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SARS-CoV-2 Positivity and Viral Load during Three Viral Waves in Mumbai, India

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Abstract: Background The SARS-CoV-2 laboratory PCR tests were generally reported only as binary positive or negative outcomes. Instead tet positivity, these results contain a great deal of epidemiological information related to viral transmission patterns in populations**.** These transmission patterns during India's SARS-CoV-2 viral waves remain largely undocumented. **Methods** We analysed 2.7 million real-time polymerase chain reaction (PCR) testing records collected in Mumbai, a bellwether for other Indian cities. We used the inverse of cycle threshold (Ct) values to determine community-level viral load. We quantified wave-specific differences by age, sex and slum population density. **Results** Overall PCR positivity was 3.4% during non-outbreak periods, rising to 23.2% and 42.8% during the Aleph (June-November 2020) and Omicron waves (January 2022), respectively, but only 9.9% during the Delta wave (March-June 2021). The community-level median Ct values fell and rose ~7-14 days prior to PCR positivity rates. Viral loads were 4-fold higher during the Delta and Omicron waves than during non-outbreak months. The Delta wave had high viral loads at older ages, in women and in areas of higher slum density. During the Omicron wave, differences in viral load by sex and for slum density had disappeared, but older adults continued to show higher viral load.**Conclusions** Mumbai's viral waves had markedly high viral loads representing an early signal of pandemic trajectory. Continue the vaccination in elderly Indians could reduce viral load in subsequent waves. Ct values are practicable monitoring tools.

Keywords: SARS-CoV-2 pandemic; PCR positivity; Aleph Delta Omicron waves; Mumbai; India

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Introduction

Various waves of SARS-CoV-2 virus infection in India has led to one of the world's highest totals of confirmed COVID-19 case totals (45 million as of Oct 1, 2022), with about 3-4 million excess deaths in independent studies analysing registered deaths or national surveys. By contrast, only 0.6 million deaths are reported by official government data sources (Coronavirus.app, 2022; Jha et al., 2022).

Three major viral waves occurred in India. The first wave of 6 months occurred from June 2020-November 2020 and peaked in late September 2020, with moderate increases in excess deaths from the virus (Coronavirus.app, 2022). We refer to this wave as "Aleph". The Aleph wave was due mostly to the original virus that originated from Wuhan Province China, with some B.1.1.7 (Alpha) and B.1.617.1 (Kappa) variants of concern. The Delta wave of 4 months occurred from March-June 2021 was due predominantly to the B.1.617.2 variant (which is known for greater transmissibility and reduced responsiveness to vaccines (Cherian et al., 2021; Mlcochova et al., 2021)) and was characterized by the largest spike in excess deaths. The Omicron wave mostly in January 2022 caused widespread infection but far lower mortality and was due predominately to the B.1.1.529 variant.

Despite India collecting large amounts of testing data in central repositories, little testing or viral load data are available publicly. In the absence of reasonably representative national data, careful examination of sub-national settings can inform our understanding of the virus and transmission

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patterns in India (Walker et al., 2021). Intergenerational households are the norm in Mumbai City, with 13 million people, and about 65% of its population live in cramped, often poorly ventilated slum housing, allowing for easy transmission of the virus (Ghosh et al., 2020). Moreover, Mumbai is considered a bellwether setting which showed the earliest increases and declines during each viral wave (Velumani et al., 2021). The Municipal Corporation of Greater Mumbai (MCGM) reports over one million real-time polymerase chain reaction (PCR) confirmed cases and 17,000 confirmed COVID deaths since March 2020 (Gov-MH, 2022; MCGM, 2022a). However, these reports underestimate true numbers as even by the end of the first wave, serosurveys suggested that about a third of adults including nearly half of adult slum dweller had SARS-CoV-2 antibodies (Velumani et al., 2021; NITI-Aayog et al., 2020).

Here, we examine data from a private but widely-used laboratory in Mumbai, capturing over 2.7 million individual PCR testing data with a subset providing information on viral load, as defined by inverse of the cycle threshold (Ct) values. Ct values are considered as a proxy for viral load, particularly if tested in early stages of the infection (He et al., 2020). We quantify differences in positivity and viral load during the three waves, and quantify each wave by sex, age, and slum population density, as well as the time lags.

Methods

Data Sources

We included 2.7 million people of all ages who underwent PCR testing between 9 April 2020 and 30 January 2022 by a large commercial laboratory (Thyrocare) with many franchises throughout Mumbai. Thyrocare has been approved by the Indian government for Covid-19 testing and conducts all testing in one facility in Navi Mumbai, with samples sent daily from franchise locations (ICMR, 2020). Participants had to pay 600-750 Rupees ($8 to 10 USD) per test. All records were anonymised with no identifiable patient information. The available data includes the date of test, age, sex, and 6-digit postal code of tested individual. Reasons for testing were not recorded, but the tested population includes those referred by physicians, hospitals, quarantine centers, and self-referrals from both public and private sectors. For each record, we extracted the PCR results, and from 22 August 2020 onwards, the Ct values among all PCR positives. We mapped individual pin codes to municipal wards (Table A2). Individuals from about 5% of pin codes that cut across municipal wards were assigned to the ward with the largest population in that pin code. We collected publicly available PCR confirmed cases and deaths, published in tabular form by age, sex, and municipal ward from the MCGM Covid-19 portal (MCGM, 2022a; 2022b; Banaji, 2022). MCGM provides estimates of the sex-specific total population and proportion living in slums (Table A1) for each ward. Research Ethics approval was provided by Unity Health Toronto.

Laboratory Methods

Nasopharyngeal or oropharyngeal or combined specimens from each person were collected into a barcoded sterile tube containing transport medium and transported within one day to the central Thyrocare laboratory. All six commercial PCR kits used for diagnosis were approved by the Indian Council of Medical Research (ICMR; Table A3), with independent validation to establish each assay's high sensitivity and specificity. The Ct value was defined as the number of cycles required for the fluorescent signal to cross the detection threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater the amount of target nucleic acid; each unit of Ct represents a doubling of viral load). Ct values between 0 to 35 ranges were considered as test positive (Das et al., 2021) and values <30 were considered strongly positive (Das et al., 2021; PHE, 2020; Service et al., 2022; Bayat et al., 2021).

Statistical Methods

Usually laboratory PCR test results were generally used only as binary positive or negative outcomes. However, these test results contain a great deal of information than that. Although there

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are challenges to relying on single Ct values for individual-level decision-making, even a limited aggregation of data from a population can inform on the trajectory of the pandemic (Hay et. al., 2021). We calculated the daily PCR positivity rate (percent positive among total tested) and median Ct values from Thyrocare data. We plotted the 7-day moving average of community-level PCR positivity rates and median Ct values from Thyrocare data along with official PCR confirmed cases and death counts from MCGM's COVID-19 dashboard. We used Pearson correlation coefficients to determine the simple agreements between these time series.

We used generalized additive models (Wood, 2017; Andriamandimby et al., 2022) to model changes in the daily median Ct value and in PCR positivity over time. We modelled daily median Ct value with a normal distribution, and applied a logistic model for the number of daily positive/negative PCR tests. We included a day-of-week effect and a smoothly varying time trend having a second derivative penalty. The model included a daily (normally distributed) overdispersion term. Model parameters were estimated with maximum likelihood.

The age distributions of MCGM confirmed cases and Thyrocare cases were slightly varying between the outbreak periods (Tables A4 and A5), thus we adjusted the ages to the 5-yearly age distribution of 2011 census (ORGCC, 2021) in all calculations of PCR positivity rates and median Ct values.

Given that the PCR positivity and the median Ct values varied by age, sex, and municipal wards over 3 pandemic waves, we created a multivariate logistic model to investigate the effects of age (10 yearly classified with 20-29 years as the reference), sex (males as the reference) and the slums population density (high: more than 60% of the population living in slums; medium: 33%-60%; and low: up to 32% with low slum density as the reference) on PCR positivity for each wave. We created a similar proportional logistic model with the same explanatory variables to examine high versus medium viral load groups for each viral wave by classifying the groups using Ct quartile ranges (high: 25% or less, medium: 25%-75%, low: 75% or more), comparing the highest viral load (i.e., low Ct) to the medium range as the reference. Comparisons of low Ct to the high Ct value quartiles yielded similar results (data not shown). We used R software version 4.1.0 to fit the generalized additive models and SAS software version 9.4 to perform all other statistical analyses (R Core Team, 2017; SAS Inc, 2015).

Results

Over 2.7 million PCR tests for SARS-CoV-2 were conducted at Thyrocare from April 2020 to January 2021, with 2.2 million during the non-outbreak months. The overall PCR positivity was 5.3%, ranging from 3.4% during the non-outbreak months, rising to 23.2% and 42.8% during the Aleph and Omicron waves, respectively, but a surprisingly small increase to 9.9% during the Delta wave. PCR positivity was generally higher in women than in men and higher at older ages and in areas with higher slum density (Table 1).

Table 1. Summary of official case counts, community-level PCR positivity rates and median Ct values from MCGM Covid-19 dashboard and Thyrocare tested population during the pandemic period from Apr 2020 to Jan 2022.

