clear; however, it is assumed to be responsible for the enhanced transmissibility of the B.1.1.7 variant similar to the P681R. Also, D614G mutation in spike protein is known to be responsible for augmented transmissibility of the SARS-CoV-2 (Korber et al., 2020). Therefore, it is imperative to track existing circulating variants and dominant mutations to quickly identify developing novel variants to ensure a better decision-making system for public health policies and management of COVID-19 outbreaks.

Since COVID-19 patients excrete virus particles in the feces (Crank et al., 2022), RT-qPCR has been used to detect and quantify SARS-CoV-2 RNA in wastewater around the world (Kumar et al., 2021a, b; 2022; Hata et al., 2021; Albastaki et al., 2021; Fitzgerald et al., 2021; Chavarria-Miró et al., 2021; Ahmed et al., 2021; Wu et al., 2022; Wade et al., 2022). The wastewater-based epidemiology surveillance is getting recognition worldwide due to its potential for early detection, larger population coverage, coverage of asymptomatic carriers, and reduced expense compared to large-scale clinical testing (Polo et al., 2020). The WHO recognized the environmental wastewater surveillance strategies to monitor and detect the viral pathogens in circulation. The tracking of SARS-CoV-2 genomic variants from wastewater could also provide a better insight into their origin, pathogenicity, and transmission. However, variant screening in wastewater is challenging due to the heterogeneity (different sources) and complex nature (pollutant load, drug residues, and physicochemical properties) of the wastewater from which very specific fragmented nucleic acids must be accurately identified. Genomic surveillance of wastewater may prove its worthiness as a powerful tool for detecting, identifying, predicting, and developing an early warning system for identifying VOCs in circulation to support public health interventions. Only a few reports are available that have sequenced the SARS-CoV-2 genome from wastewater samples to identify variants in different parts of the world, including Montana, USA (Nemudryi et al., 2020), California, USA (Crits-Christoph et al., 2021), Switzerland (Jahn et al., 2021), London (Wilton et al., 2021), Canada (Landgraff et al., 2021), etc. However, none have been performed in India, which we report. The goal here is to show the value of wastewater variant screening to flag the early appearance of new VOCs and circulation of known variants as a key component of future health care protection and management systems.

The second wave of COVID-19 badly affected all of India, but Gujarat was one of the most affected states, with a total of ~0.5 million new cases and deaths of ~5 thousand people from Apr 1, 2021 to Jun 1, 2021 (COVID 19 INDIA). To address this emergency, we performed SARS-CoV-2 genome sequencing in freshwater/wastewater samples during the first wave and before the second wave of COVID-19 in India and compared sequences with the reference variant (Wuhan/Hu-1/2019, EPI_ISL_402125), with three objectives: i) determine existing circulating variants and prevalent mutations among Gujarat populations; ii) relate dominant variants and pandemic in the region; iii) assess the potential of genomic surveillance sequencing of wastewater as an early warning system to detect rapidly emerging new variants.

2. Methodology

2.1. Study area and sample collection

Ahmedabad is the seventh-largest city in India and the second biggest trade center in the western Indian region, with an estimated population of ~8.25 million in 2021 (UN world urbanization prospects, 2018). In the present study, six samples were collected, including freshwater and wastewater for analysis. Two samples were collected from the Sabarmati River in the month of Sep 2020. Likewise, two untreated wastewater samples were collected from the Vinzol wastewater treatment plant (70 MLD, Activated Sludge Process) in Ahmedabad in Nov 2020. In Feb 2021, two samples (Untreated and treated WW) from the Vinzol treatment plant were collected for analysis. The operational parameters of the Vinzol WWTP have been provided in a tabular form as supplementary (Table S1).

The samples were collected by grab hand sampling using 250 mL sterile bottles (Tarsons, PP Autoclavable, Wide Mouth Bottle, Cat No. 582240, India). Simultaneously, blanks in the same type of bottle were examined to know any contamination during the transport. The samples were kept cool in an ice-box until further process. The analysis was performed on the same day after bringing the samples to the laboratory. All the analyses were performed in Gujarat Biotechnology Research Center (GBRC), a Gujarat state government-funded research Institute equipped with high-end Next generation sequencing (NGS) and computing facility. Further, GBRC is also an Indian Council of Medical Research (ICMR), New Delhi approved SARS-CoV-2 testing laboratory.

