REVIEWS

The emergence of SARS-COV-2 Omicron subvariants: current situation and future trends

Running title: **The emergence of SARS-COV-2 Omicron subvariants**

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SUMMARY

The SARS-CoV-2 Omicron variant (B.1.1.529) has been the most recent variant of concern (VoC) established by the World Health Organization (WHO). Because of its greater infectivity and immune evasion, this variant quickly became the dominant type of circulating SARS-CoV-2 worldwide. Our literature review thoroughly explains the current state of Omicron emergence, particularly by comparing different omicron subvariants, including BA.2, BA.1, and BA.3. Such elaboration would be based on structural variations, mutations, clinical manifestation, transmissibility, pathogenicity, and vaccination effectiveness. The most notable difference between the three subvariants is the insufficiency of deletion (Δ69-70) in the spike protein, which results in a lower detection rate of the spike (S) gene target known as (S) gene target failure (SGTF). Furthermore, BA.2 had a stronger affinity to the human Angiotensin-converting Enzyme (hACE2) receptor than other Omicron sub-lineages. Regarding the number of mutations, BA.1.1 has the most (40), followed by BA.1, BA.3, and BA.3 with 39, 34, and 31 mutations, respectively. In addition, BA.2 and BA.3 have greater transmissibility than other sub-lineages (BA.1 and BA.1.1). These characteristics are primarily responsible for Omicron's vast geographical spread and high contagiousness rates, particularly BA.2 sub-lineages.

Keywords: SARS-CoV-2, COVID-19, BA.2 sub-lineages, immune evasion, Omicron variant, spike protein, transmissibility

INTRODUCTION

Coronavirus disease (COVID-19) has become a worldwide pandemic since it was declared by the World Health Organization (WHO) on March, 2020. It is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and appeared first in Wuhan, Hubei, China. The pandemic led to large numbers of deaths and severe morbidities worldwide.

Fortunately, the number of cases and deaths has exceeded 632 million and 6.6 million, respectively, as of October 22, 2022, with more than 12.8 billion vaccine doses administered [1]. SARS-CoV-2 has mutated and undergone antigenic variations. As of May 2021, four variants have been discovered, including Alpha, Beta, Gamma and Delta [2]. WHO classified the new SARS-COV-2 mutation, found in Botswana on November 11, 2021, as the Omicron subvariant (B.1.1.529) on November 26, 2021 [3]. Omicron showed a significant mutation ability with increased mutations compared to other variants [2]. Interestingly, Uddin et al. highlighted the Omicron's high infectivity and antibody resistance. They reported that Omicron could be 10-fold more transmissible and infectious than the early original ancestor and 2.8-fold more than the Delta variant. Furthermore, Omicron is 88% able to evade recent COVID-19 vaccines [4]. Accordingly, the number of COVID-19 patients has rapidly increased after this variant's appearance [5]. It quickly spread to South Africa as the average number of patients per day increased from 280 to 800 in one week [6]. Therefore, neighboring countries such as Mozambique, Swaziland, Zimbabwe, Namibia, and Botswana were alerted [3]. On December 13, 2021, a Canadian patient was documented as having the Omicron subvariant (B.1.1.529). Guangdong, a Chinese city, recorded 65 foreign omicron cases imported from 16 countries. They were discovered through the second sequencing generation on December 31, 2021 [5].

Interestingly, after analyzing the sequences of viruses that affected a fraction of infected people globally in March 2022, Omicron has been revealed in 100% of cases in many countries, including Argentina, Cambodia, Colombia, Croatia, Ecuador, Greece, New Zealand, and Russia [7]. However, this result may not indicate the complete eradication of other variants since only a sample of all patients was sequenced. Also, actively monitored or recently discovered variants may be overrepresented because their suspected cases are sequenced preferentially or faster than other cases [7]. As of October 19, 2022, the Omicron variant is evident in at least 206 countries. The United States of America (USA) and the United Kingdom (UK) are the most affected countries, with 1,827,027 and 1,316,923 cases, respectively, followed by Germany (446,143), Denmark (304,947), France (283,549), Japan (251,145), and Canada (189,819) [8]. As of October 19, 2022, the number of recent omicron variant cases globally is shown in Figure 1. The evolution over time indicates that Omicron became the predominant and exclusive VOC circulating globally during 2022 (Figure 2).

Furthermore, the Omicron revealed several sub-variants, including BA.1, the most spread type of Omicron; BA.2.12.1, a sub-variant identified in New York and circulated quickly in the US in May; B.4 and B.5, which were first discovered in January and February and frequently found in South Africa during May [9]. Finally, B.5 entered Portugal and made the sixth wave, leading to a peak in the number of cases and deaths number. The cases number was 2888 per million during the last week of May, compared to 373 new cases per million in Spain [10].

BA.2, another omicron sub-variant, has been the most recent and common variant of SARS-CoV-2 globally [9]. BA.2 counted around 86% of all analyzed sequences globally and more than 50% of new cases in the USA in March 2022. Further, it has appeared in China, the UK, and Germany [9,11]. Finally, BA.3, a subvariant containing 1276 amino acids, was detected in Gauteng Province, South Africa. It was found that BA.3 has 34 mutations, including R216, which is a unique mutation. Further, compared to BA.1, BA.3 showed a higher capability to transmit among people [12].

Our literature review will thoroughly explain the current state of Omicron emergence, particularly by comparing different omicron subvariants, including BA.2, BA.1, and BA.3. Such elaboration would be based on structural variations, mutations, clinical manifestation, transmissibility, pathogenicity, and vaccination effectiveness.

