

Original Article

Wastewater-based genomic surveillance of SARS-CoV-2 in vulnerable communities in Mumbai

Pratibha Prakash Kadam, Tejal Mistry, Nerges Mistry & Kayzad Soli Nilgiriwala

Department of Tuberculosis, The Foundation for Medical Research, Mumbai, India

Received February 16, 2024; Accepted August 30, 2024; Ahead of print January 8, 2025; Published ***** **, 2024

Background & objectives: The global impact of COVID-19, with over 45 million cases and 533,300 deaths in India alone, necessitates effective surveillance methods. Traditional approaches face challenges in detecting pre-symptomatic and asymptomatic cases, prompting the exploration of wastewater-based epidemiology (WBE). This study focuses on Mumbai's vulnerable slums, aiming to assess the potential of WBE as an alternative surveillance method.

Methods: Genomic surveillance of SARS-CoV-2 was conducted in Mumbai's vulnerable settings (slums) for 11 months (August 2022 to June 2023). Wastewater samples from open drains and sewage treatment plants were correlated with reported COVID-19 cases in the city. Early detection of emerging viral variants and seasonal variations in viral load were explored.

Results: Correlations were identified between wastewater samples and reported COVID-19 cases in Mumbai's vulnerable slums, with early detection occurring three weeks before clinical diagnoses, underscoring the potential utility of WBE. Genomic sequencing provided insights into the viral variants, identifying shifts in predominant variants. Seasonal variations showed higher viral concentrations in summer and monsoon, potentially associated with accelerated droplet evaporation in early summer and droplet-based transmission during mid-summer and monsoon.

Interpretation & conclusions: Wastewater-based epidemiology emerges as a cost-effective and rapid early warning system, providing crucial insights into virus behaviour and evolution. Particularly significant for countries like India, WBE aids in outbreak monitoring and targeted interventions. The global integration of wastewater surveillance emphasizes its importance in comprehensive pandemic monitoring, establishing it as an integral component of public health strategies worldwide.

Key words COVID-19 - early detection - genomic surveillance - open drains - SARS-CoV-2 - seasonal variations - viral variants - wastewater-based epidemiology (WBE)

The global impact of the COVID-19 pandemic caused by SARS-CoV-2 has been profound, affecting nations, including India, with over 45 million cases and 533,300 deaths¹. Understanding the distinct waves of the

disease is crucial for an effective response². Traditional methods focus on symptomatic cases, leaving pre-symptomatic and asymptomatic infections undetected^{3,4}, casting doubt on solely relying on clinical surveys⁵.

Research has revealed that SARS-CoV-2 is excreted in various bodily fluids, including saliva, sputum, and faeces, introducing the virus into sewage water⁶⁻⁸. Analyzing wastewater becomes pivotal due to high viral loads in the gastrointestinal tract, supplementing respiratory-based testing^{8,9}. Wastewater-based epidemiology (WBE) has gained recognition globally, offering real-time insights into COVID-19 transmission¹⁰. Wastewater-based epidemiology enables early detection of SARS-CoV-2 RNA, often preceding clinical cases, proving effective even in low-prevalence scenarios, serving as a sensitive tool for monitoring virus circulation and detecting early stages of outbreaks^{3,6,11}.

In India, only Ahmedabad, Bangalore, Jaipur, Chennai, and Pune conducted sequencing-based SARS-CoV-2 surveillance^{5,10,12-14}. For instance, the presence of B.1.617.2 in Jaipur in early March 2021 correlated with local clinical patient data¹². However, Mumbai City relied solely on RT-PCR-based wastewater surveillance, lacking active sequencing-based surveillance^{15,16}. This study aimed to fill the gap through genomic surveillance in Mumbai's densely populated slums. Over 11 months, genomic surveillance of drains and sewage revealed temporal variations in SARS-CoV-2 RNA concentration, correlating with active COVID-19 cases and enabling early detection. The study identified predominant variants, advancing understanding of the virus' behaviour and evolution within communities. This approach enhances public health measures in densely populated and resource-limited settings, contributing to global efforts to control and mitigate the impact of the ongoing pandemic.

Materials & Methods

Wastewater sample collection and RNA isolation: Wastewater samples were systematically collected twice weekly from eight open drain sites in Mumbai's slums [Dharavi: (D1, D2, D3), Kherwadi: (K4, K5), Behrampada: (B6, B7), Siddharth Nagar: (S8)] between August 17, 2022 and June 19, 2023. This was done with permission from the Municipal Corporation of Greater Mumbai (MCGM) and following guidance from the National Institute of Virology's (NIV) polio surveillance programme. The collection sites were selected based on major slum locations in consultation with MCGM. To expand the analysis citywide, two new collection sites at sewage treatment plants (STPs) – Bandra STP and Worli STP – were introduced in March 2023. The Institutional Research Ethics

Committee granted a waiver from ethics approval, as the study did not directly involve human participants. Additionally, the Institutional Biosafety Committee approved handling the live virus.

Sampling was conducted using 500 ml polypropylene bottles, with 400 ml collected between 8 AM and 11 AM to ensure efficient recovery. After sealing, bottles were wiped with 70% ethanol, placed in ziplock bags, and stored in an ice-packed cooler. Samples were processed within 24 h at a Biosafety Level 2 facility to obtain near real-time information on viral concentrations in sewage. Following pasteurization at 60°C for 1 h, 45 ml of wastewater (in duplicates) was centrifuged at 5,000 x g for 10 min at 4°C. The 40 ml supernatant was filtered first through two Whatman Grade 1 filter papers and then through a 0.22 µm PES membrane filter. The filtrate solution was combined with 3.2 g of PEG (8,000 MW) and 0.68 g of NaCl (1.7%) in a sterile 50 ml falcon bottle and incubated for 16 h at 48°C until PEG completely dissolved (SOP provided by Science and Engineering Research Board-SERB, <https://serb.gov.in/>).

After PEG dissolution, the solution was centrifuged at 10,000 rpm for 30 min at 4°C. Following supernatant removal, 140 µl of elution (TE) buffer was added to resuspend the pellets; one set of resuspended pellets was stored at -80°C for future use. The solution was vortexed to dissolve the pellets, and viral RNA was isolated using the QiaAmp viral RNA mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol.

SARS-CoV-2 RT-qPCR detection: Real-time quantitative PCR (RT-qPCR) was conducted using the Bio-Rad CFX96 system (Bio-Rad, California, USA) and GenePath Dx CoViDx One v2.1.1TK kit (GenePath Diagnostics), targeting SARS-CoV-2 specific genes (N, RdRp, E). The kit provided qualitative outcomes, and viral load quantification was done using the COVID-19 viral load Calculation tool (RUO). Each sample was tested in duplicate to reduce false negatives, with any replicate positive for at least two genes considered positive. Tap water served as the negative control.

SARS-CoV-2 genome amplification and sequencing: Positive samples with Ct values lower than 35-40 (before February 2023 <35, after was <40) were sequenced either by Illumina (n=38) or OxfordNanopore (n=85) sequencing. Pooled libraries were prepared using the Illumina COVIDSeq protocol for Illumina sequencing. cDNA was amplified using

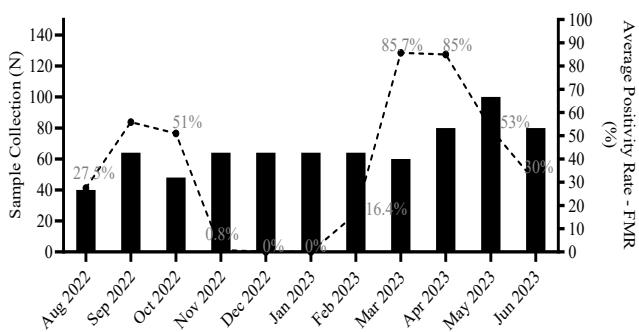


Fig. 1. Monthly average positivity rate (%) among collected wastewater samples.

ARTIC primers in two pools, followed by sequencing on the NextSeq™ 2000 platform (Illumina, California) with 116 bp read length.

Nanopore sequencing used LunaScript RT SuperMix Kit (New England Biolabs) for cDNA synthesis and MIDNIGHT primers for amplification. Barcoding and adaptor ligation followed the rapid MIDNIGHT Protocol, and sequencing was performed on the MinION MK1B with the SpotON flow cell.

Early warning SARS-CoV-2 detection strategy: A correlation analysis between viral RNA concentrations in wastewater and positive cases in the city was done to generate an early warning for impending SARS-CoV-2 waves.

Bioinformatics pipeline and analyses: Wastewater samples comprised a mix of actively circulating (sub-) variants. The raw reads were aligned to the SARS-CoV-2 reference genome (NC_045512.2) using the Burrows-Wheeler Alignment tool (BWA) MEM¹⁷. Coverage statistics were obtained using SAM tools¹⁸ and primer trimming for Illumina sequenced samples was performed with iVar¹⁹. Aligned reads were used for single nucleotide variants (SNVs) and INDELs calling using BCFtools¹⁸.

For lineage abundance prediction, a deconvolution matrix was generated using Freyja²⁰—a bioinformatics pipeline for estimating lineage abundance from wastewater. Freyja utilizes SNV frequency and sequencing depth to estimate accurate lineage abundance within samples.

For the comparison of viral load across different seasons, the specific months were selected based on the weather patterns of the current research year. The monsoon periods were defined as August 17 to October 27, 2022, and June 19 to June 28, 2023. The winter

period extended from November 2022 to February 2023, while the summer period was from March 14 to June 14, 2023. Statistical analyses and graph plotting were performed using GraphPad Prism version 9.1.2 (<https://www.graphpad.com/features>).

Results

Sample collection overview and positivity rate: We analysed SARS-CoV-2 RNA variation in wastewater samples over 11 months (August 2022 to June 2023) to assess the pandemic's status in vulnerable settings of Mumbai. Among the 728 samples processed (672 from open drains and 56 from STPs), 292 tested positive for at least two out of three targeted genes (E-gene, N-gene and RdRp). No SARS-CoV-2 genes were detected in negative control samples. Positive samples' Ct values ranged between 30 and 40. The average positivity rate among all the samples collected during the study period was determined to be 36.5 per cent.

