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REVIEW

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"Is Omicron mild"? Testing this narrative with the mutational landscape of its three lineages and response to existing vaccines and therapeutic antibodies

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Abstract

SARS-CoV-2 Omicron with its lineages BA.1, BA.2, and BA.3 has triggered a fresh wave of Covid-19 infections. Though, Omicron has, so far, produced mild symptoms, its genome contains 60 mutations including 37 in the spike protein and 15 in the receptor-binding domain. Thirteen sites conserved in previous SARS-CoV-2 variants carry mutations in Omicron. Many mutations have shown evolution under positive selection. Omicron's giant mutational leap has raised concerns as there are signs of higher virus infectivity rate, pathogenesis, reinfection, and immune evasion. Preliminary studies have reported waning of immunity after two-dose primary vaccine regime, need for the boosters, folds reduction in vaccine effectiveness and neutralizing antibodies even after boosting and significant neutralization resistance with the therapeutic monoclonal, polyclonal, and convalescent antibodies against Omicron. The narrative that "Omicron is mild," therefore, needs time to be tested with a deeper, scientific dwelling into the facts.

KEYWORDS

immune evasion, neutralization resistance, Omicron, Omicron lineages, SARS-CoV-2 mutations, vaccine effectiveness (VE), viral evolution

1 | INTRODUCTION

Since its emergence in December 2019, SARS-CoV-2 induced Covid-19 disease has spread to about 240 countries and territories of the world with about 435 million (435, 626, 514) confirmed cases and 5.9 million (5, 952, 215) deaths as on March 1st, 2022. Among the worst Covid-19 affected nations, the United States has reported a maximum number of SARS-CoV-2 infections and Covid-19 related deaths followed by India and Brazil.[1] Since the beginning of the pandemic, the reports of multiple sites in the genome of SARS-CoV-2 (ORF1a, ORF1b, ORF3a, ORF8, *N*, and *S* genes) under positive selection, gave early signs of tremendous genome plasticity of this virus[2–4] that resulted in the emergence of many variants each with their characteristic set of mutations.

A novel SARS-CoV-2 variant detected in mid-November 2021 in Botswana and South Africa was named B.1.1.529 "Omicron" and designated as a variant of concern (VOC) by the World Health Organization (WHO).[1] It is the fifth SARS-CoV-2 VOC to be detected after Alpha (B.1.1.7/United Kingdom), Beta (B.1.351/South Africa), Gamma (P.1/Brazil), and Delta (B.1.617.2/India) variants. In the three nomenclature systems proposed by phylogenetic assignment of named global outbreak lineages, Nextstrain and global initiative on sharing all influenza data (GISAID), Omicron belongs to Pango lineage B.1.1.529 with BA.1, BA.2, and BA.3 included as its three descendent lineages. BA1.1 has been identified as a sub-lineage under BA.1. Next strain nomenclature has assigned "clade 21M" to Omicron; "21K to BA.1; "21L" to BA.2, while clade "GRA" has been assigned to Omicron by GISAID.[1]

Although, Omicron has emerged at a time when vaccine immunity is increasing in the world, still, it has raised concerns by triggering a fresh wave of Covid-19 infections even among people who had previously received two doses and even boosters of Covid-19 vaccines. Preliminary evidences suggest an increased risk of reinfection associated with this variant.[1] It is even gripping regions where the Delta variant is still prevalent. Due to a short doubling time of 2–3 days, and many unique mutations that may confer it higher transmissibility and immune escape potential than its predecessors,[5] the likelihood of global spread of Omicron is high. At present, Omicron has been detected in 149 countries with an exponential increase in the cases.[1] Although, symptoms produced by Omicron are apparently milder than Delta variant,[6] the ongoing research on the durability of immunogenicity acquired by vaccinations or previous infections and the efficacy of therapeutic antibodies approved for clinical use against SARS-CoV-2 virus will shed light to better understand the long-term effects of this novel variant.

2 | MUTATIONAL LANDSCAPE OF OMICRON VARIANT

It is common for viruses to mutate during their replication. Overall, coronaviruses' replication is highly fidel and shows a low mutational frequency due to 3′–5′ exonuclease activity of their NSP_{14} protein.[7] SARS-CoV-2 diversity and mutation rate is half of the influenza virus,[8] but several genes including *ORF1a*, *ORF1b*, *ORF3a*, *ORF8*, *N* and *S* with a high mutational rate[2-4,9] have resulted in new mutations that offer survival or selective advantage by improving the "viral fitness." This has led to the emergence of new SARS-CoV-2 variants by modulation of receptor binding efficiency, transmission, severity of disease, reinfection, immune evasion, and resistance to neutralizing and therapeutic antibodies among others.[10] The reported modes of evolution of SARS-CoV-2 variants explained in detail in Section 3 include recombination, epistasis, pervasive, episodic, and directional selection.[4,11-15]

The latest SARS-CoV-2 variant "Omicron" is heavily mutated[13,16] and has accumulated an unprecedented high number of mutations. Omicron shares some of its mutations with other SARS-CoV-2 variants but carries a large number of unique mutations (detailed in Section 2.2), some of which have been shown to be linked to higher transmissibility and immune escape, suggesting a significant shift in the evolutionary trajectory of the SARS-CoV-2 virus. In addition, 13 sites previously observed to be conserved in SARS-CoV-2 variants have been found to harbor mutations in Omicron. These sites have been divided into 3 clusters.[4] Cluster 1 (sites 339, 371, 373, 375), cluster 2 (sites 493, 496, 498, 505) and cluster 3 (764, 856, 954, 969, and 981) mutations have shown a decrease in the neutralization of target classes 4, 1, and 2 antibodies and increased interactions between S1 and S2 subunits and reduced S1 shedding, respectively.[4] It is interesting to note that the emergence of various VOIs and VOCs of SARS-CoV-2 including the current Omicron variant has largely been supported by induction of substitution, deletion, and sparingly insertion mutations and ins214EPE mutation has been seen for the first time in Omicron.

2.1 | Omicron lineages BA.1, BA.2, and BA.3

BA.1 is predominant and represents about 99% of the Omicron sequence available at GISAID database.[17] Although, the majority of Omicron mutations have occurred on the spike, other proteins have also been shown to act as pharmacologically-targetable epitopes (such as the nucleocapsid protein) or as immuno-evasion driving and clinically relevant components (such as ORF8), ORF3a and others (Table 1, Figures 1 and 2). In comparison to the ancestral reference SARS-CoV-2 Wuhan Hu-1 genome, BA.1 Omicron genome contains a total of 60 mutations (Table 1, Figures 1 and 2), with 37 (6 deletions + 1 insertion + 30 substitutions) mutations located in the spike protein, and remaining 23 mutations spread in other parts of the genome (Figures 1 and 2). Sub-lineage BA1.1 with a total of 61 mutations differs from BA.1 in possessing an additional "R346K" mutation[1] (Figure 1). BA.1 and BA.1.1 are unique in containing an ins214EPE spike mutation.

The BA.2 lineage is popularly known as "Stealth Omicron" and has a total of 59 genomic mutations and 31 spike mutations (3 deletions + 28 substitutions) (Table 1 and Figure 1). Besides, lineage BA.3 supposedly similar to BA.1 in its antigenicity contains a total of 56 mutations along with 33 mutations in its spike protein (6 deletions + 27 substitutions) (Table 1 and Figure 1). BA.2 and BA.3