MATERIALS AND METHODS

We searched on PubMed, Scopus, Web of Science, Google Scholar, and accredited international websites using keywords including "Coronavirus", "SARS-CoV-2", "Variants", and "Omicron". In addition, all study designs (*i.e.*, retrospective, and prospective observational studies, letters, and reviews) related to our search strategy have been included in our literature review.

Structure

SARS-CoV-2, one of the Coronaviridae family members, possesses a positive, encapsulated, and unsegmented single-stranded ribonucleic acid (RNA). The genomic structure of the Coronavirus consists of three key regions: 1) Open reading frames (ORFs) 1a; 2) ORF 1b as the non-structural proteins component; 3) structural proteins, which account for one-third of the viral gene sequence. These structures are composed of the membrane (M), envelope (E), spike protein (S), and nucleocapsid (N) [13,14]. Coronavirus virulence is linked to its structural and non-structural proteins [15,16].

Some virus variants have emerged during the recent COVID-19 spread due to the SARS-CoV-2 dissemination. According to their impact on epidemiological and clinical status, these variants are designated as variants of concern (VOC), variants under monitoring (VUM), and variants of interest (VoI) [17]. Previously, the following four variants, Alpha, Gamma, Beta, and Delta, have been known as VOCs. These variants introduce unique genetic variations, primarily in the spike protein. There have been numerous mutations described for these variants, including (1) alpha (spike mutations: Δ69-70, Δ144, N501Y, A570D, D614G, P681H, T716I, S928A, D1118H), (2) beta (L18F, D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G, A701V), (3) gamma (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F), and (4) delta (T19R, G142D, Δ156–157, R158G, L452R, T478K, D614G, P681R, D950N) [18, 19].

Nowadays, the Omicron variant is the only VOC classified by the WHO. Four commonly identified sub-lineages of Omicron are the most common type globally, including BA.2, BA.3, BA.1, and BA.1.1 [17, 20]. BA.2 had 1270 amino acids, the same as the original Omicron version (BA.1, discovered in November 2021), but slightly more than BA.3 (1267 amino acids). The BA.1 sub-lineages have the highest molecular weight (141,328.11) than other subvariants, possessing an additional R346K spike protein mutation [21].

Recently, several new Omicron sub-lineages have emerged, including BA.2.12.1 (North America), BA.4, and BA.5 (South Africa mainly), as well as BA.5.1 (Portugal particularly), possibly driving the pandemic further [22, 23]. The origin of the Omicron variant is still unconfirmed. However, it is thought to be related to three distinct theories of circulation: in a hidden population, in immunocompromised patients, and as an adaptation in animal reservoirs that were transmitted to humans [24-26].

The general structure of the Omicron variant is like the previously described genomic pattern of SARS-CoV-2. However, in comparison with the wild type (Wuhan-Hu-1), BA.1 variant has 28 substitutions in its amino acids, one insertion, and three deletions of the spike protein (A67 V, Δ69–70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371 L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F) and 50 mutations in total (including membrane protein, envelope protein, nucleocapsid protein, and non-structural protein). Meanwhile, BA.2 sub-lineages have shown subtly different alterations in

the spike protein, with 29 substitutions in its amino acids and one insertion (T19I, L24S, ins25PPA, D142D, V213G, G339D, S371 L, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K), with 51 total mutations observed in the whole genome. Meanwhile, BA.3 has most of its mutations (27 mutations of the spike protein, 43 in total) with BA. 2 and BA.1, except for a single mutation on NSP6 (A88V) [26-29].

BA.2.12.1 has five more mutations in comparison with BA.2 sub-lineages, located at the L452Q and S704L, g.11674 C > T (ORF1ab), g.15009 T > C (ORF1ab), and g.21721 C > T (S) [29,30]. Meanwhile, BA.4 and BA.5 pose identical spike proteins with BA.2 variant, with some additional mutations, namely del69/70, L452R, F486V, Q493 reversion, and N658S. These two lineages are frequently discussed in a companion [17,29,31]. For a complete description, BA.4 has 30 amino acid mutations in total (V3G, T19I, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K) with additional five deletions in the spike protein (L24del, P25del, P26del, H69del, V70del) [32]. Henceforth, BA.5 subvariants possessed 34 nonsynonymous mutations, including T19I, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, L24del, P25del, P26del, H69del, and V70del, which is generally similar with BA.4 type [33].

Undoubtedly, any variability in the amino acid side chain (*i.e.*, charge, size, and hydrophobicity) can change the intrinsic properties of the affected protein along with its interplay with other proteins or molecules [34]. The most significant distinction between BA.2 and BA.1 and BA.3 is the absent deletion (Δ69-70) in the primer target site of the spike protein, indicating that the BA.2 viral genome lacks the spike (S) gene target failure (SGTF) characteristic [35]. It complicates real-time detection of this subvariant using commonly available reverse transcriptase-polymerase chain reaction (RT-PCR) [36].

Furthermore, based on the comparison between BA.2, BA.1, and BA.3, it is identified that BA.1 poses eight specific mutations (A67V, ins214EP, R216E, S371L, G496S, T547K, N856K, and L981F), the same amount as BA.2 (T19I, L24del, P25del, P26del, A27S, V213G, T376A, and

R408S). Meanwhile, BA.3 possesses a lower amount of distinct mutation (one, R214del) [25, 28, 37].

Each of the Omicron sub-lineages has 21 common mutations in spike protein (42D, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K) (Figure 3) [25].