The calculation of the average positivity rate (%) for the wastewater samples collected is depicted below:

$$\text{Average Positivity Rate (\%)} = \frac{\text{total number of positive samples per time point} * 100}{\text{total number of collection sites per time point}}$$

A gradual decrease in positivity rate occurred from Oct 2022, with no (very low in November 2022) positivity between November 2022 and January 2023. The positivity rate in the samples increased from February 2023 to April 2023, followed by a decline from May 2023 to June 2023 (Fig. 1).

A bar plot analysis (Supplementary Fig. 1) illustrated the distribution of viral load (number of copies) across different sites. Siddharth Nagar open drains exhibited the highest average viral load (mean: 4,585 copies/ml), followed by Kherwadi (mean: 2,440 copies/ml). Among the STPs, Worli STP showed a high viral load (mean: 3,358 copies/ml). A paired t-test revealed no significant difference ($P=0.53$) in viral load between STPs and open drains from the 57th time point (Supplementary Fig. 2).

Temporal variation in SARS-CoV-2 RNA concentration and early warning detection: We compared the average positivity rate (%) of samples to Mumbai's COVID-19 case load during surveillance. A temporal trend analysis (Fig. 2) correlated SARS-CoV-2 RNA in wastewater with Mumbai's positive cases. Viral load peaked twice during the SARS-CoV-2 waves in August 2022 and end of February 2023. The peak viral load coincided

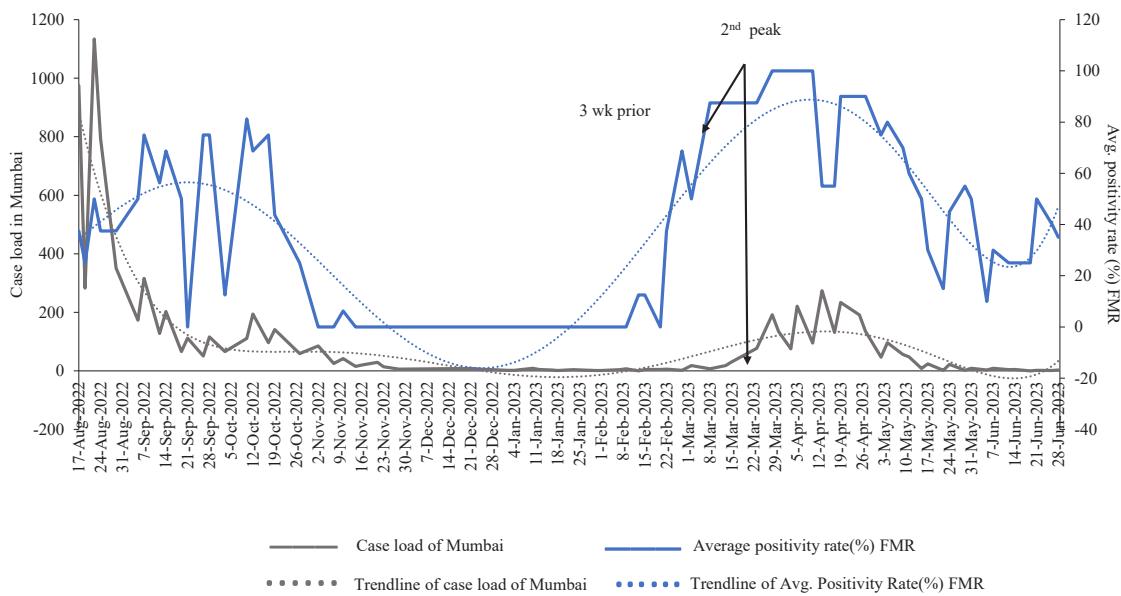


Fig. 2. Trend of COVID-19 case load in Mumbai and positivity rate (%) in wastewater samples processed at The Foundation for Medical Research.

Table I. Pre-dominant lineages determined based on sequencing (August 2022 – June 2023)

Month, yr	No. of samples sequenced (n=103)	Pre-dominant lineages
August, 2022	12	BA.2.75
September, 2022	30	BA.2.75, BA.2.75*
October, 2022	2	BA.2.10, XBB.2
November, 2022	0	-
December, 2022	0	-
January, 2023	0	-
February, 2023	12	XBB.1.16, XBB.1.16.1
March, 2023	27	XBB.1.5*
April, 2023	6	XBB.1.16.1, XBB.1.16
May, 2023	12	XBB.1.16*, BA.2*
June, 2023	2	BA.2*, XAS, BA.2.65, BA.4*

with the rise and decline of positive cases in Mumbai. From November 2022 to the second week of February 2023, no positive samples were found, aligning with Mumbai's trendline of positive cases. A rise in viral load from February 24, 2023, preceded the surge in Mumbai's cases by around 2-3 wk. Mumbai's positive cases increased until May 24, 2023, and then declined in June 2023. Hence, the detection of SARS-CoV-2

RNA in wastewater signalled the early stages of local outbreaks.

Sequencing statistics: Of the collected samples, 123 (17%) were sequenced by either Illumina or Nanopore sequencing. Statistics and lineage abundance were determined for 103 samples (Illumina: n=38; Nanopore: n=65). Sequenced reads ranged from 0.001 to 1.8 million, with a median sequence coverage of 209x. Genome coverage varied from 6 per cent to 98 per cent (Supplementary Table I).

Detection of variants: In August 2022, BA.2.75 and its sub-variants predominated (70%) among other Omicron sub-variants. BA.2.75 dominance continued in September 2022 (80%), alongside BA.2.10 (5%) and the recombinant variant XBB.1* and XBB.2* (8%). From October 2022, fewer samples were sequenced, revealing XBB.2* and other Omicron sub-variants (Table I and Fig. 3).

During the second peak observed in this study between February and May 2023, XBB.1.16.1 (25%) and XBB.1.16 (15%) were dominant, followed by various sub-variants. These trends continued through May 2023, showing fluctuations in dominant variants (Supplementary Table II). All the variants for each sample are provided in the supplementary table II. Comparing variants from sewage drains and STPs revealed no differences (Supplementary Table III).

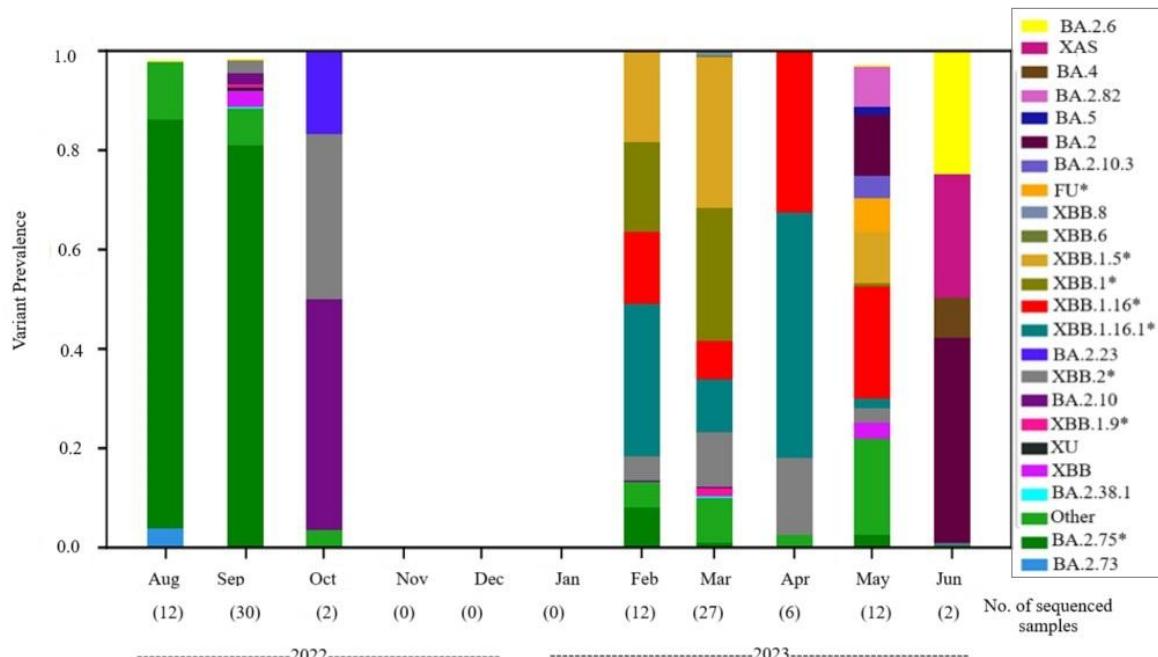


Fig. 3. SARS-CoV-2 lineage distribution in wastewater samples from August 2022 – June 2023 was predicted using Freyja (<https://github.com/andersen-lab/Freyja>).

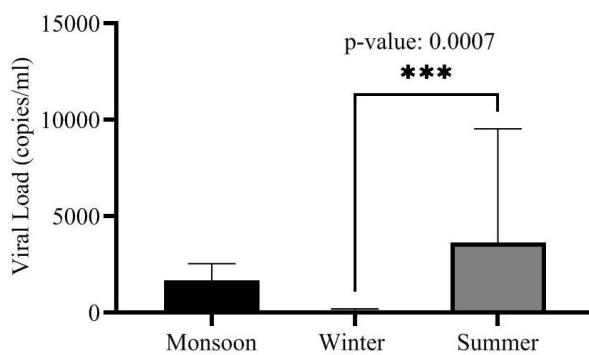


Fig. 4. Comparison of seasonal change on mean viral load (copies/ml).

