

The Omicron-transformer: Rise of the subvariants in the age of vaccines

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ABSTRACT

Introduction: Omicron is the latest SARS-CoV-2 variant of concern, the pathogen that causes COVID-19. Since its emergence in late 2021, Omicron has displaced other circulating variants and caused successive waves of infection worldwide throughout 2022. Omicron is characterised by the rapid emergence of many subvariants and high rates of infection in people with vaccine- and/or infection-induced immunity. This review article will consolidate current knowledge regarding Omicron subvariants, the role of boosters, and future vaccine development.

Method: This narrative review is based on a literature search using PubMed. Search terms related to Omicron were used and priority was given to published peer-reviewed articles over pre-prints.

Results: Studies indicate that vaccinations and boosters are important to reduce disease severity, hospitalisation, and death from Omicron. A variety of factors, such as differing host factors, circulating variants, and forces of infection, can influence the benefit of repeated booster administration. Next-generation bivalent vaccines have now been approved in some countries including Singapore and have demonstrated the ability to induce broad variant protection. Future third-generation vaccines involving mucosal vaccines and/or pan-sarbecovirus vaccines may provide broader and longer-lasting protection.

Conclusion: Due to current high levels of vaccine- and infection-induced immunity, it is likely that rates of severe illness, hospitalisation, and death due to Omicron will continue to moderate. Nevertheless, the virus is ever-changing, and public health policies, especially those related to vaccinations, will also have to continually evolve and adapt as COVID-19 transitions to endemicity.

Ann Acad Med Singap 2022;51:712-29

Keywords: Booster, COVID-19, infectious diseases, Omicron, vaccine

INTRODUCTION

The emergence and evolution of SARS-CoV-2 have been publicly tracked in unprecedented detail through a combination of intensive genomic sequencing and open-access sharing of data.¹ This surveillance information describes how waves of COVID-19 infections have been driven by the emergence of new variants of concern (VOCs) and their subvariants. While diverse genotypes are a characteristic of RNA viruses, VOCs are defined as genetic variants with evidence of phenotypic differences—either an impact on diagnostics, treatment or vaccines, or evidence of increased transmissibility or disease severity.²

Omicron (Pango lineage B.1.1.529), the most recent VOC, was first detected in South Africa and Botswana

in November 2021, where it was associated with rapidly increasing case numbers.³ Compared to previously circulating VOCs, Omicron has an unusually large number of mutations, especially in the important spike protein, with changes in 32 amino acid residues (Fig. 1).⁴ As Omicron successfully spread across the globe to become the dominant variant, it also acquired additional mutations and formed different subvariants (Fig. 2), each with its own epidemiological, clinical, and viral characteristics.

This review article aims to provide a succinct summary of the current knowledge regarding Omicron and its subvariants, including epidemiology, immune evasion, vaccine effectiveness/efficacy (mainly mRNA vaccines), and future vaccine development. In so doing, we will

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CLINICAL IMPACT

What is New

- COVID-19 vaccination is important to minimise symptoms of Omicron and reduce the probability of severe illness, hospitalisation, and death, especially for the older adults.
- Boosters offer a short-term boost for protection against Omicron infection but more importantly, their protective effect against severe outcomes declines compared to primary series.
- Evidence of benefit from repeated boosters is strongest in people with weaker immunity such as older adults.
- Bivalent and multivalent COVID-19 vaccines show great promise to induce pan-variant protection.

Clinical Implications

- This review can help guide clinicians and policymakers in deciding which patients should be prioritised for booster vaccination.

consider the role of first and second vaccine booster doses, and suggest clinical guidance on their administration, based on the latest available data.

METHOD

This narrative review is based on a literature search using PubMed. Search terms used were “Omicron epidemiology”, “Omicron emergence”, “Omicron booster”, “Omicron third dose”, “Omicron fourth dose”, “Omicron targeting vaccine”, “Omicron bivalent vaccine”, and “Omicron multivalent vaccine”. Priority was given to published peer-reviewed articles over pre-prints.

RESULTS

Epidemiology of Omicron and subvariants

After its detection in November 2021,^{5,6} Omicron rapidly replaced Delta as the dominant circulating variant worldwide.^{5,7} The first Omicron outbreaks were by subvariant BA.1, which in various countries peaked around December 2021 to February 2022.⁷⁻⁹ By March 2022, the BA.2 subvariant had displaced BA.1,^{10,11} and within different regions, divergent sublineages such as BA2.1.1 and BA2.12.1 established themselves in France and the US, respectively.^{12,13}

In April 2022, hybrid recombinant variants were detected. There were concerns these could become the

next dominant variants and combine the severity of Delta infection with the transmissibility of Omicron.¹⁴⁻¹⁶ However, that did not come to pass; by June 2022, new BA.4 and BA.5 subvariants had emerged and became increasingly prevalent.¹⁷ BA.5 is presently the dominant subvariant worldwide.¹⁷ More recently, BA.2.75.2 and BA.4.6 have emerged and there are concerns of a new wave of infections due to their immune evasion capability (Figs. 1 and 2).^{18,19} Also recently in Singapore, there has been a sharp uptick in new cases due to the Omicron hybrid subvariant XBB (Fig. 3). Such rapid emergence and establishment of different Omicron subvariants complicate the development of variant-specific vaccines, as vaccine candidates may be outdated by the time they enter clinical trials, let alone clinical use.