All Omicron subvariants share a common mutation at the Receptor-binding Motif (RBM) region, including N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, and Y505H. This mutation impacts the binding capacity of the virus to the human Angiotensin-converting Enzyme (hACE2) receptor. Interestingly, BA.2 possesses the highest affinity when it is compared with the rest Omicron sub-lineages). Other crucial mutations are L452Q (promoting human-to-human transmission, augmenting infectivity, strengthening receptor binding, and diminishing vaccine-induced protection), L452R, and F486V (participating in immune escape mechanism), in addition to mutations in H655Y, N679K, and P681H (increasing spike cleavage and facilitating virus transmission) [25, 38]. Meanwhile, the N658S mutation on the BA.4 and BA.5 subvariants (at the beginning of subvariant emergence) was responsible for a reduction in binding affinity with the hACE2 receptor, though it no longer appears [39]. The neutralizing performance of monoclonal antibodies (mAbs), convalescent plasma, and vaccines can be influenced by mutations in K417N, N440K, G446S, S4777N, T478K, and N501Y [39, 40]. The essential NSP mutations are NSP6, NSP12, and NSP14, causing an alteration in the T cell immunity and innate immune response [29, 41]. The comparison of molecular features between BA.2, BA.1, and BA.3 is depicted in Table 1.

Characteristics	**BA.1**	**BA.2**	**BA.3**
Molecular weight	141,328.11	141,185.78	140,900.61
Charged residues	111	108	109
Unique mutations (spike protein)	8	8	1
Unique mutations (RBD)	0	2	0
Unique mutations (RBM)	1	0	0
Root mean square deviation value (vs wild type)	0.68	0.68	0.68
Binding pocket area	93.87	49.03	49.03

Binding pocket volume	37.52	16.44	16.43
Deleterious spike protein mutation	6	2	5
BFE (kcal/mol)	-70.6	-72.36	-73.55
BFE changes (kcal/mol)	2.60	2.98	2.88
Docking energy with hACE2 (kcal/mol)	-943.4	-974.0	-999.3
Potential vaccine breakthrough	0.88	0.91	0.89
K_D values of hACE2 binding affinity (nM)	19.5	10.0	22.1
SGTF	Yes	No	Yes
Effective reproduction number (R_0) vs Delta variant	1.99	2.51	N/A
Generation time vs Delta variant	0.60	0.51	N/A

Table 1 - The comparison between BA.2, BA.1, and BA.3 subvariants of Omicron [25, 42-45]. Abbreviation: BFE: Binding free energy, hACE2: human Angiotensin-converting enzyme 2, RBM: Receptor-binding Motif, RBD: Receptor-binding Domain, SGTF: S-gene Target Failure

Infection with Omicron: Properties and Clinical Outcomes

Omicron is more contagious and associated with a high rate of reinfections. It can also escape vaccine-induced immune responses [6, 46]. Omicron variants have a short incubation period of about three days [47]. Computational analysis of the Delta and Omicron documented that Omicron affinity to ACE2 receptors is higher, explaining its higher transmissibility. This great affinity may be due to many mutations, including Q493R, S371L, S375F, Q498R, T478K, Asn501Tyr, K417, D614G, L452R, and N501Y [48, 49].

Compared to the SARS-COV-2 Delta variant, Omicron leads to fewer severe clinical outcomes [50-53]. Omicron replication occurs in the upper respiratory airway leading to little lung harm with a mild form of the disease [54]. Clinical manifestations induced by Omicron include flu symptoms such as fever, fatigue, headache, throat pain, and sore throat with no loss of taste. These mild manifestations lead to a low hospitalization rate in a data linkage study by Wolter et al. [55]. It is worth noting that this outcome should be interpreted with caution since analysis may be inconclusive due to the small number of included severe outcomes, the ability of S gene target failure (SGTF) to be a proxy for detection of other variants such as alpha variant and the

setting of the analysis which was in the early fourth wave with a small number of admitted individuals to hospital where individuals with milder symptoms were more likely to be admitted [55, 56].

Additionally, about 21% of the study's hospitalized South African individuals with the SARS-CoV-2 omicron variant had severe clinical outcomes [56]. However, this rate might change in other populations with different demographics and lower levels of infection or vaccine-derived immunity [56]. Interestingly, patients with Omicron infection can develop a robust immunity with a high capacity to neutralize many SARS-CoV-2 variants caused by this decrease in reinfection with Delta [57]. The Omicron is characterized by new mutations in its receptor binding site, leading to an excellent capacity for transmission and a different antibody response [58]. The existence of these mutations may also explain the implication of antibody receptor binding domains of Omicron in escaping immunity. Furthermore, such mutations may also make immunity from previous infections less effective against new reinfection [58]. Yet, more extensive investigations are needed to evaluate Omicron's rate and causes of reinfections and the possible factors of this phenomenon.

Omicron BA. 2 transmissibility, pathogenicity, and severity

In their preprint, Lyngse et al. found that BA.2 has a higher risk of infection compared with BA.1 in the unvaccinated, fully vaccinated, and booster-vaccinated individuals with Odds Ratio (OR) of (2.19; 95%-CI 1.58-3.04), (2.45; 95%-CI 1.77-3.40) and (OR 2.99; 95%-CI 2.11-4.24), respectively. Thus, they concluded that Omicron BA.2 subvariant is more transmissible than BA.1. However, it does not increase its transmissibility from vaccinated individuals [59]. Additionally, it could reinfect the persons infected with BA.1, promoting double peaks in infection rates [60]. Furthermore, BA.2 is named the stealth variant due to its challenging track as BA.2 and BA.3, requiring genomic sequences to be tracked, unlike BA.1, which could be detected by PCR test [60].