Impact of seasonal change on viral load: We conducted a comparative analysis of viral load concentrations across various seasons (monsoon, winter and summer) to understand the role of seasonal changes in facilitating transmission. The average viral load in monsoon, winter and summer was determined to be 1,695 copies/ml, 72.5 copies/ml and 3,657 copies/ml, respectively. Summer exhibited a notably high average viral load (after excluding one outlier data point). One-way ANOVA test for viral load variations among different seasons showed statistically significant differences between viral loads of winter and summer ($P=0.0007$) (Fig. 4). Further, seasonal viral load was compared for each open drain site using one-way ANOVA, and

for STPs, a t-test was used as the viral load was only available for the monsoon and summer seasons (Table II). All the open drains showed significant differences ($P\leq 0.0001$) in seasonal viral loads except Siddharth Nagar (S8) which had zero viral load in winter ($P=0.1$). The viral load difference between monsoon and summer for both the STPs was non-significant ($P=0.29, 0.15$). Winter and summer showed significant differences in viral load at all sites except S8. The viral load between monsoon and summer was significant for all sites except Dharavi (D2) and S8 (Table II).

Discussion

This study underscores the significance of WBE for monitoring SARS-CoV-2 in Mumbai's urban slums. By showing a temporal correlation between viral loads in wastewater and city-wide COVID-19 case trends, with early detection three weeks prior to detection of clinical cases, WBE proves to be a cost-effective tool for early outbreak detection and public health intervention²¹. Additionally, genomic sequencing of wastewater samples helps track emerging variants, providing insights into viral evolution and transmission dynamics. These findings emphasize WBE's value in public health response, particularly in resource-limited settings, making it vital for pandemic monitoring and preparedness.

Table II. Statistical comparison of seasonal viral load (copies/ml) across all sample collection sites

Locations	Mean Monsoon	Mean Winter	Mean Summer	Monsoon vs. Winter	Monsoon vs. Summer	Winter vs. Summer	P value
D1	1314	136	2422	*	*	****	<0.0001
D2	1674	71.7	3720	ns	*	****	<0.0001
D3	1063	71.2	1924	*	ns	****	<0.0001
K4	2084	36.5	2786	***	ns	****	<0.0001
K5	3304	173	4256	**	ns	****	<0.0001
B6	1425	28.6	1712	****	ns	****	<0.0001
B7	1338	71.2	1506	****	ns	****	<0.0001
S8	1274	0	13227	ns	ns	ns	0.1
T-test							
B9	684.2	-	2901	-	ns	-	0.29
L10	766.8	-	3790	-	ns	-	0.15

D1, Dharavi-1; D2, Dharavi-2; D3, Dharavi-3; K4, Kherwadi-4; K5, Kherwadi-5; B6, Bandra-6; B7, Bandra-7; S8, Siddharth Nagar-8
Monsoon: Aug-Oct 2022, June 19-June 28, 2023; Winter: Nov-Feb 2022; Summer: March-June 14, 2023

Our longitudinal study on SARS-CoV-2 viral concentration in Mumbai's vulnerable areas indicates early shedding in asymptomatic or non-reporting patients. Wastewater surveillance, complementing clinical testing, effectively predicts COVID-19 trends²². Over 11 months, we analysed 728 samples from open drains and sewage treatment plants (STPs), with 292 testing positive via RT-PCR, showing higher sensitivity for the E-gene. Significant viral load variations were observed among sites, with Siddharth Nagar-8 and Worli STP displaying the highest loads. No notable difference was found between open drain and untreated STP wastewater, affirming sampling consistency and data reliability (Supplementary Fig. 3).

The study revealed dynamic changes in SARS-CoV-2 lineages. BA.2.75 dominated from August-September 2022, shifting to XBB in October 2022. A temporary absence of positive samples from November 2022 to January 2023 was followed by a resurgence in February 2023 with XBB.1.16. Viral loads increased in Mar 2023, with a decline in XBB.1.16 and XBB.1.16.1 dominance by May 2023. Mixed sub-lineages were noted in later months, showcasing WBE's impartial overview of viral diversity²³. Our findings align with a similar study across Maharashtra, corroborating the prevalence of BA.2.75 and XBB.1.16 during comparable periods²⁴. The SARS-CoV-2 lineages showed temporal but not geographical variation, validated against city-wide clinical sample data and the GISAID database, underscoring the Freyja tool's effectiveness²⁰ for identifying mixed SARS-CoV-2 variants.

Understanding the impact of seasonal changes on SARS-CoV-2 transmission is crucial. Our study found significant seasonal differences in viral loads, with higher concentrations in summer (average 3,657 copies/ml) compared to winter ($P=0.0007$) (Fig. 4). SARS-CoV-2 primarily spreads through droplets larger than 10 μm ²⁵. Increased transmission in March-April 2023 could be due to higher temperatures and dry air, which promote droplet evaporation, allowing them to stay airborne longer²⁶. Conversely, high humidity in May 2023 and the monsoon season (June-September 2023) also influenced transmission patterns, with significant differences between summer and winter ($P<0.0001$) and marginally significant differences between monsoon and winter, likely due to rain diluting wastewater (Fig. 4).

Wastewater-based monitoring is a global strategy for predicting COVID-19 outbreaks. Lamba *et al*¹⁰ detected SARS-CoV-2 trends in wastewater 8-14 days before the emergence of clinical data. Our findings align globally, highlighting wastewater analysis for revealing viral patterns and detecting emerging variants. Despite dilution challenges, deep sequencing overcomes genome coverage limitations. Expertise in identifying new variants is essential for enhanced monitoring in India.

The global adoption of wastewater surveillance, from Europe to North America, Australia, and Asia, underscores its role in forewarning potential COVID-19 spikes. Similar to polio surveillance, monitoring SARS-CoV-2 through WBE and correlating it with local clinical data is crucial for tracking outbreaks and

providing a comprehensive view of the situation²⁷. Our study's correlation between wastewater viral trends and actual infection rates reflects a global sentiment, emphasizing the integration of WBE into pandemic monitoring frameworks. Real-time genomic surveillance is essential for understanding viral load patterns and identifying new variants, contributing to pandemic preparedness for future health crises.

Data availability: The genomic data produced in this research is accessible and downloadable through GISAID accession numbers (EPI_ISL_18216418 - EPI_ISL_18216453 and EPI_ISL_18226307 - EPI_ISL_18226373) on the GISAID repository (<https://gisaid.org/>).

Financial support & sponsorship: This study received funding from the Science and Engineering Research Board (SERB) (SERB approval No. CVD/2022/000008), which was allocated to The Foundation for Medical Research, Mumbai, India.

Conflicts of Interest: None.

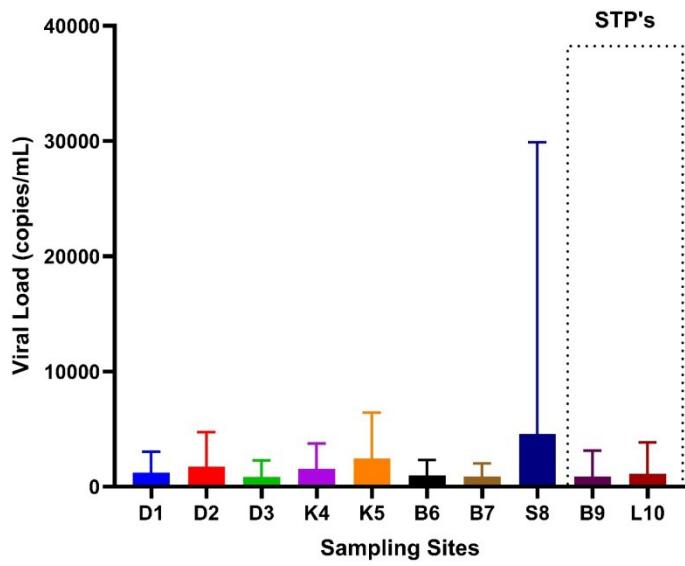
Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