Pathological characteristics of Omicron compared to Delta

There is currently good evidence that Omicron causes significantly less severe disease than Delta. For example, in a study involving patients admitted from Paris emergency departments, Omicron was independently associated with better hospital outcomes compared to Delta, with a decrease in intensive care unit (ICU) admission by 11.4%, mechanical ventilation by 3.6%, and mortality by 4.2% (differences were adjusted for the number of vaccine doses).²⁰ Overall, the rates of severe disease with Omicron among vaccinated individuals are comparable to seasonal influenza, though the public health impact has remained significant due to the extraordinarily high number of cases. The lower virulence has been partially attributed to changes in virus receptor binding that reduced Omicron's efficiency at infecting cells in the lungs and gut, but not the upper airways.²¹

The higher number of Omicron cases can be attributed to the higher transmissibility and infectivity compared to previous variants, with an effective reproduction number of 4.20 (BA.1/2), which is triple that of Delta.²² Viral factors such as increased angiotensin-converting enzyme-2 receptor affinity might contribute to this increased transmissibility.²¹ Furthermore, Omicron is effective at evading host immunity and can infect vaccinated individuals (vaccine escape) or recovered individuals infected with non-Omicron variant (re-infection).^{3,23} As the average healthcare burden per infected individual is reduced, many countries in the midst of re-opening borders and stepping down public health measures continued to do so.²⁴ These policy shifts also facilitated rapid transmission of the Omicron subvariants.

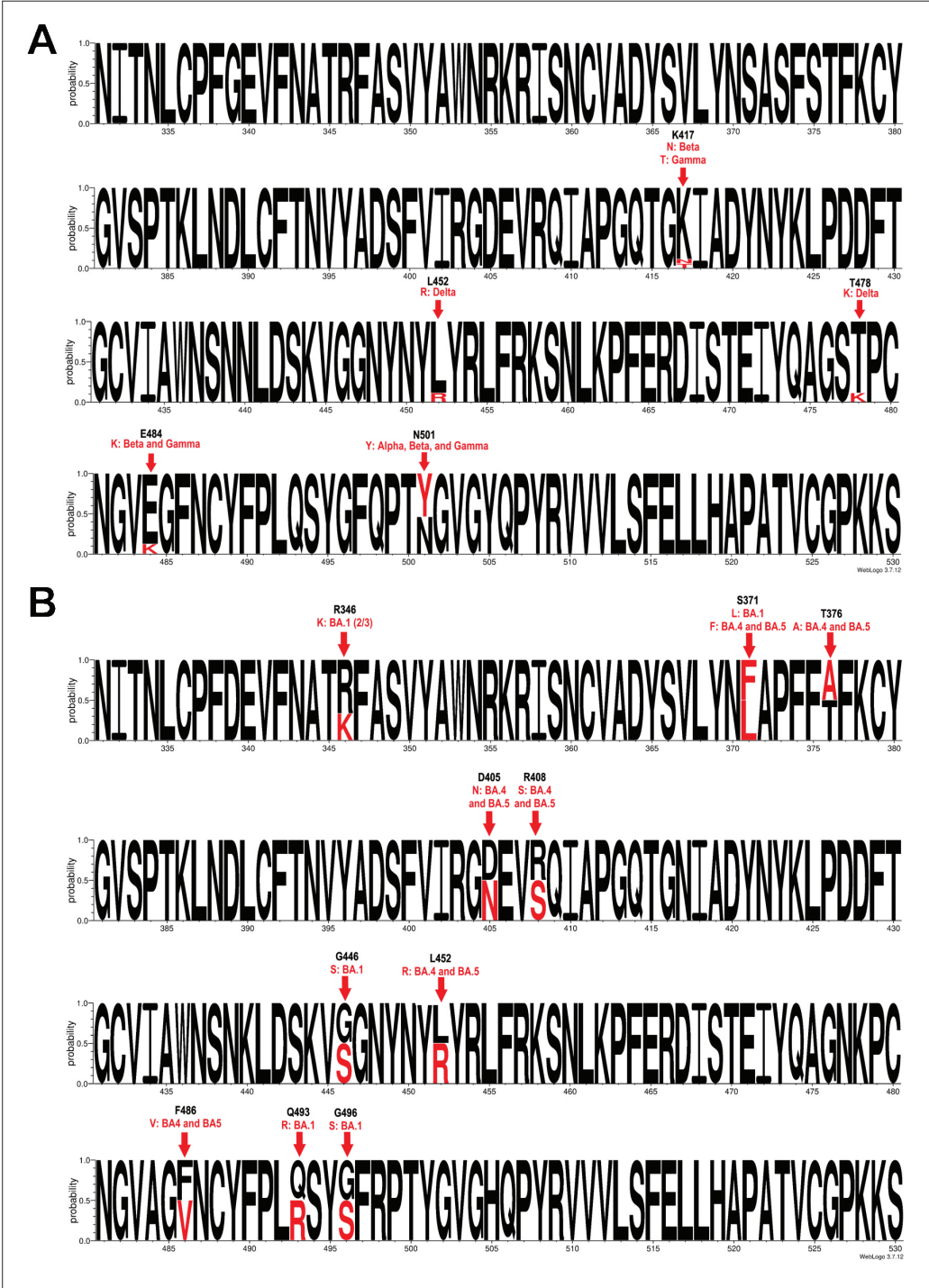


Fig. 1. Variation in receptor-binding domain of COVID-19 spike glycoprotein. Receptor-binding domains (residues 331 to 524) of COVID-19 spike glycoprotein sequences from the National Center for Biotechnology Information, US data package (accessed 4 August 2022) were separated into non-Omicron (A) and Omicron (B) for analysis (extracted by searching for the variant name and Pango lineage). Sequences without a complete receptor-binding domain were excluded from the analysis. Black letters represent original wildtype sequence while red letters represent mutations. (A) Inter-variant variation in receptor-binding domain across wildtype, Alpha, Beta, Gamma and Delta are highlighted in red (total positions with inter-variant variation: 5). (B) Intra-variant variation in receptor-binding domain across Omicron subvariants are highlighted in red (total positions with intra-variant variation: 10). In total, 31 wildtype, 37 Alpha, 5 Beta, 6 Gamma and 10 Delta sequences were analysed for non-Omicron (total 89) while 3 BA.1, 1 BA.4 and 2 BA.5 sequences were analysed for Omicron (total 6). Y-axis represents the percentage of sequences analysed that possessed a particular amino acid residue at that position. There is greater intra-variant variation in the receptor-binding domain of the spike glycoprotein of Omicron than inter-variant variation in non-Omicron variants despite analysing far fewer Omicron sequences than non-Omicron.