Its transmissibility and pathogenic potency differ from BA.1 and BA.3 subvariants. This difference in biological properties also interests resistance to antiviral drugs and vaccine-enhanced immunity [61]. In contrast to BA.1 subvariant transmission, Omicron BA.2 sub lineage's spread and transmission are faster [59, 62, 63]. In addition to this, BA.2 may have a link with susceptibility to infection among unvaccinated, vaccinated, and booster-vaccinated patients compared to BA.1. The study of docking energy of Omicron receptor-binding domain

(RBD) showed that Omicron subvariant BA.2 RBD possesses a greater affinity regarding binding to ACE2 receptor than BA.1 RBD. Also, mutations at Receptor-binding Motif (RBM) residues may affect the affinity to the ACE2 receptor. Together, these data may explain the difference in infectivity among Omicron subvariants [25]. Furthermore, its immune-evasive actions may influence vaccine effects, but this does not enhance its potency of transmission among vaccinated patients [59].

A report highlighted the comparable pathogenicity of BA.2 to B.1.1 but higher than BA.1 in a hamster model [61]. Yet, another study found also that the pathogenicity and replication properties of both BA. 1 and BA.2 were similar in rodents [64]. The fusogenicity of the SARS-CoV-2 variant is closely related to its pathogenic capacities. Studies exploring the virological properties of BA.2 showed it has higher fusogenicity and pathogenicity than BA.1. Results from cell culture experiments suggested that the Delta variant is more fusogenic than both B.1.1 and BA.1 [65, 66]. However, unlike the Delta variant, the BA.2 fusogenicity did not increase S protein cleavage efficiency [61, 66]. Therefore, deep clinical and virological investigations should be done to understand BA.2 pathogenicity and determine the different factors that influence the invasive characteristic of the different subvariants. Further, the rising in BA.2 could be attributed to the cessation of the public health interventions that occurred globally simultaneously, and it may be just the version that spread when the people lifted the masks [13].

Symptoms caused by omicron subvariants BA.2 and BA.1 are similar [67]. In a report, patients having BA.2 had more risk of developing cold-like clinical symptoms and a higher rate of daily activities disruption than those having BA.1. In general, patients having BA.2 infection had more risk of being symptomatic than those with BA.1. This may be due to the lower neutralizing antibodies present against BA.2 and its capacity to evade vaccine protection [68, 69]. Infection with the BA.1 subvariant may be a protective factor for reinfection with BA.2 [69]. In a recently published cohort, the proportion of patients with an age of 65 years or more was more in the BA.2 (15%) group than in the BA.1 group (8.8%). Hospitalized individuals' proportion was higher among patients with BA.2 (6.3% versus 1.4%), but no transfer to ICU was registered [70]. Further analysis found that old age and infection with BA.2 were associated with increased odds of hospitalization, while only age was identified as a risk factor for mortality, particularly in higher age groups [>80 years: adjusted OR= 36.78; 95%CI: 16.64-81.33] [70]. In a cohort from the Apulia region, the median age of subjects having BA.2 infection (42 years) was higher than

the reported age in Denmark, which was 32 years. That may be explained by the region's population distribution characteristics [69]. Reports showed that sex, age, hospitalization, mortality, and COVID-19 reinfection rates did not differ among subjects infected with BA.2 or BA.1 [67, 69]. A current study from California revealed that the risk of severe outcomes (including symptomatic hospital admission, ICU admission, use of mechanical ventilation, and death) in BA.2 infection was not different from that reported in infections with BA.1 or BA.1.1 [71]. The German national surveillance data analysis suggested that the risk of hospitalization of patients aged 35 years or more due to infections caused by BA.2 or BA.1 is 80 per cent lower than the Delta-associated risk. They also showed that, among patients having Omicron infection, the rate of vaccinated patients was lower than in the Delta cohort, with proportions of 2.3% and 4.4% for both lineages, respectively. In total, the hospitalization rate among patients infected with Delta was threefold higher than that of the Omicron group [72].

Co-Infection and Recombination of Omicron and Delta

Co-infections with different variants enhance the risk of viral recombination and the production of new variants that could potentially be VOC. Therefore, co-infection detection and identification are essential for determining their risk in vulnerable patients, known as incubators of evolutionary events in the SARS-CoV-2 journey [73]. Few studies explored the possibility of concurrent infection between Omicron subvariants and the Delta variant. Some studies identified Omicron and Delta co-infection cases in geographically distinct areas [74, 75]. Several shared mutations have been detected among VOCs and Omicron sublineages. The omicron subvariants genome analysis showed various recombinations of VOC and these variants, including Deltacron-like variants [76]. However, data on the influence of co-infection on Omicron and Delta on infection outcomes and the efficacy of current vaccines are scarce. Also, the existence of recombinants resulting from different variants like "Demicron" and "Deltacron" is controversial since there is no evidence whether the detected viruses were a real new variant or they were due to a sequencing error [77]. In front of the inconsistency of the current data, prevention of Omicron spread in unvaccinated and vulnerable patients seems to be an essential step to control recombination events and prevent further waves of COVID-19.

