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Supplementary appendix

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Supplement to: Sheward DJ, Kim C, Fischbach J, et al. Omicron sublineage BA.2.75.2 exhibits extensive escape from neutralising antibodies. *Lancet Infect Dis* 2022; published online Oct 13. https://doi.org/10.1016/S1473-3099(22)00663-6.

Appendix

Omicron sublineage BA.2.75.2 exhibits extensive escape from neutralising antibodies

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Supplementary Figures

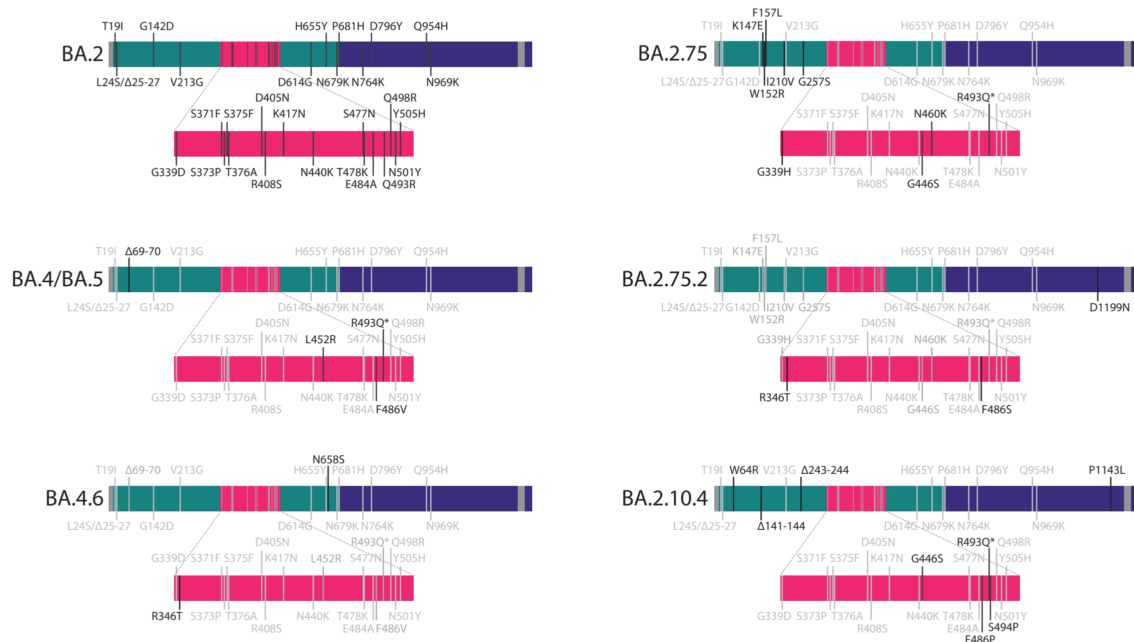

Fig S1. Spike mutations in omicron sublineages. Novel substitutions compared to the corresponding parental lineage are shown in black, with shared substitutions shown in grey. Depicted are BA.2, BA.4 and BA.5 (which have an identical spike amino acid sequence), BA.4.6[1], BA.2.75[2], BA.2.75.2[3], and BA.2.10.4[4].

Fig S2. Lineage growth analysis. Bayesian multinomial (softmax) regression growth advantage estimates (and 95% Bayesian credible intervals) for emerging and established SARS-CoV-2 lineages, estimated separately for different geographical regions (see Appendix page 5). BA.2.75.2 (and the closely related BM.1.1, which shares T346 and S486 but not N1199) are among the most rapidly growing lineages in all regions. Estimates for variants with <50 sequences in each region are greyed out, and those with <5 sequences are excluded.