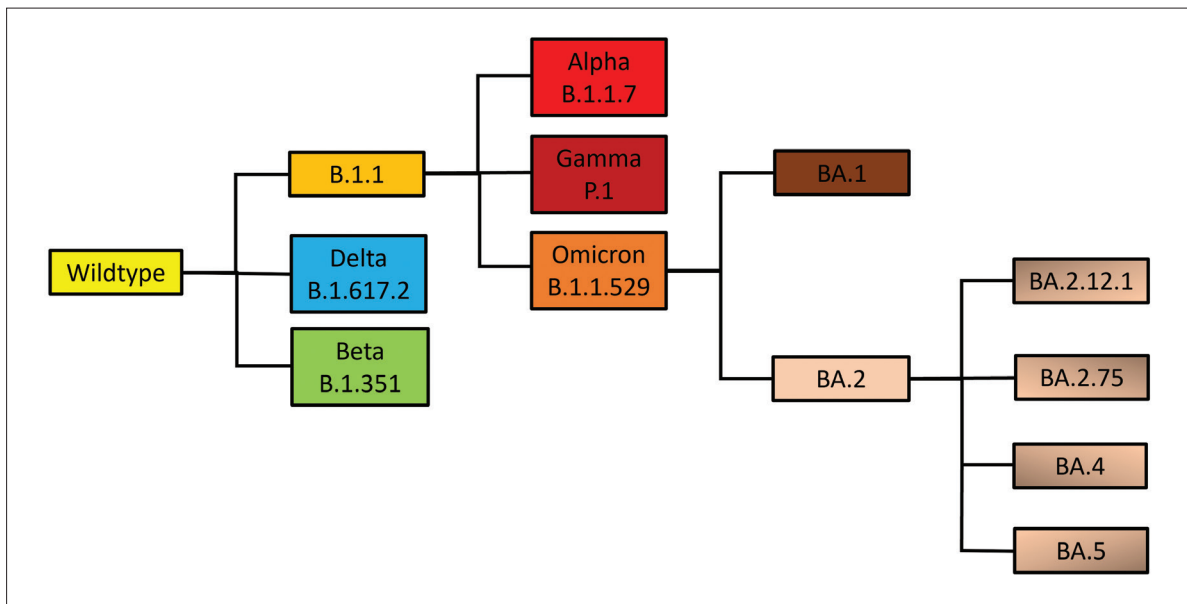


Fig. 2. Phylogeny of the spike glycoprotein of COVID-19 variants of concern. How the various COVID-19 variants of concern are related to each other based on data from NextStrain.

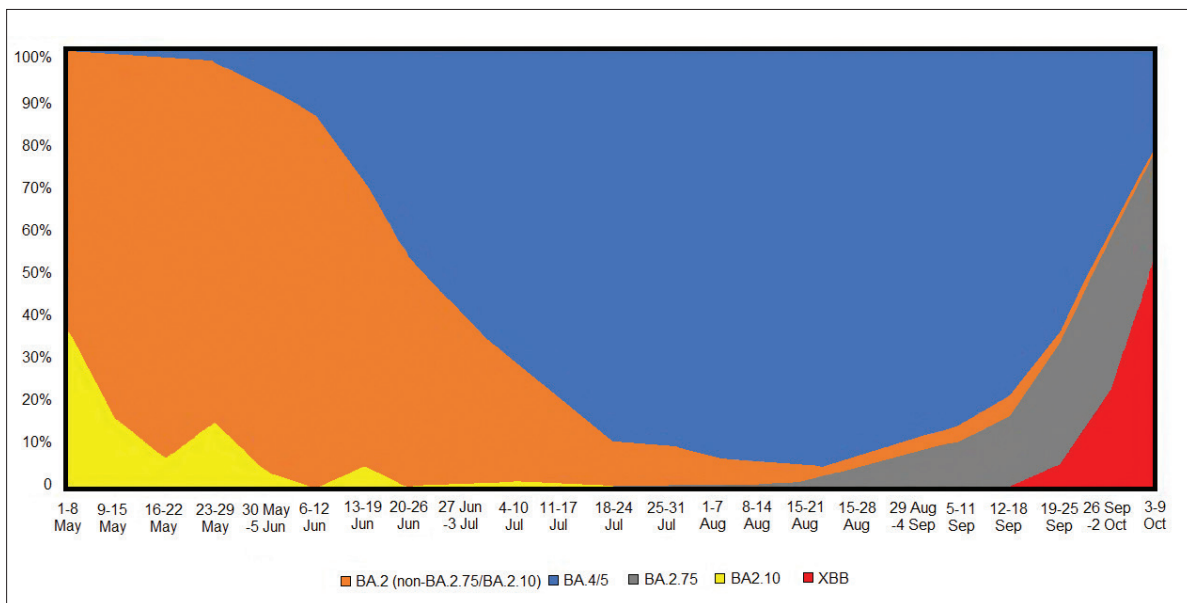


Fig. 3. Relative prevalence of Omicron subvariants in Singapore from May to October 2022.

