

An Early and Preliminary Assessment of the Clinical Severity of the Emerging SARS-CoV-2 Omicron Variants in Maharashtra, India

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Abstract

Background: The SARS-CoV-2 Omicron variants BA.2.74, BA.2.75, and BA.2.76 have appeared recently in India and have already spread to over 40 countries. They have acquired additional mutations in their spike protein compared to BA.2, branching away on the SARS-CoV-2 phylogenetic tree. These added mutations have raised concerns about the impact on viral pathogenicity, transmissibility, and immune evasion properties of the new variants.

Material and methods: A total of 990 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) positive SARS-CoV-2 samples, with a cycle threshold value (Ct) less than 25, were processed for SARS-CoV-2 whole genome sequencing between June 3, 2022 to August 7, 2022. All corresponding demographic and clinical data were recorded and analyzed using Microsoft® Excel.

Results: Out of 990 samples sequenced, BA.2.75 (25.03%) was the predominant Omicron sublineage, followed by BA.2.38 (21.01%), BA.5 (9.70%), BA.2 (9.09%), BA.2.74 (8.89%) and BA.2.76 (5.56%). A total of 228 cases of BA.2.74, BA.2.75, and BA.2.76 were contacted by telephone, of which 215 (94.30%) were symptomatic with mild symptoms, and 13 (5.70%) had no symptoms. Fever (82.02%) was the most common symptom, followed by cough (49.12%), cold (35.97%), fatigue (27.19%), headache (21.05%), and myalgia (20.61%). Of the 228 cases, 195 (85.53%) cases recovered at home, and 33 (14.47%) required institutional quarantine. Recovery with conservative treatment was observed in 92.98% of cases, while 4.83% required additional oxygen therapy. Only three (1.32%) cases had poor outcomes resulting in death, and the remaining 225 (98.68%) survived. Among the 228 cases, 219 (96.05%) cases were vaccinated with the COVID-19 vaccine; of these, 72.60% had received both doses, 26.03% had also received the precautionary booster dose, while 1.37% were incompletely vaccinated with a single dose of vaccine.

Conclusion: The current study indicates that the three BA.2 sublineages are causing mild disease in India. However, BA.2.75 has key mutations that are notable for accelerated growth and transmission and require close and effective monitoring.

Categories: Infectious Disease

Keywords: sars-cov-2 whole-genome sequencing, ba.2.76, ba.2.75, ba.2.74, omicron

Introduction

Since the first case of severe acute respiratory syndrome coronavirus (SARS-CoV-2) in March 2020, India has witnessed three pandemic waves. The original SARS-CoV-2 strain with D614G mutation (Clade G, Pangolin lineage B.1), Clade GH (Pangolin lineage B.1), and GR (Pangolin lineage B.1.1.1) dominated the first wave followed by the Delta (B.1.617.2) and its sublineages (AY. *) caused the second wave, and Omicron (B.1.1.529) and its sublineages (BA.1 and BA.2) are driving the third wave [1].

The first Omicron variant, B.1.1.529 was first reported from South Africa on November 24, 2021 [2] and was designated as a Variant of Concern (VoC) on November 26, 2021 by World Health Organization. By late January 2022, the Omicron variant had been identified in 171 countries across all six WHO regions. Due to its substantial growth advantage over Delta, the Omicron variant rapidly replaced the Delta variant globally. Initially, the Omicron variant (B.1.1.529) comprised three sister lineages, B.1.1.529.1 (BA.1), B.1.1.529.2 (BA.2) and BA.1.1.529.3 (BA.3). Characteristic constellation of mutations, particularly 26-32 mutations in its spike protein, have been responsible for its increased transmissibility and its ability to evade immunity established by natural infection or vaccination [3].

India witnessed its third wave from late December 2021 to late February 2022 [4], with BA.1 and BA.2

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dominating the early and the latter half of the wave, respectively [3]. Since then, the Omicron variant has evolved continuously to give rise to BA.4/ BA.5 in South Africa [5] and BA.2.12.1 in the USA [6], causing global outbreaks. Unlike the Delta variant, Omicron caused less severe disease and decreased hospitalization rates and deaths. However, unvaccinated individuals with advanced age and underlying disease conditions suffered from the severe disease [7].