References

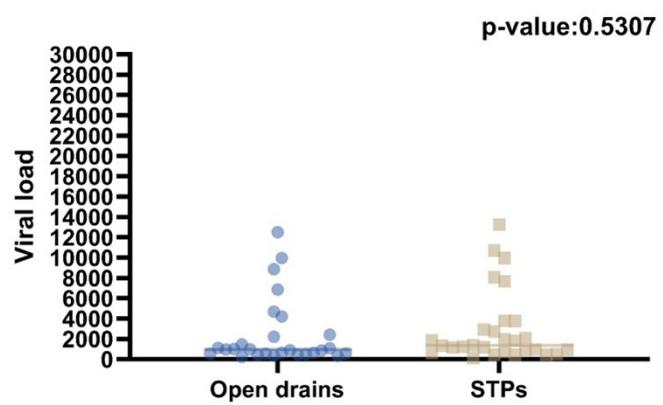
1. World Health Organization 2023. WHO COVID-19 dashboard. Available from: <https://covid19.who.int/region/searo/country/in>, accessed on February7, 2024.
2. Ayala A, Villalobos Dítrans P, Elorrieta F, Castillo C, Vargas C, Maddaleno M. Identification of COVID-19 waves: Considerations for research and policy. *Int J Environ Res Public Health* 2021; **18** : 11058.
3. Marceca F, Rocha Viegas L, Pregi N, Barbas MG, Hozbor D, Pecci A, *et al*. Pool strategy for surveillance testing of SARS-CoV-2. *Science Reviews - from the end of the World* 2021; **2** : 41-54.
4. Calvetti D, Hoover AP, Rose J, Somersalo E. Metapopulation network models for understanding, predicting, and managing the coronavirus disease COVID-19. *Frontiers in Physics* 2020; **8** : 261.
5. Kumar M, Joshi M, Patel AK, Joshi CG. Unravelling the early warning capability of wastewater surveillance for COVID-19: A temporal study on SARS-CoV-2 RNA detection and need for the escalation. *Environ Res* 2021; **196** : 110946.
6. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, *et al*. 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. *Sci Total Environ* 2020; **728** : 138764.
7. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: A systematic review and meta-analysis. *Lancet Microbe* 2021; **2** : e13-e22.
8. Atoui A, Cordevant C, Chesnot T, Gassilloud B. SARS-CoV-2 in the environment: Contamination routes, detection methods, persistence and removal in wastewater treatment plants. *Sci Total Environ* 2023; **881** : 163453.
9. Zhang Y, Cen M, Hu M, Du L, Hu W, Kim JJ, *et al*. Prevalence and persistent shedding of fecal SARS-CoV-2 RNA in patients with COVID-19 infection: A systematic review and meta-analysis. *Clin Transl Gastroenterol* 2021; **12** : e00343.
10. Lamba S, Ganeshan S, Daroch N, Paul K, Joshi SG, Sreenivas D, *et al*. SARS-CoV-2 infection dynamics and genomic surveillance to detect variants in wastewater - a longitudinal study in Bengaluru, India. *Lancet Reg Health Southeast Asia* 2023; **11** : 100151.
11. Bivins A, North D, Ahmad A, Ahmed W, Alm E, Been F, *et al*. Wastewater-based epidemiology: Global collaborative to maximize contributions in the fight against COVID-19. *Environ Sci Technol* 2020; **54** : 7754-7.
12. Nag A. Monitoring of SARS-CoV-2 variants by wastewater-based surveillance as a sustainable and pragmatic approach – a case study of Jaipur (India). *Water Switz* 2022; **14** : 1-19.
13. Chakraborty P, Pasupuleti M, Jai Shankar MR, Bharat GK, Krishnasamy S, Dasgupta SC, *et al*. First surveillance of SARS-CoV-2 and organic tracers in community wastewater during post lockdown in Chennai, South India: Methods, occurrence and concurrence. *Sci Total Environ* 2021; **778** : 146252.
14. Dharmadhikari T, Rajput V, Yadav R, Boargaonkar R, Patil D, Kale S, *et al*. High throughput sequencing based direct detection of SARS-CoV-2 fragments in wastewater of Pune, West India. *Sci Total Environ* 2022; **807** : 151038.
15. Wani H, Menon S, Desai D, D' Souza N, Bhathena Z, Desai N, *et al*. Wastewater-based epidemiology of SARS-CoV-2: assessing prevalence and correlation with clinical cases. *Food Environ Virol* 2023; **15** : 131-43.
16. Sharma DK, Nalavade UP, Kalgutkar K, Gupta N, Deshpande JM. SARS-CoV-2 detection in sewage samples: Standardization of method & preliminary observations. *Indian J Med Res* 2021; **153** : 159-65.
17. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009; **25** : 1754-60.
18. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, *et al*. Twelve years of SAM tools and BCF tools. *Gigascience* 2021; **10** : giab008.
19. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, De Jesus JG, Main BJ, *et al*. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol* 2019; **20** : 8.
20. Karthikeyan S, Levy JI, De Hoff P, Humphrey G, Birmingham A, Jepsen K, *et al*. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. *Nature* 2022; **609** : 101-8.

21. Ngwira LG, Sharma B, Shrestha KB, Dahal S, Tuladhar R, Manthalu G, et al. Cost of wastewater-based environmental surveillance for SARS-CoV-2: Evidence from pilot sites in Blantyre, Malawi and Kathmandu, Nepal. *PLOS Glob Public Health* 2022; 2 : e0001377.
22. Wu F, Zhang J, Xiao A, Gu X, Lee WL, Armas F, et al. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *mSystems* 2020; 5 : e00614-20.
23. Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res* 2020; 181 : 115942.
24. Matra S, Ghode H, Rajput V, Pramanik R, Malik V, Rathore D, et al. Wastewater surveillance of open drains for mapping the trajectory and succession of SARS-CoV-2 lineages in 23 class-I cities of Maharashtra State (India) during June 2022 to May 2023. *Research Square* 2024. DOI: 10.21203/rs.3.rs-4609404/v1.
25. La Rosa G, Fratini M, Della Libera S, Iaconelli M, Muscillo M. Viral infections acquired indoors through airborne, droplet or contact transmission. *Ann Ist Super Sanita* 2013; 49 : 124-32.
26. Kulkarni RD. Why is COVID-19 rising in summer? *Indian J Med Microbiol* 2021; 39 : 564.
27. Wilkinson AL, Diop OM, Jorba J, Gardner T, Snider CJ, Ahmed J. Surveillance to track progress toward polio eradication - worldwide, 2020-2021. *MMWR Morb Mortal Wkly Rep* 2022; 71 : 538-544.

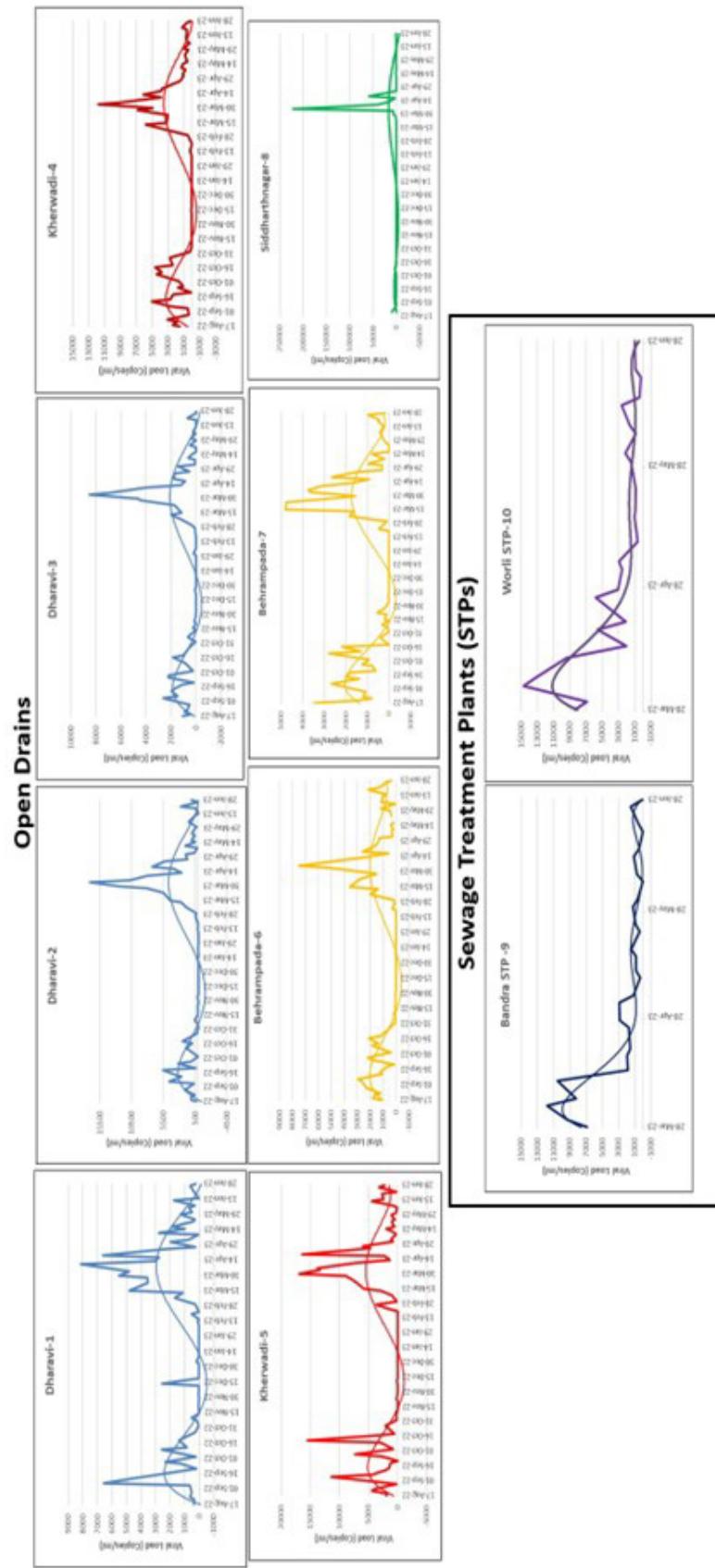
For correspondence: Dr Kayzad Soli Nilgiriwala, Department of Tuberculosis, The Foundation for Medical Research, Mumbai 400 018, Maharashtra, India
e-mail:fmr@fmrindia.org



Supplementary Fig.1. Distribution of SARS-CoV-2 Viral load per sampling sites (Open drains and STPs).



Supplementary Fig. 2. Statistical Analysis of open drains and STPs using paired end t-test for groups in GraphPad Prism.



Supplementary Fig. 3. Trendline of Viral load for each location (8 open drains & 2 STPs).