Source: Ministry of Health, Singapore. Update on COVID-19 situation and measures to protect healthcare capacity, Annex, updated 15 October 2022. <https://www.moh.gov.sg/docs/librariesprovider5/default-document-library/annexad79528af5784a1b8c95c986c82e3131.pdf>. Accessed 24 November 2022.

Immune evasion potential of more recent Omicron subvariants

Current epidemiological evidence indicates that BA.4 and BA.5 exhibit higher transmissibility compared to BA.1 and BA.2.^{6,8,25} This may be due to BA.4 and BA.5 evading neutralising antibodies induced by BA.1 infection, resulting in an increased risk of re-infection.²⁶⁻²⁸ Vaccinated persons with previous SARS-CoV-1 infection (the causative agent of the 2002–2004

severe acute respiratory syndrome epidemic) possess protective antibodies against BA.1, but these same antibodies conferred markedly reduced protection against BA.2.12.1, BA.4 and BA.5.^{26,27} BA.2.12.1, BA.4 and BA.5 also display stronger neutralisation evasion of 3-dose vaccination regimens than BA.1 and BA.2.^{26,27} Similar to how the initial Omicron subvariants (BA.1 and BA.2) had increased immune escape compared to Delta, the new Omicron subvariants have superseded

their predecessors. Given the increase in vaccination and infection rates over time and thus increase in positive selection pressure, it is unsurprising that new “successful” COVID-19 variants have stronger immune escape ability.

Benefits of primary mRNA vaccine series in the context of Omicron immune evasion

Despite the lower severity of Omicron infection and its propensity to evade vaccine- and infection-induced immunity, vaccination remains a cornerstone of the COVID-19 public health response. For unvaccinated individuals with infection-derived immunity, cellular immune responses are comparable to vaccinated individuals, even though humoral immunity is lower.²⁹⁻³¹ This robust T-cell response may provide protection against severe illness in the event of re-infection. The breadth of the immune response in unvaccinated individuals infected with Omicron BA.1 is however narrower than in vaccinated individuals, particularly against non-Omicron variants.³² Unvaccinated BA.1 convalescent individuals, compared to vaccinated individuals, also demonstrated a greater decrease in neutralisation against BA.4 and BA.5 (both of which are capable of evading BA.1 induced immunity).²⁸ As such, vaccination of Omicron-convalescent individuals remains beneficial.

Vaccinations are also important to minimise symptoms and reduce the risk of severe disease, critical illness and death, especially for older adults.^{33,34} Long-term symptoms of COVID-19 (long COVID) are also a major public health concern. A UK study found that vaccination sharply reduces the probability of long COVID even after 6 months post-second dose (0.24 to 0.5 time as likely to get long COVID)³⁵ in spite of a likely significant antibody titre decay.³⁶⁻³⁹

Decay in circulating antibody titres after vaccination or infection is inevitable. However, it is important to consider other facets of the immune system. Firstly, cellular immunity is likely to be important for protection against severe disease (but is more complex to measure than antibody levels). Secondly, mucosal immune response following infection may offer additional protection against re-infection but may not correlate with systemic measures of immunity.⁴⁰⁻⁴² Finally, both humoral and cellular immune memory are likely to be long-lasting. Studies have demonstrated that the 2-dose primary series of mRNA vaccines continue to offer >70% protection against hospitalisation, severe illness and death even if the last dose was administered more than 6 months prior to breakthrough

infection.^{43,44} Protection against severe disease lasts significantly longer and decays at a slower rate compared to protection against infection.^{36,45,46} Collectively, completing the primary vaccination series provides significant benefits despite Omicron’s lower mortality and higher immune evasion ability.

Role of the first booster

Repeated antigen exposures through vaccination and infection result in immune response maturation. Seven months after the second dose, Omicron variant neutralising antibodies were only detected at significant levels in 55% of patients, and were markedly lower among older adults and men.^{37,39} The first booster after a 2-dose primary mRNA series significantly boosts circulating antibody levels, and induces a more effective response against Omicron, when compared to no booster.^{37,47-51} In a clinical trial of mRNA boosters, Omicron neutralisation (measured by a multiplex surrogate virus neutralisation test) increased from 26.2% to 82.5% 28 days after homologous boosting (28.6% to 84.2% for heterologous boosting),³⁹ even though neutralisation activity elicited against Omicron is still comparatively lower than that against the ancestral and Delta variants.^{48,52-54} Whether boosting with a heterologous vaccine to the primary series offers significant additional benefits remains unclear.^{38,39,49,50}

The mRNA vaccines are effective at inducing cellular immunity, and mRNA boosters significantly increase the number of virus-specific memory B- and T-cells.³⁸ This increase in both humoral and cellular immunity by the first booster may account for the 90% lower mortality rate in people who received the booster.⁵⁵ As such, the evidence for the benefits of the first booster is highly compelling for all individuals.

Evidence for the second booster

The role of second boosters on the other hand is more complicated. An in vitro study showed that the second mRNA booster dose induces broadly neutralising antibodies (bNAbs) that are effective against BA.1, BA.1.1, BA.2 and BA.3 Omicron subvariants.⁵⁶ A UK clinical trial, COV-BOOST, demonstrated similar findings; the second mRNA booster significantly increases both cellular and humoral immunity to a level that is equal to or exceeds the first mRNA booster and is well-tolerated.⁵⁷ However, there was also evidence of limited additional boost from the second booster when pre-booster immunity is already high. This was particularly evident among individuals with prior infection. Crucially, this is not “immune exhaustion”

(where pre-existing low immunity cannot be increased due to prior vaccinations) but rather an “immune ceiling” (a maximum limit to the extent humoral and cellular immunity can be boosted). Such ceiling effects have also been observed in influenza wherein subjects with high pre-vaccination antibody titres do not experience significant increase in protection.⁵⁸ This also has to be distinguished from immune tolerance where there is modulation in the immune response to minimise damage to the host and is also observed for influenza vaccines.^{59–61} Since significant boosting can still be induced in subjects with low levels of protection and no significant improvement is seen when pre-booster immunity is already high, this is more likely to be due to the ceiling effect.⁵⁷ Therefore, repeated boosters can offer significant improvement in protection if timed when existing immunity has waned.