After the waning of the third wave, India saw a surge in COVID-19 cases from the fourth week of May 2022 [4]. On sequencing, these variants were characterized as BA.2 by Pangolin COVID-19 Lineage Assigner, a web application designed by the Centre for Genomic Pathogen Surveillance to assign lineages. However, the predominance of BA.2 after the waning of the third COVID-19 wave was unexplainable. This issue was discussed in detail on the GitHub repository, cov-lineages/pango-designation, a repository for suggesting new lineages that should be added to the current scheme for naming the SARS-CoV-2 virus. Subsequently, the Indian isolates of BA.2 were further classified into sub-lineages BA.2.74 (Issue #775) [8], BA.2.75 (Issue #773) [9], and BA.2.76 (Issue #787) [10]. The BA.2.75 subvariant has been classified as an Omicron subvariant under monitoring by the World Health Organization (WHO) due to its increasing numbers in India and its identification in dozen countries, including Nepal, Singapore, Martinique, China, New Zealand, Cambodia, and Indonesia [11]. Only three months after its designation, BA.2.75 subvariant has acquired several mutations to give rise to new sublineages (BA.2.75.1, BA.2.75.2, BA.2.75.3, BA.2.75.4, BA.2.75.6, BL.1, BM.1 and BN.1) that can compete with the circulating lineages [12].

With the emergence of new variants, data on the clinical severity of the disease caused by them are essential to guide public health planning and response. Therefore, the current study aimed to describe the severity and clinical presentation of these emerging SARS-CoV-2 variants sequenced at Byramjee Jeejeebhoy Government Medical College, Pune, Maharashtra.

Materials And Methods

This study was carried out at the Department of Microbiology of the Byramjee Jeejeebhoy Government Medical College (BJGMC), Pune, Maharashtra. It was part of the Indian SARS-CoV-2 Genomics Consortium (INSACOG) sequencing activity for epidemiological and clinical surveillance of SARS-CoV-2 in Maharashtra. INSACOG is a Pan-India network of 54 laboratories to monitor the genomic variations in the SARS-CoV-2 virus and to study the linkages between the variants and epidemiological trends. The present study was conducted for rapid assessment of the clinical severity of the newly identified sub-lineages of BA.2 in India.

The study falls within the research activities approved by the BJGMC Institutional Ethics Committee, Pune, Maharashtra, India. This article was previously posted to the medRxiv preprint server on September 9, 2022.

Sample acquisition

The sequencing laboratory at the Department of Microbiology, BJGMC, Pune, is the coordinating laboratory for the surveillance of the SARS-CoV-2 virus in the community in Maharashtra. It receives samples for sequencing from various SARS-CoV-2 RT-PCR swab collection and testing centers in Pune and other districts of Maharashtra. It also receives samples from patients admitted to its dedicated COVID-19 hospital. Thus, the samples adequately represented hospital-admitted and community cases of COVID-19.

Nasopharyngeal samples collected in Viral Transport Medium, between 3rd June 2022 to 7th August 2022 with a cycle threshold (Ct) value of less than 25, were transported to the laboratory, maintaining a cold chain at 2 to 8°C. The samples were stored at -80°C until further processing.

The viral RNA was extracted using the MagMax™ Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific Inc., Waltham, US), following the manufacturer's instructions. The RNA samples were processed for SARS-CoV-2 whole genome sequencing, and its analysis was done at the Centre of Excellence for Genomics, Department of Microbiology, BJGMC, Pune.

Library preparation, next-generation sequencing, and lineage analysis

Libraries for COVID-19 were prepared using Rapid Barcoding and Midnight RT-PCR Expansion kits (Midnight protocol) (Oxford Nanopore Technologies (ONT), Littlemore, United Kingdom). Sequencing was conducted on the R9.4 flow cell by ONT using a GridION sequencer (ONT, United Kingdom). Primary data acquisition was performed using MinKNOW, version 22.05.7, the operating software that operates nanopore sequencing devices. Base-calling was performed using Guppy, version 6.1.5, in fast base calling mode. The data were further processed using the wf-ARTIC workflow, a repository that contains the nextflow workflow for running the ARTIC SARS-CoV-2 workflow on multiplexed GridION runs, installed in MinKNOW software. Lineage identification was carried out using Pangolin COVID-19 Lineage Assigner, version 4.1.1, pangolin-data version 1.12, and University of California Santa Cruz (UCSC) Genome Browser USHER: Ultrafast Sample placement on Existing tree. The clade analysis was done using the Nextclade software, version 2.3.0.

Data collection

A set of individual-level data was obtained, corresponding to the samples received from various RT-PCR laboratories sending samples for sequencing. Each sample's unique identification number (ICMR ID) was also recorded. Additional information on the presence of any symptoms, hospitalization, treatment, comorbidities, and vaccination status was collected via a telephonic interview with each patient.

Statistical analysis

All demographic and clinical data were recorded and analyzed using Microsoft® Excel.

Results

Between June 3, 2022 and August 7, 2022, 990 RT-PCR-positive SARS-CoV-2 samples were processed and sequenced at BJGMC, Pune. The study population included cases from all age groups with a median age of 36 years. The male-to-female ratio was 1.39:1. Table 1 shows the geographical distribution of the sequenced samples.

