

Supplementary Information

Integrated Genomic and Virological SARS-CoV-2 Surveillance in Germany 2022/23: Monitoring Viral Evolution and Epidemiological Characteristics

Authors

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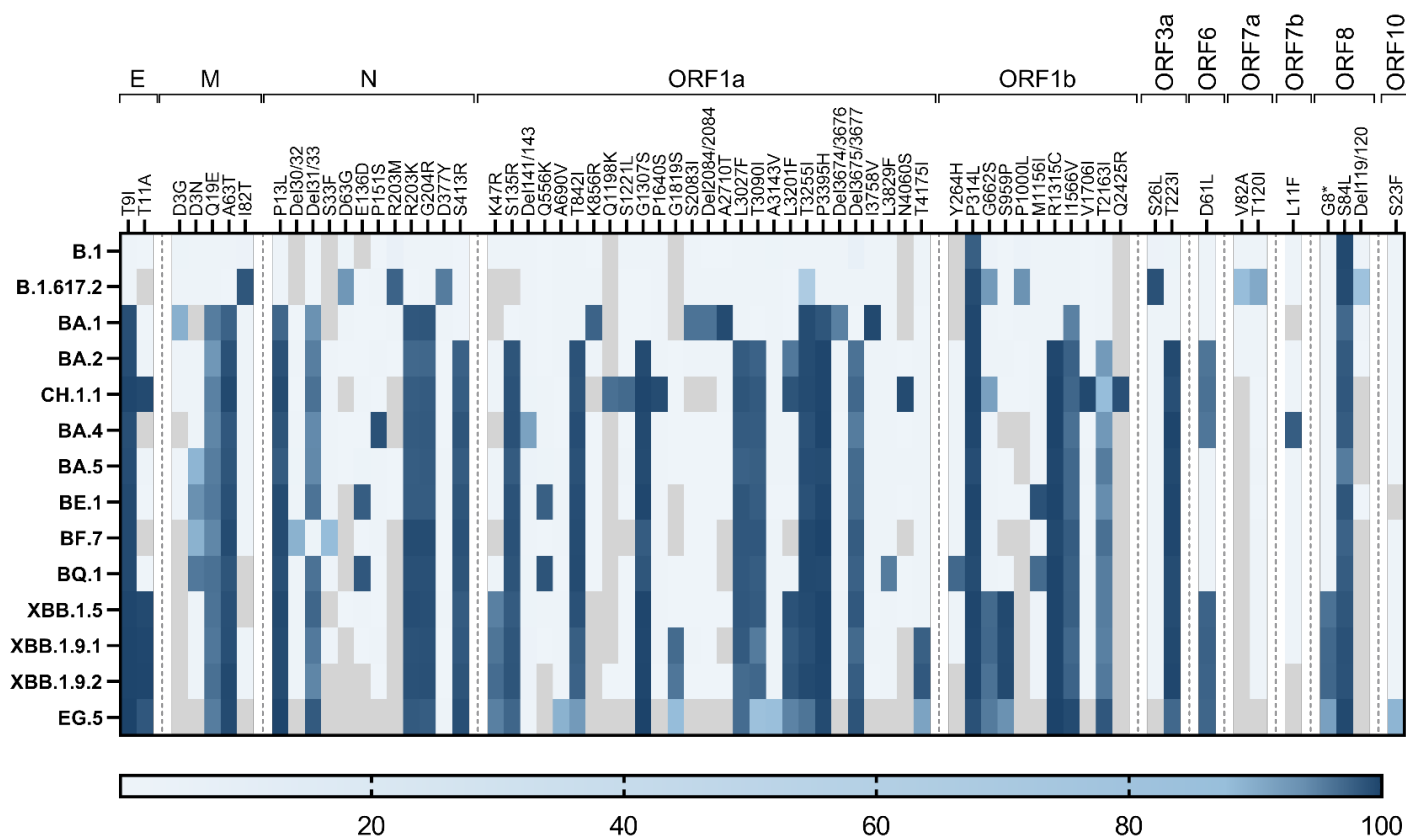
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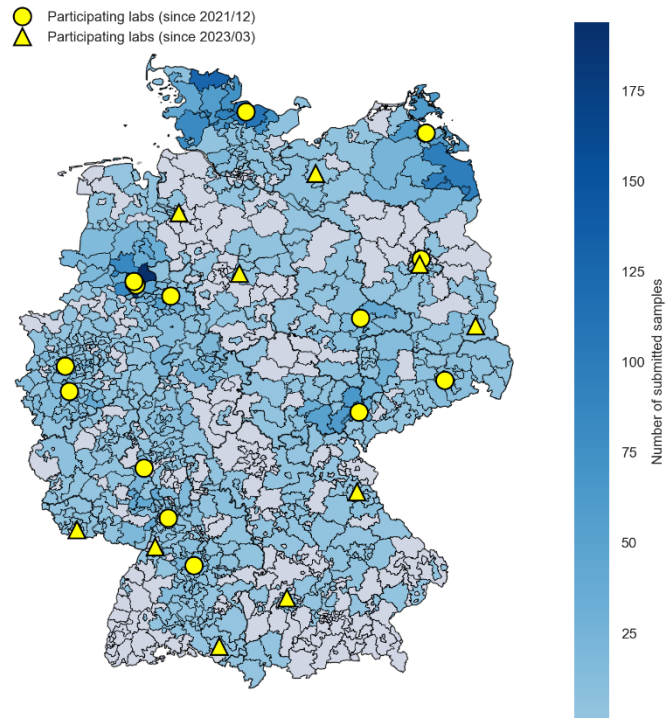
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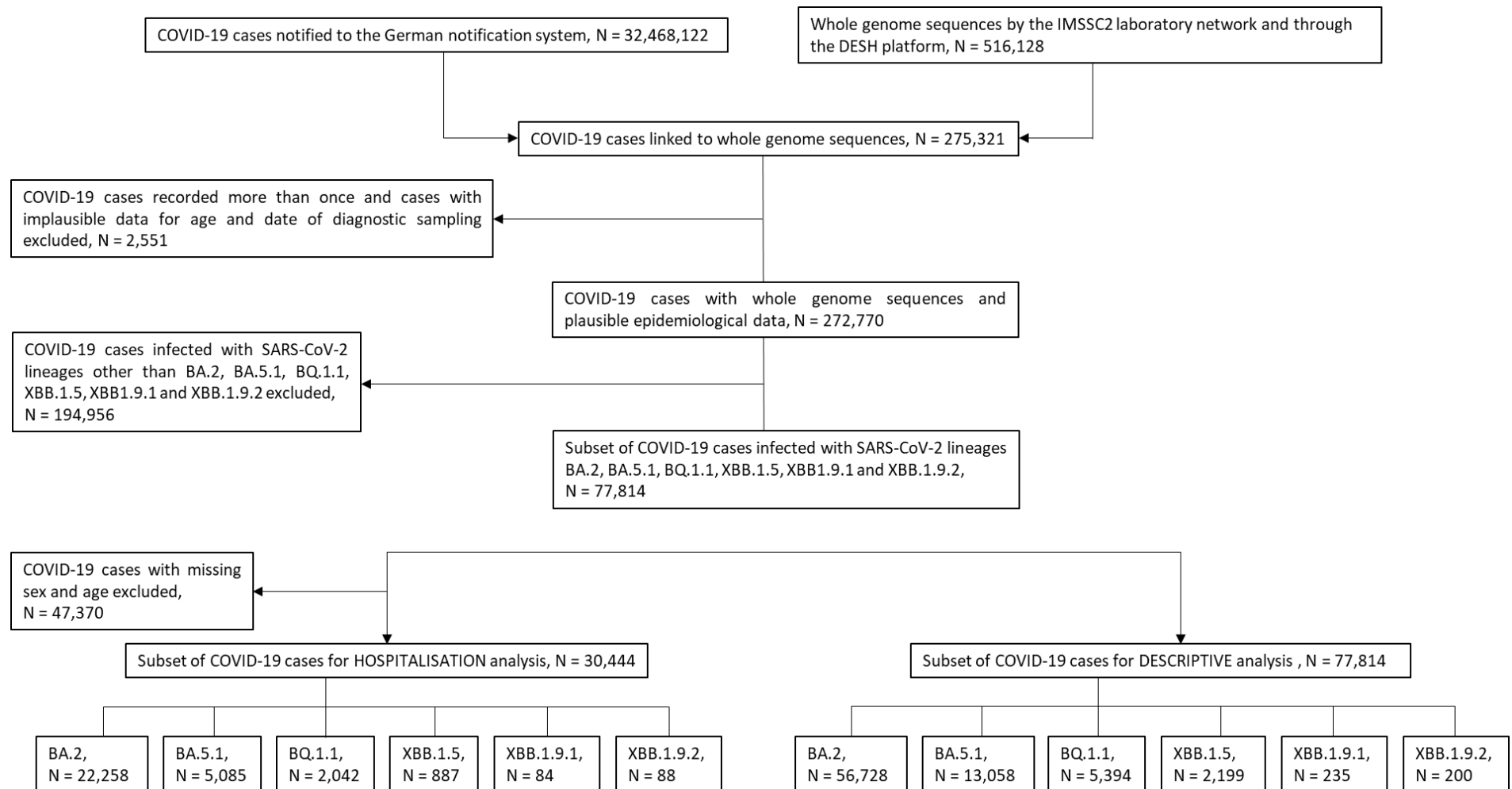