Diagnostic measures of Omicron

Many diagnostic tests have been used to detect SARS-CoV-2, including Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for viral RNA detection, immunoglobulin assay, and viral

antigen detection, with the RT-PCR as the most reliable and widely used tool. It is the gold standard diagnostic test with more than 97% specificity and more than 95% sensitivity [78]. However, the role of RT-PCR in detecting the new subvariant (BA.2) is still controversial, warranting the development of new variant-specific PCR assays for detecting the newly emerging variants [79]. To correctly identify the VOCs, genome sequencing is key [80-101].

Indeed, a novel RT-qPCR assay has been developed to differentiate between Omicron sub-lineages (BA.2, BA.1, and BA.3) [80]. Furthermore, using flow cytometry in conjunction with RT-qPCR has enhanced the detection of the Omicron variant beside other VOCs [81]. However, further investigations are needed to evaluate the possibility of implementing such flow cytometric techniques into the standard RT-PCR testing of Omicron, considering its additional cost and the need for the expertise required to run such methods. Other diagnostic modalities, including rapid antigen and rapid antibody assay, provide a quick and low-cost alternative to PCR. That is particularly useful in surveillance programs where many people must be screened [82, 83]. Recent evidence suggests a similar performance of rapid antigen testing in detecting the Omicron variant compared with previous variants [84].

Nasopharyngeal swabs versus combined oropharyngeal/nares swabs

At the pandemic's beginning, RT-PCR of nasopharyngeal (NP) swabs was the gold standard diagnostic approach in community and hospitalized patients [85]. An alternative sampling method was combined oropharyngeal/nares (OPN) swabs, which showed comparable performance levels to NP swabs [86]. One study demonstrated that the OPN route might be preferable to NP due to increased SARS-CoV-2 viral shedding through the saliva [87]. That is particularly the case for Omicron compared to other variants like Delta [88]. Another study suggested NP swabs are superior to OPN swabs and saliva in detecting Omicron by finding higher viral loads in NP swabs compared to saliva [89]. Such discrepancy in the results of the two types of swabs among different studies may be attributed to other factors, such as the technical difficulty of performing NP swabs compared to the relative ease of performing OPN swabs or the problem of obtaining enough saliva, particularly in older patients. But whenever possible, combined OPN and NP swabs should be considered. That also warrants re-evaluation of the standards of diagnostic testing as the Omicron variant continues to spread rapidly worldwide.

Immunization against Omicron

Various studies reported consistent findings about immunization efficacy against the Omicron subvariants. Initially, the two primary doses lead to mild-to-moderate protection. Booster doses have also been found to enhance protection substantially. However, vaccine effectiveness is rapidly waning over time [101]. Boosters can also provide higher levels of protection against severe disease, as evident by the increased effectiveness from 70-80% at the time of the second dose to more than 90% after the booster dose, resulting in lower rates of hospitalizations and death, highlighting the importance of raising awareness of the public about the benefits of booster doses [90, 91]. Compared to the Delta variant (from 89 to 80%), there is lower vaccine effectiveness against Omicron (from 36 to 1%) after two doses. However, booster doses showed similar efficacy against Omicron (95%) and Delta (99%) variants regarding severe outcomes [92]. Andrews et al. reported a similar reduction over time in vaccine effectiveness for the omicron variant for two BNT162b2 doses (from 65.5% to 15.4%) and two mRNA-1273 doses (from 75.1% to 14.9%) [91].

Recommendations and Future Directions

The escalating pandemic of COVID-19 has put healthcare in turmoil across the globe due to its geographical expansion and high contagiousness rates. On the same wavelength created a bow-wave of distress and apprehension among general masses. It is considered the brutal pandemic and human tragedy that swept across the borders.

The virus causing COVID-19 has the potential to change consistently. Since the beginning of the outbreak, several notable variants have been seen. Co-infection associated with various SARS-CoV-2 variants, especially Omicron subvariants, becomes conceivable when numerous variants circulate in the same region simultaneously, which could open the way for new variants to emerge by viral homologous recombination [93]. Although emerging variants are a normal part of virus evolution, monitoring each appears of prime importance to ensure countries are ready. That is particularly obvious in omicron variants, which are more aggressive, exceptionally transmissible, vaccine-resistant, equipped to cause more severe disease, or compared to the original strain of the virus.

This co-infection has extended the unsettling of SARS-CoV-2 acquiring new mutations even more swiftly. Tracking this perspective allows main stakeholders to track the surfacing of these new VOCs and know and respond to any changes in their transmission or vaccine efficacy. Unfortunately, it seems that nations unable to contain the spillover of COVID-19 and new

contagious variants, including Omicron, continue to emerge [94]. Therefore, we need a global public health system to attack spillover from wild animals, and WHO should consider the concept of OneHealth more than ever.

Policy and decision-makers should have learned the lesson, and now, they should recognize the cost of not preventing diseases. Passive and active surveillance of diseases is of paramount importance, especially for newly emerging infectious diseases. Campaigns and awareness are crucial for healthcare workers and populations at the individual level. Vaccination for the whole population and the continuous complementary work with the commitment to international health regulations to prevent the spread of epidemics globally. New limited outbreaks might be seeded at increasing immunization rates, and infections occur again. Thus, applying and concordance of public health preventive strategies could still be helpful.

Nevertheless, the infection risk could be controllable in the case of immunized individuals in places with a low spread of SARS-COV-2 variants or a low case ratio. Furthermore, COVID-19 symptoms may be similar to the common cold due to increased worldwide immunization over the long term, leading to the emerging period of seasonal coronaviruses, which is a sign that COVID-19 may likely become an endemic disease [95]. Nevertheless, the COVID-19 pandemic has not stopped yet, and certain European countries are presenting new peaks and waves as of October 2022 (Figure 4).