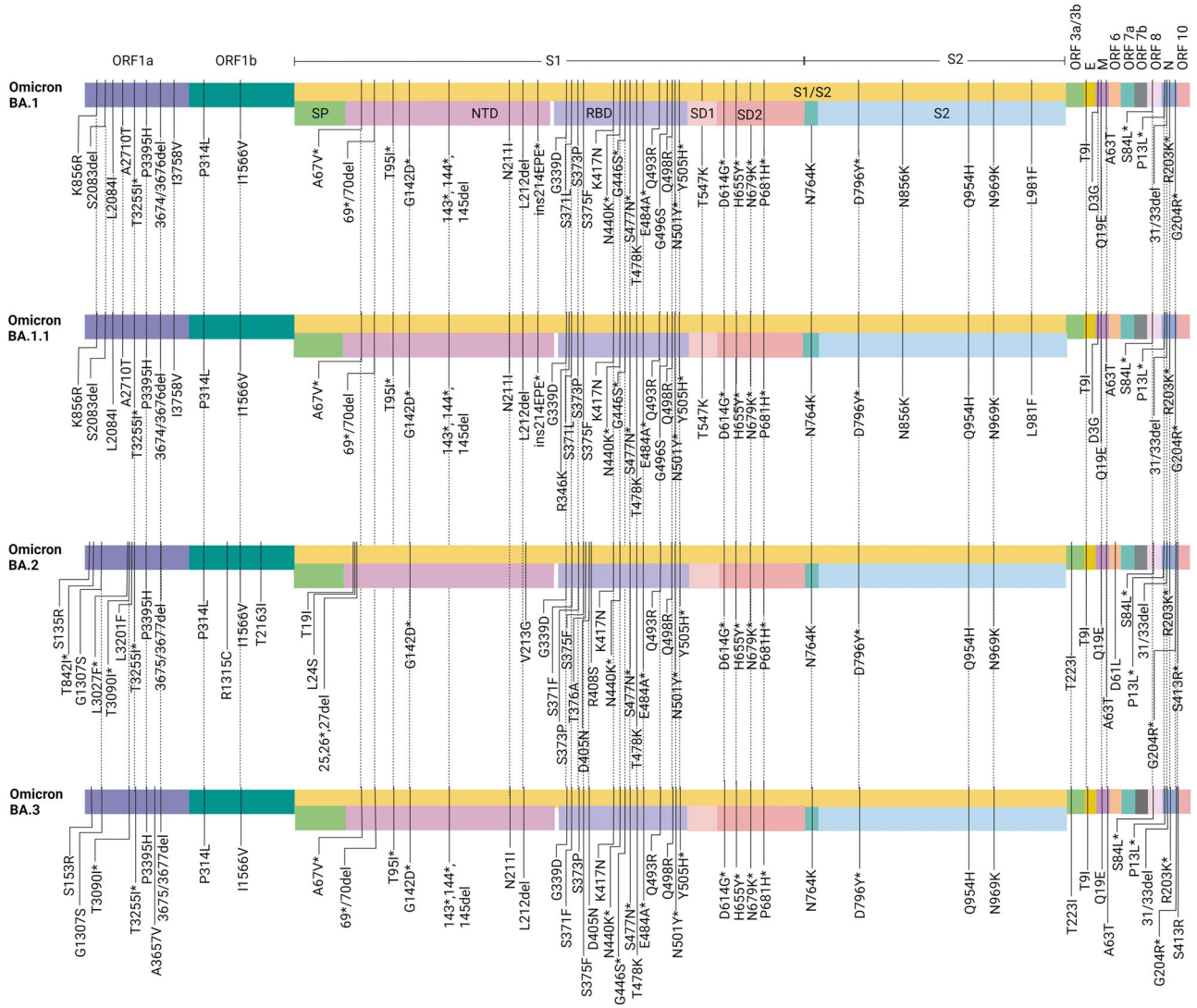

FIGURE 1 Schematic representation of distribution and sharing of mutations across genomes of Omicron lineages BA.1 with sub-lineage BA1.1, BA.2, and BA.3. ORF1a and 1b: Open reading frame 1a and 1b; Different domains of Spike (S) protein are SP: Signal peptide; NTD: N-terminal domain; RBD: Receptor-binding domain; SD1 and SD2: Sub-domain 1 and 2; S1/S2: Protease cleavage site; S1 and S2: Spike subunit 1 and 2; E: Envelope protein; M: Membrane protein and N: Nucleocapsid protein. *Shows the positive selection

have been reported in about 0.1% and 0.01% of GISAID entries,[17] respectively.

2.1.1 | Comparison of mutation profile in the whole genome in Omicron lineages

Upon comparison of the mutation profiles of whole virus genomes in Omicron BA.1, BA.2, and BA.3 lineages, 37 mutations were observed to be common with 10 and 7 mutations being shared between BA.1-BA.3 and BA.2-BA.3 lineages, respectively (Figure 3). While only spike protein mutations were observed to be shared between BA.1-BA.3, ORF1a (G1307S, T3090I, and 3677del), spike protein (S371F, D405N), ORF3a (T223I), and N protein (S413R) showed common mutations between BA.2-BA.3 (Figure 1 and Table 1).

Likewise, many mutations were observed to be specific to each lineage. For instance, thirteen BA.1 mutations (ORF1a-K856R, S2083del, L2084I, A2710T, L3674del, I3758V; Spike protein—ins214EPE, S371L, G496S, T547K, N856K, L981F; M protein—D3G) and fifteen BA.2 mutations (ORF1a—S135R, T842I, L3027F, L3201F; ORF1b—R1315C, T2163I; Spike protein—T19I, L24S, P25del, P26del, A27del, V213G, T376A, R408S; and ORF6—D61L) were found to be unique. For BA.3 lineage, two unique mutations listed were ORF1a-S153R and A3657V mutations (Figure 3). Omicron lineage BA.1 has been found to be more pathogenic than ancestral Wuhan virus and Delta VOC.[18] On the other hand, BA.2 shows a 1.4 times higher effective reproduction number than BA.1 and its furogenicity and pathogenicity have also been reported to be higher than BA.1.[19]

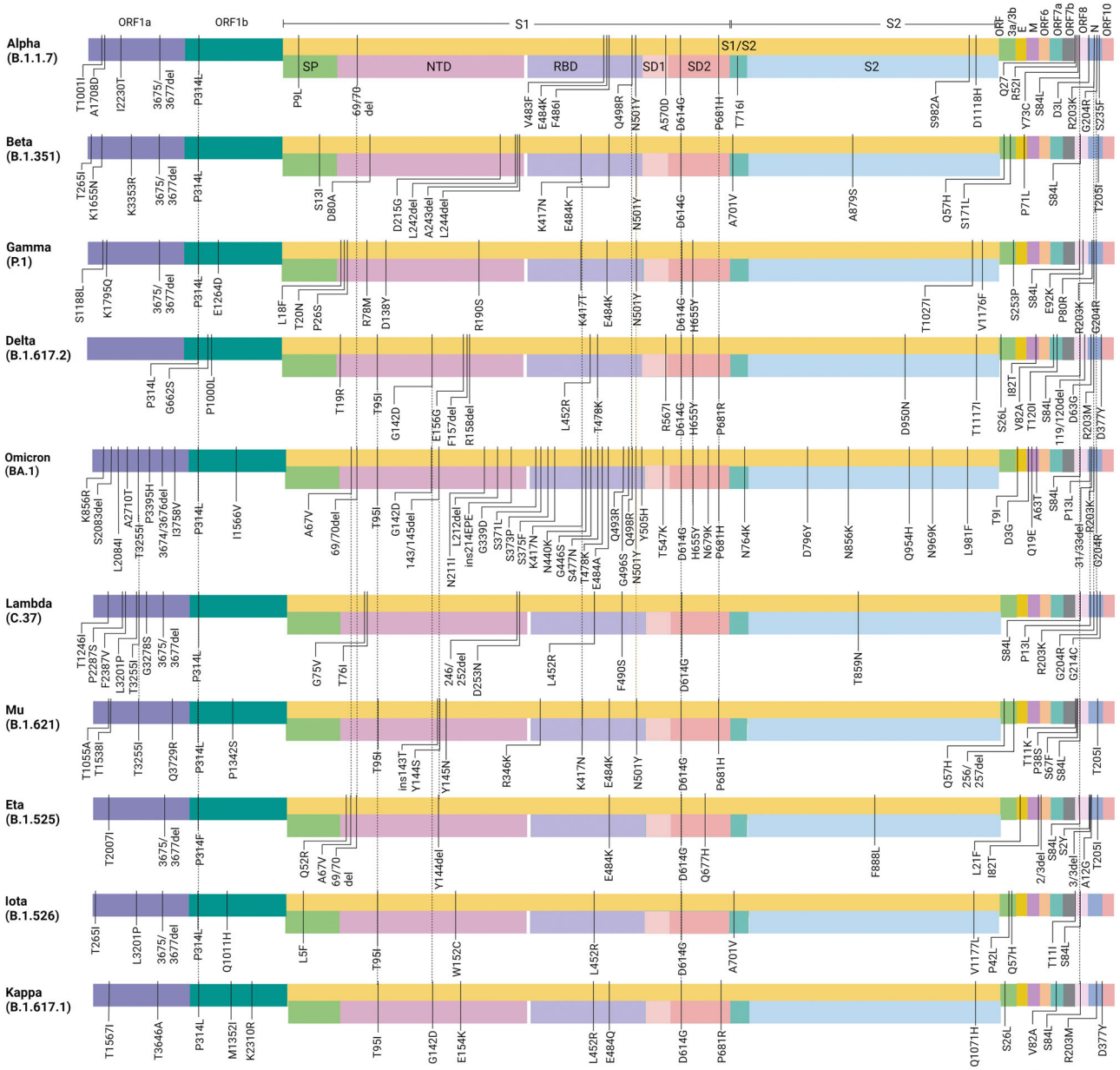

FIGURE 2 Schematic representation of distribution and sharing of mutations across genomes of SARS-CoV-2 VOCs (Alpha, Beta, Gamma, Delta, Omicron BA.1), VOIs (Lambda and Mu), and VUMs (Eta, Iota, and Kappa). ORF1a and 1b: Open reading frame 1a and 1b; Different domains of Spike (S) protein are SP: Signal Peptide; NTD: N-terminal domain; RBD: Receptor-binding domain; SD1 and SD2: Sub-domain 1 and 2; S1/S2: Protease cleavage site; S1 and S2: Spike subunit 1 and 2; E: Envelope protein; M: Membrane protein and N: Nucleocapsid protein

2.1.2 | Comparison of mutation profile in the spike protein in Omicron lineages

Spike protein serves as the fundamental domain in SARS-CoV-2 pathogenesis as it facilitates viral entry into the human host. It has been the main target for neutralization of antisera and therefore, Covid-19 diagnostics, therapeutics, and vaccines are largely based on the spike protein.[1] A comparison between the 4 Omicron lineages with respect to the shared and unique spike mutations is shown in Figure 1. A total of 21 spike mutations were found to be common between the 3 lineages, while 10 and 2 mutations were shared between BA.1-BA.3 and BA.2-BA.3, respectively. While 6 spike mutations (ins214EPE, S371L, G496S, T547K, N856K, L981F) were unique to BA.1, 8 mutations (T19I, L24S, P25del, P26del, A27del, V213G, T376A, and R408S) were observed to be unique to lineage BA.2. BA.3 lineage contained a total of 33 spike mutations that were an amalgamation of 21 common mutations between the 3 lineages along with 10 and 2 mutations shared with BA.1 and BA.2 lineages, respectively (Figure 1).