Suppl. Figure 1. Mutational Profiles of Omicron Sublineages. Mutation patterns of the indicated SARS-CoV-2 variants in the structural viral proteins E, M, and N, as well as the open reading frames (ORF) ORF1a, ORF1b, ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, based on a minimum mutation prevalence of 75% according to outbreak.info.org.

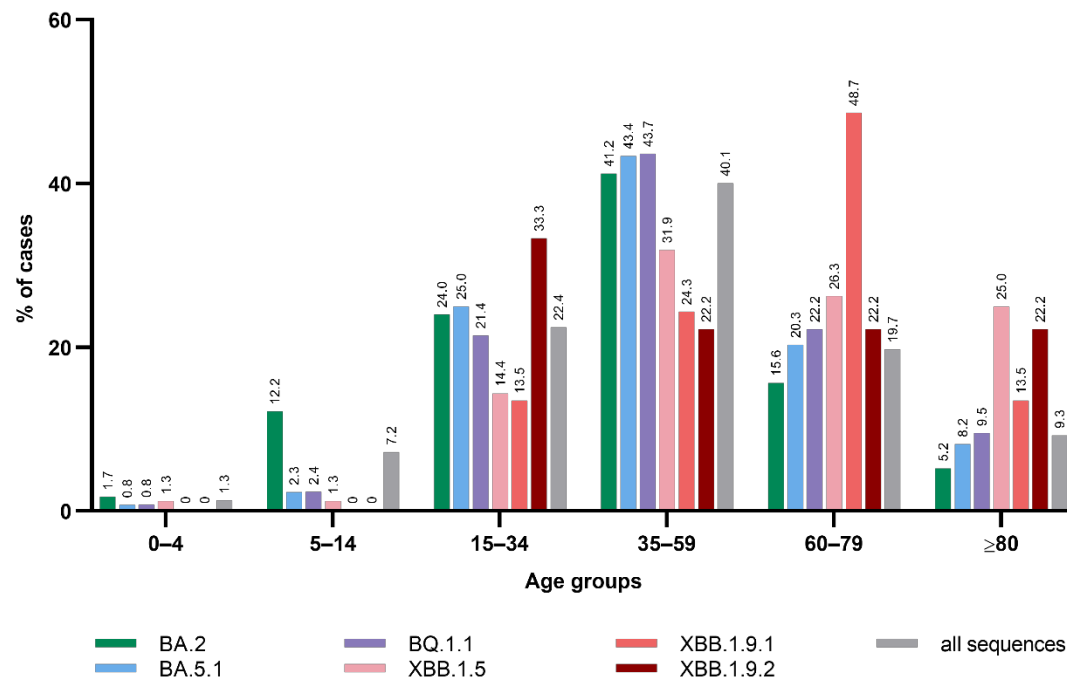


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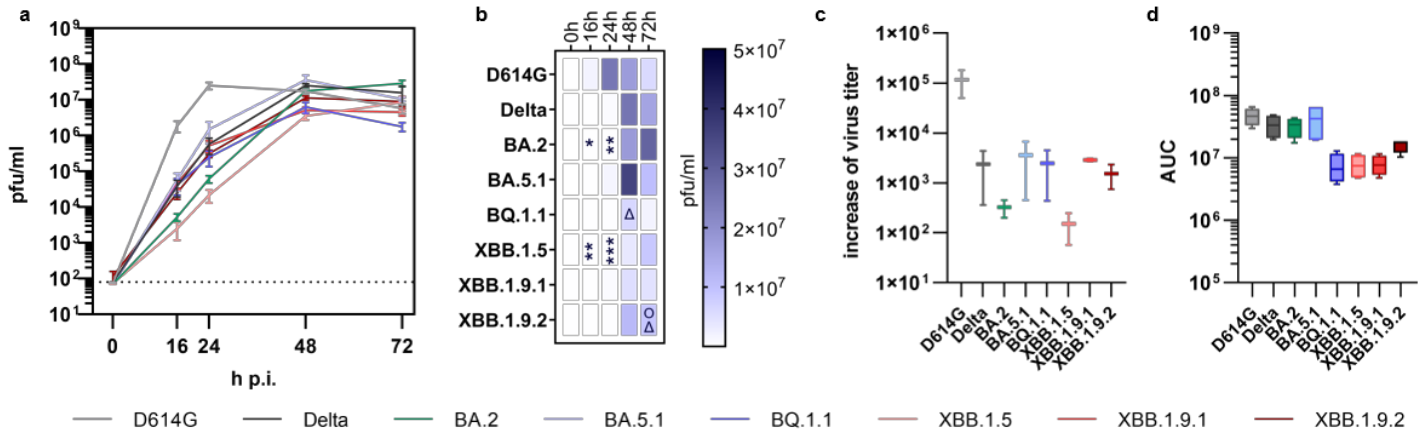
34 **Suppl. Figure 2. Geographical Distribution of SARS-CoV-2 Sequences Captured by the IMSSC2 Laboratory**
 35 **Network (12/2021 - 04/2023).** Map of Germany showing the geographic distribution of SARS-CoV-2 positive
 36 samples included in the IMSSC2 genomic surveillance from December 1st, 2021, to April 30th, 2023 (n=4,595).
 37 Shades of blue indicate sample numbers per the 3-digit zip code region of origin, while IMSSC2 network
 38 laboratories are represented by yellow points (participating since 12/2021) or triangles (participating since
 39 03/2023). The map was created with a custom Python script using the GeoPandas package version 0.10.2⁶⁷.



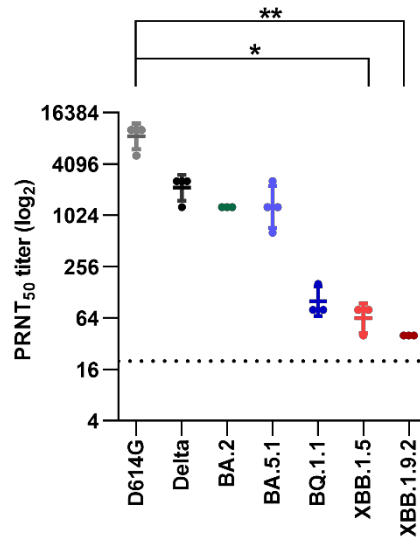
Suppl. Figure 3. Flow Chart of the Selection of COVID-19 Cases included in the Descriptive Analysis and in the Sub-Analysis on Hospitalization in Germany, December 1st, 2021, to April 30th, 2023.