Geographical Area	Number (%)
Pune	792 (80%)
Mumbai	143 (14.44%)
Solapur	27 (2.73%)
Latur	16 (1.62%)
Jalgaon	7 (0.71%)
Sindhudurg	5 (0.50%)
Grand Total	990 (100%)

TABLE 1: Geographical distribution of 990 RT-PCR-positive SARS-CoV-2 samples

Of these 990 samples sequenced, BA.2.75 (23.03%) was the predominant Omicron sublineage followed by BA.2.38 (21.01%), BA.5 (9.70%), BA.2 (9.09%), BA.2.74 (8.89%) and BA.2.76 (5.56%) (Table 2).

SARS-CoV-2 Lineage	Number (%)	
BA.2.38	BA.2.38	193
	BA.2.38.1	13
	BH.1 (BA.2.38.3.1)	02
BA.2.75	BA.2.75	215
	BA.2.75.1	13
BA.2.74		88 (8.89%)
BA.2		90 (9.09%)
BA.5	BA.5	07
	BA.5.1	03
	BA.5.2	33
	BA.5.2.1	27
	BA.5.2.3	12
	BA.5.3	01
	BA.5.3.1	01
	BA.5.5	01
	BE.3	02
		96 (9.70%)

	BF.3	08	
	BF.5	01	
BA.2.76			55 (5.56%)
	BA.4	08	
BA.4	BA.4.1	01	12 (1.21%)
	BA.4.1.8	02	
	BA.4.6	01	
BA.2.10			10 (1.01%)
BA.2.18			08 (0.81%)
B.1.1.529			06 (0.61%)
BA.2.79	BA.2.79	01	03 (0.30%)
	BA.2.79.1	02	
BA.2.15			02 (0.20%)
BA.2.40.1			02 (0.20%)
BA.2.12	BA.2.12	01	02 (0.20%)
	BA.2.12.1	01	
BA.2.7			01 (0.10%)
BA.2.23			01 (0.10%)
BA.2.43			01 (0.10%)
BA.2.56			01 (0.10%)
BA.2.61			01 (0.10%)
Samples with poor coverage			175 (17.68%)
Total			990 (100%)

TABLE 2: Variant distribution among the 990 SARS-CoV-2 samples sequenced

The distribution of variants to date of sample collection is shown in Figure 1.

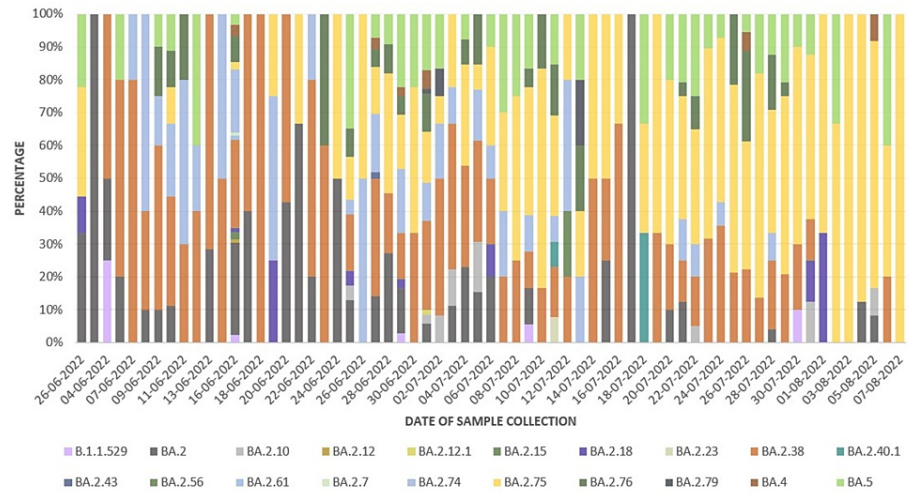


FIGURE 1: Distribution of variants to date of sample collection (from June 6, 2022 to August 7, 2022)

Demographic characteristics of the BA.2 sublineage confirmed cases (BA.2.74, BA.2.75, and BA.2.76)

The demographic and epidemiological characteristics of 371 BA.2 sub-lineage (BA.2.74, BA.2.75 and BA.2.76) confirmed patients are summarised in Table 3. Of these 371 cases, 212 (57.14%) were male, and 159 (42.86%) were female. The median age of the cases is 36 years, and the age group 21-40 years was predominantly affected.