Data source/ Characteristics	Overall period Apr 2020 to Jan 2022 (22 months)	Pandemic periods			
		Non outbreak periods (11 months)	Aleph wave (Jun-Nov 2020) 6 months	Delta wave (Mar-Jun 2021) 4 months	Omicron wave (Jan 2022) 1 month
Government Covid dashboard					

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Official PCR confirmed cases	**1,022,979**	**160,288**	**232,484**	**376,528**	**253,679**
Case rate per 1000 population*	**191.6**	**12.8**	**33.9**	**82.4**	**222.1**
Age <40 years	130.5	7.6	18.1	54.3	159.1
Age >= 40 years	334.9	24.8	71.0	148.4	369.9
Female	n.a.	n.a.	29.0	76.1	191.6
Male	n.a.	n.a.	38.1	87.8	248.1
Low slum areas	261.2	16.4	45.1	123.5	262.5
Medium slum areas	173.8	13.1	32.8	77.4	195.7
High slums	151.5	8.2	25.9	68.8	164.3
Thyrocare PCR testing					
Total No. tested in 000	2,717.3	2,181.7	64.2	445.1	26.2
Test positive in 000	155.0	78.8	17.9	46.6	11.7
Overall PCR Positivity (%)†					
All ages	5.3	3.4	23.2	9.9‡	42.8
Age < 40 years	5.1	3.4	18.8	9.5	41.3
Age ≥ 40 years	6.5	3.9	36.9	11.7	49.2
Female	5.75	3.75	25.87	11.46	44.06
Male	4.98	3.2	21.91	8.98	41.82
Low slum areas§	4.2	3.3	20.4	7.2	44.3
Medium slum areas§	21.7	8.3	28.8	25.3	42.2
High slum areas§	17.7	5.2	25.8	19.9	42.8
Median Ct value†					
Overall	24.0	25.0	26.0	23.0	23.2
Inter quartile range (Q1, Q3)	(19.0,28.0)	(20.0, 28.0)	(21.0,31.0)	(18.0, 28.0)	(19.5, 27.6)
Age < 40 years	24.0	25.0	25.0	23.0	23.1
Age ≥ 40 years	24.0	24.0	26.0	22.0	21.9
Female	24.0	25	25.0	23.0	23.1
Male	24.0	24	26.0	23.0	23.2
Low slum areas§	24.0	25.0	26.0	24.0	22.9
Medium slum areas§	22.3	23.0	25.0	21.0	23.0
High slum areas§	23.0	23.1	26.0	22.0	23.4

Notes:

1. * Case rates were adjusted to duration (number of months) of each pandemic wave as shown in column label headings to reflect the annualized rate.
2. † Crude PCR positivity per 100 tested and median Ct values were varying by age. For comparison of PCR positivity between viral waves, we adjusted the ages of tested population to 5-yearly age distribution of census population in 2011. Details of Government Covid dashboard data, overall PCR positivity and Ct values are shown in Web Tables A4–A6**.**
3. ‡ The reported lower test positivity during the Delta wave from March to June 2021 was likely because of many people frequently get tested for work and travel purposes as required by the government.
4. § Slum population density - high: more than 61% of the population living in slums, medium: 33% to 60%, and low: up to 33%.

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The median Ct value for period from September 2020 to January 2022 was 24.0 (interquartile range 19.0-28.0). Median Ct values were similar during the non-outbreak months (25.0) and the Aleph wave (26.0) but notably lower during the Delta (23.0) and Omicron waves (23.2). The differences of about two Ct units from non-outbreak months represents an approximately 4-fold higher viral load. During the Aleph and Omicron waves, the median Ct values were similar in areas with low, medium or high slum density, but during the Delta wave, medium and high slum density areas had lower Ct values by two or three units in absolute Ct values (or 4-fold to 8-fold higher viral load).

The PCR testing data covered all 24 wards and 83 of 91 postal pin codes within Mumbai and suburban districts (Tables A1 and A2). The age distribution for men and women testing PCR positive in the Thyrocare laboratories was similar to the overall distributions among 830 000 confirmed cases, a subset of data reported by the MCGM (Figure 1). In Thyrocare and MCGM data, the Aleph wave was characterized by a dual peak of PCR positivity around age 30 and at age 60 years, whereas PCR positivity during the Delta and Omicron waves peaked around age 30 years.

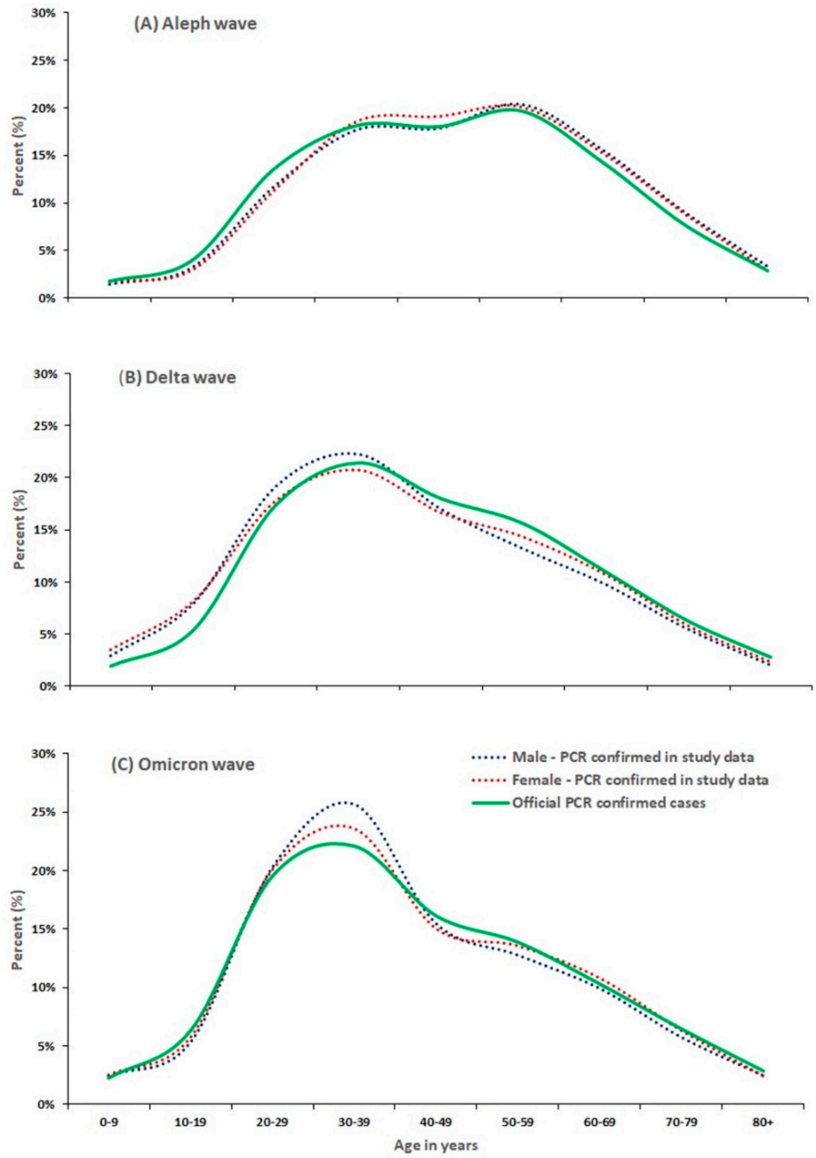

Figure 1. Age distribution of PCR confirmed cases between MCGM Covid dashboard and Thyrocare study data during the three viral waves. Aleph wave (Jun 2020-Nov 2020), (B) Delta wave (Mar 2021-Jun 2021), (C) Omicron wave (Jan 2022). This figure compares the similarity of age-sex distributions of PCR confirmed Covid cases (863,000 individuals; solid green line) reported in MCGM dashboard data (Banaji, 2022; IDFC Ins, 2022) and the PCR tested population in Thyrocare study data

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(76,000 individuals; dotted red are males, dotted blue are females). P-values of Kruskal-Wallis similarity test for age distributions were 0.53, 0.31 and 0.73 respectively.

The 7-day averages of MCGM reported PCR confirmed cases correlated temporally with Thyrocare PCR positivity rates and the inverse of the median Ct values (Figure A2; with higher viral load shown to rise vertically). The Pearson correlation coefficients showed agreement between PCR confirmed case counts in MCGM and PCR positivity and median Ct values for each wave were: Aleph (0.46, n.a.), Delta (0.94, -0.67) and Omicron (0.85, -0.88) respectively. Daily deaths, which are likely with substantial undercounts, were also correlated with Thyrocare results (data not shown).

The rise in median viral load (inverse of Ct values) was substantial during the Delta wave, with a six-unit drop in Ct value representing a 64-fold increase in community viral load. By contrast, during the Omicron wave, the viral load rose less sharply with an absolute Ct value difference of about three, representing an 8-fold higher viral load, and the period of higher viral load was of a shorter duration that during the Delta wave. The predicted smoothed daily time series of PCR positivity and median Ct values from generalized additive models (Figure 2) were similar to the 7-day daily median counterparts (Figure A2). The 95% confidence intervals of the daily predicted values show higher uncertainty for daily median Ct value in September 2020 to February 2021 and for both daily median Ct value and PCR positivity in November to December 2022, largely reflecting reduced testing volumes during this period.