Suppl. Figure 4. Distribution of COVID-19 Cases among Age Groups in the IMSSC2 Laboratory Network. Relative frequency (%) of laboratory confirmed BA.2, BA.5.1, BQ.1.1, XBB.1.5, XBB.1.9.1, and XBB.1.9.2 cases among age groups calculated based on year of birth of COVID-19 case, in Germany between December 1st, 2021, to April 30th, 2023. The grey bars (all sequences) within the age groups refer to all notified COVID-19 cases with epidemiological information and linked to WGS data from the IMSSC2 laboratory network (n = 4,595).



Suppl. Figure 5. Infection of Non-Human Vero E6 Cells with Omicron Sublineages. Vero E6 cells were infected with indicated SARS-CoV-2 viruses: D614G, Delta and selected Omicron sublineages (BA.2, BA.5.1, BQ.1.1, XBB.1.5, XBB.1.9.1 or XBB.1.9.2) at MOI 0.1. **a** Progeny viruses were collected from supernatant at indicated time points and titrated using standard plaque assay on Vero E6 cells. Replication analysis was performed for $n=2$ in technical duplicates and is shown as mean \pm SEM. **b** Heatmap represents viral titers from replication analysis (a) at corresponding time points. **c** Increase of viral titers in early phase of infection was calculated from linear regression between 0 and 16 h p.i. from replication analysis (a). **d** Area under the curves (AUCs) were calculated from replication analysis in (a). Data are shown as mean \pm SEM. Data are shown as boxplots (min to max; box extends from the 25th to the 75th percentile, center line represents median). Statistical analyses were performed using non-paired, non-parametric Kruskal-Wallis test, statistical significances are displayed compared to SARS-CoV-2 D614G (*), BA.2 (Δ) or XBB.1.5 (O) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