Characteristics	Number of cases (%)			
	BA.2.74	BA.2.75	BA.2.76	Total
Age Group (in years)				
0-10	03 (3.41%)	04 (1.75%)	04 (7.27%)	11 (2.96%)
11-20	02 (2.27%)	15 (6.58%)	03 (5.45%)	20 (5.39%)
21-30	20 (22.73%)	65 (28.51%)	08 (14.54%)	93 (25.07%)
31-40	21 (23.86%)	51 (22.37%)	14 (25.46%)	86 (23.18%)
41-50	15 (17.05%)	32 (14.04%)	14 (25.46%)	61 (16.44%)
51-60	11 (12.50%)	24 (10.53%)	02 (3.64%)	37 (9.97%)
61-70	11 (12.50%)	20 (8.77%)	04 (7.27%)	35 (9.43%)
71-80	04 (4.55%)	11 (4.82%)	06 (10.91%)	21 (5.66%)
>80	01 (1.14%)	06 (2.63%)	00 (00%)	07 (1.89%)
Gender				
Male	50 (56.82%)	131 (57.46%)	31 (56.36%)	212 (57.14%)
Female	38 (43.18%)	97 (42.54%)	24 (43.64%)	159 (42.86%)
Area of Residence				
Pune	69 (78.40%)	216 (94.74%)	48 (87.27%)	333 (89.76%)
Mumbai	17 (19.32%)	02 (0.88%)	07 (12.73%)	26 (7.01%)
Solapur	0 (00%)	10 (4.38%)	0 (00%)	10 (2.69%)
Latur	01 (1.14%)	0 (00%)	0 (00%)	01 (0.27%)
Sindhudurg	01 (1.14%)	0 (00%)	0 (00%)	01 (0.27%)
Calendar week of sample collection				
22 nd week of the year 2022 (30 th May to 5 th June 2022)	00 (00%)	00 (00%)	00 (00%)	00 (00%)
23 rd week of the year 2022 (6 th to 12 th June 2022)	18 (20.45%)	01 (0.44%)	06 (10.91%)	25 (6.74%)
24 th week of the year 2022 (13 th to 19 th June 2022)	20 (22.73%)	03 (1.32%)	07 (12.73%)	30 (8.09%)
25 th week of the year 2022 (20 th to 26 th June 2022)	03 (3.41%)	09 (3.95%)	04 (7.28%)	16 (4.31%)
26 th week of the year 2022 (27 th June to 3 rd July 2022)	28 (31.82%)	36 (15.79%)	14 (25.45%)	78 (21.02%)
27 th week of the year 2022 (4 th to 10 th July 2022)	07 (7.95%)	26 (11.40%)	05 (9.09%)	38 (10.24%)
28 th week of the year 2022 (11 th to 17 th July 2022)	04 (4.55%)	12 (5.26%)	03 (5.45%)	19 (5.12%)
29 th week of the year 2022 (18 th to 24 th July 2022)	06 (6.82%)	42 (18.42%)	03 (5.45%)	51 (13.75%)
30 th week of the year 2022 (25 th to 31 st July 2022)	02 (2.27%)	62 (27.19%)	13 (23.64%)	77 (20.76%)
31 st week of the year 2022 (1 st August to 7 th August 2022)	00 (0%)	37 (16.23%)	00 (0%)	37 (9.97%)

TABLE 3: Demographic characteristics of the emerging Omicron variants

Clinical characteristics of the BA.2 sublineage cases (BA.2.74, BA.2.75 and BA.2.76)

Of the 371 cases, 228 (61.46%) could be contacted to obtain information regarding symptoms,

hospitalization status, treatment and vaccination status. Table 4 describes the clinical characteristics and vaccination status of the confirmed cases of BA.2.74, BA.2.75 and BA.2.76.

Characteristics	Number of cases (%)			
	BA.2.74	BA.2.75	BA.2.76	Total
Total number of cases with history available	59 (67.05%)	132 (57.89%)	37 (67.27%)	228 (61.46%)
History of previous COVID-19 infection	13 (22.03%)	15 (11.36%)	04 (10.81%)	32 (14.04%)
Symptom status at the time of sample collection				
Asymptomatic	03 (5.08%)	07 (5.30%)	03 (8.11%)	13 (5.70%)
Symptomatic	56 (94.92%)	125 (94.70%)	34 (91.89%)	215 (94.30%)
Comorbidity Score				
0 (No Comorbidity)	48 (81.36%)	119 (90.15%)	33 (89.19%)	200 (87.72%)
1 (Presence of one condition)	09 (15.25%)	10 (7.58%)	03 (8.11%)	22 (9.65%)
2 (Presence of two or more conditions)	02 (3.39%)	03 (2.27%)	01 (2.70%)	06 (2.63%)
Initial presenting symptoms				
Fever	47 (79.66%)	111 (84.09%)	29 (78.38%)	187 (82.02%)
Cough	35 (59.32%)	60 (45.45%)	17 (45.95%)	112 (49.12%)
Fatigue/ Weakness	20 (33.90%)	32 (27.06%)	10 (27.03%)	62 (27.19%)
Myalgia	13 (22.03%)	24 (24.24%)	10 (27.03%)	47 (20.61%)
Cold	23 (38.98%)	49 (37.12%)	10 (27.03%)	82 (35.97%)
Headache	14 (23.73%)	30 (22.73%)	04 (10.81%)	48 (21.05%)
Running Nose/ Coryza	05 (8.47%)	14 (10.61%)	06 (16.22%)	25 (10.97%)
Diarrhoea	02 (3.39%)	01 (0.76%)	00 (00%)	03 (1.32%)
Breathlessness	01 (1.70%)	09 (6.82%)	10 (27.03%)	20 (8.77%)
Vomiting	01 (1.70%)	02 (1.52%)	00 (00%)	03 (1.32%)
Sore throat	01 (1.70%)	13 (9.85%)	01 (2.70%)	15 (6.58%)
Confusion	00 (00%)	01 (0.76%)	00 (00%)	01 (0.44%)
Chest Pain	00 (00%)	00 (00%)	01 (2.70%)	01 (0.44%)
Loss of Taste	00 (00%)	01 (0.76%)	00 (00%)	01 (0.44%)
Type of Quarantine				
Home Quarantine	54 (91.53%)	107 (81.06%)	34 (91.89%)	195 (85.53%)
Institutional Quarantine/ Required Hospitalization	05 (8.47%)	25 (18.94%)	03 (8.11%)	33 (14.47%)
Treatment				
Need for Conservative Treatment	57 (96.62%)	119 (90.15%)	36 (97.30%)	212 (92.98%)
Need for Low Flow Oxygen	01 (1.69%)	07 (5.30%)	00 (00%)	08 (3.51%)