Supplementary Table I: All 103 sequenced sample genomic metadata

Sample Site	Sample name	Collection date	Ct value	Sequencing by	Total reads	Reads aligned	Average read length	Average depth (X)	Genome coverage %
Dharavi-1	FMR-D1-4a	24-08-2022	35.4	Illumina Miseq	1315600	588648	107.9	2124X	56.89
	FMR-D1-6b	05-09-2022	23.0	Illumina Miseq	1631198	1305146	106.6	4653X	64.86
	FMR-D1-7b	07-09-2022	21.3	Illumina Miseq	1213338	1212381	109.1	4423X	97.21
	FMR-D1-8a	12-09-2022	21.6	Illumina Miseq	897640	860868	109.3	3147X	88.2
	FMR-D1-9a	14-09-2022	34.0	Illumina Miseq	1166873	1110466	108.9	4044X	91.21
	FMR-D1-15a	10-10-2022	35.1	Nanopore MinION	40332	2023	590.6	40X	96.44
	FMR-D1-51a	27-02-2023	25.4	Nanopore MinION	55453	13302	404.1	180X	54.27
	FMR-D1-52a	02-03-2023	24.8	Nanopore MinION	31639	4468	343.5	51X	52.43
	FMR-D1-53b	08-03-2023	23.0	Nanopore MinION	46435	2536	340.2	29X	50.38
	FMR-D1-54a	13-03-2023	23.9	Nanopore MinION	28742	5830	387.3	76X	61
	FMR-D1-55a	15-03-2023	22.2	Nanopore MinION	34582	10358	341.1	118X	59.71
	FMR-D1-67b	02-05-2023	22.4	Nanopore MinION	5555	2234	400.5	30X	8.2
	FMR-D1-77b	05-06-2023	24.1	Nanopore MinION	11450	6782	412.8	94X	56.24
	FMR-D2-2b	19-08-2022	2.0	Nanopore MinION	3890	2481	334.8	28X	89.82
Dharavi-2	FMR-D2-10a	19-09-2022	32.5	Illumina Miseq	1503072	1052561	109.3	3847X	93.23
	FMR-D2-5b	29-08-2022	35.2	Illumina Miseq	1765744	1263383	108	4563X	42.66
	FMR-D2-7b	07-09-2022	33.3	Illumina Miseq	1239510	704725	109.2	2574X	95.33
	FMR-D2-8a	12-09-2022	22.1	Illumina Miseq	1185893	1097028	109.4	4339X	91.74
	FMR-D2-9b	14-09-2022	33.7	Illumina Miseq	1163873	1094666	109.2	3998X	89.74
	FMR-D2-12a	26-09-2022	33.2	Nanopore MinION	30805	2243	394.4	30X	95.17
	FMR-D2-50b	22-02-2023	24.4	Nanopore MinION	1397	204	314.5	2X	63.71
	FMR-D2-51a	27-02-2023	24.8	Nanopore MinION	13169	3679	344.9	42X	39.87
	FMR-D2-52a	02-03-2023	24.0	Nanopore MinION	36276	4713	405	64X	38.38
	FMR-D2-53b	08-03-2023	22.8	Nanopore MinION	29602	13884	413.8	192X	73.42
	FMR-D2-54a	13-03-2023	22.2	Nanopore MinION	36831	2986	393.8	39X	65.06
	FMR-D2-55a	15-03-2023	21.8	Nanopore MinION	58402	8726	385.2	112X	57.87
	FMR-D2-59b	03-04-2023	32.0	Nanopore MinION	9940	6386	464.5	99X	74.75
	FMR-D2-67a	02-05-2023	22.7	Nanopore MinION	7726	526	350.7	6X	13.83
	FMR-D2-77b	05-06-2023	24.3	Nanopore MinION	4380	1532	248.1	13X	41.55
Dharavi-3	FMR-D3-10a	19-09-2022	35.0	Illumina Miseq	1553333	1200152	109.1	4379X	90.16
	FMR-D3-1b	17-08-2022	34.3	Illumina Miseq	1508602	819755	108.6	5479X	46.18
	FMR-D3-6a	05-09-2022	21.7	Illumina Miseq	1325037	294399	107.8	1061X	52.21
	FMR-D3-7b	07-09-2022	22.1	Illumina Miseq	1135859	586412	109.1	2140X	90.73
	FMR-D3-8b	12-09-2022	34.0	Illumina Miseq	1254574	1097977	109.3	4013X	91.98
	FMR-D3-9a	14-09-2022	22.6	Illumina Miseq	1239199	1233673	109	4497X	89.6
	FMR-D3-12b	26-09-2022	34.5	Nanopore MinION	21485	2006	548.9	37X	90.62
	FMR-D3-51b	27-02-2023	25.8	Nanopore MinION	53729	1343	315.8	14X	61.85
	FMR-D3-53b	08-03-2023	23.1	Nanopore MinION	38649	2577	390.9	34X	54.8
	FMR-D3-54a	13-03-2023	35.9	Nanopore MinION	32712	6970	378.8	88X	46.89
	FMR-D3-55a	15-03-2023	23.3	Nanopore MinION	44482	1473	343.8	17X	52.31

Contd...

Sample Site	Sample name	Collection date	Ct value	Sequencing by	Total reads	Reads aligned	Average read length	Average depth (X)	Genome coverage %
Kherwadi-4	FMR-K4-1b	17-08-2022	22.1	Illumina Miseq	1144569	1036528	110	3715X	87
	FMR-K4-10a	19-09-2022	3.0	Illumina Miseq	1240945	1074531	109.1	3920X	92.61
	FMR-K4-2a	19-08-2022	34.2	Illumina Miseq	1188547	1104407	105.8	4205X	61.31
	FMR-K4-3b	22-08-2022	36.5	Illumina Miseq	1014488	884185	107	3164X	50.79
	FMR-K4-4a	24-08-2022	36.5	Illumina Miseq	1589981	610092	107.6	2195X	85.28
	FMR-K4-7a	07-09-2022	21.9	Illumina Miseq	1304828	1223011	107.9	4413X	48.17
	FMR-K4-8a	12-09-2022	21.8	Illumina Miseq	1110171	524589	109.1	1914X	92.85
	FMR-K4-9b	14-09-2022	22.7	Illumina Miseq	789271	753734	109.1	2750X	90
	FMR-K4-48a	15-02-2023	25.2	Nanopore MinION	4193	245	463.4	4X	49.1
	FMR-K4-51a	27-02-2023	24.1	Nanopore MinION	44918	10288	403.2	139X	66.89
	FMR-K4-52b	02-03-2023	23.6	Nanopore MinION	11912	3696	455.4	56X	43.27
	FMR-K4-53a	08-03-2023	22.2	Nanopore MinION	63099	8805	387.2	114X	57.23
	FMR-K4-54a	13-03-2023	33.1	Nanopore MinION	40924	14678	426.1	209X	66.35
	FMR-K4-55a	15-03-2023	22.1	Nanopore MinION	52274	8304	416.7	116X	69.97
Kherwadi-5	FMR-K4-59b	03-04-2023	32.0	Nanopore MinION	3078	417	429.4	6X	48.1
	FMR-K4-67a	02-05-2023	23.4	Nanopore MinION	5707	409	403	6X	9.95
	FMR-K4-76b	31-05-2023	24.0	Nanopore MinION	10575	6391	382.8	82X	49.76
	FMR-K5-10a	19-09-2022	22.8	Illumina Miseq	1378412	1126586	109	5024X	89.84
	FMR-K5-6a	05-09-2022	21.0	Illumina Miseq	1102878	1061216	107	3797X	67.28
	FMR-K5-7a	07-09-2022	22.4	Illumina Miseq	779904	702081	107.9	2533X	66.84
	FMR-K5-8a	12-09-2022	22.4	Illumina Miseq	1542010	663793	109.2	2424X	89.17
	FMR-K5-13b	28-09-2022	33.1	Nanopore MinION	42394	14724	418.1	206X	97.75
	FMR-K5-50b	22-02-2023	23.8	Nanopore MinION	1173	110	544.2	2X	53.02
	FMR-K5-51b	27-02-2023	21.9	Nanopore MinION	21265	4146	359	50X	42.02
	FMR-K5-52a	02-03-2023	25.7	Nanopore MinION	40812	1187	452.3	18X	56.32
	FMR-K5-53a	08-03-2023	23.7	Nanopore MinION	61452	6413	416.7	89X	61.3
	FMR-K5-54a	13-03-2023	22.3	Nanopore MinION	40881	6390	446.1	95X	54.95
	FMR-K5-55a	15-03-2023	22.0	Nanopore MinION	64061	14911	430.8	215X	58.76
	FMR-K5-76b	31-05-2023	24.8	Nanopore MinION	29087	8967	370.6	111X	57.91
Bhehrampada-6	FMR-B6-3a	22-08-2022	34.6	Illumina Miseq	1287713	1204244	110	4320X	93
	FMR-B6-6a	05-09-2022	21.8	Illumina Miseq	1429788	1109797	107.5	3990X	78.46
	FMR-B6-7b	07-09-2022	33.6	Illumina Miseq	1056282	842112	108.6	3058X	88.52
	FMR-B6-50a	22-02-2023	24.2	Nanopore MinION	1806	330	354.7	4X	73.66
	FMR-B6-51a	27-02-2023	25.2	Nanopore MinION	20611	6686	337.6	76X	44.01
	FMR-B6-52b	02-03-2023	24.4	Nanopore MinION	51388	6801	331.9	76X	76.91
	FMR-B6-53a	08-03-2023	23.1	Nanopore MinION	28775	9796	442.4	145X	58.5
	FMR-B6-54a	13-03-2023	23.6	Nanopore MinION	52699	15377	413.6	213X	44.82
	FMR-B6-55a	15-03-2023	22.1	Nanopore MinION	35528	10332	433.4	145X	68.29
	FMR-B6-60a	05-04-2023	32.9	Nanopore MinION	5479	1985	394.6	26X	69.92
Contd...	FMR-B6-67a	02-05-2023	23.2	Nanopore MinION	7291	365	351.9	4.3X	19.57
	FMR-B6-76b	31-05-2023	24.0	Nanopore MinION	8938	5300	346.3	61.4X	58.32