Suppl. Figure 6. Serum Neutralization Titers against Selected Omicron Sublineages. Neutralization titers of pooled pre-Omicron sera against the indicated viral variants are expressed as PRNT50 titers. Neutralizing activity of pooled pre-Omicron sera against authentic SARS-CoV-2 viruses including D614G, Delta and selected Omicron sublineages BA.2, BA.5.1, BQ.1.1, XBB.1.5 or XBB.1.9.2. PRNT50 of negative serum was below detection limit in all assays (data not shown). Data are shown as geometric mean PRNT50 titer and 95% confidence intervals. Statistical analyses were performed using non-paired, non-parametric Kruskal-Wallis test (* $p < 0.05$; ** $p < 0.01$).

Table S1. Characteristics of COVID-19 cases notified to the German national surveillance system through public health authorities and COVID-19 cases with random diagnostic samples and whole-genome sequencing (WGS), as well as a subset of WGS linked cases with additional epidemiological data included in the analysis, Germany, December 1st, 2021, to April 30th, 2023. Cases for which epidemiological data were not reported are categorized as missing and are presented in italics.

Characteristic	COVID-19 cases notified to German national surveillance system		COVID-19 cases linked with WGS		COVID-19 cases linked with WGS and epidemiological data	
	Number	%	Number	%	Number	%
Total	32468122	100	516128	1.6	272770	0.8
Sex^a						
Female	16891676	52.0	146039	28.3	144765	53.1
Male	15299244	47.1	128349	24.9	127082	46.6
<i>Missing</i>	<i>277202</i>	<i>0.9</i>	<i>241740</i>	<i>46.8</i>	<i>923</i>	<i>0.3</i>
Age groups (years)						
0–4	861707	2.7	4437	0.9	4393	1.6
5–14	3808849	11.7	22868	4.4	22538	8.3
15–34	9423171	29.0	79009	15.3	78241	28.7
35–59	12760500	39.3	110565	21.4	109590	40.2
60–79	4323041	13.3	41553	8.1	41290	15.1
≥ 80	1262350	3.9	16821	3.3	16651	6.1
<i>Missing</i>	<i>28504</i>	<i>0.1</i>	<i>240875</i>	<i>46.7</i>	<i>67</i>	<i>0.02</i>
Hospitalization						
Yes	598026	1.8	9249	1.8	9182	3.4
No	13687958	42.2	105990	20.5	105161	38.5
<i>Missing</i>	<i>18182138</i>	<i>56.0</i>	<i>400889</i>	<i>77.7</i>	<i>158427</i>	<i>58.1</i>
Intensive Care Unit	n/N*	%	n/N*	%	n/N*	%
Yes	36162/598026	6.0	830/9249	9.0	829/9182	9.0
No	534776/598026	89.4	8030/9249	86.8	8021/9182	87.4
<i>Missing</i>	<i>27088/598026</i>	<i>4.5</i>	<i>389/9249</i>	<i>4.2</i>	<i>332/9182</i>	<i>3.6</i>
Deaths						
Yes	65036	0.2	1296	0.3	1285	0.5
No	30856636	95.0	262401	50.8	259930	95.3
<i>Missing</i>	<i>1546450</i>	<i>4.8</i>	<i>252431</i>	<i>48.9</i>	<i>11555</i>	<i>4.2</i>
Vaccination						
Yes	5681654	17.5	59167	11.5	58913	21.6
No	1881425	5.8	16328	3.2	16219	5.9
<i>Missing</i>	<i>24905043</i>	<i>76.7</i>	<i>440633</i>	<i>85.4</i>	<i>197638</i>	<i>72.5</i>

^a Sex at birth.

* n = COVID-19 cases in intensive care unit, N = number of hospitalized COVID-19 cases

Table S2. COVID-19 cases with hospitalization among age groups after infection with SARS-CoV-2 Omicron BA.2, BA.5.1, BQ.1.1 and with recombinant lineages XBB.1.5, XBB.1.9.1, and XBB.1.9.2, Germany, December 1st, 2021, to April 30th, 2023 (n=1,896). Hospitalized cases for which information on age was not reported were categorized as missing and are presented in italics.