N-terminal domain (NTD), receptor-binding domain (RBD), and receptor binding motif (RBM) are three important regions of spike protein that mediate viral binding to angiotensin-converting enzyme 2 (ACE2)

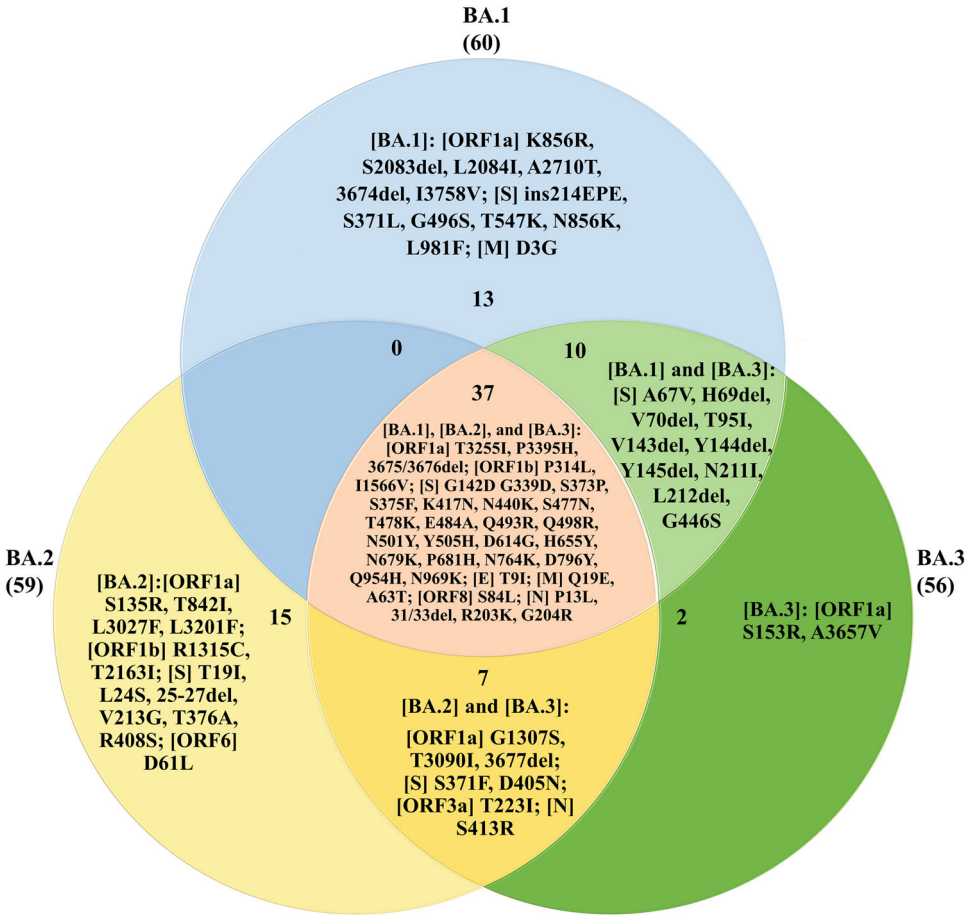

FIGURE 3 Venn diagram showing shared and unique mutations in the genomes of Omicron lineages BA.1, BA.2, and BA.3

receptor for effective transmission. NTD and RBD mostly have been the primary target of neutralizing antibodies and vaccines reportedly harbor many mutations in Omicron. BA.1 showed 11 NTD and 15 RBD (10 in the RBM) mutations. While BA.2 showed 7 NTD and 16 RBD (8 in the RBM) mutations. BA.3 lineage, on the other hand, showed 10 NTD and 15 RBD (9 in the RBM) mutations (Table 1 and Figure 4A). Multiple mutations identified in the NTD, RBD, and RBM domains raise concerns on enhanced transmission, reduced antibody response, and immune evasion.[1] Spike protein of lineage BA.1 is less efficiently cleaved at furin site and is less fusogenic than the ancestral virus and VOC Delta.[18]

2.2 | Comparison of Omicron mutational profile with other VOCs, variants of interest (VOIs), and variants under monitoring (VUMs) of SARS-CoV-2

2.2.1 | Comparison of genomic mutational profile of Omicron (BA.1) versus other VOCs (Alpha, Beta, Gamma, and Delta)

Against a total of 60 mutations observed in Omicron BA.1 lineage, 29, 24, 26, and 27 genomic mutations were observed in SARS-CoV-2 VOCs Alpha, Beta, Gamma, and Delta, respectively (Table 1 and Figure 2). Omicron variant was observed to share 10 and 5 common mutations with Alpha and Beta variants, while 7 mutations each were shared with Gamma and Delta variants, respectively (Tables 1 and 2, Figure 2). The genomic regions ORF1b, spike protein, ORF8, and N protein showed common Omicron mutations in Alpha and Gamma, and ORF1b, spike protein, and ORF8 regions showed Omicron shared mutations in Beta and Delta variants.

2.2.2 | Comparison of genomic mutational profile of Omicron (BA.1) versus other VOIs (Lambda and Mu)

A total of 29 and 24 genomic mutations were observed in VOIs Lambda and Mu, respectively against a total of 60 mutations observed in Omicron BA.1 (Tables 1 and 2, Figure 2), out of which seven and eight mutations were common between Lambda and Mu when compared with Omicron BA.1. The ORF1a, ORF1b, Spike protein, ORF8, and N protein were the regions in Lambda that shared mutations with Omicron. While, in Mu, barring N protein the remaining four regions (ORF1a, ORF1b, Spike protein, and ORF8) showed common mutations with Omicron (Tables 1 and 2, Figure 2). The unique "R346K" mutation possessed by sub-lineage BA1.1 is shared with VOI mu (Tables 1 and 2).

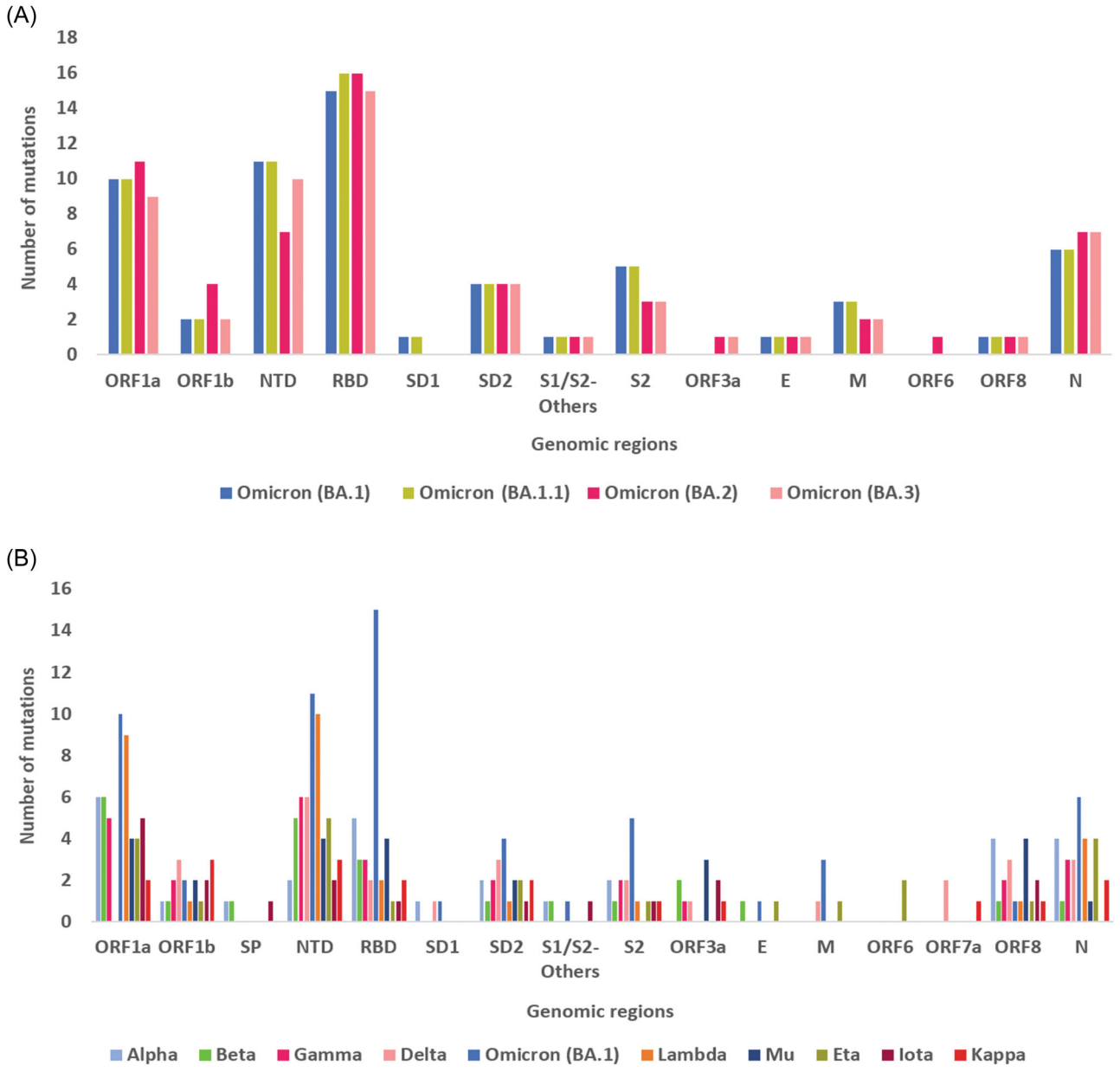

FIGURE 4 (A) Number of mutations recorded in different genomic regions of SARS-CoV-2 Omicron lineages BA.1, BA. 2, BA.3, and sub-lineage BA.1.1. (B) Number of mutations recorded in different genomic regions of VOCs (Alpha, Beta, Gamma, Delta, and Omicron BA.1); VOIs (Lambda and Mu); and VUMs (Eta, Iota, and Kappa) of SARS-CoV-2