A recent observational study in Israel showed that while a second mRNA booster provided significant increased protection against infection by the Omicron variant, this protection peaked at fourth week post-vaccination and waned thereafter.^{45,62} Protection against severe illness however, is expected to wane more slowly, similar to previous studies on the first mRNA booster.⁴⁵

Another recently published study showed that a second booster dose of BNT162b2 vaccine offers strong protection against hospitalisation (64%) and deaths (72%),⁶³ and a mild-to-moderate protection (34%) against infection (Table 1). While this vaccine effectiveness is apparently lower than previous reported effectiveness for the primary vaccines series (e.g. 90% for BNT162b2 against hospitalisation or death),⁶⁴ change in comparator groups should be recognised, where current comparator groups likely have background immunity from prior vaccination or infection and are not immunologically naive. In addition, with the extremely high community prevalence of COVID-19 during Omicron waves, a substantial proportion of infected individuals are hospitalised with Omicron infection rather than because of it (“incidental COVID”). Finally, estimates of the effectiveness of boosters have to date relied on observational studies, rather than randomised clinical trials powered to determine vaccine efficacy. Therefore, one must be cautious when comparing vaccine efficacy/effectiveness across different studies. It would also be prudent to differentiate laboratory endpoints, such as neutralisation titres, from clinical outcomes that are arguably more meaningful.

Repeated boosters however may incur socioeconomic costs that need to be weighed against potential benefits

when deciding the frequency of boosters. For young and healthy individuals, the benefits of a second booster may be marginal as demonstrated by a healthcare worker study in Israel where vaccine effectiveness against symptomatic diseases was only 31–43%.⁶⁵

For older adults, vaccination and boosters consistently provide significant protection.^{33,34} Data from the US Centers for Disease Control and Prevention currently indicate that in June 2022, a second booster for people over 50 years old decreases risk of COVID-19 mortality by a factor of 3 compared to those with a single booster.⁶⁶ This is corroborated by a study from Arbel et al., demonstrating a substantial reduction in hospitalisation and deaths among people aged 60 and older who have received 2 boosters compared to those who have only received one.⁶⁷ One possible reason why older adults gain more from repeated boosters is the “immune ceiling” effect as previously discussed, coupled with the observed trend that protection in older adults tends to wane faster.⁶⁸ As such, the evidence for second boosters for older adults is stronger compared to the younger population.

Overall, current evidence for vaccination including boosters of the vulnerable population is strong. Nonetheless, any public health policy on vaccination should factor in predicted levels of community cases, given that such protection wanes over time, and monitor closely changes in pathogenicity and virulence of dominant circulating strains. Given the lower virulence of circulating Omicron subvariants, mandatory repeated vaccinations of non-vulnerable populations are not supported by currently available evidence.

Omicron-targeting vaccine strategies

Much work is currently being conducted to develop an Omicron-targeting vaccine. A booster matched to Omicron spike protein successfully boosted rhesus macaques after completion of the 2-dose primary vaccine regimen (Table 2).⁶⁹ However, other Omicron-targeted vaccine strategies have been less successful; these vaccines induced neutralising antibodies in mice specific to Omicron but few-to-none against other variants.^{70–73} This corresponds with another study of unvaccinated BA.1 convalescent individuals, wherein the neutralising antibodies demonstrated lower cross-reactivity against non-Omicron variants and Omicron BA.2, when compared to vaccinated individuals.³² Interestingly, vaccines targeting Delta-induced neutralising antibodies were active against all variants tested in mice^{70,71} (Table 2). Collectively, these studies suggest Omicron-targeting vaccine strategies may be challenging.

Table 1. Representative comparison of vaccine effectiveness against SARS-CoV-2 infection across different studies during period of predominant Omicron infection

Vaccine dosage	Comparator group	Population	Number of weeks since last dose	Reported vaccine effectiveness (%)	Reference
BNT162b2 (Primary)	COVID-negative	Adult (27 Nov 2021 to 12 Jan 2022)	2–4	65.5	68
			>25	8.8	
BNT162b2 (First booster, primary: ChAdOx1 nCoV-19)			2–4	62.4	
			>10	39.6	
mRNA-1273 (First booster, primary: ChAdOx1 nCoV-19)			2–4	70.1	
			5–9	60.9	
mRNA-1273 (First booster, primary: ChAdOx1 nCoV-19)			2–4	73.9	
			5–9	64.4	
BNT162b2 (Second booster)	BNT162b2 (First booster)	Long-term care facility resident (10 January 2022 to 31 March 2022)	>1	34	63

Superscript reference numbers: Refer to REFERENCES

Bivalent and multivalent vaccines

There has been modest success in the development of pan-variant vaccines and these target antigens from multiple SARS-CoV-2 variants (Table 3). For example, a trivalent vaccine developed by combining the Sinopharm HB02 antigen, and Delta- and Omicron-targeting antigens, could induce bNABs in mice against all variants tested including HB02, Beta, Delta and Omicron.⁷⁴ Likewise, another trivalent vaccine targeting ancestral, Beta and Delta spike proteins induced the production of bNABs in mice against all COVID-19 variants tested.⁷⁵ The vaccine candidates most advanced in the development pathway are from Moderna and Pfizer-BioNTech.

