

Supplementary Table S1. Details of all identified positive cases.

Patient ID*	Area	Collection date	Delay ¹	Age (yr)	Sex	RT PCR	NsP3 ²	RdRp ²	Spike A ²	Spike B ²	IgM ³	IgA ³	IgG ³	PRNT 80% ⁴
Control cohort														
	Milan	24/10/2018	9	80	M	-	-	-	-	-	-	+	-	NA
	East Milan	14/03/2019	0	5	F	-	-	-	-	-	-	-	+	NA
	Milan	24/03/2019	2	55	M	-	-	-	-	-	-	-	+	NA
	Brescia	21/05/2019	2	48	F	-	-	-	-	-	+	-	-	NA
	North-West Milan	27/05/2019	1	1	M	-	-	-	-	-	+	-	-	NA
	Milan	24/06/2019	1	32	F	-	-	-	-	-	-	+	-	NA
	Milan	12/07/2019	8	2	F	-	-	-	-	-	-	+	-	54%
	East Milan	12/07/2019	3	51	M	-	-	-	-	-	-	+	-	50%
	Brescia	20/07/2019	1	48	F	-	-	-	-	-	-	+	-	-
	North Milan	01/08/2019	1	24	M	-	-	-	-	-	-	+	-	52%
Pre-pandemic cases														
	Milan	29/08/2019	1	24	F	-	-	-	-	-	-	+	-	59%
	North-East Milan	01/09/2019	1	35	M	-	-	-	-	-	-	+	-	-
1	Milan	12/09/2019	3	0-67	M	-	-	-	-	+(U)	+	-	+	-
	Milan	28/09/2019	0	42	M	-	-	-	-	-	+	-	-	-
2	East Milan	12/10/2019	0	18	F	-	-	-	-	+(S)	-	-	-	-
3	Brescia	17/10/2019	1	9	F	-	-	+(U)	-	-	-	-	-	-
4	North-West Milan	19/10/2019	1	1	M	-	-	+(S)	-	-	-	+	+	-
5	Brescia	22/10/2019	1	1	F	-	+(S)	-	-	-	+	-	-	62%
6	East Milan	23/10/2019	NA	1	F	-	-	+(S)	-	-	+	-	-	-
7	North-West Milan	22/11/2019	7	2	M	-	+(S)	-	-	-	NA	-	-	NA
	Milan	01/12/2019	1	14	M	-	-	-	-	-	-	+	-	-
8	West Milan	05/12/2019	4	4	M	-	-	-	-	+(S)	NA	-	-	NA
9	South-East Milan	15/12/2019	2	25	F	-	-	+(U)	+(U)	+(U)	-	-	-	-
10	Milan	09/01/2020	1	53	M	-	-	-	-	+(U)	-	-	-	-
11	Brescia	14/01/2020	4	40	M	-	-	-	-	+(S)	-	-	-	NA

Pandemic cases														
12	North-West Milan	15/01/2021	1	65	F	-	-	-	-	-	-	+	+	1:60
13	Lodi	16/01/2021	9	32	F	-	+	(U)	-	+	(U)	-	-	-
14	North Milan	25/01/2021	4	1	F	-	-	-	-	-	-	+	+	1:160
	Milan	02/02/2021	1	1	F	-	-	-	-	-	-	+	+	-
15	Milan	26/02/2021	5	47	M	-	-	-	-	-	+	(U)	-	-
	North Milan	24/03/2021	6	32	M	-	-	-	-	-	-	-	+	+
														NA

*Referred to Table 3 in the main text.

¹Days from exanthema onset to sample collection. ²U: urine, S: oropharyngeal swab. ³NA: serum/data not available. ⁴PRNT: plaque-reduction neutralization test. Either the neutralization dilution of serum that results in 80% plaque reduction or the percentage of plaque reduction (at a dilution of 1:10) for partially neutralizing samples are shown.

Supplementary Table S2. Details of the mutations identified in this study.

ID	Area	Collection date	Sequenced region (nt)	Nucleotide change¹	Amino acid change¹	Mutation type²
1	Milan	12/09/2019	22904-23330	none	none	/
2	Milan	12/10/2019	22904-23330	none	none	/
3	Brescia	17/10/2019	14366-14655	C14408T	P323L	β_3
4	Milan	19/10/2019	14366-14655	C14408T	P323L	β_3
5	Brescia	22/10/2019	2977-3185	C3037T, T2987C, T3012C	F924F, F908L, L916S	β_1
6	Milan	23/10/2019	14366-14655	C14408T	P323L	β_3
7	Milan	22/11/2019	2977-3185	C3037T	F924F	β_1
8	Milan	05/12/2019	22904-23330	none	none	/
9	Milan	15/12/2019	22904-23681	A23403G	D614G	β_2
			14366-14655	C14408T	P323L	β_3
10	Milan	09/01/2020	22904-23330	23000Y, 23222R		/
11	Brescia	14/01/2020	22904-23330	none	none	/
13	Lodi	16/01/2021	2977-3185	C3037T	F924F	β_1
			23310-23681	A23403G	D614G	β_2
16	Milan	26/02/2021	22904-23330	none	none	/

¹Referred to Wuhan HU-1 (NC_045512.2); ²Referred to Kumar et al. Mol Biol Evol 2021

Supplementary Figure S1. Electropherograms obtained from sample ID. #10.