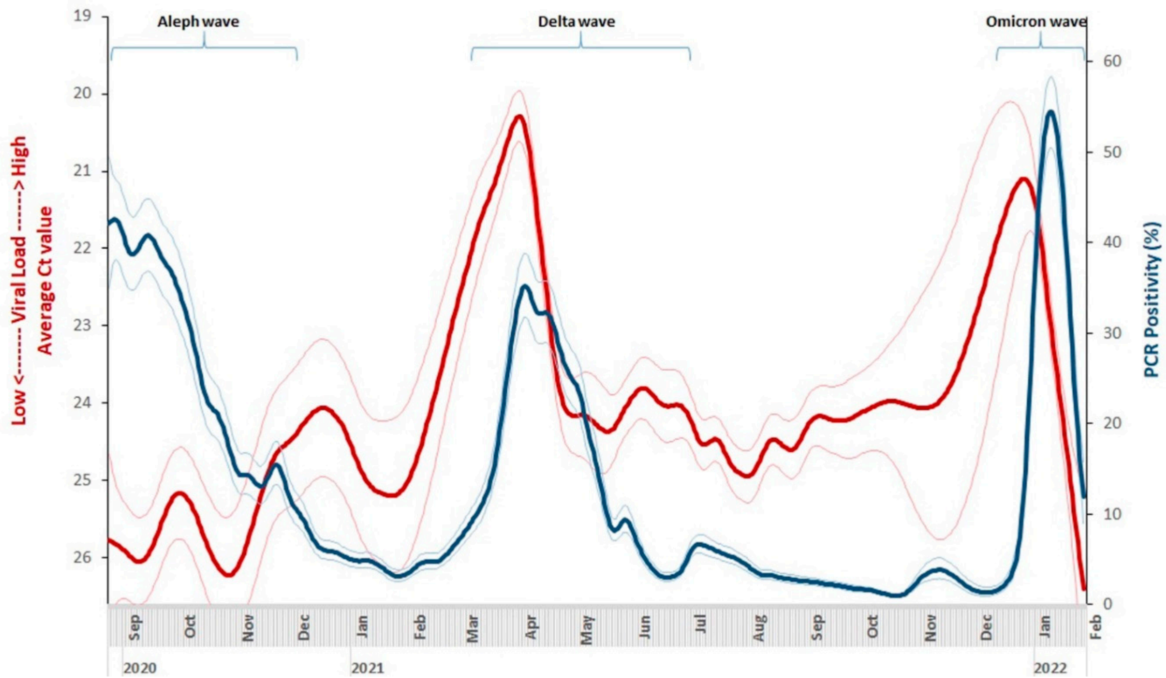

Figure 2. Trends in smoothed daily viral loads (inverse of cycle thresholds) and polymerase chain reaction (PCR) positivity in Thyrocare laboratory data in Mumbai, India. The trend graphs apply generalized additive models to examine changes in the daily median Ct value and PCR positivity over time. The thick red line represents the smoothed daily Ct values and surround pink lines are the corresponding 95% confidence intervals; the thick dark blue line represents the smoothed daily PCR positivity and the surround light blue lines are the corresponding 95% confidence intervals. Daily Ct values are plotted on reverse y-axis on the left, with lower Ct value correspond to higher viral load. The 95% confidence intervals of the daily predicted values show higher uncertainty for daily median Ct value in September 2020 to February 2021 and also show higher uncertainty both Ct daily median Ct value and PCR positivity in November to December 2022. The graph is restricted to the time period of August 24, 2020 to January 30, 2022, therefore only partially covered the Aleph wave which occurred from June – November 2020.

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The beginning, peak, and the end of each COVID wave was determined from the rise and fall of individual curves of viral load (Ct values) and PCR positivity using the 7-day averages as shown in Figure A2. In each wave, the first detected change was in the viral load, followed by PCR positivity and lastly by MCGM reported deaths (Table A7). Ct values also declined first from the peaks. During the Delta wave, the increase in viral load began on February 1, 2021 and peaked on March 31, 2021, which were about 5 and 17 days earlier than the rise and peak in PCR positivity, respectively. During the Omicron wave, the increase in viral load (December 11, 2021) and the peak (January 2, 2022) also preceded the rise in cases and peak PCR positivity about 10 days. For the beginning-to-peak duration, the Delta wave lasted twice as many days (46-58) than the Omicron wave (20-22). For the peak-to-end duration for the Delta wave, viral load increases lasted for 49 days, PCR positivity for 72 days, MCGM reported confirmed cases for 90 days, and MCGM reported deaths lasted longest with 99 days.

Because of the strong age, sex, and slum density patterns of both PCR positivity and median Ct values, multivariate analyses considering each is required. Among the Thyrocare tested population, PCR positivity was higher in females in all three viral waves (Figure 3), and there was a notable age gradient in the multivariate odds ratio of being PCR positive when compared to the reference age group of 20-29 years (age test for trend for each has $p < 0.0001$; Figure A1). During the early Aleph and Delta waves, areas with high or medium slum density had higher odds of PCR positivity than low slum areas, but these differences were not seen during the Omicron wave.

Figure 4 shows similar examination of the multivariate predictors of viral load, comparing the highest quartile to the middle two quartiles during the Delta and Omicron waves, which correspond to an absolute difference of 5 and 3.7 in Ct values (or 32-fold and 13-fold differences in community viral load). Females tended to have higher viral load during the Delta wave but not during the Omicron wave. Similarly, areas with high or medium slum density had higher odds ratio of high viral load during the Delta wave, but these differences disappeared during the Omicron wave. During the Delta wave, ages 30 years or older had somewhat higher odds ratio of high viral load, but during Omicron, the higher odds ratio were seen at ages 60 years and older.

Covariate	Category	Cases / Total	RT−PCR Positivity	OR (95% CI)
(A)				
Aleph wave: Jun−Nov 2020				
Sex	Female	6,789 / 22,537	30.1	1.1 (1.0−1.1)
	Male	11,102 / 41,662	26.6	1.0
Age (years)	0−9	258 / 893	28.9	2.1 (1.8−2.5)
	10−19	571 / 2,275	25.1	1.8 (1.6−2.0)
	30−39	3,158 / 15,659	20.2	1.4 (1.3−1.4)
	40−49	3,187 / 11,135	28.6	2.1 (2.0−2.3)
	50−59	3,640 / 9,158	39.7	3.5 (3.3−3.7)
	60−69	2,778 / 6,483	42.9	4.0 (3.7−4.2)
	70−79	1,607 / 3,712	43.3	4.1 (3.8−4.4)
	80+	596 / 1,448	41.2	3.7 (3.3−4.2)
	20−29	2,096 / 13,436	15.6	1.0
Slum density	High	5,173 / 16,622	31.1	1.4 (1.4−1.5)
	Medium	3,964 / 11,772	33.7	1.4 (1.3−1.5)
	Low	8,754 / 35,805	24.4	1.0
(B)				
Delta wave: Mar−Jun 2021				
Sex	Female	12,425 / 48,341	25.7	1.2 (1.2−1.2)
	Male	17,073 / 80,859	21.1	1.0
Age (years)	0−9	518 / 2,625	19.7	1.1 (1.0−1.2)
	10−19	1,585 / 6,578	24.1	1.4 (1.4−1.5)
	30−39	6,663 / 33,978	19.6	1.1 (1.1−1.2)
	40−49	5,140 / 22,988	22.4	1.3 (1.3−1.4)
	50−59	4,290 / 15,741	27.3	1.7 (1.6−1.8)
	60−69	3,102 / 9,377	33.1	2.2 (2.1−2.4)
	70−79	1,891 / 4,961	38.1	2.8 (2.6−3.0)
	80+	796 / 1,960	40.6	3.1 (2.8−3.4)
	20−29	5,513 / 30,992	17.8	1.0
Slum density	High	10,749 / 48,044	22.4	1.5 (1.5−1.6)
	Medium	8,551 / 30,850	27.7	1.2 (1.2−1.2)
	Low	10,198 / 50,306	20.3	1.0
(C)				
Omicron wave: Jan 2022				
Sex	Female	5,553 / 12,107	45.9	1.1 (1.0−1.1)
	Male	6,174 / 14,114	43.7	1.0
Age (years)	0−9	284 / 738	38.5	0.9 (0.8−1.1)
	10−19	620 / 1,532	40.5	1.0 (0.9−1.1)
	30−39	3,015 / 7,033	42.9	1.1 (1.0−1.2)
	40−49	1,815 / 3,995	45.4	1.2 (1.1−1.3)
	50−59	1,497 / 3,065	48.8	1.4 (1.3−1.5)
	60−69	1,162 / 2,229	52.1	1.6 (1.5−1.8)
	70−79	670 / 1,198	55.9	1.9 (1.7−2.1)
	80+	286 / 512	55.9	1.9 (1.6−2.3)
	20−29	2,378 / 5,919	40.2	1.0
Slum density	High	5,352 / 12,052	44.4	1.0 (0.9−1.0)
	Medium	4,119 / 9,222	44.7	1.0 (0.9−1.1)
	Low	2,256 / 4,947	45.6	1.0