2.2.3 | Comparison of genomic mutational profile of Omicron (BA.1) versus other VUMs (Eta, Iota, and Kappa)

VUMs Eta, Iota, and Kappa viral genomes showed a total of 23, and 18 for each mutation (Figure 2, Tables 1 and 2). Six (Eta; Spike protein, ORF8), Four (Iota; ORF1b, Spike protein, and ORF8), and five (Kappa; ORF1b, Spike protein, and ORF8) mutations were shared between the analyzed VUMs with Omicron BA.1 variant.

A comparison of the number of incurred mutations in various genomic regions in the 10 analyzed SARS-CoV-2 VOCs. VOIs and VUMs are shown in figure 4B. Further, the present whole-genome mutation analysis of 10 SARS-CoV-2 VOCs, VOIs, and VUMs indicates that Omicron BA.1 shares maximum (10) mutation with VOC Alpha variant followed by 8 (VOI Mu) and 7 each with VOCs Gamma, Delta and VOI Lambda (Figure 2).

2.2.4 | Comparison of spike mutations of Omicron (BA.1) versus other VOCs, VOIs, and VUMs

The Omicron spike protein has 1270 AAs as compared to 1271 AAs in the Delta variant and 1273 AAs in the ancestral Wuhan-Hu-1

TABLE 1 Total genomic mutations recorded in SARS-CoV-2 VOCs (Alpha, Beta, Gamma, Delta, Omicron BA.1, BA. 1.1, BA. 2, and BA.3), VOIs (Lambda and Mu) and VUMs (Eta, Iota, and Kappa)

S. No.	Variant name (WHO and PANGO lineage)	Mutations
1.	VOC Alpha (B.1.1.7)	**[ORF1a]**T1001I, A1708D, I2230T, 3675/3677del; **[ORF1b]** P314L; **[S]** P9L, H69del, V70del, V483F, E484K, F486I, Q498R, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H; **[ORF8]** Q27*, R52I, Y73C, S84L; **[ORF8]** S84L; **[N]** D3L, R203K, G204R, S235F
2.	VOC Beta (B.1.351)	**[ORF1a]**T265I, K1655N, K3353R, 3675/3677del; **[ORF1b]** P314L; **[S]** S13I, D80A, D215G, L242del, A243del, L244del, K417N, E484K, N501Y, D614G, A701V, A879S; **[ORF3a]** Q57H, S171L; **[E]** P71L; **[ORF8]** S84L; **[ORF8]** S84L; **[N]** T205I
3.	VOC Gamma (P.1)	**[ORF1a]**S1188L, K1795Q, 3675/3677del, **[ORF1b]** P314L, E1264D; **[S]** L18F, T20N, P26S, R78M, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F; **[ORF3a]** S253P; **[ORF8]** S84L, E92K; **[ORF8]** S84L; **[N]** P80R, R203K, G204R
4.	VOC Delta (B.1.617.2)	**[ORF1b]**P314L, G662S, P1000L; **[S]** T19R, T95I, G142D, E156G, F157del, R158del, L452R, T478K, R567I, D614G, H655Y, P681R, D950N, T1117I; **[ORF3a]** S26L; **[M]** I82T; **[ORF7a]** V82A, T120I; **[ORF8]** S84L, 119/120del; **[ORF8]** S84L; **[N]** D63G, R203M, D377Y
5.	VOC Omicron BA.1 (B.1.1.529)	**[ORF1a]**K856R, S2083del, L2084I, A2710T, T3255I, P3395H, 3674/3676del, I3758V; **[ORF1b]** P314L, I1566V; **[S]** A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211I, L212del, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F; **[E]** T9I; **[M]** D3G, Q19E, A63T; **[ORF8]** S84L; **[N]** P13L, 31/33del, R203K, G204R
6.	VOC Omicron BA.1.1	**[ORF1a]**K856R, S2083del, L2084I, A2710T, T3255I, P3395H, 3674/3676del, I3758V; **[ORF1b]** P314L, I1566V**; [S]** A67V, 69/70del, T95I, G142D, 143/145del, N211I, L212del, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F; **[E]** T9I; **[M]** D3G, Q19E, A63T; **[ORF8]** S84L; **[N]** P13L, 31/33del, R203K, G204R
7.	VOC Omicron BA.2	**[ORF1a]**S135R, T842I, G1307S, L3027F, T3090I, L3201F, T3255I, P3395H, 3675/3677del; **[ORF1b]** P314L, R1315C, I1566V, T2163I; **[S]** T19I, L24S, 25/27del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; **[ORF3a]** T223I; **[E]** T9I; **[M]** Q19E, A63T; **[ORF6]** D61L; **[ORF8]** S84L; **[N]** P13L, 31/33del, R203K, G204R, S413R
8.	VOC Omicron BA.3	**[ORF1a]**S153R, G1307S, T3090I, T3255I, P3395H, A3657V, 3675/3677del; **[ORF1b]** P314L, I1566V; **[S]** A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211I, L212del, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; **[ORF3a]** T223I; **[E]** T9I; **[M]** Q19E, A63T; **[ORF8]** S84L; **[N]** P13L, 31/33del, R203K, G204R, S413R
9.	VOI Lambda (C.37)	**[ORF1a]**T1246I, P2287S, F2387V, L3201P, T3255I, G3278S, 3675/3677del; **[ORF1b]** P314L; **[S]** G75V, T76I, R246del, S247del, Y248del, L249del, T250del, P251del, G252del, D253N, L452R, F490S, D614G, T859N; **[ORF8]** S84L; **[N]** P13L, R203K, G204R, G214C
10.	VOI Mu (B.1.621)	**[ORF1a]**T1055A, T1538I, T3255I, Q3729R; **[ORF1b]** P314L, P1342S; **[S]** T95I, ins143T, Y144S, Y145N, R346K, K417N, E484K, N501Y, D614G, P681H; **[ORF3a]** Q57H, 256/257del; **[ORF8]** T11K, P38S, S67F, S84L; **[N]** T205I
11.	VUM Eta (B.1.525)	**[ORF1a]**T2007I, 3675/3677del; **[ORF1b]** P314F; **[S]** Q52R, A67V, H69del, V70del, Y144del, E484K, D614G, Q677H, F888L; **[E]** L21F; **[M]** I82T; **[ORF6]** 2/3del; **[ORF8]** S84L; **[N]** S2Y, 3/3del, A12G, T205I
12.	VUM Iota (B.1.526)	**[ORF1a]**T265I, L3201P, 3675/3677del; **[ORF1b]** P314L, Q1011H; **[S]** L5F, T95I, W152C, L452R, D614G, A701V, V1177L; **[ORF3a]** P42L, Q57H; **[ORF8]** T11I, S84L
13.	VUM Kappa (B.1.617.1)	**[ORF1a]**T1567I, T3646A; **[ORF1b]** P314L, M1352I, K2310R; **[S]** T95I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H; **[ORF3a]** S26L; **[ORF7a]** V82A; **[ORF8]** S84L; **[N]** R203M, D377Y

Abbreviations: PANGO, phylogenetic assignment of named global outbreak lineages; WHO, World Health Organization.