Vaccine accessibility should be ensured along with tackling the hesitancy challenges. Reinfection in vaccinated individuals is likely to occur, and thus, vaccination and receiving a booster shot is the best protection against Omicron, as proposed by CDC. Similarly, CDC put forward "layered prevention strategies" for both the groups vaccinated and the non-vaccinated. Experts in the field also advised masking, social distancing, and other mitigation strategies.

Researchers should focus on environmental drivers that lead to zoonosis and the social behaviours associated with the spreading of these diseases. Environmental drivers such as climate change and air pollution complicate the situation, as we have seen during the COVID-19 pandemic [96]. However, it is unclear how climate change impacts the evolution of the viruses, how they play a role in the spread of such viruses from animals to humans, and to which extent climate drivers assist in the mutations of viruses such as Omicron. As a result, researchers need more data from several cases and information at the molecular level, what is happening at the genetic level, and how other factors facilitate this transformation. Moreover, researchers should

look for resources that help them track epidemics rapidly, such as social media platforms and website information, to follow and track diseases and develop guidelines. Another critical point is the use of well-structured methods with the help of machine learning and natural language processing [97].

The concept of one health should be generalized [98]. The focus of this concept needs an interdisciplinary research team from fields such as veterinary medicine, public health, ecology, and environment, together with epidemiologists to further understand what kind of practices lead to this rapid spread of viruses and how can public health professionals intervene to stop this spread. Furthermore, the concept of one health and climate change is essential to study the drivers of the propagation of diseases [99]. Finally, factors such as the mobility of humans and the exotic trade of wild animals should be considered to have a complete picture of all the interactions and drivers for the spread of zoonotic diseases [100].

Even though the role of public health professionals is significant in prevention, healthcare providers and clinicians should increase their preparedness and update their knowledge about new viruses and their different mutations. They should also spread health knowledge in their surroundings and to their patients. Furthermore, infection control protocols and guidelines and dealing with any patient could be a suspected case.

Finally, improving the diagnostic ability among healthcare providers and supporting them with the necessary tools can remedy diagnostic errors. Increased monitoring and sequencing activities are required to understand better the current variants of SARS-CoV-2, especially Omicron. Field investigations such as household transmission studies, contact follow-up, and laboratory evaluations when capacity exists should be performed to deepen comprehension of Omicron's features. Because S-gene Target Failure (SGTF) from a commonly used PCR test (ThermoFisher® TaqPath®) is suggested for Omicron, it can be utilized as a marker for this variant, potentially leading to more effective Omicron identification. It should have been noted that specific sequences do not have this deletion. As a result, SGTF can be utilized as a valuable Omicron proxy marker for surveillance purposes. However, because this loss can also be seen in other a variant of concern, sequencing should be used to confirm it (*e.g.*, Alpha and subsets of Gamma and Delta). That can be achieved through workshops to teach them about the clinical presentations of the diseases and to avoid any possible diagnostic biases that might occur. The proper use of diagnostic tools to take specimens with caution is also very crucial.

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RAF, TPU, AAE, AYB, KA, IA, TA, and AJRM: acquired information, drafted the article, designed the figures, and approved the final version. RAF, AA, BA, and AJRM: the conception and design of the study and final approval of the version to be submitted. RAF, AA, BA, RS, and AJRM: interpretation of data and revising it critically for important intellectual content. All the authors gave final approval of the version to be submitted.

DECLARATION OF COMPETING INTEREST

Authors have no conflict of interest, except AJ Rodriguez-Morales, speaker/consultant for Amgen, AstraZeneca, and Valneva, concerning COVID-19 vaccines.

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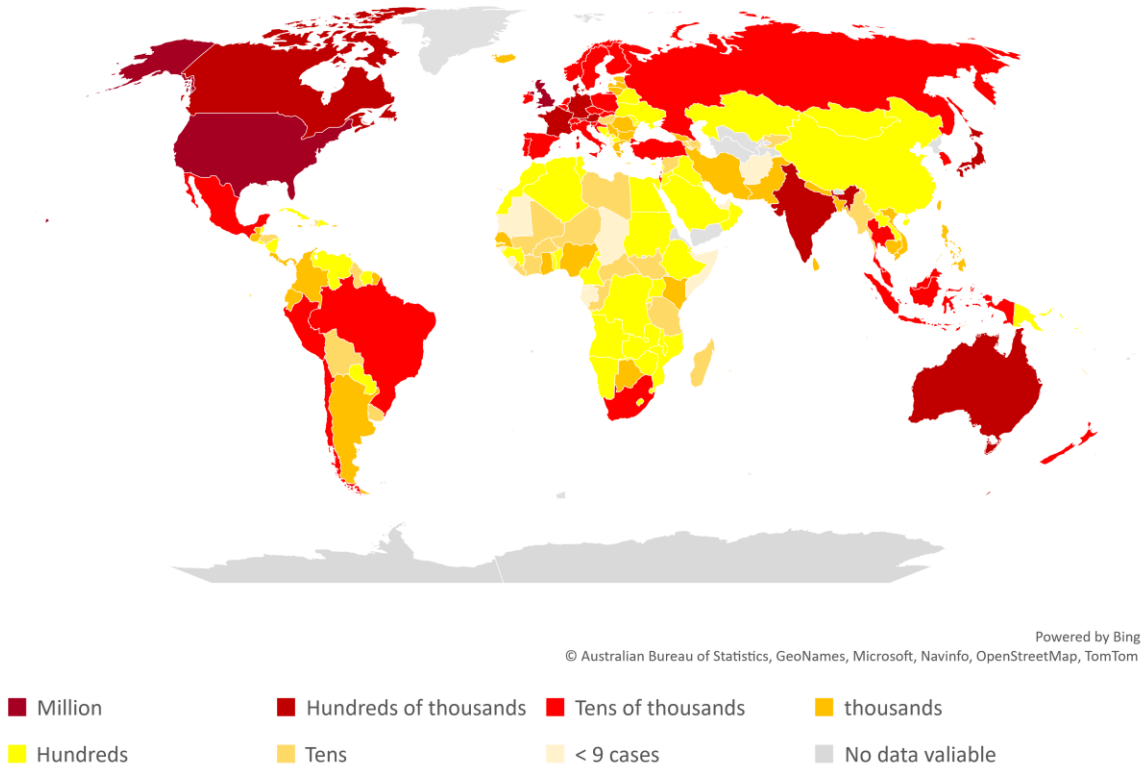