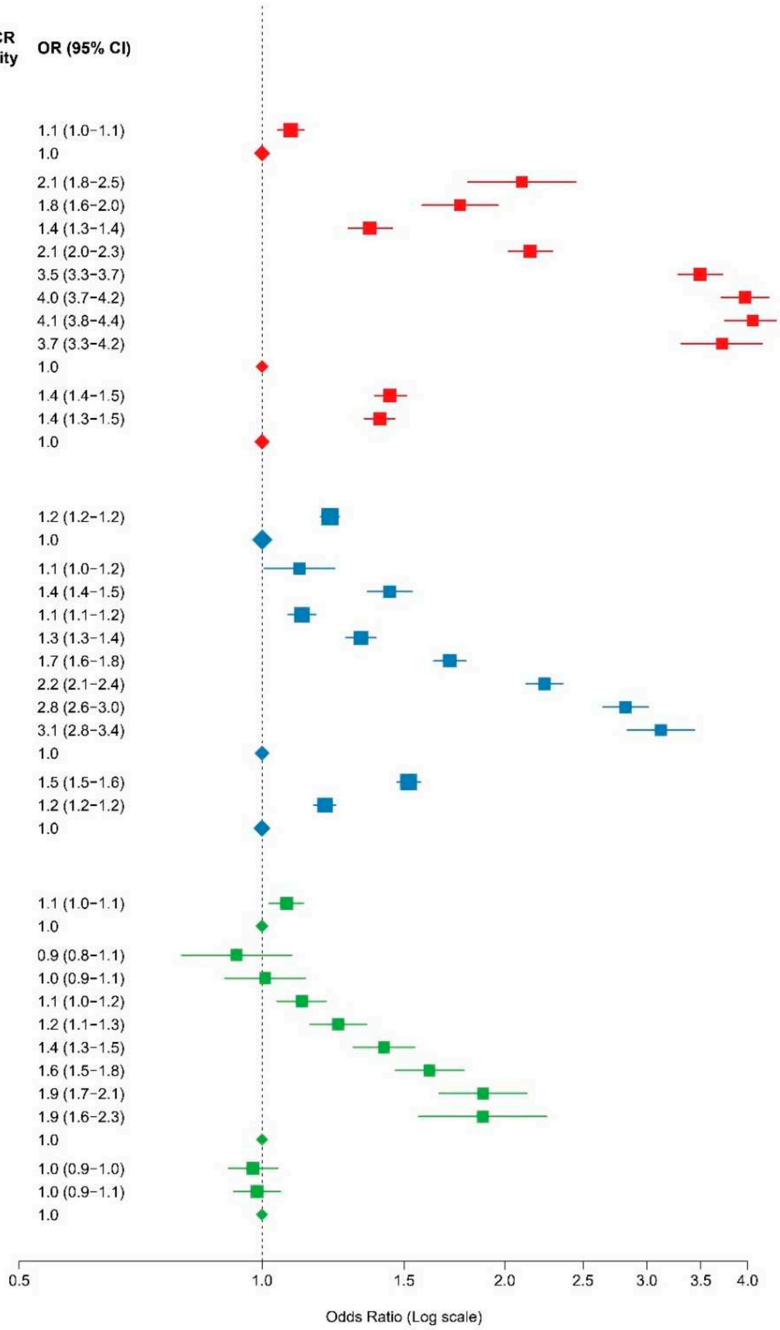

Figure 3. Adjusted odds ratios of PCR positivity at 3 viral outbreak periods in Thyrocare data. For each pandemic outbreak period, a separate multivariable binary logistic model was fitted on PCR positivity in study data to assess the independent effects of covariates age (10 yearly groups), sex, and slum status of residence ward. The age group of 20-29 years usually had the lowest reporting PCR positivity among age groups, male in sex, and areas of low slum density that represents higher social status of living were selected as the relevant reference groups.

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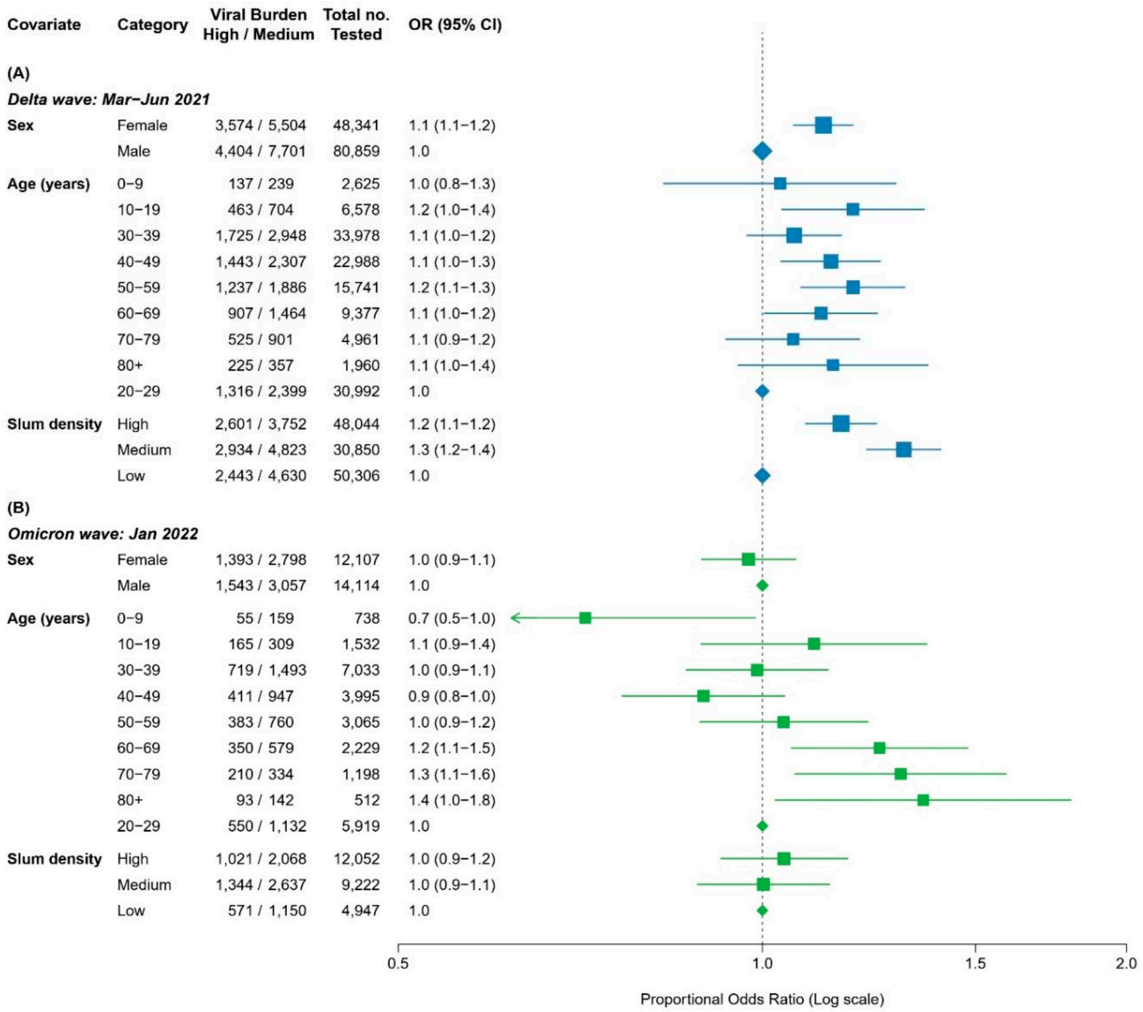

Covariate	Category	Viral Burden High / Medium	Total no. Tested	OR (95% CI)
(A)				
Delta wave: Mar–Jun 2021				
Sex	Female	3,574 / 5,504	48,341	1.1 (1.1–1.2)
	Male	4,404 / 7,701	80,859	1.0
Age (years)	0–9	137 / 239	2,625	1.0 (0.8–1.3)
	10–19	463 / 704	6,578	1.2 (1.0–1.4)
	30–39	1,725 / 2,948	33,978	1.1 (1.0–1.2)
	40–49	1,443 / 2,307	22,988	1.1 (1.0–1.3)
	50–59	1,237 / 1,886	15,741	1.2 (1.1–1.3)
	60–69	907 / 1,464	9,377	1.1 (1.0–1.2)
	70–79	525 / 901	4,961	1.1 (0.9–1.2)
	80+	225 / 357	1,960	1.1 (1.0–1.4)
	20–29	1,316 / 2,399	30,992	1.0
Slum density	High	2,601 / 3,752	48,044	1.2 (1.1–1.2)
	Medium	2,934 / 4,823	30,850	1.3 (1.2–1.4)
	Low	2,443 / 4,630	50,306	1.0
(B)				
Omicron wave: Jan 2022				
Sex	Female	1,393 / 2,798	12,107	1.0 (0.9–1.1)
	Male	1,543 / 3,057	14,114	1.0
Age (years)	0–9	55 / 159	738	0.7 (0.5–1.0)
	10–19	165 / 309	1,532	1.1 (0.9–1.4)
	30–39	719 / 1,493	7,033	1.0 (0.9–1.1)
	40–49	411 / 947	3,995	0.9 (0.8–1.0)
	50–59	383 / 760	3,065	1.0 (0.9–1.2)
	60–69	350 / 579	2,229	1.2 (1.1–1.5)
	70–79	210 / 334	1,198	1.3 (1.1–1.6)
	80+	93 / 142	512	1.4 (1.0–1.8)
	20–29	550 / 1,132	5,919	1.0
Slum density	High	1,021 / 2,068	12,052	1.0 (0.9–1.2)
	Medium	1,344 / 2,637	9,222	1.0 (0.9–1.1)
	Low	571 / 1,150	4,947	1.0

Figure 4. Proportional odds ratios of high (lowest 25% of Ct values) vs medium (25%-75% of Ct values) viral load groups. (A) Delta wave: March-June 2021, (B) Omicron wave: January 2022. We fitted two separate multivariable proportional logistic models for Delta wave and for Omicron wave to assess the effects of age, sex, and slum density of residence ward between viral load categories (distingwished by Ct value quartile ranges high viral load: lowest 25% of Ct, medium: 25%-75%, low: highest 75% of Ct). The median quartile range was chosen as the reference group. The related (25%, 50%, 75%) quartile values of each viral wave were: Delta wave: (18.0, 23.0, 28.0) and Omicron wave: (19.5, 23.2, 27.6). We assess the effects of each independent covariates including age (10 yearly groups), sex, and slum density of residence ward on high viral loads vs medium viral loads using the proportional odds ratios. The age group of 20-29 years usually had the lowest reporting PCR positivity among age groups, male in sex, and areas of low slum density that represents higher social status of living were selected as the relevant reference groups.