Sample Site	Sample name	Collection date	Ct value	Sequencing by	Total reads	Reads aligned	Average read length	Average depth (X)	Genome coverage %
Bhehrampada-7	FMR-B7-1c	17-08-2022	33.6	Illumina Miseq	999225	837769	110	3005X	90
	FMR-B7-6a	05-09-2022	22.3	Illumina Miseq	1070380	744848	107.2	2670X	59.43
	FMR-B7-7a	07-09-2022	22.1	Illumina Miseq	914310	881326	109.5	3227X	93.51
	FMR-B7-8b	12-09-2022	34.0	Illumina Miseq	1206039	1148898	109.4	4203X	92.13
	FMR-B7-9b	14-09-2022	22.8	Illumina Miseq	1087282	1039291	109.4	3802X	89.18
	FMR-B7-15b	10-10-2022	22.3	Nanopore MinION	38038	2765	513.5	47X	93.63
	FMR-B7-50a	22-02-2023	24.2	Nanopore MinION	1924	252	243.4	2X	63.96
	FMR-B7-51b	27-02-2023	24.5	Nanopore MinION	86788	15603	363.9	190X	58.55
	FMR-B7-52b	02-03-2023	25.7	Nanopore MinION	49729	13527	388.5	176X	46.27
	FMR-B7-53a	08-03-2023	23.2	Nanopore MinION	30359	12613	416.4	176X	63.21
Siddharth Nagar-8	FMR-B7-54a	13-03-2023	22.9	Nanopore MinION	27991	13189	474.6	209X	37.43
	FMR-B7-55a	15-03-2023	21.8	Nanopore MinION	44669	16273	413.9	225X	58.31
Bandra STP	FMR-B7-76a	31-05-2023	26.1	Nanopore MinION	8030	2900	379	37X	34.91
	FMR-S8-3a	22-08-2022	34.2	Illumina Miseq	1183100	1136446	110	4080X	90
	FMR-S8-4a	24-08-2022	34.4	Illumina Miseq	1620400	1121984	107.7	4041X	76.69
Love Grove STP	FMR-S8-60b	05-04-2023	28.2	Nanopore MinION	21429	17316	539.7	313X	81.54
	FMR-B9-59b	03-04-2023	33.0	Nanopore MinION	10399	6911	455.9	105X	62.33
	FMR-B9-67a	02-05-2023	23.9	Nanopore MinION	5234	352	376.9	4X	6.04
	FMR-B9-76a	31-05-2023	25.2	Nanopore MinION	16290	8449	406.1	115X	48.96
Love Grove STP	FMR-L10-60a	05-04-2023	32.6	Nanopore MinION	6291	1657	282.1	16X	63.65
	FMR-L10-67a	02-05-2023	23.0	Nanopore MinION	9657	797	450	12X	7.09
Love Grove STP	FMR-L10-76a	31-05-2023	23.9	Nanopore MinION	16956	9833	405.2	133X	47.23

Supplementary Table II: Detected SARS-CoV-2 Variants in the 103 samples

Sample Name	Collection date	Proportion	Abundance
FMR-B7-1c	17-08-2022	BA.2.75.2 BA.2.73 BA.2.75 BA.2.75.5 BA.2.74 BA.2.64 BA.2.9.4 BA.5.2.11 BN.2 BA.2.75.4 BL.1 BA.2.75.1 BA.2.9.1 BF.9	0.39959417 0.18120365 0.17876783 0.17876783 0.00970874 0.00637568 0.00483092 0.00322581 0.00281955 0.00203978 0.00140548 0.00140548 0.00116189 0.00113347
FMR-K4-1b	17-08-2022	BA.2.75.2 BL.1 BM.1.1 BA.2.74 BA.4.2 BA.2.75.4 BN.2 BA.2.10.4 BA.5.2.11 BA.2.9.1 BA.4.6.1 BA.2.66 BF.9 BA.2.48	0.44828400 0.31766000 0.16703290 0.02370870 0.00505679 0.00415196 0.00242653 0.00239808 0.00217549 0.00143609 0.00136017 0.00126149 0.00117601 0.00102564
FMR-S8-3a	22-08-2022	BL.1 BA.2.75.2 BA.2.38 BA.2.12 BA.2.17 BA.2.74 BA.2.9.4 BA.2.14 BA.2.63 BA.4.2 BA.2.37 BA.2.64 XU BA.2.58 BN.2 BA.2.70 BA.2.59 BA.2.29 BA.2.41 BA.2.9.1 BA.2.27 BF.9 BA.2.21 BA.3.1 BA.2.48 BA.2.12.2	0.42198600 0.34146296 0.03046837 0.03046837 0.02899200 0.01869970 0.01739130 0.01711880 0.01225720 0.00912168 0.00746269 0.00646092 0.00288864 0.00273437 0.00246778 0.00239977 0.00236072 0.00208452 0.00202716 0.00175159 0.00149309 0.00133914 0.00128653 0.00123982 0.00113080 0.00100799
FMR-B6-3a	22-08-2022	BL.1 BM.2 BA.2.75 BA.2.75.2 BA.2.74 BA.2.38 BA.2.37 BA.2.17 BA.4.2 BA.2.63 BA.2.66 BA.2.75.4 BA.2.9.4 BA.2.64 XN BA.2.76.1 BN.2 BA.2.9.3 BA.2.58 BA.2.41 BA.2.59 BF.9 BA.5.2.3 BA.4.6.1 BA.2.61 BA.2.30 BA.2.9.6 B.1.618 BA.4.1.7 BA.2.19 BA.2.3.8 BH.1 BF.11	0.44721200 0.13753182 0.10163469 0.08013178 0.04572970 0.02106408 0.02106408 0.02095810 0.01783860 0.01434770 0.01063270 0.00596817 0.00515109 0.00470685 0.00310937 0.00300661 0.00239206 0.00211416 0.00211020 0.00206513 0.00187477 0.00169300 0.00154560 0.00141176 0.00132236 0.00129962 0.00122750 0.00116369 0.00108434 0.00107296 0.00106169 0.00100338 0.00100150
FMR-D2-2b	19-08-2022	BA.2.75	1
FMR-B6-6a	05-09-2022	BA.2.75 BA.2.9.4 BA.2.74 BA.4.2 BA.5.2.2 BA.2.64 BA.2.53 BA.2.59 BA.3.1 XAG XAK BF.5 BA.2.3.15	0.88421126 0.02919247 0.02489930 0.01072393 0.00729929 0.00509614 0.00402955 0.00317628 0.00265322 0.00205719 0.00166113 0.00117537 0.00103931
FMR-B6-7b	07-09-2022	BA.2.75 BF.4 BF.9 B.1.1.528 B.1.160.13 BA.4.1.4	0.97148043 0.00468861 0.00354750 0.00210453 0.00193986 0.00136612
FMR-B7-6a	05-09-2022	BA.2.75 BA.2.64 BA.4.2 BA.4.1.2 BF.9 BA.2.9.1 BA.2.77 BG.2 BA.2.33 P.1.2	0.94112544 0.01252133 0.01044933 0.00437637 0.00198341 0.00194342 0.00161850 0.00142045 0.00139907 0.00130116
FMR-B7-7a	07-09-2022	BA.2.75 BA.2.74 BA.4.2 BA.2.21 BA.2.64 BA.2.3.8 BA.3.1 BA.2.9.1 BA.2.80	0.89720350 0.04659849 0.01179660 0.00777040 0.00311851 0.00230947 0.00220798 0.00207039 0.00105666
FMR-B7-8b	12-09-2022	BA.2.75 BA.2.38.1 BA.4.2 BA.2.9.1 BA.3.1	0.77868844 0.18991487 0.01252810 0.00124069 0.00114255
FMR-B7-9b	14-09-2022	BA.2.75 BA.2.74 BA.2.64 BA.2.21 BA.4.2 BA.5.2.3 BA.2.59 BA.3 XU BA.2.77 BA.2.66	0.89884198 0.03845953 0.01224481 0.01212120 0.00586206 0.00412141 0.00343053 0.00281751 0.00175489 0.00152439 0.00113766
FMR-D1-4a	24-08-2022	BA.2.75 BA.2.66 BA.4.2 BA.2.3.4 BA.2.53 BF.5 BA.5.2.3 BA.2.29 BA.2.77 BA.2.3.15 XAG B.1.618 BA.2.46 B.1.1.389 XW BA.2.59 BA.2.38.1	0.92100877 0.01865330 0.00969755 0.00386473 0.00384551 0.00332622 0.00328587 0.00227943 0.00178162 0.00176678 0.00170068 0.00146606 0.00138323 0.00136799 0.00128961 0.00128700 0.00100692
FMR-D1-6b	05-09-2022	BA.2.75 BA.2.21 BA.4.1.1 BG.1 BA.2.9.1 XAG AM.3 BA.5.2.5 BA.2.3.6 Q.2 BA.4.1.2	0.94977417 0.01491280 0.00218711 0.00212557 0.00183814 0.00158910 0.00149065 0.00142248 0.00126775 0.00108633 0.00107680

Contd...

Contd...