TABLE 2 Mutations shared between SARS-CoV-2 Omicron lineages BA.1, BA. 2, BA.3, and sub-lineage BA.1.1 with other VOCs (Alpha, Beta, Gamma, Delta), VOIs (Lambda and Mu) and VUMs (Eta, Iota, and Kappa)

SARS-CoV-2 variants of concern (VOC)					SARS-CoV-2 variants of interest (VOI)		SARS-CoV-2 variants under monitoring (VUM)		
Omicron (B.1.1.529)	**Alpha (B.1.1.7)**	**Beta (B.1.315)**	**Gamma (P.1)**	**Delta (B.1.617.2)**	**Lambda (C.37)**	**Mu (B.1.621)**	**Eta (B.1.525)**	**Iota (B.1.526)**	**Kappa (B.1.617.1)**
Omicron BA.1									
[ORF1a]K856R, S2083del, L2084I, A2710T, T3255I, P3395H, 3674/3676del, I3758V; **[ORF1b]** P314L, I1566V; **[S]** A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211I, L212del, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F; **[E]** T9I; **[M]** D3G, Q19E, A63T; **[ORF8]:** S84L; **[N]** P13L, 31/33del, R203K, G204R	**[ORF1b]**P314L; **[S]** H69del, V70del, Q498R, N501Y, D614G, P681H; **[ORF8]:** S84L; **[N]** R203K, G204R	**[ORF1b]** P314L;**[S]** K417N, N501Y; D614G; **[ORF8]:** S84L	**[ORF1b]**P314L; **[S]** N501Y, D614G, H655Y; **[ORF8]:** S84L; **[N]** R203K, G204R;	**[ORF1b]**P314L; **[S]** T95I, G142D, T478K, D614G, H655Y; **[ORF8]:** S84L	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** D614G; **[ORF8]:** S84L; **[N]** P13L, R203K, G204R	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** T95I, K417N, N501Y, D614G, P681H; **[ORF8]:** S84L	**[S]**A67V, H69del, V70del, Y144del, D614G; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** T95I, D614G; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** T95I, G142D, D614G; **[ORF8]:** S84L
Omicron BA.1.1									
[ORF1a]K856R, S2083del, L2084I, A2710T, T3255I, P3395H, 3674/3676del, I3758V; **[ORF1b]** P314L,	**[ORF1b]**P314L; **[S]** T95I, G142D, T478K, D614G, H655Y; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** N501Y, D614G, H655Y; **[ORF8]:** S84L;	**[ORF1b]** P314L;**[S]** K417N, N501Y; D614G;	**[ORF1b]**P314L;**[S]** H69del, V70del, Q498R, N501Y, D614G, P681H; **[ORF8]:** S84L;	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** D614G; **[ORF8]:** S84L; **[N]**	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** T95I, R346K, K417N, N501Y,	**[S]**A67V, H69del, V70del, Y144del, D614G; **[ORF8]:** S84L	**[ORF1b]** P314L; **[S]** T95I, D614G; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** T95I, G142D, D614G;

TABLE 2 (Continued)

SARS-CoV-2 variants of concern (VOC)					SARS-CoV-2 variants of interest (VOI)		SARS-CoV-2 variants under monitoring (VUM)		
Omicron (B.1.1.529)	**Alpha (B.1.1.7)**	**Beta (B.1.315)**	**Gamma (P.1)**	**Delta (B.1.617.2)**	**Lambda (C.37)**	**Mu (B.1.621)**	**Eta (B.1.525)**	**Iota (B.1.526)**	**Kappa (B.1.617.1)**
I1566V; **[S]** A67V, 69/70del, T95I, G142D, 143/145del, N211I, L212del, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F; **[E]** T9I; **[M]** D3G, Q19E, A63T; **[ORF8]:** S84L; **[N]** P13L, 31/33del, R203K, G204R		**[N]** R203K, G204R	**[ORF8]:** S84L	**[N]** R203K, G204R	P13L, R203K, G204R	D614G, P681H; **[ORF8]:** S84L			**[ORF8]:** S84L
Omicron BA.2									
[ORF1a]S135R, T842I, G1307S, L3027F, T3090I, L3201F, T3255I, P3395H, 3675/3677del; **[ORF1b]** P314L, R1315C, I1566V, T2163I; **[S]** T19I, L24S, 25/27del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R,	**[ORF1b]**P314L; **[S]** G142DT478K, D614G, H655Y; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L; **[S]** N501Y, D614G, H655Y; **[ORF8]:** S84L; **[N]** R203K, G204R	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** K417N, N501Y, D614G; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** Q498R, N501Y, D614G, P681H; **[ORF8]:** S84L; **[N]** R203K, G204R	**[ORF1a]**T3255I, 3675/3677del; **[ORF1b]** P314L;**[S]** D614G; **[ORF8]:** S84L; **[N]** P13L, R203K, G204R	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** K417N, N501Y, D614G, P681H; **[ORF8]:** S84L	**[ORF1a]**3675/3677del;**[S]** D614G; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** D614G; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** G142D, D614G; **[ORF8]:** S84L

(Continues)

TABLE 2 (Continued)

SARS-CoV-2 variants of concern (VOC)					SARS-CoV-2 variants of interest (VOI)		SARS-CoV-2 variants under monitoring (VUM)		
Omicron (B.1.1.529)	**Alpha (B.1.1.7)**	**Beta (B.1.315)**	**Gamma (P.1)**	**Delta (B.1.617.2)**	**Lambda (C.37)**	**Mu (B.1.621)**	**Eta (B.1.525)**	**Iota (B.1.526)**	**Kappa (B.1.617.1)**
Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; **[ORF3a]** T223I; **[E]** T9I; **[M]** Q19E, A63T; **[ORF6]** D61L; **[ORF8]:** S84L; **[N]** P13L, 31/33del, R203K, G204R, S413R									
Omicron BA.3									
[ORF1a]S153R, G1307S, T3090I, T3255I, P3395H, A3657V, 3675/3677del; **[ORF1b]** P314L, I1566V; **[S]** A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211I, L212del, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; **[ORF3a]** T223I; **[E]** T9I; **[M]** Q19E, A63T; **[ORF8]:** S84L; **[N]** P13L, 31/33del, R203K, G204R, S413R	**[ORF1b]**P314L; **[S]** T95I, G142D, T478K, D614G, H655Y; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** N501Y, D614G, H655Y; **[ORF8]:** S84L; **[N]** R203K, G204R	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** K417N, N501Y, D614G; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** H69del, V70del, Q498R, N501Y, D614G, P681H; **[ORF8]:** S84L; **[N]** R203K, G204R	**[ORF1a]**T3255I, 3675/3677del; **[ORF1b]** P314L;**[S]** D614G; **[ORF8]:** S84L; **[N]** P13L, R203K, G204R	**[ORF1a]**T3255I; **[ORF1b]** P314L;**[S]** T95I, K417N, N501Y, D614G, P681H; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[S]** A67V, H69del, V70del, Y144del, D614G; **[ORF8]:** S84L	**[ORF1a]**3675/3677del; **[ORF1b]** P314L;**[S]** T95I, D614G; **[ORF8]:** S84L	**[ORF1b]** P314L;**[S]** T95I, G142D, D614G; **[ORF8]:** S84L

strain,[20] indicating that the evolution of spike in the novel variants is accompanied with loss of sequences.

The Omicron variant shares many spike mutations with other VOCs, VOIs, and VUMs of SARS-CoV-2 (Figure 2 and Table 2). Eight BA.1 and BA.2 common mutations (G142D, K417N, T478K, Q498R, N501Y, D614G, H655Y, and P681H) and three BA.1 mutations (H69del, V70del, and T95I) overlap the other four VOCs (Alpha, Beta, Gamma, and Delta). Likewise, five BA.1 and BA.2 common mutations (G142D, K417N, N501Y, D614G, and P681H) and five BA.1 mutations (A67V, H69del, V70del, T95I, and Y144del) overlap the VOIs (Lambda, Mu) and VUMs (Eta, Iota, and Kappa).

2.3 | Functional characterization of omicron spike mutations

The characteristics properties of each of the SARS-CoV-2 variants are due to the specific set of mutations contained in their genome. For instance, due to their characteristic mutations, VOCs Alpha and Delta showed higher transmission rates and spread globally and VOCs Alpha and Beta were discovered to be resistant to neutralizing antibodies, thereby, affecting the effectiveness of vaccines.[21,22] Many of Omicron spike mutations that overlap with other VOCs have been previously characterized to confer increased transmissibility and higher antibody escape.[23]

Mutations K417N, T478K, N501Y, D614G, and others have been found to be associated with reinfection, partial resistance to vaccines and increased transmissibility.[5,24] S1/S2 cleavage site mutations H655Y, N679K, and P681H result in increased S1/S2 furin cleavage and facilitate efficient viral entry into the host.[25] While, S477N, Q498R, and N501Y mutations affect the ACE-2 binding affinity, S477N and E484A are responsible for immune evasion.[20,26] Further, K317T/N, L452R, Y453F, S477N, E484K, and N501Y mutations were shown to evade neutralization by 11 of the 13 analyzed monoclonal antibodies (mAbs).[27] Multiple substitutions have been observed at 477 (S477G, S477N, and S477R) and 484 positions (E484A, E484D, and E484K) in the spike protein of various variants with all the resulting variants showing resistance towards convalescent sera.[23,24] N439K has been observed to enhance the binding affinity with ACE2 receptor and to neutralize the activity of monoclonal and polyclonal antibodies in people who recovered from infection.[23,28]

Omicron Spike protein (all three lineages) carries ten deletion mutations (L24del, P25del, P26del, H69del, V70del, V143del, Y144del, Y145del, N211del, and L212del) in its NTD. Deletion mutation Y144del has been observed to modulate the effects of neutralizing antibodies.[22,29] N211del is unique to Omicron and might be responsible for enhanced transmissibility. The PRRA spike insertion mutation in S1/S2 cleavage site has been responsible for the introduction of a polybasic furin cleavage site.[30] Insertion mutation ins214EPE was found to be identical to the sequence $TMEM_{245}$ in the human genome or ORF S in the human coronavirus hCoV-229E, suggesting a human origin for Omicron.[15] Further, Miller et al.[31] showed that Omicron eludes immune response due to mutations in its RBD as well as in classes 1, 3, and 4 antibody epitopes. In this context, the most significant immune escape spike RBD mutations that have been listed include K417N/T, N439K, L452R, Y453F, S477N, E484K, and N501Y.[9]