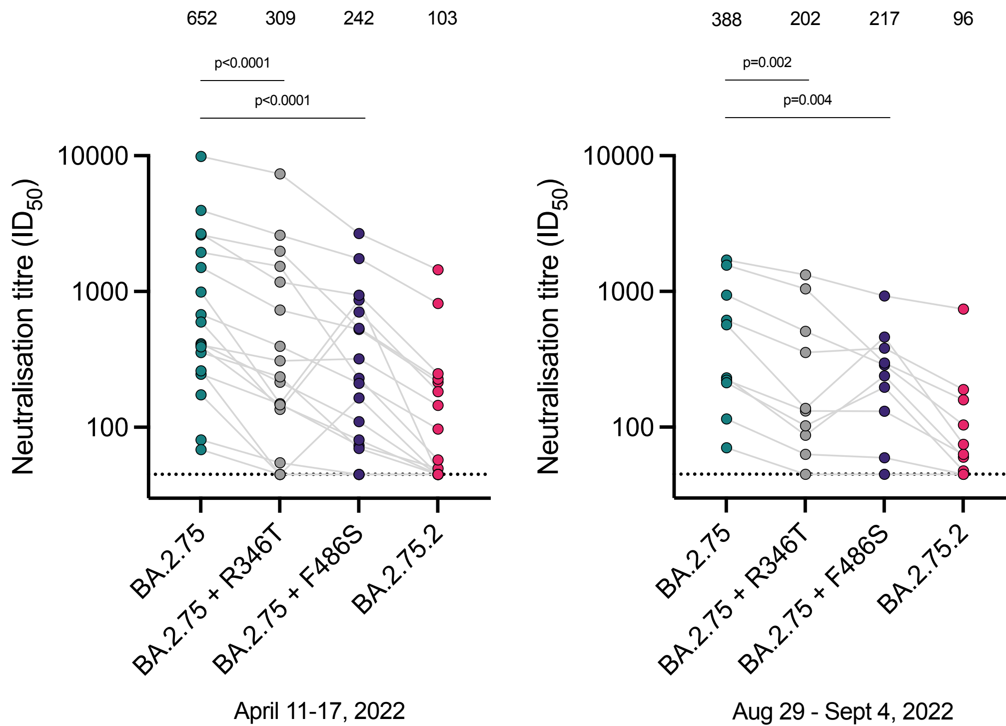

Fig S3. Both R346T and F486S contribute to BA.2.75.2 resistance. Neutralisation of BA.2.75 and mutants carrying changes present in BA.2.75.2 by serum samples from 11-17 April 2022 (n=18) and from Aug 29 - Sept 4 (n=10) highlights that both R346T and F486S contribute to the resistance of BA.2.75.2 relative to BA.2.75. Summarised above are the geometric mean neutralisation titres for each variant.