supplemental oxygen	Intubation	00 (00%)	02 (1.52%)	01 (2.70%)	03 (1.32%)				
Need for antiviral agents		01 (1.69%)	04 (3.03%)	00 (00%)	05 (2.19%)				
Need for steroid or immunomodulatory drugs		00 (00%)	00 (00%)	00 (00%)	00 (00%)				
Outcome of Disease									
Alive		59 (100%)	130 (98.49%)	36 (97.30%)	225 (98.68%)				
Dead		00 (00%)	02 (1.51%)	01 (2.70%)	03 (1.32%)				
Vaccination Status									
Vaccinated	Single Dose		00 (00%)	03 (2.31%)	00 (00%)	03 (1.37%)			
	Two doses	54 (91.53%)	45 (83.33%)	130 (98.49%)	90 (69.23%)	35 (94.59%)	24 (68.57%)	219 (96.05%)	159 (72.60%)
	Booster Dose		09 (16.67%)		37 (28.46%)		11 (31.43%)		57 (26.03%)
Not vaccinated		04 (6.78%)	02 (1.51%)	02 (5.41%)	08 (3.51%)				
Data Not available		01 (1.69%)	00 (00%)	00 (00%)	01 (0.44%)				

TABLE 4: Clinical characteristics and outcome of patients infected with emerging Omicron BA.2 sub-lineages

Among the 228 cases, 219 (96.05%) were vaccinated with at least a single dose of the COVID-19 vaccine, and the remaining eight (3.51%) were unvaccinated. Table 5 describes the type of vaccine administered to the study population. Of the 219 vaccinated cases, 159 (72.60%) have received two doses of vaccine and 57 (26.03%) have received the precautionary dose. Three (1.37%) cases were vaccinated with a single dose only. All the unvaccinated cases were children less than 18 years.

Name of the Vaccine	Number of cases (%)			
	BA.2.74	BA.2.75	BA.2.76	Total
Covaxin (BBV152A- a whole inactivated virus-based COVID-19 vaccine)				46 (21%)
One Dose	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Two doses	20 (37.04%)	11 (8.46%)	6 (17.14%)	37 (16.90%)
Booster Dose	2 (3.70%)	7 (5.38%)	0 (0%)	9 (4.11%)
Covishield (ChAdOx1 nCoV-19 Corona Virus Vaccine- a recombinant vaccine)				145 (66.21%)
One Dose	0 (0%)	2 (1.54%)	0 (0%)	2 (0.91%)
Two doses	20 (37.04%)	73 (56.15%)	13 (37.14%)	106 (48.40%)
Booster Dose	4 (7.41%)	29 (22.31%)	4 (11.43%)	37 (16.90%)
Sputnik V (Gam-COVID-Vac- a recombinant vaccine)				02 (0.91%)
One Dose	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Two doses	0 (0%)	2 (1.54%)	0 (0%)	2 (0.91%)
Booster Dose	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vaccinated, but patient does not remember the name of the vaccine administered				26 (11.87%)
One Dose	0 (0%)	1 (0.77%)	0 (0%)	1 (0.46%)
Two doses	5 (9.26%)	4 (3.08%)	5 (14.29%)	14 (6.39%)
Booster Dose	3 (5.56%)	1 (0.77%)	7 (20%)	11 (5.02%)
Total	54 (100%)	130 (100%)	35 (100%)	219 (100%)