As part of the ongoing COVE clinical trial (NCT04927065), Moderna has been developing bivalent vaccines targeting various COVID-19 variants. Their bivalent vaccine combining the currently approved mRNA-1273 vaccine antigen with an Omicron BA.1-targeting antigen induced high titres of bNABs against all COVID-19 variants and Omicron subvariants tested

(BA.4/BA.5).⁷⁶ Interestingly, their bivalent vaccine utilising Beta-targeting antigen instead of Omicron also showed similar effectiveness.⁷⁷ Both vaccines have similar safety profiles as the currently approved Moderna vaccine.^{76,77}

Pfizer/BioNTech has also been able to develop their version of a bivalent vaccine that targets the ancestral variant and BA.4/BA.5;⁷⁸ it was reported to successfully neutralise all strains including various Omicron subvariants (BA.1, BA.2, BA.2.12.1, and BA.4/5). Another bivalent vaccine targeting the ancestral strain and BA.1, administered as a 2-dose primary series in mice, also successfully neutralised all strains tested. However, when used as a first booster, the neutralisation activity was reduced against BA.4 and BA.5. Given their success in animal models, it is unsurprising that both Moderna and Pfizer's bivalent vaccines have been approved in some countries under emergency use. As more countries around the world authorise the use of these next-generation vaccines, it is hoped that subsequent broader protection can reduce the pool

Table 2. Summary of neutralisation assay results of COVID-19 variant-targeting vaccine studies

Vaccine target	Vaccine regimen	Population	Test target	Result*	Reference
mRNA-1273	2-dose primary series taken 4 weeks apart	Rhesus macaques	Ancestral	Neutralising	69
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
Beta			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
BA.1			Ancestral	Neutralising, lower than BA.1 test target	
			Beta	Neutralising, lower than BA.1 test target	
			Delta	Neutralising, lower than BA.1 test target	
			BA.1	Neutralising	
mRNA-1273	First booster taken 35 weeks since last dose of 2-dose primary series mRNA-1273		Ancestral	Successfully boosted	
			Beta	Successfully boosted	
			Delta	Successfully boosted	
			BA.1	Successfully boosted	
			BA.2	Successfully boosted	
BA.1			Ancestral	Successfully boosted	
			Beta	Successfully boosted	
			Delta	Successfully boosted	
			BA.1	Successfully boosted	
			BA.2	Successfully boosted	
Ancestral	2-dose primary series taken 2 weeks apart	Mice	Ancestral	Neutralising	70
			Beta	Neutralising	
			Delta	Neutralising	
			Omicron	Low neutralisation	
Delta			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			Omicron	Neutralising	
Omicron			Ancestral	Failed to neutralise	
			Beta	Failed to neutralise	
			Delta	Failed to neutralise	
			Omicron	Neutralising	
Delta-Omicron hybrid			Ancestral	Low neutralisation	

Table 2. Summary of neutralisation assay results of COVID-19 variant-targeting vaccine studies (Cont'd)

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
			Beta	Low neutralisation	
			Delta	Low neutralisation	
			Omicron	Neutralising	
Ancestral	2-dose primary series taken 2 weeks apart	Mice	Ancestral	Neutralising	71
			Alpha	Neutralising	
			Beta	Neutralising	
			Omicron	Failed to neutralise	
Beta			Ancestral	Neutralising	
			Alpha	Neutralising	
			Beta	Neutralising	
			Omicron	Failed to neutralise	
Delta			Ancestral	Neutralising	
			Alpha	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			Omicron	Neutralising	
Omicron			Ancestral	Failed to neutralise	
			Delta	Failed to neutralise	
			Omicron	Neutralising	
Beta	First booster taken 7 weeks after last dose of 2-dose primary series targeting Delta variant		Delta	Failed to boost	
			Omicron	Failed to boost	
Delta			Delta	Successfully boosted	
			Omicron	Successfully boosted	
Omicron			Delta	Failed to boost	
			Omicron	Failed to boost	
Ancestral	2-dose primary series taken 2 weeks apart	Mice	Ancestral	Neutralising	72
			Delta	Neutralising	
			Omicron	Low neutralisation	
Omicron			Ancestral	Failed to neutralise	
			Delta	Failed to neutralise	
			Omicron	Neutralising	
Ancestral	2-dose primary series taken 2 weeks apart	Mice	Ancestral	Neutralising	73
			Alpha	Neutralising	
			Beta	Neutralising	

Table 2. Summary of neutralisation assay results of COVID-19 variant-targeting vaccine studies (Cont'd)

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
BA.1			Gamma	Neutralising	
			Delta	Neutralising	
			BA.1	Low neutralisation	
			Ancestral	Failed to neutralise	
			Alpha	Failed to neutralise	
			Beta	Low neutralisation	
Ancestral	First booster taken 2 weeks after last dose of 2-dose primary series targeting ancestral variant		Gamma	Low neutralisation	
			Delta	Failed to neutralise	
			BA.1	Neutralising	
			Ancestral	Neutralising	
			Alpha	Neutralising	
			Beta	Neutralising	
BA.1			Gamma	Neutralising	
			Delta	Neutralising	
			BA.1	Low neutralisation	
			BA.2	Low neutralisation	
			Ancestral	Neutralising	
			Alpha	Neutralising	
BA.1	First booster taken 2 weeks after last dose of 2-dose primary series targeting BA.1 subvariant		Beta	Neutralising	
			Gamma	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
			BA.2	Neutralising	
			Ancestral	Failed to neutralise	
Ancestral	2-dose primary series taken 3 weeks apart	Mice	Alpha	Neutralising	78
			Beta	Neutralising	
			Gamma	Neutralising	
			Delta	Low neutralisation	
			BA.1	Neutralising	
			BA.2	Neutralising	
Ancestral			Delta	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	