Sample Name	Collection date	Proportion	Abundance
FMR-D3-7b	07-09-2022	BA.2.75 BA.2.38.1 BA.2.74 BA.2.66 BA.4.2 BA.2.21 BA.2.64 XZ BA.5.2.3 BF.5 XAG XAH BA.2.46 BG.2 BA.2.3.18 BA.2.13.1 XU	0.86885973 0.02233010 0.02179840 0.01941750 0.01223062 0.00947867 0.00711572 0.00448943 0.00361488 0.00213966 0.00172329 0.00155823 0.00133333 0.00125565 0.00119332 0.00117555 0.00111162
FMR-D3-8b	12-09-2022	BA.2.75 BA.4.2 BA.2.74 BA.2.9.4 BA.3.1 BA.2.64 BA.2.9.3 BA.2.53 BA.2.59 BF.5 BA.5.2.2 BA.2.54 BG.2 BA.2.55 BA.2.77 BA.2.66 BA.2.68 BA.2.76	0.88382300 0.02420410 0.02170280 0.00625195 0.00561347 0.00521539 0.00507614 0.00405731 0.00247437 0.00189621 0.00171220 0.00152323 0.00129534 0.00127453 0.00115607 0.00110497 0.00109098 0.00102215
FMR-D3-9a	14-09-2022	BA.2.75 BA.2.74 BA.4.2 BA.2.64 BA.5.2.2 BA.3.1 XAJ BA.4.6 BA.2.19 BF.9 BA.2.53 BA.5.2.3 BA.2.66 BG.2 AV.1 AM.3	0.89488500 0.02664580 0.02350380 0.01478758 0.00179765 0.00165289 0.00159587 0.00154004 0.00151676 0.00147984 0.00142349 0.00128235 0.00115629 0.00111857 0.00104895 0.00103451
FMR-D3-10a	19-09-2022	BA.2.75 BA.2.74 BA.2.9.4 BA.4.2 BA.2.64 BA.2.12 BA.2.55 BA.5.1.7 XN BA.5.1.1 BG.2 XU BA.2.77 Q.8 B.1.618 BA.2.9.3	0.86236700 0.04951210 0.01988910 0.01333190 0.00794036 0.00222785 0.00209644 0.00203735 0.00192729 0.00187091 0.00179818 0.00137927 0.00131139 0.00118746 0.00109479 0.00109051
FMR-K4-2a	19-08-2022	BA.2.75 BA.2.73 BA.2.37 BA.2.74 BA.2.21 BA.2.64 BA.2.38.2 BA.2.53 BA.2.9.1 BA.3.1 BA.2.12.1 BA.2.66 B.1.632	0.60178569 0.28701299 0.02780310 0.02622950 0.01650530 0.00630199 0.00314984 0.00289452 0.00219929 0.00213577 0.00189925 0.00134409 0.00104439
FMR-K4-3b	22-08-2022	BA.2.75 BA.4.2 BA.5.2.3 BA.5.2.2 B.13 B.1.160.13 P.1.9 N.10 B.1.618	0.97175100 0.00436130 0.00391116 0.00203547 0.00190186 0.00127877 0.00112994 0.00101010 0.00100402
FMR-K4-4a	24-08-2022	BA.2.75 BA.4.2 BA.5.2.3 BA.3.1 BG.2 P.1.2 BA.5.7 B.1.160.13 BA.2.70	0.96103046 0.01241002 0.00247239 0.00180459 0.00130321 0.00115226 0.00114286 0.00108647 0.00101153
FMR-K4-7a	07-09-2022	BA.2.75	0.99285
FMR-K4-8a	12-09-2022	BA.2.75 BA.2.74 BA.2.66 BA.4.2 BA.2.64 BA.2.9.4 BA.2.53 XU BA.5.2.3 BA.3.1 XAG AV.1 BG.2 BG.1 B.1.618 BA.2.3.15 BA.2.77 BF.5 AM.3 BA.2.12.2 BA.2.48 BA.2.13.1	0.85691000 0.04950210 0.01581100 0.01290526 0.00879053 0.00841165 0.00349560 0.00337661 0.00252425 0.00225780 0.00185063 0.00167785 0.00164974 0.00164288 0.00148948 0.00128514 0.00124146 0.00117716 0.00110619 0.00106308 0.00103783 0.00102160
FMR-K4-9b	14-09-2022	BA.2.75 BA.4.2 AV.1 BA.3.1 B.1.618	0.97561000 0.00811632 0.00118343 0.00116918 0.00110059
FMR-K4-10a	19-09-2022	BA.2.75 BA.2.74 BA.4.2 BA.2.64 BA.3.1 BA.2.59 BA.2.38.1 BA.2.77 BG.2 BA.2.9.4 XU BA.2.9.1 BA.5.1.7 AV.1	0.91322100 0.02094820 0.01355578 0.00598410 0.00534122 0.00291262 0.00285199 0.00231481 0.00186788 0.00176991 0.00176036 0.00108342 0.00103022 0.00100387
FMR-K5-6a	05-09-2022	BA.2.75 BA.2.74 XZ B.1.1.524 XAH	0.96575900 0.00555951 0.00393128 0.00295421 0.00260600
FMR-K5-7a	07-09-2022	BA.2.75 BA.2.21 BA.2.9.4 BA.2.12 BA.2.4 BA.2.52 BA.2.34 BA.2.74 BA.4.2 BA.2.38.1 BA.2.66 BA.2.35 BA.2.63 BA.2 BA.2.64 BA.2.57 BA.2.17 BA.2.38 BA.2.37 BA.2.59 BA.5.2.3 BA.4.1.4 BA.2.29 Q.7 BA.2.9.1 BA.3.1 BA.5.2.4 BA.2.3.15 AV.1 BA.2.49 BA.2.26 BF.9 BA.2.76 XH	0.63092711 0.02919710 0.02828890 0.02797474 0.02681780 0.02620400 0.02517230 0.02355710 0.02330830 0.01908250 0.01740140 0.01537590 0.01261830 0.01139982 0.00823848 0.00600100 0.00600100 0.00600100 0.00600100 0.00408998 0.00398010 0.00310604 0.00217606 0.00213675 0.00210748 0.00202664 0.00186567 0.00176031 0.00171050 0.00148335 0.00143120 0.00122662 0.00107273 0.00103520

Contd...

Contd...

Sample Name	Collection date	Proportion	Abundance
FMR-K5-13b	28-09-2022	BA.2.23.1 BA.2.80 BA.2.64 BA.2.44 BA.5.6 BA.2.25.1 XL BC.2 BA.5.2.5 B.1.177.23 B.4.7 XZ BA.1.13.1 BA.2.81 BA.2.77 BA.2.40.1 BA.2.40 BA.2.9.1 BA.5.2.2 BA.1.15 BA.1.15.3 BA.1.15.2 AY.49	0.25000000 0.11111100 0.08035700 0.07692310 0.06451610 0.05882350 0.04761900 0.03846150 0.03166950 0.02952331 0.02857140 0.02631580 0.02564100 0.02000000 0.01923080 0.01923075 0.01923075 0.01785710 0.01492540 0.00519590 0.00519590 0.00519590 0.00440529
FMR-B6-50a	22-02-2023	XBB.1.16.1 XBB.1.16 XBB.1.28 XBB.1.10 B.1.619 XBB.2.3.2 XBB.3.3 XW	0.25000000 0.25000000 0.24232208 0.24232208 0.00211416 0.00176678 0.00124844 0.00103573
FMR-B6-51a	27-02-2023	XBB.2.3 XBB.2.3.2 BA.2.75.3 BA.2.75.10 BA.2.75 XBB.2.6 XBB.2.8 XBB.2 XBB.2.7.1 XBB.1.20	0.24999998 0.24999998 0.08333331 0.08333331 0.08333331 0.08125298 0.08125298 0.08125298 0.00488599 0.00135501
FMR-B6-52b	02-03-2023	XBB.1.5.28 XBB.1.9 XBB.5 B.1.258.4	0.98889438 0.00655738 0.00148300 0.00101175
FMR-B6-53a	08-03-2023	XBB.2.3.2 XBB.6 XBB.1.22.2 XBB.1.22.1 XBB.1.22 XBB.1.18 XBB.1 XBB.2 XBB.2.5 XBB.2.7 XBB.1.7 XBB.1.15 XBB.1.4 XBB.1.29 XBB.2.6 XBB.2.1 XBB.2.2 XBB BA.5.2.47	0.25000000 0.07999838 0.04440298 0.04440298 0.04440298 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.04114930 0.00185185
FMR-B6-54a	13-03-2023	XBB.1.28 XBB.1.13 XBB.7 XBB.1 XBB.2 XBB.6 XBB.2.8 XBB.2.5 XBB.5 XBB XBB.1.6 XBB.1.7 XBB.1.18 XBB.1.4 XBB.1.15 XBB.1.11 XBB.1.17 XBB.1.9.3	0.50000000 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.03090807 0.00453515
FMR-B6-55a	15-03-2023	XBB.1.28 FG.3 XBB.1.5.16 XBB.1.18.1 XBB.1.5.21 XBB.1.18 XBB.1.5.4 XBB.1.5.26 XBB.1.4.1 XBB.1.5.31 XBB.1.5.11 XBB.1.5.23 XBB.1.5.30 XBB.1.5 XBB.1.15 XBB.1.5.35 XBB.1.4 XBB.1.9.3 FE.1 XBB.1.5.27	0.50000000 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.03069613 0.00555556 0.00217391 0.00113250
FMR-B7-50a	22-02-2023	XBB.1.5.28 XBB.1.12 XBB.1.6 XBL XBB.1.5.26 XBB.1.9.3	0.97762943 0.00627615 0.00527983 0.00349162 0.00255102 0.00182698
FMR-B7-51b	27-02-2023	XBB.1.6 FH.1 XBB.1.5.30 XBB.1.5.35 XBB.1.5.2 XBB.1.5.3 XBB.1.5.33 XBB.1.5.26 XBB.1.5.14 XBB.1.5 XBB.1.14 XBB.2.6 XBB.1.21 XBB.1.4 XBB.1.7 XBB.2.5 XBB.2.4 XBB.2 XBB.1 FD.1 XBB.1.5.4 XBB.1.5.1 XBB.1.5.12 FD.2 EK.1 EK.3 XBB.1.5.20 XBB.1.5.25 XBB.1.5.6 XBB.1.5.17 XBB.1.5.15 EU.1 XBB.1.5.13 XBB.1.5.34 XBB.1.5.22 XBB.1.5.24 XBB XBB.1.5.23 XBB.6 XBB.1.19.1 XBB.1.19 BN.1.3.3	0.20000000 0.02183991 0.02183991 0.02183991 0.02183991 0.02183991 0.02183991 0.00495050 0.00227790 0.00193799 0.00193799 0.00169635
FMR-B7-52b	02-03-2023	XBB.1.16 BA.2.48 BA.2.72 BA.2.40 BA.2.40.1 XBB.2.3	0.98366583 0.00296150 0.00228311 0.00126262 0.00126262 0.00111726
FMR-B7-53a	08-03-2023	XBB.1.16.1 XBB.1.4.1 FD.2 CA.1 BN.1.11	0.82123605 0.12500000 0.04000000 0.00891720 0.00330306

Contd...

Contd...

Contd...