TABLE 5: Type of vaccine administered versus emerging Omicron BA.2 sub-lineages

Out of 228 cases, 215 (94.30%) developed mild symptoms, of which fever (82.02%) was the most common symptom, followed by cough (49.12%), cold (35.97%), fatigue (27.19%), headache (21.05%) and myalgia (20.61%). Rest 13 (5.70%) had an asymptomatic infection. There were 195 (85.53%) cases who recovered at home, and 33 (14.47%) required institutional quarantine. Of the 228 cases, 212 (92.98%) cases recovered with supportive treatment, 11 (4.83%) required supplemental oxygen therapy and five (2.19%) were given antiviral treatment. None were administered steroids, immunomodulatory drugs or monoclonal antibodies. There were three (1.32%) cases that progressed to severe disease that resulted in death, and the remaining 225 (98.68%) cases survived. Out of the three cases who succumbed to the disease, two (66.67%) had BA.2.76 infection, while one (33.33%) had BA.2.75 infection.

Discussion

One of the most characteristic features of the SARS-CoV-2 virus has been its rapid evolution during the pandemic. New variants have emerged from time to time with selective advantages, replacing the previously circulating variants and achieving global dominance. Barely weeks after the BA.2 lineage-driven surges globally, towards the third week of May 2022, India saw a surge in cases of COVID-19 [4]. In the last 90 days, 219,503 whole genome sequences of SARS-CoV-2 have been deposited on GISAID from India. Of these, BA.2.58 (15%) is the predominant lineage, followed by BA.2.76 (14%), BA.2.75 (14%), BA.2 (9%), BA.5.2 (6%), and BA.2.74 (4%) (Figure 2) [13]. To date, 4,389 sequences of BA.2.76 [14], 3,521 of BA.2.75 [15], and 1,250 sequences of BA.2.74 [16] lineages have been deposited on GISAID. The apparent cumulative prevalence is less than 0.5% worldwide for all three lineages. In India, the prevalence of BA.2.76, BA.2.75, and BA.2.74 is 6%, 3%, and 1%, respectively. Apart from India, BA.2.76 has been detected in 48 countries and 39 US states, BA.2.75 in 37 countries and 30 US states, and BA.2.74 in 32 countries and 23 US states [14-16]. When the world, particularly countries like South Africa, the United Kingdom, the US, Germany, Portugal, and Denmark, were experiencing the latest global outbreak driven by the BA.4 and BA.5 Omicron lineages [5],

India, on the other hand, did not see an exponential increase in cases due to these two lineages. Based on the sequences deposited on GISAID, the BA.5 and BA.4 Omicron lineages continue to dominate globally, with a weekly prevalence of 69.6% and 11.8%, respectively [17]. However, it is interesting to note that, in India, the prevalence of BA.5 and BA.4 lineages is 9% and less than 0.5% among the sequences deposited on GISAID, respectively [13]. This probably could be as both Delta and BA.4/ BA.5 share the L452R mutation on the receptor-binding domain (RBD) of spike protein, the convalescent sera from Delta infection may contain L452R-specific neutralizing antibodies, which could have impaired the BA.4/ BA.5 transmission in India [18]. Also, BA.2.75 has shown 57-fold higher binding affinity to ACE2 receptors when compared with BA.5, accounting for its higher transmissibility [19].

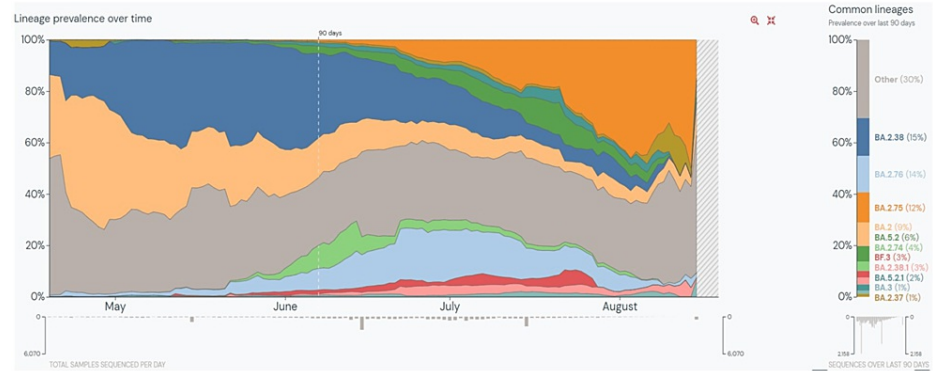


FIGURE 2: Lineage prevalence in India over the last 90 days

This graph is generated and taken from Outbreak.info as of September 9, 2022 [13].