Table 2. Summary of neutralisation assay results of COVID-19 variant-targeting vaccine studies (Cont'd)

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
BA.1			BA.1	Low neutralisation	
			BA.4/5	Low neutralisation	
			Ancestral	Low neutralisation	
			Beta	Low neutralisation	
			Delta	Low neutralisation	
Ancestral	First booster taken 104 days since 2-dose primary BNT162b2		BA.1	Neutralising	
			BA.4/5	Neutralising	
			Ancestral	Successfully boosted	
			BA.1	Lower boosting	
			BA.2	Lower boosting	
BA.1			BA.2.12.1	Lower boosting	
			BA.4/5	Lower boosting	
			Ancestral	Successfully boosted	
			BA.1	Successfully boosted	
			BA.2	Lower boosting	
BA.4/BA.5			BA.2.12.1	Lower boosting	
			BA.4/5	Lower boosting	
			Ancestral	Successfully boosted	
			BA.1	Successfully boosted	
			BA.2	Successfully boosted	
			BA.2.12.1	Successfully boosted	
			BA.4/5	Successfully boosted	

^a Neutralisation levels refer to ID₅₀ values against target variant. Boosting levels mean neutralisation levels to target variant were compared before and after boosting.

Superscript reference numbers: Refer to REFERENCES

of infected populations and thus reduce the speed at which SARS-CoV-2 evolves.

Third-generation vaccines

For the future, third-generation vaccines focusing on a pan-sarbecovirus rather than a pan-variant vaccine are in the initial stages of development. The sarbecovirus subgenus includes SARS-CoV-1, SARS-CoV-2, and other closely related viruses that have to date only been detected in animals. Pan-sarbecovirus neutralising antibodies have been detected in COVID-19 vaccinated SARS-CoV-1 survivors from Singapore, and are undergoing further characterisation.⁷⁹ Although there

are some early suggestions that BA.4 and BA.5 might be able to evade pan-sarbecovirus antibodies,²⁶ it is hoped that enough humoral and cellular protection could be provided by such vaccines to offer stronger, broader, and more long-lasting protection against future COVID-19 variants and other emerging sarbecoviruses.

Another possible direction that these third-generation vaccines could take includes mucosal vaccines for COVID-19.⁴² Similar vaccines are already in use for other respiratory pathogens such as influenza A viruses⁸⁰ and there are groups developing a nasal spray vaccine for COVID-19.⁸¹⁻⁸³ For instance, the interim review of the Patria study (NCT04871737) on the

Table 3. Summary of results of bivalent and multivalent vaccine studies using neutralisation assay

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
Delta-Omicron bivalent	2-dose primary series taken 2 weeks apart	Mice	Ancestral	Neutralising	70
			Beta	Neutralising	
			Delta	Neutralising	
			Omicron	Neutralising	
Ancestral-Omicron bivalent	Three doses of the same vaccine in 2-week intervals	Mice	Ancestral	Neutralising	73
			Alpha	Neutralising	
			Beta	Neutralising	
			Gamma	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
			BA.2	Neutralising	
Ancestral-Omicron bivalent	Second booster taken ~134–136 days (median) since first booster, 2-dose primary series and first booster are all mRNA-1273	Humans (Excludes those with SARS-CoV-2 infection within 3 months)	Ancestral	Non-inferior boosting to mRNA-1273 booster	76 COVE (NCT04927065)
			Alpha	Significantly higher boosting to mRNA-1273 booster	
			Beta	Significantly higher boosting to mRNA-1273 booster	
			Gamma	Significantly higher boosting to mRNA-1273 booster	
			Delta	Significantly higher boosting to mRNA-1273 booster	
			BA.1	Superior boosting to mRNA-1273 booster	
			BA.4/BA.5	Successfully boosted	
Ancestral-Beta bivalent	First booster taken ~8.8–9.8 months since 2-dose primary mRNA-1273 series	Humans (Excludes those with prior SARS-CoV-2 infection)	Ancestral	Superior boosting to mRNA-1273 booster	77 COVE (NCT04927065)
			Beta	Superior boosting to mRNA-1273 booster	
			Delta	Superior boosting to mRNA-1273 booster	
			Omicron	Superior boosting to mRNA-1273 booster	
Ancestral	2-dose primary series taken 3 weeks apart	Mice	Ancestral	Neutralising	74
			Beta	Low neutralisation	
			Delta	Neutralising	

Table 3. Summary of results of bivalent and multivalent vaccine studies using neutralisation assay (Cont'd)

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
Ancestral-Delta bivalent			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Low neutralisation	
Ancestral-Beta-Delta trivalent			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
Ancestral-BA.1 bivalent			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
Ancestral-Delta-BA.1 trivalent			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
Ancestral-Beta-Delta-BA.1 tetravalent			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
Ancestral	2-dose primary series taken 3 weeks apart	Mice	Ancestral	Neutralising	75
			Beta	Low neutralisation	
			Delta	Neutralising	
			Omicron	Failed to neutralise	
Delta			Ancestral	Low neutralisation	
			Beta	Low neutralisation	
			Delta	Neutralising	
			Omicron	Low neutralisation	
Ancestral-Delta bivalent			Ancestral	Neutralising	
			Beta	Low neutralisation	
			Delta	Neutralising	
			Omicron	Low neutralisation	
Ancestral-Beta-Delta trivalent			Ancestral	Neutralising	