Omicron mutations Q493 and Q498 have been shown to have enhanced binding affinity to mACE2 and are related to infectivity in animals, especially mice.[32] Similarly, Sun et al.[11] also showed that 5 key Omicron spike mutations K417, E484, Q493, Q498, and N501 have also been observed in A-501-MA-30 strain isolated from Mouse lung tissue. These observations strongly suggest that progenitor of Omicron may have jumped from humans to mice and after rapidly accumulating mutations in mice, it jumped back to humans in a reverse zoonotic cycle, indicating an inter-species evolutionary trajectory for Omicron.[11,12,33]

Reports have also shown that mutations at the ACE2-RBD interface modulate the RBD-ACE2 binding affinities. By conducting atomistic molecular dynamics simulations between ACE2 receptor and RBD of Spike protein, it has been shown that Omicron RBD binds more strongly to human ACE protein as compared to the ancestral virus.[34] In contrary, a comparison between the receptor-binding ability between Delta, Omicron variant, and ancestral virus using MD simulation and Elisa bioassay showed a much weaker receptor binding of Omicron as compared to Delta variant while its receptor binding affinity was comparable with the ancestral wild type SARS-CoV-2.[32] The binding affinity of human ACE2 receptor and RBD of Omicron and other VOCs was analyzed to show that ACE-2 binding affinity to the RBD was lower in Omicron as compared to Beta and Delta variants indicating that receptor binding affinity is not driving omicron evolution.[35] Rather, significant reduction in antibody titers against the Omicron RBD as compared to ancestral SARS-CoV-2 RBD suggest that neutralizing antibodies play an important role in the immune escape shown by Omicron.

Genetic variation in viral genes not only leads to the emergence of novel variants but also has direct implications in viral pathogenesis especially if mutations involve the RBD. Preliminary reports have shown that the Omicron variant shows a more than 10-fold increase in virus infectivity than the Delta variant.[36] The identification of various mutations in SARS-CoV-2 viral genomes, especially the novel mutations in Omicron and their functional characterization is important as it can guide future research prioritization to develop antiviral strategies to contain the spread of the virus.

3 | ORIGIN OF OMICRON VARIANT

The origin of the Omicron has been highly contested in the scientific community.[6,11,12,37,38] The likely modes of origin that have been proposed include (i) reverse zoonosis-human to animal and then animal to human transmission, (ii) from an immunocompromised person treated with antiviral drugs and antibodies, (iii) cryptic spread and circulation in a population with insufficient viral surveillance, sequencing, and vaccination, (iv) recombination arising due to co-infection by

more than one circulating strains or seasonal coronaviruses like or HCoV-229E, and (v) template switching using genomes of coinfecting viruses or from prevalent templates in the host.[2,11–15]

SARS-CoV-2 like any other virus has evolved by induction of novel mutations in its genome. The fate of newly emerged mutations largely depends on recombination, epistasis, pervasive, episodic, and directional selection.[4,11–15] The mutations conferring a competitive advantage to the virus in terms of enhanced viral replication or transmission and immune escape get positively selected and increase in frequency resulting in the establishment of novel lineages carrying these mutations as the dominant variants. Many such viral genomic sites like ORF 1ab, ORF3a, ORF8, and N protein gene among others have been identified that undergo positive selection and might have favored divergence of virus and emergence of novel variants by diversifying or directional selection, epistatic interactions, and even founder's effect.[2]

Omicron's mutational landscape of nearly 60 mutations includes 37 mutations in the spike protein (three times more than reported for other variants) out of which 30 are nonsynonymous mutations (Figures 1 and 2). Sixteen codon sites harboring these mutations have been evolving under positive selection[4] (represented with an asterisk in Figure 1). Omicron genome shows a 4-times higher fraction of positively selected sites than all of the SARS-CoV-2 spike genes that have ever shown positive selection.[4]

Based on the sequencing data available from across the world, molecular prints in preoutbreak and postoutbreak sequences point to three major/plausible pathways to explain the emergence and predominance of the Omicron variant:

(a). Silent/cryptic spread—Even though 7.5 million sequences are available in the GISAID database, several regions across the world may not be represented due to poor surveillance, economic conditions, and social and personal choices. These are potential areas where accumulation and selection of mutations could have happened and remained undetected till they spread.
(b). Single host (long infection)—Hosts with chronic infections (immunocompromised patients) provide a metabolic milieu to the adapting virus to accumulate mutations under severe stress. This mechanism though seems unlikely as the virus prefers to switch hosts rather than continue adapting to extensive changes in one host.
(c). Host jumping and adaptation—This is a plausible pathway where the mouse or rat could pick the virus through contaminated sewage and transmit it to the wild or caged animals in zoos (as several animals like wild leopard, hyenas in the zoos and pets as well have been found to be affected). This provided an opportunity for the virus to accumulate substantial mutations and with multiple intergenic interactions, selection of a wide corpus of mutations could be achieved.

Further, Omicron lineages BA.1 and BA.2 are distinct with respect to the incurred mutations and are more widespread as compared to BA.3. This implies that mutations occurred, were selected for in the specific environments and then diversified and adapted leading to the speculation that diversifying and directional selection, genome shuffling or widespread recombination or a combination of the above could have led to the emergence of Omicron lineages at nearly the same time and simultaneously with the Delta.

4 | PHYLOGENETIC RELATIONSHIP OF OMICRON WITH OTHER SARS-COV-2 VOCS, VOIS, AND VUMS

As shown in Figure 2, the Omicron variant shares many mutations with other VOCs and VOIs of SARS-CoV-2. Eight BA.1 and BA.2 common spike mutations (G142D, K417N, T478K, Q498R, N501Y, D614G, H655Y, and P681H) and three BA.1 mutations (H69del, V70del, and T95I) overlap the other four VOCs (Alpha, Beta, Gamma, and Delta). Likewise, five BA.1 and BA.2 common spike mutations (G142D, K417N, N501Y, D614G, and P681H) and five BA.1 spike mutations (A67V, H69del, V70del, T95I, and Y144del) overlap the five VOIs and VUMs (Lambda, Mu, Eta, Iota, and Kappa). Phylogenomic relationship of Omicron with the other circulating SARS-CoV-2 variants has also been worked out. While, Bansal and Kumar[13] have indicated that Omicron has phylogenetically diverged into a distinct group other than Delta and rather shares a common ancestry with VOI Lambda, Sun et al.[11] suggested that Omicron formed a monophyletic group with Gamma variant as a sister group initially and then both diverged in the mid-2020. They have supported its origin in a reverse zoonotic cycle with mouse as the most probable intermediate host.[39] Genome analysis has indicated that the Omicron variant initially evolved from 20B clade and later diverged to form two subclades,[40] while Kandeel et al.[41] have reported a separate new monophyletic clade for Omicron with some relatedness to Alpha variant.

5 | DIAGNOSTIC TESTING OF SARS-COV-2 VARIANT OMICRON

Rapid and accurate detection of SARS-CoV-2 is the first step in the effective management of Covid-19. A number of diagnostic tests including nucleic acid amplification tests (NAATs), antibody and protein-based detection, have received emergency use authorization (EUA) from Food And Drug Administration (FDA).[42–44]

5.1 | NAATs

NAATs target the SARS-CoV-2 genome and serve as a sensitive, precise and most widely used diagnostic test. The assays' targets include regions in one or more of the *E* (Envelope), *RdRP* (RNA dependent RNA polymerase), *N* (Nucleocapsid), *S* (Spike protein) and

ORF1 genes. At least two independent targets on the viral genome constitute an optimal NAAT assay and are performed using real-time reverse transcriptase PCR (rRT-PCR).[45] rRT-PCR uses naso-pharyngeal or oeso-pharyngeal fluids taken from nasal or throat swabs. The process involves isolation of viral RNA which is reverse-transcribed to synthesize a complementary DNA that is amplified by a *Taq* DNA polymerase with primers targeted at *RdRp*, *E*, *S*, and *N* genes that are amplified with a fluorogenic probe by PCR. The rRT-PCR can quantify the viral load as well which is represented as a cycle threshold (CT) value. CT value refers to the number of cycles post which the virus can be detected. The more the CT value lesser is the viral load in the body and vice versa. A CT value less than 40 is considered clinically positive.[44] Many rRT-PCR assays have been commercialized and have automated sample processing including RNA extraction, amplification and reporting[1] and are being used with rapid turnaround in regions with limited laboratory capacity.