It should be noted that a single composite sample was prepared by pooling equal concentrations of extracted RNA of Sabarmati River samples (Sept 2020). Likewise, another composite sample was prepared for wastewater samples of Nov 2020. Therefore, four final samples were used for library preparation, sequencing, and data analysis (Table 1).

2.2. SARS-CoV-2 RNA concentration method

The concentration method consisted of a PEG 9000 and NaCl precipitation protocol previously described by Kumar et al. (2020) for wastewater samples. 30 mL wastewater sample was centrifuged (Model: Sorvall ST 40 R, Thermo Scientific) at 4000 g for 30 min in a 50 mL falcon tube, followed by the filtration of the supernatant with a syringe filter of 0.2μ (Mixed cellulose esters syringe filter, Himedia). The 25 mL sample filtrated was then treated with NaCl (17.5 g/L) and PEG 9000 (80 g/L) and incubated at 17 °C, 100 rpm overnight (Model: Incu-Shaker™ 10LR, Benchmark). The sample was then transferred in an oak

Table 1
Wastewater genomic surveillance of COVID-19 in Gujarat, India.

Sample Code/ Location	Mapped Reads	On Target	Mean Depth	Uniformity	Sample Collection Date
A_Sabarmati	13,13,151	99.82%	5073	62.45%	8/22-Sept-2020
E_Vinzol_Raw	8,57,886	99.12%	4770	80.09%	19/26-Nov-2020
P_Vinzol_Inlet	10,91,811	99.98%	7204	80.43%	08-Feb-2021
T_Vinzol_Outlet	9,85,094	99.80%	4902	92.63%	08-Feb-2021

ridge tube for further centrifugation (Model: Incu-Shaker™ 10LR, Benchmark) at 14000 g for 90 min, ultimately forming the pellets. RNase-free water (300 μL) was used for the resuspension of the viral particles after discarding the supernatant. The concentrated virus samples (300 μL) were then stored in a 1.5 mL Eppendorf tube at a temperature of −40 °C for RNA isolation.

2.3. RNA extraction, library preparation, sequencing, and data analysis

RNA was extracted as described by the author's earlier studies (Kumar et al., 2021a,b) using the NucleoSpin® RNA Virus isolation kit (Macherey-NagelGmbH & Co. KG, Germany). The extraction process involved lysis of virus particles, binding of viral RNA to the column, washing, and elution of viral RNA using buffer solution. MS2 phage was used as an internal control to check any bias in the RNA extraction and the presence of inhibitors that may hinder the subsequent processes. The extracted RNA (30 μL) was subjected to cDNA synthesis using SuperScript-III First-Strand Synthesis System (Invitrogen/Thermo Fisher Scientific). We used the Ion AmpliSeq Community SARS-CoV-2 research panel and Ion AmpliSeq library kit Plus (Invitrogen/Thermo Fisher Scientific) for library preparation. The quality of the library was evaluated on Bioanalyzer (Agilent 2100) using DNA High Sensitivity (HS) Kit manufactured by the Agilent. Further, sequencing was carried out on Ion GeneStudio S5 Plus System (Thermo Fisher Scientific) on 530 Chip and 400 bp chemistry.

2.4. Data filtering, trimming, and genome assembly

All raw reads were processed using the PRINSEQ-lite v.0.20.4 program for data filtering (Schmieder and Edwards, 2011). Reads were trimmed from the right where the average quality of the 5 bp window was lower than QV25 and 5 bp from the left end was trimmed. Reads with lengths lower than 50 bp with average quality QV25 were also removed. Quality filtered data were assembled using reference-based mapping using CLC Genomics Workbench version 12.0.3. Mapping tracks were used for variant calling and identification of the mutations. Haplotyping of the assembled genomes was carried out based on the 80% (Major allele) and 20% (Minor allele) frequency. These variants were verified and confirmed using Integrative Genomics Viewer (IGV) after manual curation. Further, Pango-Lineages were identified using the Pango-lineage classification system (https://cov-lineages.org/).