The SARS-CoV-2 virus has evolved rapidly, adapting to its human hosts by developing mutations over time and resulting in the emergence of new variants. The Omicron subvariant BA.2.74 contains BA.2 mutations along with characteristic R346T and L452M mutations in its spike protein [8]. Similarly, the subvariant BA.2.76 contains BA.2 mutations with Y248N and R346T in the spike protein [10]. The subvariant BA.2.75, on the other hand, contains nine additional spike mutations compared to BA.2. The defining mutations include the BA.2 mutations with K147E, W152R, F157L, I210V, G257S in the N-terminal domain and G339H, G446S, N460K, R493Q in the RBD region of spike protein (Figure 3) [9]. The effect of the mutations in the RBD region on the virus-host interactions is described in Table 6 [20]. R493Q and N460K mutations increase the ACE-2 receptor affinity and surface RBD expression [21,22]. On the other hand, mutations G446S and G339H decrease the ACE-2 affinity and RBD expression. Such adaptive mutations can alter the pathogenic potential of the virus.

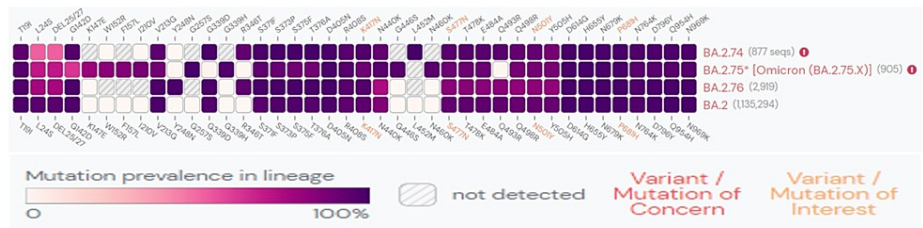


FIGURE 3: S-gene mutations in > 75% of global sequences for the three sublineages in the last 60 days

This figure is generated and taken from Outbreak.info as of September 9, 2022 [15].

Variant	Mutations	Impact on		
		ACE-2 affinity $\Delta\log_{10}$ Kd	RBD expression $\Delta\log$ (MFI)	Contact with ACE-2 receptor
BA.2.74	R346T	0.11	0.13	No
	L452M	0.11	0.24	No
BA.2.75	G339H	-0.03	-0.06	No
	G446S	-0.07	-0.18	Yes
	N460K	0.2	0.3	No
	R493Q	1.07	0.04	Yes
BA.2.76	R346T	0.11	0.13	No

TABLE 6: The defining spike mutations in the RBD region of three BA.2 sublineages and their effect on virus-host interaction

The data represented here are generated using Jbloomlab.github.io [20]

Table 7 compares the growth advantage of the common variants in India. These estimates reflect the advantage of the new variants compared to the cocirculating variants. The relative growth of a variant can be explained by three mechanisms: increased transmissibility, infectious duration, and immune evasion. The BA.2.75 sublineage has a relative growth advantage of 77% per week, with 0.08 as an assumed logistic growth rate per day. It has a 42% increase in transmissibility [23]. The spike protein mutations D339H, G446S, N460K, and R493Q allow BA.2.75 to escape neutralization by antibodies produced against different RBD epitopes in BA.2 [21]. Figure 4 shows the effect of antibodies elicited during the pre-Omicron and Omicron BA.1 period on the BA.2.75 variant. The mutations in the RBD region confer an escape fraction of 0.45 [24]. These features of BA.2.75 indicate that it might outcompete BA.4/BA.5 and can become a potential risk to global health. Therefore, the spread and frequency of these sublineages in India and countries outside India require close monitoring through sustained genomic and clinical surveillance as they possess key mutations that are notable for their accelerated growth and extensive geographical distribution.

Growth Characteristics	BA.4*	BA.5*	BA.2.38*	BA.2.74*	BA.2.75*	BA.2.76*
Relative growth advantage per week (in % with Confidence intervals)	06% (03% - 10%)	23% (22% - 24%)	02% (01% - 02%)	07% (06% - 08%)	72% (69% - 75%)	13% (12% - 14%)
Assumed logistic growth rate (per day)	0.01	0.03	0.00	0.01	0.08	0.02
Increase in transmissibility (%)	05%	15%	01%	05%	40%	09%
Reproduction Number (Re) (HPD) (As on 13.08.2022)	0.00 (0.00 - 0.01)	0.22 (0.15 - 0.28)	0.00 (0.00 - 0.00)	0.41 (0.00 - 4.68)	0.95 (0.89 - 1.01)	1.22 (0.76 - 1.68)

TABLE 7: A comparative growth advantage of BA.4*, BA.5*, BA.2.38*, BA.2.74*, BA.2.75* and BA.2.76* in India

The data represented here are as of September 5, 2022 and are generated using covSPECTRUM [23,25-29]