Besides rRT-PCR assay, reverse transcription loop-mediated isothermal amplification (RT-LAMP), clustered regularly interspersed short palindromic sequences (CRISPR) based assays, molecular microarrays and next-generation sequencing are under development or being commercialized.[44]

5.2 | Rapid diagnostic tests (RDTs) based on the detection of viral antigens

Rapid diagnostic assays have been developed to detect the presence of viral antigens[46] and/or antibodies. Most of the RDTs are based on lateral flow immunoassays (LFIs), and are less sensitive than NAATs. The advantages of antigen-based tests, however, are their low cost and rapid turnaround time. They also allow for repeat testing to quickly identify viral infections. For the antigen detecting rapid assay, viral antigen from the respiratory tract samples of infected individuals binds to antibodies adsorbed on a paper strip. Antigens are detected in acute or early infections where the virus is actively replicating.[46]

5.3 | Serological tests for antibody detection

Unlike NAATS and RDTs for antigens that are based on virus detection, serological or antibody tests are used to detect a recent or prior SARS-CoV-2 infection. They help in broad-based surveillance of Covid-19, and evaluating the immunity resulting from a previous infection or vaccination. Quantitative determination of antibodies with the passage of time helps in ascertaining the duration of vaccine-derived protection. Serological tests are taken as additional diagnostics especially in cases who are strongly suspected to have Covid-19 but where NAAT and RDT for antigens tests have produced false negatives.

Serological antibody detection is based on measuring the binding antibodies viz. total immunoglobulins (Ig), IgA, IgG, and IgM in the blood of infected individuals by enzyme-linked sorbent assay (ELISA), LFI, and chemiluminescence immunoassay. ELISA-based IgG and IgM antibody assays have more than 95% specificity for Covid-19 diagnosis.[46]

The above diagnostic methods can be used to detect all the circulating VOCs of SARS-CoV-2. Like the other SARS-CoV-2 variants, Omicron also uses the same ACE2 receptor in alliance with the host's transmembrane serine protease 2 surface protein used as a primer for entry into the human host.[47] In addition to above mentioned diagnostic assays, the Omicron variant shows S-gene Target failure (SGTF) due to spike amino acid 69–70 deletion that is absent in Delta and other variants. Therefore, failure in S gene amplification in a widely used PCR test (Thermofisher, TaqPath) can be used to identify the Omicron variant.[48]

6 | ROLE OF VACCINATION PROGRAM IN MITIGATING COVID-19

Public health and infection control measures have been supported hand-in-hand by the development of vaccines for the management of this pandemic. Vaccines' development has been fast-tracked at an unprecedented time frame, given EUA by FDA and more than 10 billion vaccine doses (March 1st, 2022) have been administered across 184 countries with about 52% of the world population now being fully vaccinated.[49] Covid-19 vaccination program has been the world's largest vaccination drive rolled out to contain the spread of the pandemic, offer protection against severe illness and restrict hospitalization and deaths. Vaccines evoke the immune system responses to produce humoral/cellular immunity and to trigger immunological memory to contain the viral spread. As on March 1st, 2022, 147 vaccines were under various phases of clinical trials, while 195 vaccines were under preclinical development phase. Ten vaccines belonging to either protein subunits, recombinant subunits, adenovirus-based vectors, messenger RNA or even inactivated whole SARS-CoV-2 virus have been approved for use by WHO as on March 1st, 2022.[49] Various approved vaccines in these categories include (i) Protein subunit vaccines (NVX-CoV2373 -Novavax; Covovax- Serum Institute of India, Novavax formulation) (ii) messenger RNA (mRNA) vaccines (mRNA-1273- Spikevax, Moderna; BNT162b2-Pfizer-BioNTech, Comirnaty) (iii) nonreplicating viral vector vaccines (Ad26.COV2.S—Janssen, Johnson and Johnson; AZD 1222/ ChAdOx1-S nCoV-19-Vaxzevria, Oxford/AstraZeneca; Covishield—Serum Institute of India, AstraZeneca formulation) and (iv) Whole inactivated virus vaccines (BBV152—Covaxin, Bharat Biotech; Coronavac—Sinovac and BBIBP-CorV, Verocell, Sinopharm, Beijing). Highest doses (approx 22%) with 65%–85% efficacy have been administered with CoronaVac (Sinovac) followed by BNT162b2/ mRNA-1273 with 90%–95% efficacy, ChAdOx1 nCoV-19 with 65%–80% efficacy, BBIBP-CorV (Sinopharm) with 65%–80% efficacy and other vaccines.[49–51]

Though, the EMA and FDA has approved 48 different vaccination regimens that vary from country to country, in general, administration of two doses (except a single dose in Ad26.COV2.S) of the same (homologous) vaccine constitutes a primary vaccine schedule. Booster

doses were included in the primary vaccination regime when waning of immunity was seen with Beta and Delta variants with the passage of time[52] and especially after the emergence and rapid spread of Omicron variant with mutations that could reduce the neutralizing antibodies and result in immune evasion.[6,13]

6.1 | Efficacy of the existing repertoire of vaccines against Omicron

Due to the presence of many mutations with known immune evasion potential in Omicron genome, data on neutralization of Omicron, vaccine effectiveness (VE) and immunogenicity comparisons by convalescent and other vaccine sera in homologous prime or homologous/heterologous prime-boost settings has been released by many research groups from more than 25 different countries.[9,20,22,26,53–76] A variety of neutralization assays and cell types including *in vitro* pseudo virus, live virus and real-life datasets have been used in these studies.

Early estimates of VE from various countries indicate a significantly reduced effectiveness of the existing vaccines in terms of a decline in neutralizing antibodies produced against Omicron[33] compared to Delta and the ancestral virus in two-dose vaccinated people, with or without a previous infection. The third booster dose has been shown to raise the antibody levels and help in reversing the declining trends of neutralizing antibodies but only to modest levels[49,59,65,74,77] that were observed to be significantly lower when compared to Wuhan-Hu-1 virus or even Delta strain.

For instance, the VE of 2-dose regime of BNT162b2 has been shown to fall from original more than 90% against the original SARS-CoV-2 virus to about 40% against Omicron in UK[55] and 33% in South Africa.[78] Waning of immunity against Omicron within a few weeks of two-dose mRNA vaccination was observed in all age groups in Israel,[79] and with either of CoronaVac/mRNA vaccines in Hongkong.[20] With four widely used Covid-19 vaccines (BNT162b2, mRNA-1273, Ad26.COV2.S, and ChAdOx1) a strikingly high levels of neutralization resistance against Omicron was observed in convalescent, two-dose vaccinated people in United States.[67] The immune responses generated in previous infection coupled with two-dose vaccination could not generate a sufficient titer to neutralize Omicron[26] and 17–22-fold fall in neutralization ability against Omicron was observed in two-dose vaccinated (ChAdOx1/BNT162b2)/convalescent individuals (Breakthrough infection). Convalescent + two-dose vaccinated (Hybrid immunity/Super immunity) individuals also showed 16-fold decline in neutralization ability.[26] Similarly, a drastic reduction or even absence of neutralization in two-dose AZD1222/BNT162b2 vaccinated individuals against Omicron has been observed.[60] VE of −38% to −42% (between 120 and 239d) against Omicron was observed in Canada after two-doses of ChAdOx1/BNT162b2.[57] A whole lot of other reports from research groups across countries (China, Canada, UAE, Denmark, India, Hongkong, Denmark, Russia, Sweden, Lebanon, Australia, Chile, Israel, Germany, and many others) have observed similar trends of significant waning (20–40-fold) or even absence of neutralization ability in two-dose vaccinated individuals with passage of time against the previous VOCs and Omicron.[21,58,60-62,64,66,80-85] Therefore, a pressing need was felt for a third booster.[86] Many reports showed a reduction in rate of infection and severe illness after administration of 3rd booster dose with mRNA BNT162b2 vaccine initially in the United Kingdom, United States, Canada, Denmark, Dominican Republic, and South Africa followed by many other countries.[56,57,60,61,67,71,80,81,85,87] Although, the 3rd booster jabs were shown to reverse the waning immunity trend set in after the two-dose vaccination regime and provided protection from severe illness, they could not protect against infections as only an incomplete neutralization of SARS-CoV-2 variants especially Omicron could be achieved even after boosting.

Nevertheless, booster vaccinations now have been administered with both homologous as well as heterologous vaccines settings. In general, mRNA vaccines have been shown to produce higher neutralization titers than other vaccines. Further, several clinical/cohort studies across laboratories in the world with different assays and vaccine types have reported that heterologous prime-boost vaccinations produce incremental increase in immunogenicity to neutralize Omicron variant better than homologous prime-booster strategies and enhance antibody and B cell and T cell mediated responses.[49,53,65,86,88–90] Many vaccine combinations have been tested for tolerance and efficacy analysis in a heterologous prime-boost regime including inactivated virus vaccine + recombinant protein vaccine/mRNA vaccine/adenovirus vector vaccine/protein subunit vaccine or adeno-virus vector vaccine + mRNA vaccines.[22,51,54,57,68,69,71] Following combinations of the currently approved vaccines have been included in the trials or cohort studies. The studies have tested two-doses of inactivated vaccines BBIBP-CorV or CoronaVac + mRNA vaccines BNT162b2 or mRNA-1273[51,69,71,91] adeno-virus vector vaccine ChAdOx1 nCoV-19/Ad26.COV2.S + mRNA vaccine BNT162b2/mRNA 1273,[63,72,89] Inactivated virus vaccine CoronaVac/BBIBP-CorV + recombinant protein vaccine ZF2001/NVSI-06-07,[54] Inactivated virus vaccine CoronaVac + adenovirus-vector vaccine Convidecia[54]; Inactivated vaccines BBIBP-CorV + Protein subunit vaccine ZF2001,[53] adenoviral viral vector vaccine SputnikV + Sputnik Light Booster.[59]

All these tested heterologous prime-boost vaccine combinations were found to be well tolerated and produced folds-increase in immunogenic response against Omicron than the corresponding homologous prime-boost vaccine regimes. This mix and match approach of vaccinations in heterologous prime-boost vaccination regime looks attractive as it appears to produce better VE than single vaccine prime-boost approach and can offer solutions to vaccine supply chain issues. Further, it can also help people migrating to different countries with different vaccination regimes.