3. Results and discussion

Non-random selection of samples for sequencing and nonhomogeneous result reporting might lead to skewed results that may fail to represent actual circulating variants concern (VOCs) and interest (VOIs). Presently, a decrease in COVID-19 diagnostic testing is predicted to delay the tracking of SARS-CoV-2 variants (Vo et al., 2022). Significant delays may also occur between sampling, sequencing, and dissemination of results to public health authorities.

Identifying the circulating variants from water and wastewater samples can provide key information about the possible origin, transmission, and epidemiology of SARS-CoV-2 at the local, national, and regional levels. Owing to its speed, representativeness, and low cost, pooled wastewater monitoring can expedite the detection of circulating variants among communities (Wang et al., 2022). Against this backdrop, we tried to identify SARS-CoV-2 genomic variants from freshwater and wastewater samples during the first COVID-19 wave (Sep/Nov 2020) and from samples prior to the second wave in India (Feb 2021) (Table 1). Specific focus was on identifying spike protein mutations in SARS-CoV-2 genome assembly compared to the reference Wuhan/Hu-1/2019 (EPI_ISL_402125) variant.

The analysis showed a total of 34 mutations in the spike protein across four samples categorized into 23 types. The key mutations included Thr19Arg, Asp614Gly (D614G) in both river water and wastewater samples of Sep and Nov 2020 (Table S2). Likewise, main mutations comprising C21618G/Thr19Arg (T19R), T22917G/Leu452Arg (L452R), C22995A/Thr478Lys (T478K), A23403G/Asp614Gly (D614G), and C23604G/Pro681Arg (P681R) were noticed in the SARS-CoV-2 genomes from the samples collected in Feb 2021 (Table 2). In addition, deletions at 22029 (6 bp), 28248 (6 bp), and 28271 (1 bp) were identified in wastewater samples collected in Feb 2021. These mutations in the SARS-CoV-2 genome were found like that of VOC-21APR-02; B.1.617.2 lineage (Delta variant). Interestingly, these mutations were absent in the samples analyzed during the first wave but showed their presence (in Feb 2021) just before the devastating second wave of COVID-19, which started in late March 2021 in India. It is worth mentioning that the present study revealed the genetic signs of the B.1.617.2 (Delta variant) in wastewater earlier in Feb 2021, more than a month in advance of the first case of novel B.1.617.2 variant (clinical sample) in the month of Mar 2021 in Gujarat.

Our results are similar to Dharmadhikari et al. (2022), who performed MinION sequencing of SARS-CoV-2 fragments in wastewater of Pune, West India, from December 2020–March 2021. The results suggested 108 mutations in six samples grouped into 39 categories and were associated with Delta variant lineage in March-2021 clinical samples. Also, S:P1140del mutation was noticed in wastewater samples in December 2020, whereas reported in clinical samples in February 2021, demonstrating the utility of wastewater data in early detection.

Jahn et al. (2021), who performed deep shotgun sequencing of wastewater samples and found key mutations corresponding to the novel B.1.1.7 variant in Switzerland two weeks before the first COVID-19 case due to this variant among the population. Similarly, sequencing by the Houston Health Department detected six Omicron-associated mutations from seven sewer sheds in Houston, Texas, on November 29, 2021, while the city's first clinical confirmation of Omicron was announced on December 1, 2021 (Kirby et al., 2022). Likewise, Vo et al. (2022) performed Amplicon-based whole-genome sequencing (WGS) of SARS-CoV-2 in wastewater samples in Southern Nevada. Results showed the presence of Alpha (B.1.1.7) and Epsilon (B.1.429) lineages in December 2020, while clinical data failed to report them until January 2021. Surprisingly, high-throughput genome sequencing can detect spike mutations (S884F, G404V, and A372T) 4 and 5 months before their clinical detections (Alba Pérez-Cataluña et al., 2022). Therefore, genomic surveillance of wastewater can clearly provide early information about novel SARS-CoV-2 variants within communities, even before the first clinical sample analysis.