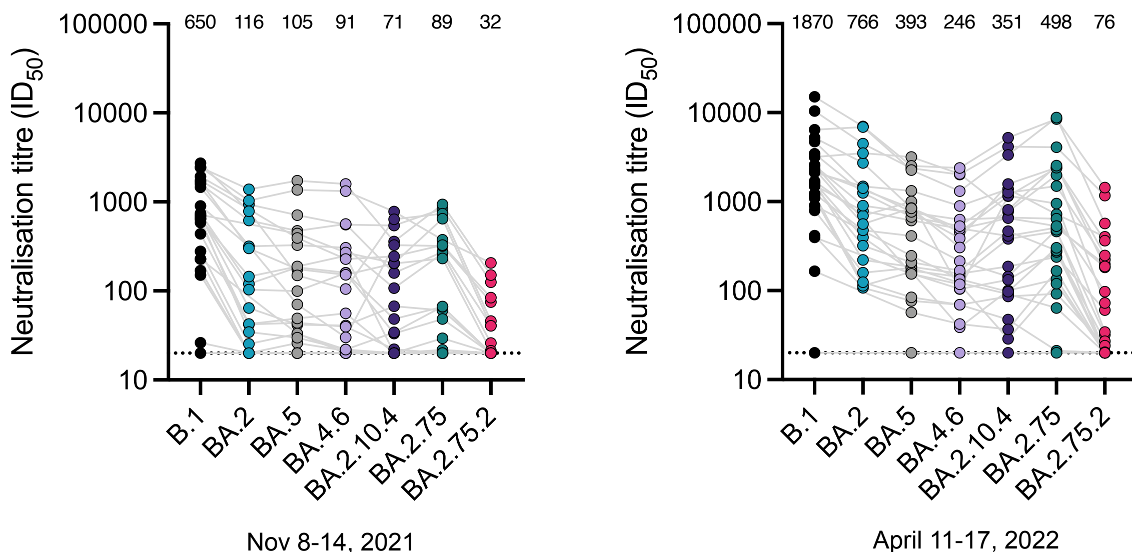

Fig S4. Neutralising antibody titres are aggregated with data from Sheward *et al*[5] highlighting the relative sensitivity of BA.2 in Nov 2021, prior to Omicron (left), and in April 2022 after a large wave of infections dominated by BA.1 and BA.2 (right).

Supplementary Text

Methods

Cell culture

HEK293T cells (ATCC CRL-3216) and HEK293T-ACE2 cells (stably expressing human ACE2) were cultured in Dulbecco's Modified Eagle Medium (high glucose, with sodium pyruvate) supplemented with 10% fetal bovine serum, 100 units/ml Penicillin, and 100 µg/ml Streptomycin. Cultures were maintained in a humidified 37°C incubator (5% CO_2).

Pseudovirus Neutralisation Assay

Pseudovirus neutralisation assay was performed as previously[6]. Briefly, spike-pseudotyped lentivirus particles were generated by co-transfection of HEK293T cells with a relevant spike plasmid, an HIV gag-pol packaging plasmid (Addgene #8455), and a lentiviral transfer plasmid encoding firefly luciferase (Addgene #170674) using polyethylenimine. Spike variants were generated by multi-site directed mutagenesis of BA.2, BA.4, or BA.2.75 expression plasmids[5], and all mutated plasmids were subsequently confirmed by Sanger sequencing.

Neutralisation was assessed in HEK293T-ACE2 cells. Pseudoviruses titrated to produce approximately 100,000 RLU were incubated with serial 3-fold dilutions of serum or monoclonal antibody for 60 minutes at 37°C in a black-walled 96-well plate. 10,000 HEK293T-ACE2 cells were then added to each well, and plates were incubated at 37°C for 44-48 hours. Luminescence was measured using Bright-Glo (Promega) on a GloMax Navigator Luminometer (Promega). Neutralisation was calculated relative to the average of 8 control wells infected in the absence of antibody. Samples were run against all variants 'head-to-head' using the same dilutions.

Monoclonal antibodies

Cilgavimab and tixagevimab were evaluated as their clinical formulations. For the rest of the monoclonal antibodies evaluated, antibody sequences were extracted from deposited RCSB entries, synthesised as gene fragments, cloned into pTWIST transient expression vectors by Gibson assembly or restriction cloning, expressed and purified, all as previously described[7].

Serum samples

75 samples from anonymized blood donors from Stockholm, Sweden, were obtained, 25 each, from week 45, 2021 (8-14 November 2021) (prior to the BA.1/BA.2 Omicron infection wave), from week 15, 2022 (11-17 April 2022) (after the BA.1/BA.2 Omicron infection wave, but prior to the arrival of BA.4 or BA.5), and from week 35, 2022 (29 August - 4 September 2022) (after the spread of BA.5). Samples from the Nov 2021 and April 2022 cohorts are a superset of samples previously studied[5]. Sera were heat inactivated at 56°C for 60 minutes prior to use in neutralisation assays.

While individual vaccination and infection histories are not available for these anonymized samples, 3rd dose "booster" vaccination rates in Stockholm went from 5% (Nov 2021) to 59% (April 2022) to 63% (Sept 2022). Case counts indicate a large BA.1/BA.2 wave prior to the April 2022 cohort[5]. While a wave of BA.5 infections occurred between the April 2022 and Sept 2022 cohort, changes in public PCR testing prevent a clear estimation of the number of infections. Changes in neutralisation between the April 2022 and Sept 2022 cohorts are therefore likely due to temporal factors (eg. immune waning) and BA.5 infections, and unlikely to be due to additional vaccinations.