Studies have also shown that SARS-Cov-2 infection before vaccination boosted the immune response (Hybrid-immunity).[92] Also, Infection after the vaccination (Break through infection) acted as a booster against the current variant Omicron.[66]

An overall compilation of VE data from various countries showed more than 19-fold drop in titers in two-dose vaccinated or

convalescent sera as compared to approximately sevenfold drop in the titers in sera from two-dose vaccinated + boosted or two-dose vaccinated + infected individuals within a maximum of 6 months of boosting.[71] As evident, although, boosting by a third homologous or heterologous vaccine dose reversed the decreasing trend of neutralizing titers but the fold-drop still was quite substantial.

It is obvious that absolute VE or neutralization data comparisons might be skewed between the countries due to various variables in the studies like methodology, considering symptomatic infection vs any infection, product used for vaccination, time interval policies for vaccinations, masking mandates, demographical differences in the countries leading to difference in number of individuals with vaccine induced or infection induced immunity, public health measures like the duration of lockdowns and curfews and hence the exposure level.[93] However, the trends suggest a general reduction in VE against Omicron variant in comparison to the original SARS-CoV-2 and Delta variant.

In nutshell, the decline in the immunogenic potential against Omicron even with this heterologous prime-boost vaccination strategy has been reported to be highly significant when compared with the ancestral and Delta strains, thereby, raising concerns about the long-term sustenance of this boosted immunity.

7 | EFFICACY OF ANTIBODY BASED THERAPEUTICS USED FOR SARS-COV-2 TREATMENT AGAINST OMICRON

Antibody-based therapeutics for Covid-19 include mAbs, polyclonal antibodies and convalescent plasma. Since, spike protein has been the target of these antibody based therapeutics, the mutations incurred in Omicron spike could affect efficacy of these treatments. A timely assessment of antibodies' efficacy against the evolving variants is very important. Studies have been carried out to test the neutralization of Omicron variant with the existing repertoire of clinically approved therapeutic antibodies.[63,82,94–96]

For instance, Cameroni et al.[80] have highlighted a major antigenic shift and immune evasion potential of the Omicron variant. While, 26 out of the 29 tested mAbs were shown to lose their neutralizing ability, mAbs S2K146, sarbecovirus mAbs sotrovimab, S2X259 and S2H97 could neutralize Omicron. They correlated in vitro neutralization of various mAbs that were directed at the RBM with the 10 incurred RBM mutations. The last three mAbs, interestingly, had their recognition sites outside the RBM.

In yet another report, a staggering 85% escape by the tested antibodies were shown by Omicron[94] by studying the RBD escaping profiles for 247 human anti-RBD neutralizing antibodies (Nabs). The analyzed Nabs were divided into six epitope groups (A-F). Spike mutations K417N, G446S, E484A, and Q493R were shown to be responsible for escape by NAbs in group A-D, which had overlapping epitopes with ACE-2 binding motif. Group E and Group F Nabs were largely less affected. In nutshell, neutralization efficiency of "LY-CoV016/Ly-CoV555," "REGN10933/REGN10987," "AZD1061/AZD8895," and "BRII-196" showed significant reduction, while "VIR-7831" and "DXP-604" showed lesser reduction. Further, a mixed response was shown by Van Blargan et al.,[96] when they tested a panel of mAbs that are approved for clinical use to neutralize Omicron variant. Several mAbs like LY-CoV555 and LY-CoV016 (Lilly); REGN10933 and REGN10987 (Regeneron); and CT-P59 (Celltrion) did not show neutralizing ability against Omicron. While, S309, the parent Mab of VIR-7831 (sotrovimab), showed minimum reduction, COV2-2196 and COV2-2130, the parent mAbs of AZD8895 and AZD1061 (AstraZeneca), showed approximately 12-fold reduction in the neutralization ability.

In an elaborate study conducted by Hoffman et al.,[63] it was shown that Omicron spike protein showed inhibition by only sotrovimab and showed resistance towards all other 18 RBD and NTD directed mAbs tested either individually or in combination. Therefore, two frequently used antibody cocktails involving casirivimab + imedevimab and etesevimab + bamlanvimab for Covid-19 treatment are no more effective against Omicron. The study highlighted the role of Omicron spike mutations G142D, V143del, Y144del, Y145del, K417N, T478K, E484A Q493R, G496S, Q498R, N501Y, and Y505H towards classes 1 and 2 mAbs. Similarly, the role of four new spike mutations S371, N440K, G446S and Q493R in Omicron variant in conferring antibody resistance against class I and 2 RBD antibodies was emphasized by Liu et al.[95] In the same study, classes 3 and 4 RBD monoclonal antibodies were also shown to have developed substantial resistance. Complete loss of neutralization of Omicron by casirivimab and imedevimab, press the need to develop variant specific therapeutic mAbs.[74] Omicron was observed to escape neutralization with a cocktail of imdevimab, and casirivimab[64] or the all the other tested Mabs (casirivimab, imdevimab, bamlanivimab, cilgavimab, and tixagevimab),[26] and only sotrovimab was observed to neutralize the virus.[26,64] In a study by Imbrechts et al.,[97] after testing various mAbs against all the five VOCs of SARS-Cov-2, however, it was concluded that three mAbs including mAb 3B8 at very low doses resulted in complete neutralization of Omicron variant. Identification of more such mAbs can give confidence to handle the continuously evolving novel variants in SARS-CoV-2.

In totality, the above initial reports of massive failure of the existing repertoire of therapeutic antibody molecules raise high concern present a vexing situation to perplex the policy makers and scientific community who must now develop alternate strategies to counter this virus.

8 | CONCLUSION

Omicron with an expanded mutational landscape has emerged as a highly transmissible SARS-CoV-2 variant that harbors many mutations showing positive selection. Besides the standard diagnostic assays including NAATs, RDTs, and ELISA based antibody detection assays, Omicron shows SGTF, which is used as an additional marker to test its presence. The origin of Omicron is contentious and the most plausible modes that could have led to the emergence of

Omicron lineages at nearly the same time and/or simultaneously with the Delta, include selection, genome shuffling or widespread recombination or a combination of above. The emergence and spread of the Omicron are due to its unique abilities that reside in the mutational landscape/edited RNA genome that provides it the flexibility for transmission. In this context, it is imperative to monitor all viruses of this group as a collective surveillance. This will indicate the likely cellular environments needed by the virus to generate modified genomes and proteomes that help it to evolve. This understanding might help in devising strategies to handle the emergence of future SARS-CoV-2 variants.

Omicron has shown significant neutralization escape with the existing vaccines and therapeutic antibodies. Although homologous/heterologous prime-boosted vaccination schedules, so far optimized to three doses have shown neutralization of Omicron, albeit to fold-reductions, it is now important to watch the duration of this boosted immunity. Future continued studies and data acquisition on the long-term immunity levels in boosted individuals, occurrence of reinfections or breakthrough infections, targeted cohorts studies in different age groups (young and elderly), immunity levels (immunity compromised group) is warranted. Whether, the neutralization escape of Omicorn with the existing vaccines is due to the waning immunity or the result of this novel variant is the most important question to answer. Undoubtedly, vaccines need to be updated to be genetically and antigenically close to the circulating viruses to provide adequate protection. Multivalent vaccines utilizing antigens from different VOCs can be an option as strain specific monovalent vaccines need time to be produced with every evolving strain. Of course, a pan SARCov-2 variant proof vaccine will be the best option, if feasible.

Further, the initial reports of escape of Omicron from unboosted vaccines and therapeutic monoclonal antibodies are disturbing. More real world data on impact of reduced neutralization and increased transmission and their correlation with rate of hospitalization and mortality in the coming months will develop a better understanding to clear the air if "Omicron is actually mild" and if our existing armor of vaccines and therapeutic molecules is sufficient to provide adequate protection. Since, the present vaccinations or therapeutics have largely targeted the spike that now has now expanded its mutational landscape, it is perhaps the time to reappropriate research prioritization and possibly look at the more conserved parts of the virus other than spike protein or tapping the T cell mediated immunity for designing the next generation therapeutics and vaccines.

AUTHOR CONTRIBUTIONS

Conceptualization, *literature search*, *writing-final draft*, *visualization*, *writing-review and editing*: Vijay Rani Rajpal. *Sequence retrieval*, *Formal analysis*, *data curation*, *writing- draft and editing*: Vijay Rani Rajpal, Shashi Sharma, Avinash Kumar. *Literature Search*, *figure illustrations*, *writing-draft*: Shweta Chand, Lata Joshi. Atika Chandra, Sadhna Babbar. *Data analysis:* Shailendra Goel. *Conceptualization*, *supervision*: Soom Nath Raina, Behrouz Shiran. All authors read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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