Discussion

Our analyses of a large dataset covering PCR positivity and viral load in Mumbai, India show the remarkably high levels of transmission occurred during the Delta and Omicron waves. The Delta wave was characterized by rapid, 64-fold increases in population medians of viral load compared to the weeks prior to the Delta wave. The rise in viral load preceded changes in PCR confirmed cases by at least 7 days in both the Delta and Omicron waves, reflecting increases in transmission caused by these high viral loads. The Delta wave was noted for high viral loads in areas of high or medium slum density, and in women. By contrast, the differences in viral load by sex and for slum density had disappeared during the Omicron wave. This may reflect the lower pathogenicity of the Omicron strain in combination with (unobserved) changes in reasons for being tested. While all three waves

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showed rising PCR positivity with age, a notably increased risk of high viral load persisted among adults over age 60 years during the Omicron wave.

India's Delta wave was particularly lethal, causing close to 3 million deaths (Jha et al., 2022). This high total arose from the biological features of the virus itself, including tropism for infecting lower respiratory tract cells that would directly influence case fatality rates. The major excess in Delta wave deaths may also reflect widespread community transmission, in particular by multiple exposures to infected people raising an individual's probability of infection, as well as the probability of becoming infected with the most infectious strain. The rapid 64-fold increase in viral load during Delta was consistent with rapid and mostly uncontrolled community transmission, including widespread intergenerational household transmission. Paradoxically, the much smaller increase in PCR test positivity rates during the Delta wave (only about 10% versus 3% during non-outbreak weeks) than seen in other waves likely reflect such widespread multi-generational infection within homes, so that many infected individuals simply did not get tested. An alternative explanation might be that Delta was not highly transmissible but extremely pathogenic.

Women had higher PCR positivity during each of the waves, but the viral load difference was seen only during the Delta wave, and not the Omicron wave. This finding and the disappearance of differences by slum population density between the Delta and Omicron wave likely reflects widespread infection during Delta and the rapid uptake of SARS-CoV-2 vaccination in Mumbai (Figure A3), which, like India's overall vaccine roll out, aimed to reach slum populations quickly (Choudhary et al., 2021). If this is the explanation, it provides some reassurance against possible future waves. Some caution is needed however, as the highest viral load during the Omicron wave was seen in the oldest age groups (above 60 years) suggesting reduced hybrid immunity (from a combination of infection and vaccination) (Brown et al., 2022). Our finding lends further argument that followup vaccine doses for the elderly are needed in India as there are now reports of emergence of Omicron variant B 2.75, and given that the Delta wave occurred several months ago. Unfortunately, India has high coverage only of two doses (Foy et al., 2021; Ghosh, 2022; Masthi et al., 2022).

Our study adds to others suggesting that use of serial trends in Ct values, even in non-representative populations is robust to track viral load, particularly in low- and middle-income countries where PCR testing access is more limited (Research in Context Panel. Most Ct values studies are from high-income countries, which differ substantially from the intergenerational transmission commonly seen in low- and middle-income countries (LMIC). In our review of the literature, we identified 67 Ct studies of which only 6 were in LMICs including two from India. None explored determinants of viral load as we do. This holds true despite some obvious limitations of our study. First, we did not have data on why people got tested in these private laboratories. Subtle biases in who is tested are a limitation of the PCR positivity rates to map trajectories of the infection (Rehman et al., 2020), and we cannot exclude that such biases also affected the Ct values. However, changes in Ct values and its early signal were so dramatic, that we believe that the signal representing higher viral load at the community level was far stronger than the inherent noise in Ct values (Tom and Mina, 2020; Cava, 2023). Similarly, various PCR machines were used, and these might affect the trends in observed Ct values. However, the ICMR and others have established the high overall sensitivity and specificity of each machines independently. Finally, the study represents only one large urban setting in India, but Mumbai has been reflective of COVID-19 patterns for urban India from the origin of the pandemic (Velumani et al., 2021; Kumar, 2020).

Our study has several strengths also, notably a large sample size with testing procedures being mostly uniform over time. Moreover, the geographic and age distribution of the Thyrocare tested population resembled that of confirmed cases reported by the MCGM.

Future studies that apply Ct values should stratify populations by past infection or vaccination status, as the sequence of these might have quite an important bearing on the relationship between infection and viral load (Abu-Raddad et al., 2022). Routine access to Ct values collected by governments may also provide a more robust early warning system to track future pandemic waves of SARS-CoV-2.

Author Contributions: Conceived the idea and developed the study design: PJ, CN. Data analysis: WS, SHF, PB, NN Literature review: WS, HG. PJ and CN, WS wrote the initial draft, and all authors were involved in commenting on subsequent revisions. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Institutional Review Board Statement: No identifiable data on any patients were used in the study. Nonetheless, we obtained Research Ethics approval from Unity Health Toronto for analyses of these anonymized data.

Informed Consent Statement: Data available to this study are anonymous. Informed consent is not applicable.

Data Availability Statement: Data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors have no competing interests to declare.

Appendix A

Table A1. Mumbai ward wise slum and non-slum population.

Ward name	Population 000					
	Total	Male	Female	Slums	Non-slums	% in slums
Low slum density wards						
A	185.0	101.2	83.8	22.3	162.7	12%
B	127.3	70.4	56.8	12.7	114.6	10%
C	166.2	98.0	68.1	16.6	149.6	10%
D	346.9	183.0	163.9	34.7	312.2	10%
E	393.3	216.1	177.2	124.2	269.1	32%
G South	377.7	331.2	267.9	124.3	253.4	33%
H West	307.6	305.9	251.4	82.6	225.0	27%
K West	748.7	401.5	347.2	215.7	533.0	29%
R Central	562.2	289.2	273.0	172.8	389.3	31%
T	341.5	176.2	165.3	85.6	255.9	25%
Medium slum density wards						
F North	529.0	285.2	243.9	238.1	290.9	45%
F South	361.0	190.7	170.2	180.1	180.8	50%
G North	599.0	208.8	169.0	361.7	237.4	60%
M West	411.9	217.6	194.3	165.0	246.9	40%
N	622.9	332.6	290.2	249.2	373.6	40%
P South	463.5	249.5	214.0	230.8	232.7	50%
R South	691.2	379.3	311.9	414.4	276.8	60%
S	743.8	398.9	344.9	408.4	335.3	55%
High slum density wards						

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H East	557.2	161.7	145.9	388.9	168.3	70%
K East	823.9	440.7	383.2	572.8	251.1	70%
L	902.2	500.6	401.6	758.1	144.1	84%
M East	807.7	436.6	371.2	686.0	121.7	85%
P North	941.4	507.5	433.8	708.2	233.1	75%
R North	431.4	233.7	197.7	281.2	150.2	65%
Total	12,442.4	6,716.0	5,726.4	6,534.5	5,907.9	53%

- Mumbai's municipal ward-wise population at 2011 government census. More than half of the population in Mumbai resides in slums.
- A, B, C, D, and E wards are in Mumbai city district and others in Mumbai suburban district.
- Wards with up to 33% of their population in slums are classified as low slum density areas. These are wards A, B, C, D, E, H-West, K-West, G-South, R-Central and T which together consist about 29% of the city's population. An average of 25% of the population of these wards lives in slums.
- Wards with 61% or more of their population in slums are classified as high slum density areas. These are the wards R-North, K-East, H-East, P-North, L and M-East, which consist about 36% of the city's population. An average of 76% of the population of these wards lives in slums.
- The remaining wards (N, M-West, F-South, F-Nort, S, R-South, G-North and P-South) consist of another 36% of the city's population are classified as the medium-slum density areas. 51% of the population of these wards lives in slums.

Source: https://github.com/muradbanaji/MumbaiData/blob/master/SlumNonslum.pdf

Table A2. Mumbai postal pin codes mapped to municipal wards.

Mumbai city district		Mumbai Suburban district	
Ward name	Pin code	Ward name	Pin code
A	400001	HE	400029
	400005		400051
	400020		400055
	400021		400098
	400032	HW	400050
	400039		400052
B	400003		400054
	400009	KE	400057
C	400002		400059
D	400004		400060
	400006		400069
	400007		400093
	400026		400096
	400034		400099
	400035	KW	400049
E	400008		400053
	400010		400056
	400011		400058

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	400027		400102
FN	400014	L	400024
	400019		400070
	400022		400072
	400031		400084
	400037	ME	400043
FS	400012		400085
	400015		400088
	400033		400094
GN	400016	MW	400071
	400017		400074
	400028		400089
GS	400013	N	400075
	400018		400077
	400025		400079
	400030		400086
		PN	400061
			400064
			400095
			400097
		PS	400062
			400063
			400065
			400104
		RC	400091
			400092
		RN	400068
			400103
		RS	400067
			400101
		S	400042
			400076
			400078
			400083
			400087
		T	400066
			400080
			400081
			400082

Notes: Out of the 91 designated pin codes in Mumbai, some straddled more than one municipal wards. We mapped those pin codes into the municipal ward based on population.