Figure 1 - The number of recent omicron variant cases globally as of October 19, 2022. This original figure was developed based on data available on the GISAID initiative [8].

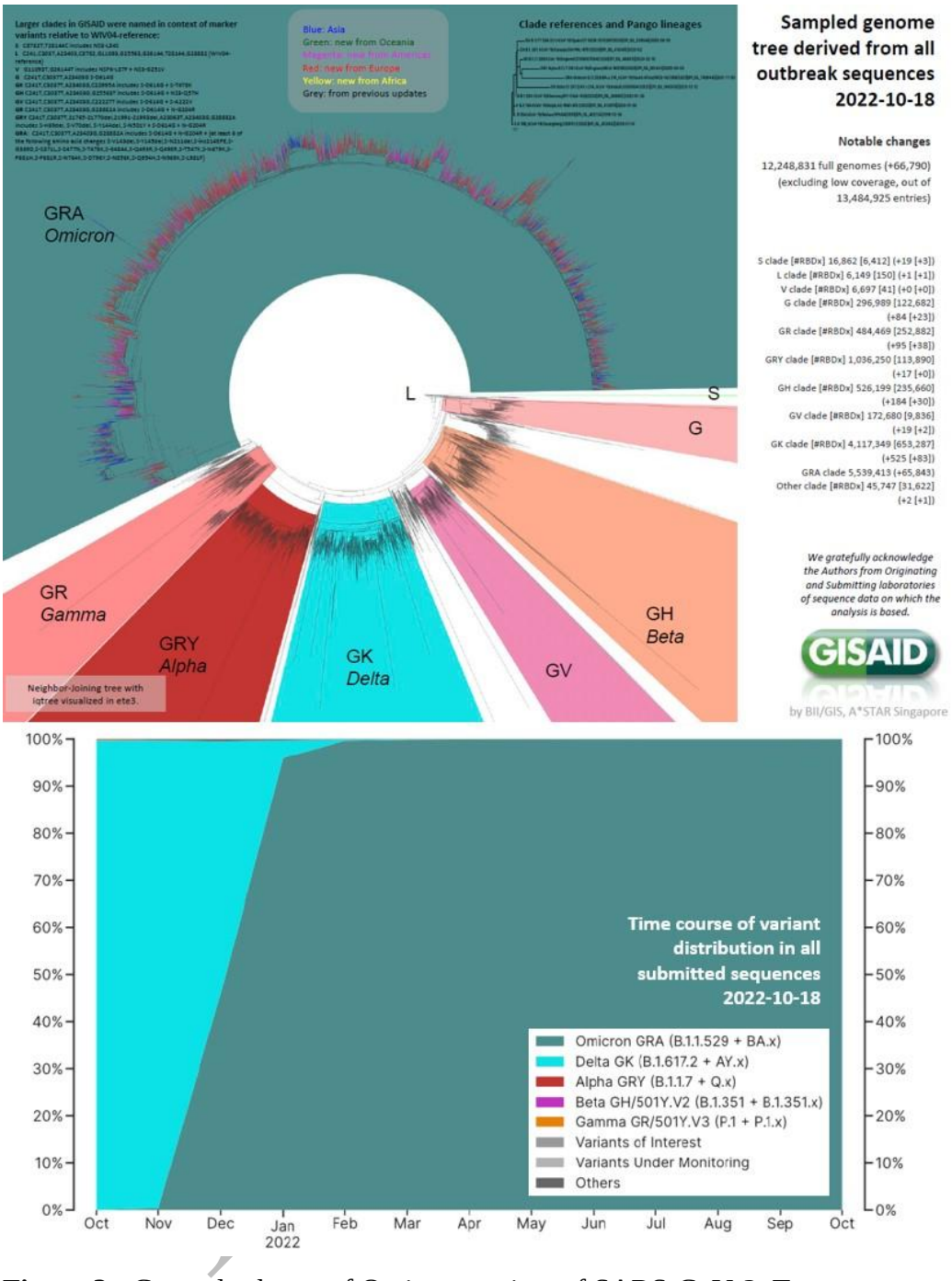

Figure 2 - General scheme of Omicron variant of SARS-CoV-2. Twenty-one common mutations