A number of international studies attempted to identify SARS-CoV-2 variants from wastewater (Table 3); For example, Nemudryi et al. (2020) identified 11 single-nucleotide variants (SNVs) in the assembled genome from wastewater samples in Bozeman, Montana (USA). These SNVs were distinct from the Wuhan-Hu-1/2019 reference sequence. Likewise, Landgraff et al. (2021) identified a near-complete SARS-CoV-2 consensus level genome sequence from untreated wastewater in Canada and reported many mutations designating the B.1.1.7 SARS-CoV-2 VOC in the sample.

Apart from the early information on VOCs in wastewater, it is important to note that we observed SARS-CoV-2 variants from the treated wastewater sample, indicating that the wastewater treatment plant (WWTP) unable to remove the virus. This finding was like those of Kumar et al. (2021a, 2021b), who reported SARS-CoV-2 RNA fragments in treated wastewater samples. Surprisingly, a low mutation rate was found in untreated wastewater compared to the treated sample in Feb 2021 (Table 2). This might be due to the high load of pollutants, resulting in high BOD, COD, TDS, TSS, etc., in untreated wastewater that might have affected the RNA quality (presence of impurities) and caused hindrance during the detection process via RT-PCR (Table S1). These pollutants may also cause PCR biases during the amplification process. Moreover, the damaged and fragmented genomes and impurities could be the possible reason that might have affected the precision of the analysis.

Table 2
Variants of the spike protein from fresh and wastewater samples: **a)** Sabarmati River (water sample dated 8th and Sep 22, 2020); **b)** Vinzol STP (untreated dated 19th and Nov 26, 2020); **c)** Vinzol STP (untreated dated February 8, 2021); **d)** Vinzol STP (treated dated February 8, 2021).

Sr No	Reference Position	Type	Length	Reference	Allele	Amino acid change	A_Sabarmati (8/22 Sept 2020)	E_Vinzol_Raw (19/26-Nov-2020)	P_Vinzol_Inlet (08-02-2021)	T_Vinzol_Outlet (08-02-2021)
1	21618	SNV	1	C	G	Thr19Arg	**109**	**2309**	**2968**	**2823**
2	21754	SNV	1	G	T	Trp64Cys	**10**	0	0	0
3	21757	INS		–	C	His 66-	**9**	0	0	0
4	21975	SNV	1	A	C	Asp138Ala	**8878**	0	0	0
5	21987	SNV	1	G	A	Gly142Asp	0	0	0	**1859**
6	22029	DEL	6	AGTTCA	–	Glu156_Arg158delinsGly	0	0	0	**1713**
7	22227	SNV	1	C	T	Ala222Val	0	0	**8**	**733**
8	22444	SNV	1	C	T	–	**8314**	0	0	0
9	22917	SNV	1	T	G	Leu452Arg	0	**5507**	**12103**	**5410**
10	22995	SNV	1	C	A	Thr478Lys	0	0	0	**3151**
11	23002	MNV	2	TA	GG	Cys480_Asn481delinsTrpAsp	**6**	0	0	0
12	23164	SNV	1	T	C	–	**17**	0	0	0
13	23403	SNV	1	A	G	Asp614Gly	**6833**	**5050**	**10612**	**5362**
14	23436	SNV	1	A	G	His625Arg	**2918**	0	0	0
15	23604	SNV	1	C	G	Pro681Arg	0	**11425**	**13271**	**7587**
16	23784	SNV	1	A	G	Tyr741Cys	**30**	0	0	0
17	23927	SNV	1	T	G	Tyr789Asp	0	**10**	0	0
18	24144	SNV	1	T	G	Leu861Trp	0	**22**	0	0
19	24173	SNV	1	G	T	Ala871Ser	**8623**	0	0	0
20	24410	SNV	1	G	A	Asp950Asn	0	0	0	**2427**
21	24532	SNV	1	A	G	–	**3475**	0	0	0
22	24775	SNV	1	A	T	Gln1071His	0	0	0	**285**
23	25101	DEL	1	A	–	Glu 1182-	0	**23**	0	0

Note: Apart from Spike protein, Vinzol STP treated WW sample dated Feb 8, 2021 showed mutations in N-Gene; Key mutations: Asp63Gly (D63G), Arg203Met (R203M), Asp377Tyr (D377Y). Where, SNV: single nucleotide variant; MNV: multi-nucleotide variants; INS: insertion; DEL: deletion.