Hospitalization	BA.2		BA.5.1		BQ.1.1		XBB.1.5		XBB.1.9.1		XBB.1.9.2		Total	
	n/N*	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Total	818/56728	1.4	398/13058	3.0	371/5394	6.9	255/2199	11.6	26/235	11.1	28/200	14.0	1896/77814	2.4
Age groups (years)														
0–4	12/1129	1.1	7/131	5.3	1/17	5.9	1/12	8.3	1/2	50.0	1/2	50.0	23/1293	1.8
5–14	16/5524	0.3	3/501	0.6	0/94	0	1/27	3.7	0/2	0	0/2	0	20/6150	0.3
15–34	68/17019	0.4	18/3641	0.5	16/1373	1.2	7/585	1.2	2/52	3.8	2/52	3.8	113/22722	0.5
35–59	107/22675	0.5	48/5508	0.9	51/2414	2.1	30/860	3.5	4/99	4.0	3/85	3.5	243/31641	0.8
60–79	273/7733	3.5	130/2423	5.4	118/984	12.0	84/411	20.4	7/47	14.9	5/31	16.1	617/11629	5.3
≥ 80	342/2633	13.0	192/854	22.5	185/511	36.2	132/304	43.4	12/33	36.4	17/28	60.7	880/4363	20.2
<i>Missing</i>	<i>0/15</i>	<i>0</i>	<i>0/0</i>	<i>0</i>	<i>0/1</i>	<i>0</i>	<i>0/0</i>	<i>0</i>	<i>0/0</i>	<i>0</i>	<i>0/0</i>	<i>0</i>	<i>0/16</i>	<i>0</i>

* n = number of hospitalizations, N = number of infected cases reported to the German notification system

Table S3. Characteristics of SARS-CoV-2 cases included in the sub-analysis on hospitalization, by SARS-CoV-2 lineages BA.2, BA.5.1, BQ.1.1, XBB.1.5, XBB.1.9.1 and XBB.1.9.2, Germany, December 1st, 2021, to April 30th, 2023 (n=30,444).

Characteristic	BA.2		BA.5.1		BQ.1.1		XBB.1.5		XBB.1.9.1		XBB.1.9.2		Total	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Sub-analysis for hospitalization	22258	73.1	5085	16.7	2042	6.7	887	2.9	84	0.3	88	0.3	30444	100.0
Sex^a														
Female	11942	53.7	2677	52.6	1117	54.7	476	53.7	50	59.5	43	48.9	16305	53.6
Male	10316	46.3	2408	47.4	925	45.3	411	46.3	34	40.5	45	51.1	14139	46.4
Age groups (years)														
0–4	415	1.9	45	0.9	4	0.2	7	0.8	1	1.2	2	2.3	474	1.6
5–14	2110	9.5	154	3.0	42	2.1	8	0.9	0	0	0	0	2314	7.6
15–34	6531	29.3	1445	28.4	470	23.0	218	24.6	22	26.2	15	17.0	8701	28.6
35–59	8745	39.3	2059	40.5	824	40.4	284	32.0	29	34.5	33	37.5	11974	39.3
60–79	3168	14.2	981	19.3	419	20.5	180	20.3	15	17.9	15	17.0	4778	15.7
≥ 80	1289	5.8	401	7.9	283	13.9	190	21.4	17	20.2	23	26.1	2203	7.2
Hospitalization														
Yes	818	3.7	398	7.8	371	18.2	255	28.7	26	31.0	28	31.8	1896	6.2
No	21440	96.3	4687	92.2	1671	81.8	632	71.3	58	69.0	60	68.2	28548	93.8
Hospitalization by age groups (years)														
	n/N*	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
0–4	12/415	2.9	7/45	15.6	1/4	25.0	1/7	14.3	1/1	100.0	1/2	50.0	23/474	4.9
5–14	16/2110	0.8	3/154	1.9	0/42	0	1/8	12.5	0/0	0	0/0	0	20/2314	0.9
15–34	68/6531	1.0	18/1445	1.2	16/470	3.4	7/218	3.2	2/22	9.1	2/15	13.3	113/8701	1.3
35–59	107/8745	1.2	48/2059	2.3	51/824	6.2	30/284	10.6	4/29	13.8	3/33	9.1	243/11974	2.0
60–79	273/3168	8.6	130/981	13.3	118/419	28.2	84/180	46.7	7/15	46.7	5/15	33.3	617/4778	12.9
≥ 80	342/1289	26.5	192/401	47.9	185/283	65.4	132/190	69.5	12/17	70.6	17/23	73.9	880/2203	39.9

* n = number of hospitalizations, N = number of infected cases reported to the German notification system