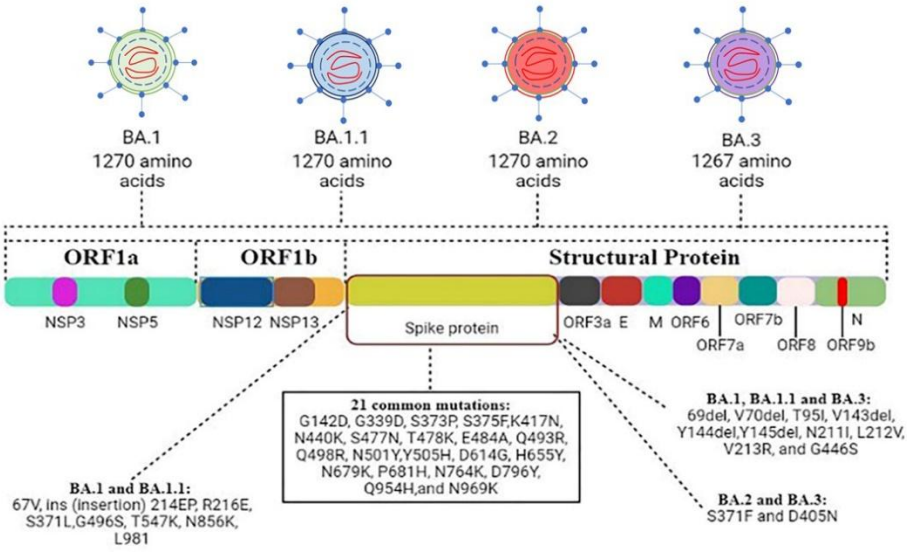

Figure 3 - General scheme of Omicron variant of SARS-CoV-2. Twenty-one common mutations are found in the spike protein shared between each subvariant.

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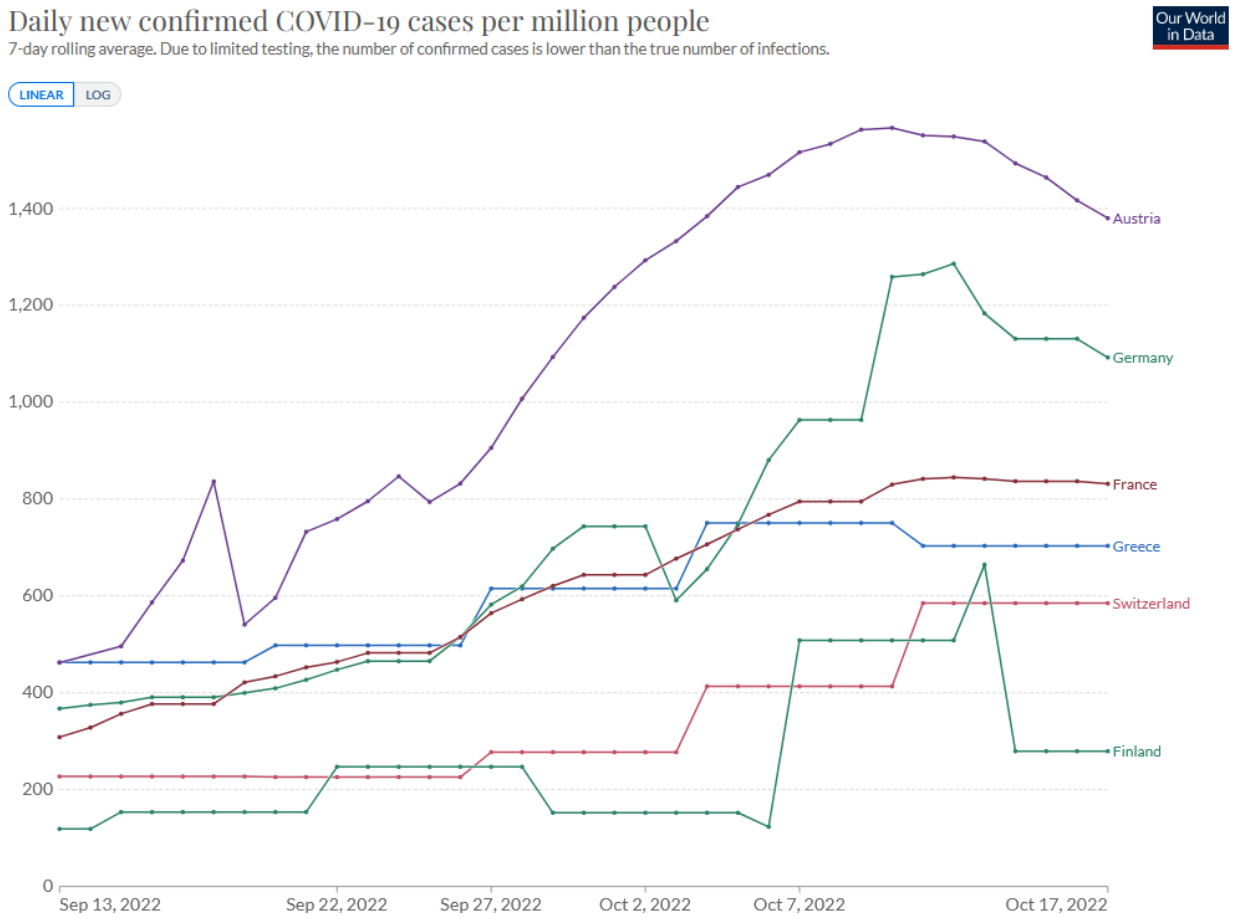

738

739 **Figure 4 -** COVID-19 cases between September 13 and October 17, 2022, in selected countries

740 of Europe.

741