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Table A3. ICMR approved testing kits used at Thyrocare central laboratory for PCR diagnosis of SARS-CoV-2 infection.

No.	Test Kit Name	Targets (Genes)*	LOD (Limit of Detection)
1	RealStar®SARS-CoV-2PCRKit	S, E	0.014,0.025 PFU/ml
2	Lab gun Siemens	RdRp, E	20 genomic RNA copies/μL
3	TaqPath™COVID-19 Combo Kit	S, ORF1ab, N	10 GCE/reaction
4	Detection expert1SSARS CoV-2 Gene store	N1, N2	100 GCE/Reaction
5	MerilCOVID-19One-stepPCRKit	N, ORF1ab	<500 RNA copies/mL
6	COVIPATH	N, ORF1ab	10 GCE/reaction

Source: Thyrocare laboratory. Abbreviations: PFU, plaque forming units; GCE, Genomic copy equivalents; mL, milliliter; RNA, Ribonucleic acid; μL, microliter. * Details on gene targets can be found at https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf. **All these kits are approved by the Indian Council of Medical Research for PCR testing purposes and the ranges of sensitivity >95% and specificity >99%. Also mentioned here LOD for each kit which varies**.

Table A4. PCR confirmed Covid cases and deaths of Mumbai Covid dashboard at different viral outbreak periods from April 2020 - January 2022.

	Cases					Deaths				
			Outbreak periods			Total	No	Outbreak periods		
Characteristics	Total since Apr 2020 to Jan 2022	Periods no major outbreaks	Aleph Wave: Jun-Nov 2020 (6 mo.)	Delta wave: Mar-Jun 2021 (4 mo.)	Omicron: Jan 2022 (till Jan17) (1 mo.)	since Apr 2020 to Jan 2022	major outbreaks	Aleph wave: May-Oct 2020 (6 mo.)	Delta wave: Mar-Jun 2021 (4 mo.)	Omicron wave: Jan 2022 (till Jan17) (1 mo.)
No. of months	22	11	6	4	1	22	11	6	4	1
PCR confirmed cases										
Total	**1,022,979**	**160,288**	**232,484**	**376,528**	**253,679**	**16,668**	**2,850**	**9,627**	**3,980**	**211**
Annualized rate per 1000 population**										
All ages	**192**	**13**	**34**	**82**	**222**	**1.5**	**0.2**	**1.4**	**0.9**	**0.2**
0-9	29	2	4	11	36	0.0	0.0	0.0	0.0	0.0
10-19	66	4	8	26	83	0.0	0.0	0.0	0.0	0.0
20-29	163	9	21	66	201	0.1	0.0	0.1	0.0	0.0
30-39	237	14	36	102	284	0.3	0.1	0.2	0.2	0.0
40-49	248	17	47	115	276	1.0	0.2	0.9	0.7	0.1
50-59	332	25	77	149	357	3.3	0.6	3.4	2.0	0.1
60-69	414	33	95	179	452	7.8	1.2	7.9	4.3	0.8
70-79	559	41	115	229	638	15.9	2.1	15.1	9.2	2.6
80+	588	39	104	247	688	23.3	3.2	19.5	14.0	6.1
Sex										

Female	n.a.	n.a.	29	76	192	n.a.	n.a.	n.a.	n.a.	n.a.
Male	n.a.	n.a.	38	88	248	n.a.	n.a.	n.a.	n.a.	n.a.
Municipal ward, Slum density										
		16.4	**45.1**	**123.5**	**262.5**					
Low slum areas	**261.2**									
A	379	23	55	132	484					
B	79	9	23	34	79	Deaths by wards not available				
C	101	8	37	46	93					
D	316	22	61	148	349					
E	155	15	33	73	162					
G South	193	16	38	81	219					
H West	470	25	53	159	625					
K West	324	17	38	116	422					
R Central	257	16	61	131	264					
T	266	22	66	138	261					
Medium slum		**13.1**	**32.8**	**77.4**	**195.7**					
areas	**173.8**									
F North	179	15	27	80	205					
F South	169	14	43	65	186					
G North	137	12	32	60	148					
M West	216	14	30	85	267					
N	149	12	34	74	152					
P South	270	14	39	123	319					
R South	209	12	41	100	231					
S	150	10	29	62	174					
		8.2	**25.9**	**68.8**	**164.3**					
High slum areas	**151.5**									
H East #	166	12	22	68	203					
K East	206	12	31	88	247					
L	99	8	16	40	119					
M East #	91	7	15	38	108					
P North	144	8	30	70	157					
R North	159	8	34	77	172					

Notes:

1. ** The denominator for case rate and death rates was the estimated 13 million Mumbai population. For convenience of comparing rates between viral waves, rates were adjusted to duration (number of months) of each pandemic wave to reflect the annual rate.
2. Data presented in this table were from Mumbai Covid dashboard data and as available for 24 wards of the Greater Mumbai municipal corporation area (MCGM (2022a)). Covid deaths by wards were not available.
3. # Ward H East and M East are home to Dharavi slums, one of the highest population density areas of Mumbai.
4. Wards with less than 33% of population lives in slums were categories as low slum density areas and more than 60% as high slum density areas. Detailed slum population shows in Table A1.
5. Rates by sex was calculated using a subset of data where male and female breakdown was available.

Table A5. Descriptive PCR positivity rates (%) of the Thyrocare tested population at different viral outbreak periods from April 2020 to January 2022.

Characteristics	Total period No. tested (000)	Aleph wave Jun-Nov 2020 (6 months) No. tested (000)	Aleph wave Jun-Nov 2020 (6 months) *PCR Positivity* % *	Delta wave Mar-Jun 2021 (4 months) No. tested (000)	Delta wave Mar-Jun 2021 (4 months) *PCR Positivity* % *	Omicron wave Jan 2022 (1 months) No. tested (000)	Omicron wave Jan 2022 (1 months) *PCR Positivity* % *	Periods no major outbreaks Apr 2020 - Jan 2022 No. tested (000)	Periods no major outbreaks Apr 2020 - Jan 2022 *PCR Positivity* % *
Total no. tested (No. positive)	2,717.3 (155.0)	64.2 (17.9)	***23.2***	445.1 (46.6)	***9.9***	26.2 (11.7)	***42.8***	2,181.7 (78.8)	***3.4***
Age in 10 years									
0-9	73.7	0.9	***28.9***	14.1	***9.8***	0.7	***38.5***	58.0	***5.3***
10-19	269.5	2.3	***25.0***	39.6	***9.3***	1.5	***40.5***	226.1	***4.1***
20-29	641.5	13.4	***15.6***	103.2	***8.7***	5.9	***40.2***	518.9	***2.7***
30-39	580.5	15.7	***20.1***	99.2	***10.5***	7.0	***42.8***	458.6	***3.6***
40-49	457.7	11.1	***28.4***	75.6	***10.6***	4.0	***45.5***	367.0	***3.6***
50-59	329.2	9.2	***39.5***	53.7	***11.3***	3.1	***48.7***	263.2	***3.7***
60-69	233.0	6.5	***42.9***	37.1	***12.2***	2.2	***51.7***	187.2	***4.2***
70-79	106.7	3.7	***43.5***	17.9	***14.0***	1.2	***55.7***	84.0	***4.4***
80+	25.3	1.4	***41.2***	4.7	***20.5***	0.5	***55.9***	18.7	***6.1***
Sex									
Female	1,105.3	22.5	***25.9***	166.4	***11.5***	12.1	***44.1***	904.2	***3.8***
Male	1,612.0	41.7	***21.9***	278.7	***9.0***	14.1	***41.8***	1,277.5	***3.2***
***District, Municipal wards and slum* density**									
Mumbai	2,567.0	33.7	***22.0***	386.7	***7.8***	12.6	***41.3***	2,134.0	***3.4***
Mumbai Suburban	150.3	30.5	***24.7***	58.5	***23.5***	13.6	***44.2***	47.8	***6.5***
Low slum areas	**2,519.1**	**35.8**	**20.4**	**366.2**	**7.2**	**4.9**	**44.3**	**2,112.1**	**3.3**
A	2,386.8	6.1	7.8	321.9	5.6	0.3	35.2	2,058.5	3.3
B	12.6	4.1	46.6	5.5	19.7	0.0	43.5	2.9	5.2
C	2.7	0.6	11.4	0.8	15.5	0.2	35.0	1.0	4.9
D	48.2	6.0	16.0	14.7	12.8	0.8	42.1	27.5	4.2