Lineage growth analysis

Data processing: All sequences obtained from GISAID[8] EpiCoV™ (bulk fasta download, 2022/10/03) were processed with Nextclade[9] (version 2.5.0), using the "sars-cov-2-21L" reference dataset to perform lineage assignment. Sequences from the last 100 days (after 2022/06/26) with "good" overall QC status and sequence coverage >90% were retained. Countries were selected that had more than 200 sequences in total, with more than 90 in the last 30 days. Lineages with more than 40 sequences were kept and sequences from lineages with frequencies below our cutoff were aggregated to those of their ancestral lineages. Countries were grouped into three *regions* based on their location and volume of recent sequence data: Asia, Europe, and North America. Australia, with high sequence volumes, was merged into Asia because no other countries from Oceania remained after the selection process.

Model: The dynamics of the ratio of competing variants is well-modelled by a multinomial (softmax) regression[10]. The sequence counts over lineages at a country are modelled with a Bayesian multinomial regression, similar but not identical to PyR_0[11]. Formally, the sequence count n_{ij} at time t_i and country j is to follow a multinomial distribution with parameter $softmax(a \times t_i + b_j)$, where a refers to a vector of lineage-specific slopes (i.e., growth rates) and b_j a vector of lineage- and country-specific intercepts. The intercept parameter b_j is indexed by country j to reflect the fact that the introduction time of a lineage depends on the country, while the slope parameter a is shared among countries. Lineages inherit the growth rates of their ancestors, adjusted for new mutations, so the slope a_k of lineage k is the sum of growth adjustment parameters θ of its ancestors and itself. The intercept b_k of lineage k is the sum of a global intercept μ_k and a local intercept δ_{jk}. For the purpose of Bayesian inference, we put priors on these parameters such that $\theta_k \sim Normal(0, 0.1)$, $\mu_k \sim Normal(0, 5)$, and $\delta_{jk} \sim Normal(0, 2)$. The "Daily growth advantage (relative to BA.2)" depicted in Fig S2 is $exp(a_k)$.

Inference: The parameters of the model were inferred using Markov chain Monte Carlo, sampled by Hamiltonian Monte Carlo in Stan[12] (version 2.30.1). We ran six independent sampling chains for each region with Stan's default sampling parameters. The number of warm-up iterations is 1000 for Asia+Australia and Europe, and 3000 for North America, because the convergence was slower in North America.

Convergence diagnostics: The effective sample sizes were estimated with Stan's stansummary program. The estimated effective sample sizes for a_k for BA.2.75.2 are 2162, 9811, and 9791 for North America, Asia+Australia, and Europe, respectively, and 3058, 7051, and 8784 for BM.1.1.

Ethical Statement

The blood donor samples were anonymized, and not subject to ethical approvals, as per advisory statement 2020–01807 from the Swedish Ethical Review Authority.

Statistical analysis

Individual ID_{50} and IC_{50} values for each sample against each variant were calculated in Prism v9 (GraphPad Software) by fitting a four-parameter logistic curve to neutralisation by serial 3-fold dilutions of serum/antibody. Neutralising titres between groups were compared using a Wilcoxon matched-pairs signed rank test using Prism v9. Fold change between any pair of variants was calculated excluding samples where either variant's ID_{50} was < 20.

Author contributions

Conceptualization, D.J.S., T.P.P., B.M.;
Formal analysis, D.J.S., K.S., B.M;
Conducted the assays, D.J.S., C.K., J.F., T.P.P.;
Designed the methodology, D.J.S., C.K., R.E., K.S., T.P.P, B.M.;
Responsible for figures and tables, D.J.S., K.S., T.P.P., B.M.;
Resources, S.M., R.E., S.R, N.K.B., G.B.K.H., J.A., B.M.;
Oversaw the study, D.J.S., G.B.K.H., S.T.R., J.A., B.M;
Funding Acquisition, S.T.R., G.B.K.H., J.A., T.P.P., B.M.
Writing – original draft, D.J.S., B.M.;
Writing – review & editing, D.J.S., G.B.K.H., J.A., T.P.P., B.M.
D.J.S and B.M. were responsible for the decision to submit the manuscript for publication.

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