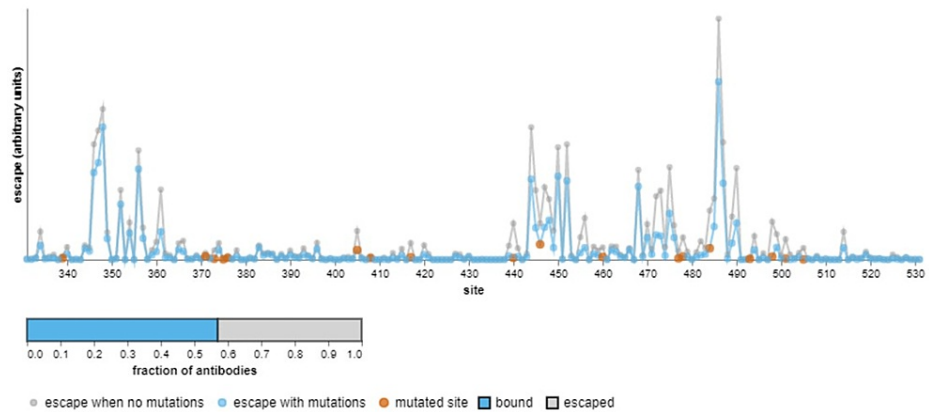


FIGURE 4: Effect of antibodies elicited during pre-Omicron SARS-CoV-2 and Omicron BA.1 period on neutralization of BA.2.75 variant*

*Escape fraction ranges from 0 to 1, where 0 means no escape and 1 means complete escape. Orange dots represent the site of mutation. Blue dots represent the escape in the presence of a mutation. Grey dots represent escape in the absence of a mutation [24]

The current study indicates that these BA.2 sublineages caused mild disease with reduced need for hospital admission. In animal models, BA.2.75 replicated more efficiently in the lungs of hamsters than other Omicron variants causing focal pneumonia characterized by patchy inflammation in alveolar regions. These findings suggest that the Omicron subvariant BA.2.75 can cause severe respiratory disease and may affect the clinical outcome in infected humans [30]. However, it is still unclear to what extent the intrinsic virulence of the virus and the immunity due to vaccination or previous infections could have contributed to mild disease in India. Further, data from other clinical settings will be essential to assess the behavior of these BA.2 sublineages in countries with different levels of previous infections and vaccination.

The fact that subvariant BA.2.75 contains mutations greater than BA.2 and BA.4/BA.5 raises concern regarding the possibility that it might have significantly reduced sensitivity to therapeutic monoclonal antibodies and antibodies developed by vaccination/natural infection. It is important to note that these subvariants have emerged when the world is about to achieve global immunity against the SARS-CoV-2 virus through various vaccines available for COVID-19. India has conducted 200 crore vaccinations and has vaccinated 73.02% of its population with at least one dose of vaccine and 66.85% of the population with two doses of vaccine. Around 7.67% of the total population has received the precautionary dose [31]. Various studies on the evasion of neutralizing antibodies have found that the Omicron subvariant BA.2.75, which has a local growth advantage in India, is 1.8 and 1.1 times more resistant to sera from vaccinated individuals than BA.2 and BA.2.12.1, respectively. However, it is 0.6 times more sensitive than BA.4/BA.5. It has increased resistance to class 1 and 3 monoclonal antibodies but is sensitive to class 2 monoclonal antibodies like Casirivimab (REGN10933), Tixagevimab (COV2-2196) and S2E12. This increased resistance may be due to the spike protein's G446S and R460K mutations. It is also 3.7 times more resistant to Bebtelovimab, the only monoclonal antibody potent against all Omicron subvariants [22]. Another study in a small sample of plasma from post-vaccination Delta infection shows that the BA.2.75 subvariant is more immune evasive than the BA.4/BA.5 lineages in the Delta-stimulated immune background, which probably may explain why BA.2.75 has a growth advantage over BA.4/BA.5 in India [18]. Also, a study found that BA.2.75 has a higher resistance to BA.5-induced immunity. This property may make BA.2.75 variant spread efficiently in areas where BA.5 has been widely circulating [32]. Close monitoring and following these variants effectively and investigating their development as early as possible is crucial.

Conclusions

To conclude, this study provides essential and early evidence of the severity of the disease in patients infected with BA.2.74, BA.2.75, and BA.2.76 sublineages. Most individuals suffered from mild illnesses like fever, cough, cold, fatigue, and headache. Currently, there is no evidence of an increased risk of hospital admission or severe disease due to these sublineages in India. Despite these second-generation variants being detected only recently, they have the potential to be successfully transmitted across several countries due to the presence of critical mutations and significant growth advantages. The ability of the SARS-CoV-2 virus to evolve continuously and achieve increased transmission and immune evasion reinforces the importance of vaccination and sustained epidemiological surveillance to detect emerging new variants.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Institutional Ethics Committee, BJGMC, Pune issued approval BJGMC/IEC/Pharmac/0721233-233. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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