Table 3. Summary of results of bivalent and multivalent vaccine studies using neutralisation assay (Cont'd)

Vaccine target	Vaccine regimen	Population	Test target	Result ^a	Reference
Ancestral-Beta-Gamma-Delta tetravalent			Beta	Neutralising	
			Delta	Neutralising	
			Omicron	Neutralising	
			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
Ancestral-BA.1 bivalent	2-dose primary series taken 3 weeks apart	Mice	Omicron	Neutralising	78
			Ancestral	Neutralising	
			Beta	Neutralising	
			Delta	Neutralising	
			BA.1	Neutralising	
			BA.4/5	Neutralising	
Ancestral-BA.1 bivalent	First booster taken 1 month since 2-dose primary BNT162b2		Ancestral	Successfully boosted	
			BA.1	Less boosting compared to ancestral target	
			BA.4/5	Less boosting compared to BA.1 target	
Ancestral	Second booster taken ~6.3 months (median) since first booster, 2-dose primary series and first booster are all BNT162b2	Humans	BA.1	Successfully boosted	
			BA.4/5	Less boosting compared to BA.1 target	
			BA.1	Superior boosting to BNT162b2 booster	
Ancestral-BA.1 bivalent			BA.4/5	Less boosting compared to BA.1 target	
Ancestral-BA.4/5 bivalent	First booster taken 104 days since 2-dose primary BNT162b2	Mice	Ancestral	Successfully boosted	
			BA.1	Successfully boosted	
			BA.2	Successfully boosted	
			BA.2.12.1	Successfully boosted	
			BA.4/5	Successfully boosted	

^a Neutralisation levels refer to ID₅₀ values against target variant. Boosting levels mean neutralisation levels to target variant were compared before and after boosting. If another vaccine is mentioned, it means that vaccine was used as a comparator. Superiority and non-inferiority mentioned here refers to pre-specified analysis as part of a clinical trial.

Superscript reference numbers: Refer to REFERENCES

usage of a nasal spray vaccine in Mexico found it to be safe and immunogenic when followed by an intramuscular dose.⁸⁴ Given that mucosal surfaces are often the first point of contact between SARS-CoV-2 viral particles and host cells, a strong mucosal immunity may prevent infection.^{40,42} These mucosal vaccines may cover a gap, as some studies have indicated that the BNT162b2 vaccine does not significantly boost mucosal immunity.⁴¹ This may also account for why current available vaccines are much better at preventing severe illness than infection.^{36,45,46} Inducing strong mucosal immunity in addition to systemic immunity may provide better protection against COVID-19 infections and moderate subsequent transmission. Reducing COVID-19 transmission would better protect the unvaccinated population in the community.

These strategies for the third-generation vaccines are not mutually exclusive; mucosal vaccines still require a spike protein target⁴² and can utilise a multivalent or even pan-sarbecovirus targeting strategy.⁷⁹ Furthermore, varying the administration route may provide better overall protection and warrants further investigation.⁸⁴ Nonetheless, a multipronged approach to the development of future COVID-19 vaccines would be important not only as COVID-19 transitions to endemicity, but also to reduce the risk of future pandemics with new emerging sarbecoviruses.

Clinical implications of the available evidence

Drawing a firm conclusion on the optimal COVID-19 vaccine booster strategy is limited by evolving evidence and variants. The following statements reflect the authors' opinion and are in line with the Singapore Ministry of Health guidance:

1. Unvaccinated individuals should be vaccinated with any of the available licensed COVID-19 vaccines, regardless of COVID-19 infection history.
2. For individuals fully vaccinated with the primary series and first booster, who have no history of COVID-19, a second booster should be offered based on the individual's risk of severe illness. Risk factors include older age or comorbid conditions including diabetes, hypertension, chronic heart or lung disease, and active cancer. In this uninfected group, administration of bivalent vaccines that include an Omicron component (of whichever subvariant) is preferable. For individuals assessed to be at low risk, there is likely to be limited benefit from an early second booster. Annual vaccination (similar to influenza) may be optimal to re-boost the immune system after antibody waning.

3. For fully vaccinated individuals (both the primary series and first booster) who have a history of COVID-19 in the past year, protection against severe illness due to Omicron is likely to be high. In this population, early booster doses are unlikely to offer significant benefit, except in high-risk individuals, including older adults and those with immunocompromising conditions. The frequency of repeated boosters that will be required is uncertain. Again, annual vaccination may be beneficial, but in low-risk individuals, the immune effects of infection should not be ignored, and re-vaccinating one year from either last vaccine dose or infection is reasonable. This suggestion may evolve if new variants emerge with different virulence or immune evasion characteristics.

CONCLUSION

With high levels of "hybrid" immunity from vaccination and/or prior infection among much of the world's population, rates of severe illness and death are expected to continue to decline. Nevertheless, maintaining high levels of population immunity through vaccination remains a key tool for moderating the effects of the pandemic. Current vaccine candidates that are most advanced in clinical trials (and approved in some countries like Singapore) are bivalent and multivalent vaccines targeting more than one variant at once,^{70,74-77} and these are hoped to offer broader and sustained protection against SARS-CoV-2 infection. As the COVID-19 pandemic transitions to endemicity,⁸⁵ it is important that vaccine formulations as well as public health policy continue to adapt and evolve with the virus to reduce morbidity, mortality and the public health burden of this disease.

Disclosure

Dr Barnaby Young reports personal fees from AstraZeneca, Gilead, Roche and Novacyt outside the scope of this work.

Acknowledgements

We would like to thank Dr Poh Xuan Ying for providing useful advice for the article.

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