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E	11.8	0.9	9.4	5.8	17.3	-	-	4.3	3.3
GS	9.1	3.1	15.0	2.3	18.9	0.4	45.1	3.3	7.1
H West	6.1	1.2	18.6	2.5	20.6	0.3	47.5	2.1	6.8
K West	24.8	8.6	23.1	6.9	23.7	1.5	42.7	7.8	8.8
R Central	9.3	3.1	33.6	3.0	24.5	0.3	50.9	2.9	11.6
T	7.8	2.0	15.9	2.9	35.2	1.2	48.8	1.7	8.9
Medium slum areas	**79.1**	**11.8**	**28.8**	**30.9**	**25.3**	**9.2**	**42.2**	**27.3**	**8.3**
F North	11.8	3.2	38.4	4.4	24.6	0.9	47.0	3.3	21.7
F South	6.0	0.5	25.9	2.4	16.2	0.3	32.6	2.8	2.2
G North	21.2	3.7	26.3	7.0	20.5	2.7	37.0	7.9	7.3
M West	5.8	0.8	24.8	2.5	24.2	0.5	45.9	1.8	4.4
N	10.5	0.6	38.2	4.2	23.5	1.8	43.8	4.0	7.4
P South	10.0	1.6	15.1	4.4	30.5	1.3	38.6	2.7	8.0
R South	10.3	1.1	32.4	5.0	35.9	1.3	55.0	2.9	8.6
S	3.6	0.3	29.5	1.0	21.2	0.5	32.6	1.7	3.9
High slum areas	**119.0**	**16.6**	**25.8**	**48.0**	**19.9**	**12.1**	**42.8**	**42.3**	**5.2**
H East	36.0	5.9	21.6	16.2	16.4	2.4	36.2	11.5	3.7
K East	26.1	2.8	12.4	10.0	20.9	2.9	43.3	10.4	4.7
L	24.0	1.5	20.4	9.1	15.7	2.7	41.0	10.7	5.2
M East	8.2	1.7	23.8	2.8	19.4	0.9	43.9	2.8	8.1
P North	17.9	3.3	50.8	7.2	29.3	2.3	47.0	5.1	8.3
R North	6.8	1.4	34.9	2.7	29.2	0.8	56.0	1.9	6.5

Notes:

1. This table presents PCR test positivity of 2.7 million self-tested population in Mumbai. The last column "Periods no major outbreaks" are the remaining 10 months without major outbreaks.
2. * To compare PCR positivity rates between viral waves, we standardized the age of tested population to age distribution of census population in 2011.

Table A6. Descriptive Ct values of the Thyrocare tested population during different viral waves from April 2020 to January 2022.

Characteristics	Total period	Aleph wave Jun-Nov 2020 (6 mo.)		Delta wave Mar-Jun 2021 (4 mo.)		Omicron wave Jan 2022 (1 mo.)		Periods no major outbreaks Apr 2020 - Jan 2022	
	No. tested (000)	No. tested (000)	Median Ct value	No. tested (000)	Median Ct value	No. tested (000)	Median Ct value	No. tested (000)	Median Ct value
Total	**2,717.3**	**64.2**	**26.0**	**445.1**	**23.0**	**23.2**	**23.6**	**2,181.7**	**25.0**
Age in 10 years									
0-9	73.7	0.9	24.0	14.1	24.0	0.7	22.9	58.0	25.0

10-19	269.5	2.3	25.0	39.6	23.0	1.5	23.1	226.1	25.0
20-29	641.5	13.4	25.0	103.2	23.0	5.9	23.7	518.9	25.0
30-39	580.5	15.7	26.0	99.2	23.0	7.0	23.2	458.6	24.0
40-49	457.7	11.1	26.0	75.6	23.0	4.0	23.0	367.0	25.0
50-59	329.2	9.2	26.0	53.7	22.0	3.1	22.8	263.2	25.0
60-69	233.0	6.5	26.0	37.1	22.0	2.2	21.9	187.2	24.0
70-79	106.7	3.7	26.0	17.9	22.0	1.2	21.8	84.0	24.0
80+	25.3	1.4	26.0	4.7	22.0	0.5	21.4	18.7	24.0
Sex									-
Female	1,105.3	22.5	25.0	166.4	23.0	12.1	23.1	904.2	25.0
Male	1,612.0	41.7	26.0	278.7	23.0	14.1	23.2	1,277.5	24.0
District, municipal wards and slum density									
Mumbai	2,567.0	33.7	26.0	386.7	23.0	12.6	23.2	2,134.0	25.0
Mumbai Suburban	150.3	30.5	25.0	58.5	22.0	13.6	23.2	47.8	23.5
	2,519.1	**35.8**	**26.0**	**366.2**	**24.0**	**4.9**	**22.9**	**2,112.1**	**25.0**
Low slum areas									
A	2,386.8	6.1	29.0	321.9	24.0	0.3	23.6	2,058.5	25.0
B	12.6	4.1	26.0	5.5	22.0	0.0	20.8	2.9	26.0
C	2.7	0.6	26.0	0.8	23.0	0.2	25.5	1.0	22.1
D	48.2	6.0	28.0	14.7	24.0	-	-	27.5	26.0
E	11.8	0.9	27.0	5.8	23.0	0.8	23.3	4.3	22.8
G South	9.1	3.1	27.0	2.3	22.0	0.4	22.6	3.3	22.2
H West	6.1	1.2	25.0	2.5	22.0	0.3	23.5	2.1	22.0
K West	24.8	8.6	25.0	6.9	22.0	1.5	22.9	7.8	23.0
R Central	9.3	3.1	24.0	3.0	22.0	0.3	22.1	2.9	22.0
T	7.8	2.0	26.0	2.9	21.0	1.2	22.7	1.7	22.0
Medium slum areas	**79.1**	**11.8**	**25.0**	**30.9**	**21.0**	**9.2**	**23.0**	**27.3**	**23.0**
F North	11.8	3.2	26.0	4.4	21.0	0.9	23.1	3.3	22.0
F South	6.0	0.5	27.0	2.4	22.0	0.3	24.4	2.8	23.0
G North	21.2	3.7	25.0	7.0	21.0	2.7	23.3	7.9	23.0
M West	5.8	0.8	28.0	2.5	24.0	0.5	23.0	1.8	25.0
N	10.5	0.6	23.0	4.2	21.0	1.8	22.8	4.0	22.0
P South	10.0	1.6	25.0	4.4	21.0	1.3	22.7	2.7	22.0
R South	10.3	1.1	25.0	5.0	20.0	1.3	22.6	2.9	23.0
S	3.6	0.3	26.0	1.0	22.0	0.5	23.4	1.7	22.3
High slum areas	**119.0**	**16.6**	**25.5**	26.0	**22.5**	22.0	**23.7**	23.4	
H East	36.0	5.9	26.0	16.2	23.0	2.4	25.0	11.5	24.0
K East	26.1	2.8	25.0	10.0	22.0	2.9	23.1	10.4	22.0
L	24.0	1.5	26.0	9.1	22.0	2.7	23.2	10.7	23.0
M East	8.2	1.7	26.0	2.8	22.0	0.9	23.3	2.8	21.0
P North	17.9	3.3	25.0	7.2	22.0	2.3	22.8	5.1	24.0

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R North	6.8	1.4	26.0	2.7	21.0	0.8	22.9	1.9	25.0

Notes: This table presents PCR Ct values of 2.7 million self-tested population data from Thyrocare laboratory network. Total study period is 22 months from April 2020 to January 2022 and during this period 3 pandemic outbreaks were experienced in Greater Mumbai area. The last column "Periods no major outbreaks" is the remaining 11 months of the pandemic period where no major outbreaks occurred.

Table A7. The begin, peak and end of viral waves observed in Ct values, PCR positivity, confirmed Covid case and deaths at Delta and Omicron outbreaks.

Pandemic period/ Time series	The earliest date of change observed at			No. of days from the earliest observed date ¥			Number of days beginning to peak and peak to end		
	Beginning	Peak	End	Beginning	Peak	End	Beginning to peak	Peak to end	Overall
Delta wave (Mar-Jun 2021)									
Thyrocare study data									
Ct value	1-Feb-2021	31-Mar-2021	19-May-2021	0	0	0	58	49	107
PCR Positivity	18-Feb-2021	5-Apr-2021	16-Jun-2021	17	5	28	46	72	118
MCGM official PCR confirmed									
Cases	9-Feb-2021	5-Apr-2021	4-Jul-2021	8	5	46	55	90	145
Deaths	13-Mar-2021	1-May-2021	8-Aug-2021	40	31	81	49	99	148
Omicron wave (Jan 2022)									
Thyrocare study data									
Ct value	11-Dec-2021	2-Jan-2022	30-Jan-2022*	0	0	*	22	28*	50*
PCR Positivity	22-Dec-2021	11-Jan-2022	30-Jan-2022*	11	9	*	20	19*	39*
MCGM official PCR confirmed									
Cases	18-Dec-2021	10-Jan-2022	29-Jan-2022*	7	8	*	23	19*	42*
Deaths	4-Jan-2022	24-Jan-2022	30-Jan-2022*	24	22	*	20	6*	26*

Notes: This table presents wave properties of the earliest dates observed in the beginning, peak and end of Ct values, PCR positivity, MCGM's confirmed Covid case prevalence and death time series during Delta and Omicron SARS-CoV-2 outbreak periods in Mumbai. Each time series was recorded daily and used 7-day average for this analysis. The relevant daily time series data are illustrated in Figure A2. Aleph wave (Jun - Nov 2020)

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was excluded here because Ct values were not available for the complete outbreak period. * In Omicron wave, end date would not be fully accurate because data collection terminated prematurely at end of January 2022. ¥ No. of days were calculated using the difference of days between the earliest observed date of any of the time series.