Overall, the genomic surveillance of SARS-CoV-2 variants in wastewater samples offers the information of circulating novel variants and their cryptic transmission in advance with the following advantages:

a) It is useful for detecting and identifying VOCs, variants of interest (VOIs), and variants under investigation (VUIs) within a population.
b) A continuous and large-scale time-series monitoring of wastewater can identify disease outbreaks and clustering of VOCs, VOIs & VUIs, and explain their genesis, virulence, transmission, and spread within a population.
c) It can give more detailed and less biased data as it covers a broader population, whereas clinical samples only represent a subset of those who went through sequencing tests.
d) Wastewater sequencing data can also reveal genomic variants which are not reported as dominant (low frequencies) in clinical data (Pérez-Cataluña et al., 2022).
e) It can give information about novel muations that are not previously described/reported (Pérez-Cataluña et al., 2022).
f) It can help in identifying regions with a greater prevalence of the virus/variants in circulation among populations which may help in zoning the city. This data can further be used to help with non-pharmaceutical interventions (NPIs).
g) It can help in assessing the success of containment and the efficacy of NPIs
h) This approach is comparatively less time-consuming, low budget, and less manpower requiring than large-scale clinical testing and sequencing.

Although among the primary goals of this work is to show the value of wastewater sampling in health protection, there are challenges in SARS-CoV-2 genomic surveillance**.** For example, enrichment and concentration are needed for wastewater samples because SARS-CoV-2 concentration can be low, resulting in potentially damaged and fragmented RNA. Further, sample collection timing, methods, and intervals are critical for optimal surveillance, some of which have not been optimized. Physicochemical phenomena in wastewater can lead to false-positive and negative signals, and primer biases and sensitivity issues exist. Poor amplification of target amplicons and partial genome coverage are also possible and false negatives in variants with subtle mutations.

Despite such reservations, work here shows the huge value of wastewater for VOC identification and early detection, which grossly overweighs any limitations, and, in fact, such limitations will diminish as more information and methods are developed. In our case, early warning data was not available early enough, but we suggest that our approaches be considered on a wide scale as part of the global health protection infrastructure in the future.

4. Conclusion

Genomic surveillance of wastewater enables researchers to identify recent introductions of SARS-CoV-2 lineages prior to their detection by local clinical sequencing. All along, the monitoring and presence of SARS-CoV-2 variants in wastewater offer a better picture of the dominant variant, transmission, and epidemiology. In the present study, a total of 34 mutations in the spike protein across four samples were noticed, categorized into 23 types. The study concludes that this approach is not only beneficial for detecting and identifying VOCs, VUIs, transmission, and epidemiology of SARS-CoV-2 but also aids in assuring adequate and resilient public health responses. The study concludes that wastewater monitoring for VOCs using high-throughput sequencing can provide more timely surveillance data than clinical sequencing data.

Author Contribution

Madhvi Joshi: Supervision, Conceptualization, Visualization, Data interpretation, Writing -review and editing, Project administration. **Manish Kumar**: Supervision, Conceptualization, Visualization, Data interpretation, Writing -review and editing, Project administration. **Vaibhav Shrivastava**: Data interpretation, Writing -original draft and editing. **Dinesh Kumar**: Data interpretation, Writing -original draft and editing. **Dalip Singh Rathore**: Methodology, sample collection and processing. **Ramesh Pandit**: Data interpretation, Writing -review and editing. **David W. Graham**: Writing -review and editing. **Chaitanya G.**

Table 3
Detection of SARS-CoV-2 variant of concerns (VOCs) in environmental samples employing different molecular approaches.