Sample Name	Collection date	Proportion	Abundance
FMR-K4-53a	08-03-2023	XBB.1.16 XBB.1.22.1 XBB.1.5.36 XBB.1.5.1 XBB.1.5.3 XBB.1.5.26 XBB.1.5.7 XBB.1.11.1 XBB.1.5 XBB.1.30 XBB.2.7.1 XBB.2.6 XBB.1.21 XBB.1.11 XBB.1.18 XBB.1.7 XBB.1.13 XBB.2.7 XBB.2.5 XBB.2.4 XBB.2 XBB.1 XBB EK.2.1 XBB.1.5.28 XBB.1.5.17 EK.2 EK.3 XBB.1.5.20 XBB.1.5.32 XBB.1.5.25 XBB.1.5.6 XBB.1.5.39 XBB.1.5.8 XBB.1.5.19 XBB.1.5.23 XBB.1.5.15 EU.1 XBB.1.22 XBB.1.5.13 XBB.1.5.10 XBB.1.5.34 EM.1 XBB.1.18.1 EK.1 BN.1.4.2 BE.2 CA.5 BN.1.5 XAC BN.1.9 BA.2.3.2 XBB.1.23 XBB.1.4.1 B.1.1.258	0.04235670 0.02137747 0.02137747 0.02137747 0.02137747 0.02137747 0.02137747 0.02137747 0.02137747 0.02137747 0.00220264 0.00202020 0.00193798 0.00159617 0.00138313 0.00132802 0.00113507 0.00113250 0.00109890 0.00102354
FMR-K4-54a	13-03-2023	XBB.1.10 XBB.1.13 BF.34 XBB.1.28	0.98822737 0.00509165 0.00135501 0.00104384
FMR-K4-55a	15-03-2023	XBB.1.16 XBB.1.5.24 XBB.1.5.32 XBB.1.5.8 XBB.1.16.1 XBL XBB.1.9.4	0.69293066 0.16997998 0.12500000 0.00345423 0.00322211 0.00183824 0.00150602
FMR-K5-50b	22-02-2023	XBB.1.16 XBB.1.16.1 XBB.1.22.1 XBB.1.5.36 XBB.1.5.14 XBB.1.5.31 XBB.1.5.26 XBB.1.5.33 XBB.1.5.3 XBB.1.5.2 XBB.1.5.30 XBB.1.5.12 XBB.1.5.23 XBB.1.22 XBB.1.5.24 XBB.1.18.1 XBB.1.5.34 XBB.1.5.15 XBB.1.5.17 XBB.1.5.39 XBB.1.5.25 XBB.1.5.20 XBB.1.5	0.25000001 0.25000000 0.02380952
FMR-K5-51b	27-02-2023	XBB.1.16.1 XBB.2.3 XBB.1.9.3	0.99429400 0.00222900 0.00106200
FMR-K5-52a	02-03-2023	XBB.1.16.1 XBB.1.27 XBB.1.13 B.1.1.153 XBB.1.21 XBB.1.10	0.94349777 0.04479187 0.00578035 0.00172700 0.00156039 0.00153139
FMR-K5-53a	08-03-2023	XBB.1.16.1 XBB.1.16	0.83200000 0.16621300
FMR-K5-54a	13-03-2023	BA.2.75.1	0.99401
FMR-K5-55a	15-03-2023	XBB.1.5.32 XBB.1.5.24	0.50000000 0.50000000
FMR-B6-60a	05-04-2023	XBB.1.16.1	0.9901
FMR-B9-59b	03-04-2023	XBB.1.16.1 XBB.1.16 XBB.2.3.3 XBB.2.3.4 XBB.2.3 B.1.321	0.34691000 0.28315500 0.12262167 0.12262167 0.12262167 0.00128205
FMR-D2-59b	03-04-2023	XBB.1.16.1 XBB.2.4 XBB.2.3 XBB.2.3.3 XBB.1.5.28 XBB.1.6 FD.2 B.26 XBB.2.3.1 BA.2.50 BF.11.4 BF.11	0.32337000 0.19516327 0.18002245 0.18002245 0.09446840 0.01534530 0.00303030 0.00268500 0.00173010 0.00165017 0.00125628 0.00125628
FMR-K4-59b	03-04-2023	XBB.1.16	1
FMR-L10-60a	05-04-2023	XBB.1.16.1 XBB.1.16 FE.1	0.49608822 0.49332978 0.01058200
FMR-S8-60b	05-04-2023	XBB.1.16.1 XBB.1.16 XBB.1.25 XBB.6	0.80018800 0.16647900 0.03129600 0.00203700

Contd...

Contd...

Contd...

Contd...

Sample Name	Collection date	Proportion	Abundance
FMR-B7-76a	31-05-2023	BA.3 BA.2.23 BA.2.10.2 BA.2.10.3 BA.2.33 BA.2.11 BA.2.40 BA.2.50 BA.2.30 BA.2.41 BA.2.72 BA.2.43 BA.2.24 BA.2.85 BA.2.10 BA.2.1 BA.2.31 BA.2.27 BA.2.58 BA.2.65 BA.2.29 BA.2.5 BA.2.56 BA.2 BA.2.10.1 BA.2.18 BA.2.47 BA.2.81 XW BA.2.82 BA.2.31.1 BA.2.55 BA.2.22 BA.2.46 BA.2.13 BA.2.45 BA.2.14 BA.2.23.1 XAS BA.2.59 BA.2.40.1 BA.2.15 BA.2.19 BA.2.36 BA.2.51 BA.2.69 BA.2.25 BA.2.71 BA.2.13.1 BA.4.8 BA.2.3.7 BQ.1.13	0.02325600 0.01996624 0.01996624 0.01996624 0.01996624 0.01996624 0.01996624 0.01996624 0.01996624 0.01996624 0.00367647 0.00306000
FMR-B9-76a	31-05-2023	BA.2.10.3 BA.2.10.2 BA.2.4 BA.2.5 BA.2.65 BA.2.58 BA.2.27 BA.2.31 BA.2.10 BA.2.23 BA.2.72 BA.2.41 BA.2.30 BA.2.50 BA.2.62 BA.2.33 BA.2 BA.2.57 BA.2.55 BA.2.31.1 BA.2.17 BA.2.67 BA.2.68 BA.2.73 BA.2.12 BA.2.10.1 BA.2.15 BA.2.2 BA.2.59 XN BA.2.28 XAP XAT BA.2.21	0.03386748 0.03386748 0.03386748 0.03386748 0.03386748 0.03386748 0.03386748 0.03386748 0.03386748 0.00628931 0.00485437 0.00397498 0.00167502 0.00104932
FMR-D1-77b	05-06-2023	XAS BA.2.65 BL.1.1 B.1.502	0.49485702 0.49485702 0.00666667 0.00170358
FMR-D2-77b	05-06-2023	BA.2.40.1 BA.2.33 BA.2.19 BA.2.16 BA.2.36 BA.2.51 BA.2.25 BA.2.71 BA.2.34 BA.2.14 BA.2.18 BA.2.10.1 BA.2.10.2 BA.2.10.3 BA.2.11 BA.2.32 BA.2.40 BA.2.30 BA.2.72 BA.2.23 BA.2.3 BA.2.10 BA.2.31 BA.2.27 BA.2.65 BA.2.29 BA.2.5 BA.2.4 BA.2.15 BA.2.56 BA.2.3.6 BA.2.70 BA.2.13.1 BA.2.3.15 BA.2.3.7 BA.2.31.1 BA.2.55 BA.2.3.13 BA.2.3.18 BA.2.35 BA.2.13 BA.2.45 BA.2.47 BA.2.23.1 BA.2.3.5 BA.2.3.16 BA.2.3.10 BA.2.3.9 BA.2.3.2 BN.3 BA.4.2 XBR BA.4.1 BA.4.1.5 BA.4.1.9 BA.4.1.4 BA.4.1.1 BA.4.1.8 XBF.3	0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01887243 0.01538460 0.01520538 0.00396825 0.00396825 0.00396825 0.00396825 0.00396825 0.00396825 0.00227272
FMR-K4-76b	31-05-2023	BA.2.10.2 BA.2.10.1 BA.2.29 BA.2.65 BA.2.31 BA.2.10 BA.2.24 BA.2.23 BA.2.10.3 BA.2 BA.2.19 BA.2.15 BA.2.55 BA.2.31.1 BA.2.6 XQ BA.2.33 XAH XAP XG BA.2.64	0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.06807550 0.00826446 0.00816308 0.00719424 0.00694449 0.00694449 0.00562991 0.00380226
FMR-K5-76b	31-05-2023	BA.2.82 BA.2.62 BA.2.3 XAP XAH BA.3	0.97566202 0.00709220 0.00543478 0.00512057 0.00512057 0.00156986
FMR-L10-76a	31-05-2023	BA.2.48 BA.2.71 BA.2.55 BA.2.31 BA.2.40.1 BA.2.15 BA.2.19 BA.2.36 BA.2.51 BA.2.25 BA.2.14 BA.2.27 BA.2.18 BA.2.85 BA.2.10.1 BA.2.10.3 BA.2.33 BA.2.40 BA.2.30 BA.2.23 BA.2.31.1 BA.2.65 BA.2.1 BA.2.29 BA.2.4 BA.2.5 BA.2.10 BF.31.1 BE.8 BE.12 XN BN.3 BA.5.2.41 BA.5.2.8 BA.5.2.35 BF.26 XBP BA.5.2.9 BF.32 BN.1.5 BN.1.11 DH.1 DY.1 DY.1.1 DY.3 BA.5.2.48 DY.4 XAK DQ.1 BA.1.14.1 BA.2.2.1	0.03380789 0.03380789 0.03380789 0.03380789 0.03380789 0.03380789 0.03380789 0.03380789 0.03380789 0.00937500 0.00386100 0.00314465 0.00251889 0.00216344 0.00208098 0.00195312 0.00165837 0.00153257 0.00153257 0.00153257 0.00153257 0.00153257 0.00148651 0.00145773 0.00141044 0.00118064

Supplementary Table III: Correlation of Clinical and Wastewater sample data from Mumbai

Date	Lineages of Clinical samples (MCGM)	Date	Lineages of Wastewater samples (FMR)
March 7, 2023	XBB.1.9.1	February 22, 2023	XBB.1.16.1, <u>XBB.1.16</u> , XBB.1.28, XBB.1.5, XBB.1.18, XBB.1.9.5, XBB.1.9.1, <u>XBB.1.9</u> , XBB.2.3
March 9, 2023	XBB.1.16		
March 12, 2023	XBB.1	February 26, 2023	XBB.2.3.2, XBB.1.10, XBB.2.2, XBB.5, XBB.2.7, XBB.2.5, XBB.3, XBB.6, XBB.4, XBB.2, <u>XBB.1</u> , XBB