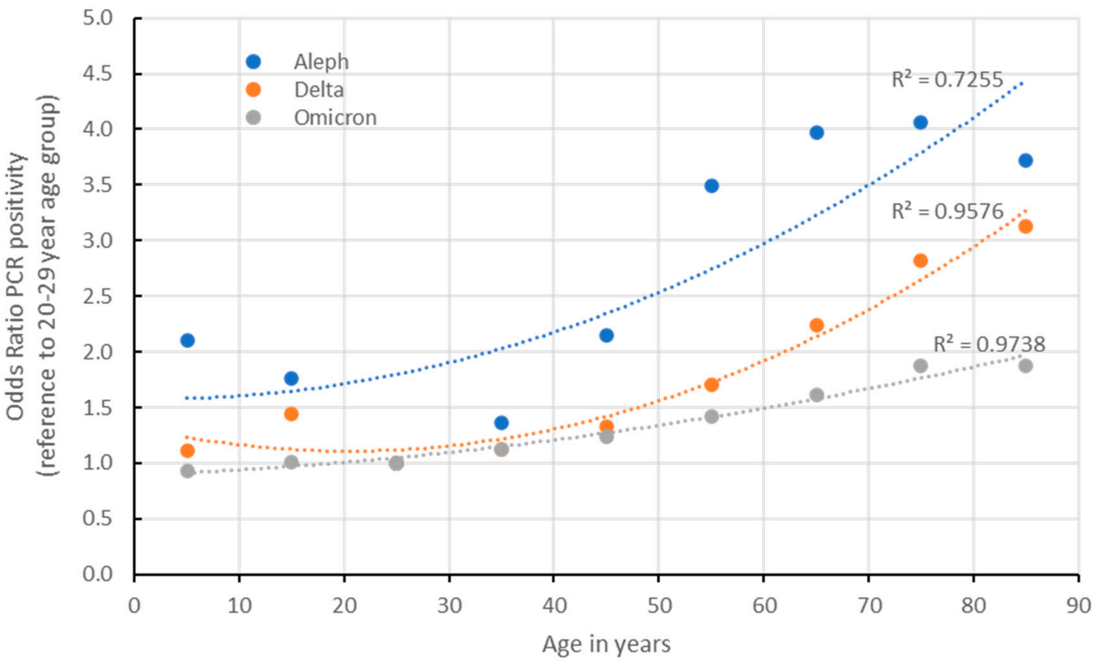

Figure A1. Age trend of PCR positivity during Aleph, Delta, and Omicron viral waves. The dotted scatter represents the gender and slum status adjusted odds ratios of PCR positivity for 10 yearly age groups. Odds ratios were measured relative to the age 20-29 year group whom always had the lowest positivity rate. The dotted curves are the polynomial trends for each viral wave. R-squared for each viral wave: Aleph 0.73, Delta 0.96 and Omicron 0.97. The original odds ratios used in this figure were extracted from Figure 3, forest plot. We tested for trend of age using Cochran-Armitage Trend Test: Aleph (p<0.0001), Delta (p<0.0001) and Omicron (p<0.0001).

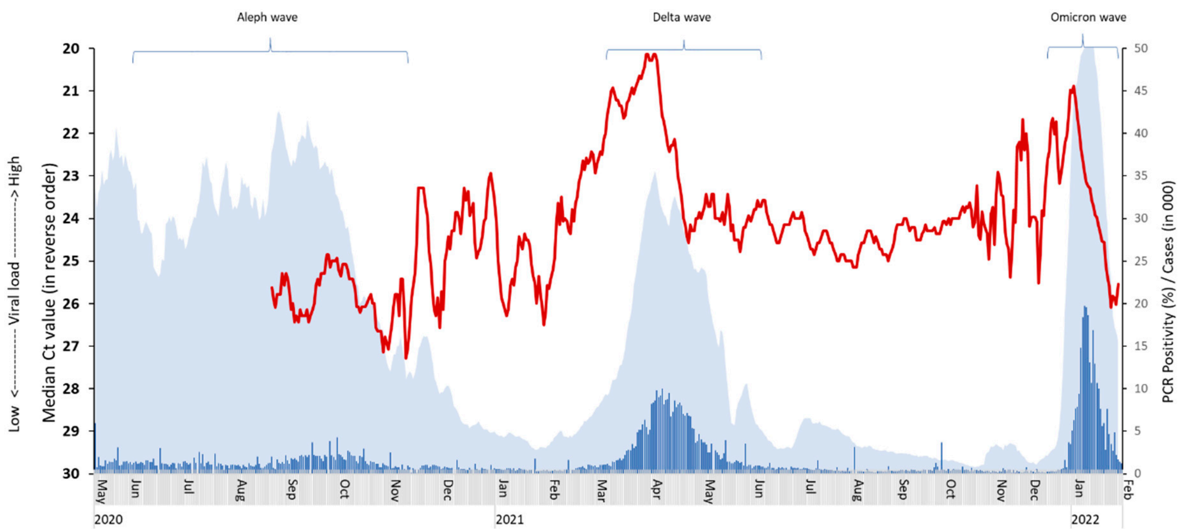

Figure A2. Overview of Mumbai SARS-CoV-2 viral outbreaks - daily time series of MCGM Covid-19 dashboard and Thyrocare study data. First pandemic outbreak was from Jun-Nov 2020 (Aleph wave), second from Mar-Jun 2021 (Delta wave), and third in January 2022 (Omicron wave). Daily PCR confirmed case counts in 000 (blue bar) are as reported in Mumbai Covid dashboard. 7-day average

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PCR positivity (gray area) and daily median Ct values (red curve) are from Thyrocare data. The red curve represents the median Ct values refers to Y-axis on the left and all other time series refer to Y-axis on the right. Left Y-axis is shown in reverse order so a lower Ct value implies a higher viral load.

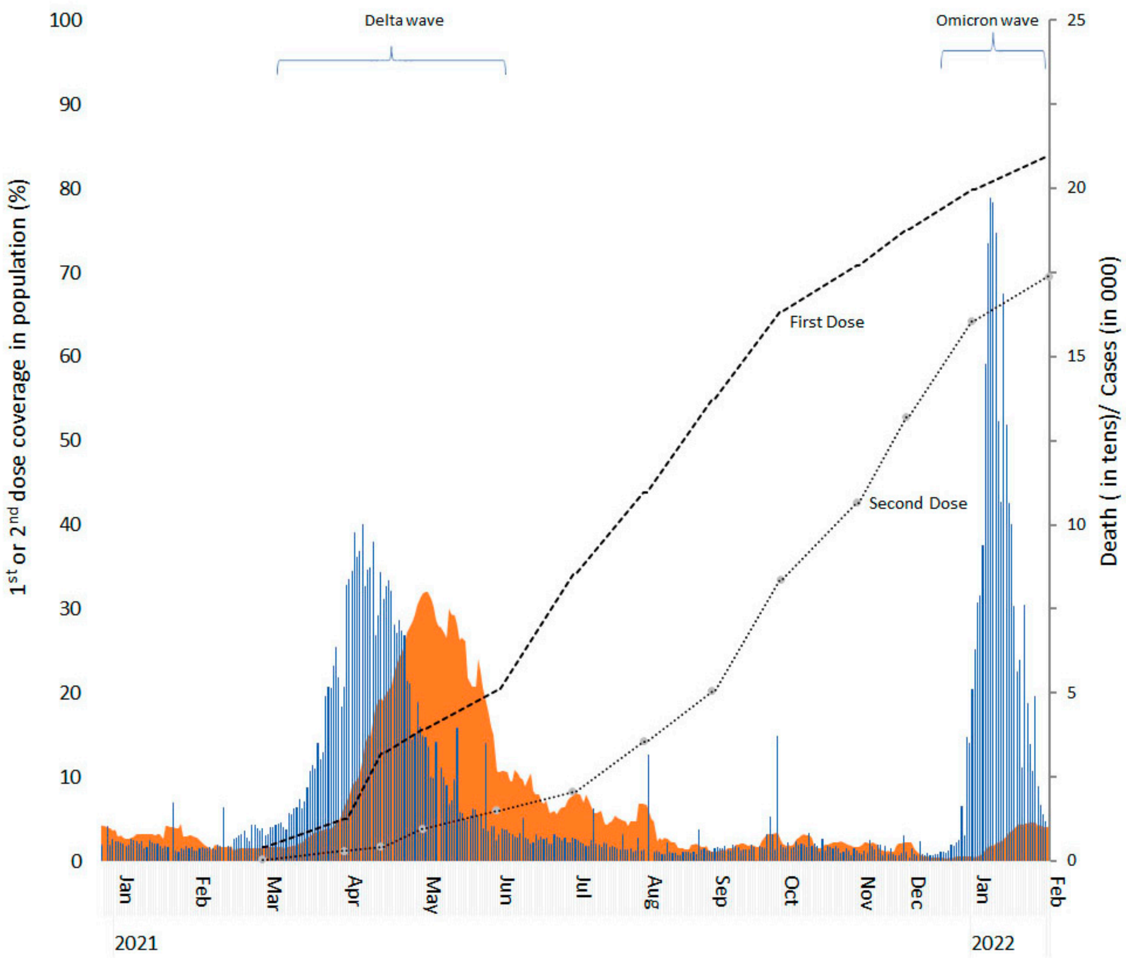

Figure A3. Vaccine coverage, daily time series of PCR confirmed cases and deaths from COVID-19 from January 2021 - January 2022 in Mumbai. The first pandemic outbreak wave was from Jun-Nov 2020 (Aleph wave), second from Mar-Jun 2021 (Delta wave), and third in January 2022 (Omicron wave). First pandemic wave is not shown here because vaccine coverage was not substantial at the period. Daily PCR confirmed case counts in 000 (blue bar), deaths in tens (orange area), percentage of vaccine coverage (thick dotted line - 1st dose, thin dotted line - 2nd dose) are as reported in Mumbai Covid dashboard. The vaccine coverage refers to Y-axis in left and all other time series refer to Y-axis in right.

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