Country	Time	Sample type	Approach	Key Mutations	SARS-CoV-2 variant	Reference
Ahmedabad, India	Sep and Nov 2020; Feb 2021	River water and wastewater	cDNA synthesis/Sequencing using Ion GeneStudio S5 Plus	Spike protein: T19R, L452R, T478K, D614G, & P681R and deletions at 22029 (6 bp), 28248 (6 bp), & 28271 (1 bp)	Delta (B.1.617.2)	*Present Study*
Queensland, Australia	Aug 30, 2021 to September 1, 2021	Wastewater	cDNA synthesis/Tiling amplicon-based sequencing technology (ATOPlex)	a) Sample 1: 5 AA substitution on the spike protein (F342L, I358M, S359R, S399P, & K417N) b) Sample 2.24 mutations on ORF1a, ORF1b, ORF3a, ORF7a, ORF8, and spike protein c) Sample 3.16 AA mutations on ORF1a and ORF1b	a) Beta b) Beta c) Delta	Wang et al. (2020)
New York City (NYC), USA	Jan to June 2021 (fort weekly)	Wastewater	cDNA synthesis, iSeq/MiSeq sequencing (Targeted sequencing)	Q493K, Q498Y, E484A, and T572N	Omicron (B.1.1.529)	Smyth et al. (2022)
California, USA	Nov 25 and Nov 30, 2021	Wastewater	Mutation-specific reverse transcription–polymerase chain reaction (RT-PCR) and sequencing	delHV69–70, del 143–145	Omicron (B.1.1.529)	Kirby et al. (2022)
Italy	Nov 11 to Dec 25, 2021	Sewage	Nested RT-PCR amplification/ cDNA synthesis/Sanger sequencing	H655Y, N679K and P681H	Omicron (B.1.1.529)	La Rosa et al. (2022)
Southern Nevada, USA	Dec 2020	Wastewater	cDNA synthesis/Amplicon-based whole genome sequencing (WGS); Illumina NextSeq 500 sequencer	N501Y, ΔH69/ΔV70, and A570D mutations; Spike Y144 deletion and SNVs at P681H, T716I, and S982A	Alpha (B.1.1.7)	Vo et al. (2022)
20 European countries	Mar 10 to Mar 30, 2021	Sewage	cDNA synthesis/ library preparation using the Ion AmpliSeq SARS-CoV-2 Research Panel	a) D614G was most abundant, followed by: P681H, T716I, A570D, S982A, H69del, Y144del, D1118H, N501Y, K417N, E484K b) Only six out of the 27 AA mutations (i.e. D1118H, D614G, H69del, N501Y, P681H, S982A, and T716I) were present in all the samples c) A570D and Y144del were identified in 53 samples.	Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2), and Gamma (P.1)	Agrawal et al. (2022)
Pune, India	Dec 2020 to Mar 2021	Wastewater	cDNA synthesis/nanopore library preparation (MinION sequencing)	P1140del, L452R E484Q, D614G; Q1071H, C480R, D950N, N801, P681R	Delta (B.1.617.1)	Dharmadhikari et al. (2022)
Spain	Apr 2020 to Jan 2021	Sewage (76 samples)	cDNA synthesis/Illumina MiSeq platform by paired-end reads (2 × 200)	A total of 627 nucleotide substitutions and 20 deletions. a) During first and second wave: 8 deletions detected, with 5 of them in the (Δ21–23, Δ82–84, Δ84–86, Δ141–143, and Δ682); one in the spike glycoprotein (Δ385); and two in the ORF3a (Δ80 and Δ11–20). b) In third wave: deletion in spike glycoprotein (Δ69/70 and Δ144	Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and others	Pérez-Cataluña et al. (2022)
Canada	Jan 26, 2020	Wastewater	cDNA and Amplicon Preparation/ Nanopore Library Preparation and Sequencing	a) Mutations in spike gene N501Y, 570 S, P681H and Orf8 (Q27*) b) A deletion of 6 nucleotides in the spike gene (21765–21770 nt)	Alpha (B.1.1.7)	Landgraff et al. (2021)

Joshi: Supervision, Visualization, Data interpretation, Writing -review and editing, Project administration.

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

The raw sequencing data have been deposited in the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under Bio-project accession number PRJNA736211. The SARS-CoV-2 Genomes were submitted to Global Initiative on Sharing All Influenza Data (GISAID) with accession numbers (EPI_ISL_2484893, EPI_ISL_2484894, EPI_ISL_2484895, EPI_ISL_2484896, EPI_ISL_2484897, EPI_ISL_2484898, and EPI_ISL_2484899).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119757.

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