



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
9th Report
(Study period: 2 - 6 May 2022)

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Main findings:

- Overall, 158 wastewater samples were collected in the week 2-6 May from 18 Regions and 2 Autonomous Provinces (A.P.).
- Only mutations characteristic of the Omicron variant were detected, found in 17 Regions and two A.P. (no sequences were obtained from the remaining three Regions).
- Aminoacid substitutions of sublineage BA.2 were detected in 100% of the sequences (17 Regions/A.P.) by Sanger sequencing.
- NGS results confirmed the predominance of sublineage BA.2, detected in all the NGS pools.
- Sublineages BA.4/BA.5 were not detected.

Introduction

On 17 March 2021, the “EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU”, strongly encouraged Member States to put in place, no later than 1 October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants¹.

Following the above EU Recommendation, the Istituto Superiore di Sanità (ISS) instituted “flash surveys”, i.e. periodic (monthly) sampling campaigns to be held in different locations in Italy over the course of a brief period, aimed at assessing the diversity of SARS-CoV-2 in wastewater in the country.

Aim

The aim of this report is to summarize the results of the ninth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 2-6 May 2022.

Methodology

The survey included sewage samples collected at wastewater treatment plants (WTPs) located in 17 regions and 2 autonomous provinces (A.P.):

- North-West Italy: Liguria, Lombardia, Piemonte and Valle d’Aosta;
- North-East Italy: Emilia-Romagna, Veneto, A.P. of Bolzano and A.P. of Trento, Friuli-Venezia Giulia;
- Central Italy: Abruzzo, Lazio, Marche, Toscana and Umbria;
- Southern Italy and Islands: Calabria, Campania, Basilicata, Molise, Puglia and Sicilia.

Overall, 158 wastewater samples were collected between 2 and 6 May 2022 (**Table 1**).

¹ Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. (<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472&qid=1628798981209>)

Table 1. Sampling sites and characteristics of the WTPs studied

Sample ID ISS	Region/A. P.	City	Sampling Date	WTP	Population equivalent
SARI9994	Abruzzo	Pescara	03/05/2022	Via Raiale	160000
SARI9995	Abruzzo	Pescara	03/05/2022	Villa Carmine	140000
SARI9997	Abruzzo	Chieti	03/05/2022	S. Martino	114500
SARI9998	Abruzzo	L'Aquila	03/05/2022	Pile	48000
SARI10000	Abruzzo	Teramo	03/05/2022	Villa Pavone	41824
SARI10089	Basilicata	Potenza	03/05/2022	Tiera di Vaglio	95000
SARI10090	Basilicata	Matera	03/05/2022	Pantano	24000
SARI9950	Calabria	Cosenza	03/05/2022	Cosenza - Code di volpe	191000
SARI9951	Calabria	Cosenza	03/05/2022	Cosenza - Sant'Angelo	45000
SARI9952	Calabria	Reggio Calabria	03/05/2022	Ravagnese - località Aeroporto	120000
SARI9953	Calabria	Catanzaro	03/05/2022	Catanzaro - Zona industriale	120000
SARI9954	Calabria	Crotone	03/05/2022	Crotone - località Papaniciaro	60000
SARI9955	Calabria	Catanzaro	03/05/2022	Catanzaro Lido - Loc. Verghello	90000
SARI10046	Campania	Napoli	03/05/2022	Napoli OVEST - Ingresso Principale	950000
SARI10048	Campania	Salerno	03/05/2022	Salerno	700000
SARI10049	Campania	Caserta	03/05/2022	Villa Literno	631714
SARI10055	Campania	Avellino	05/05/2022	Manocalzati	140000
SARI10057	Campania	Salerno	05/05/2022	Nocera Sup	299121
SARI10058	Campania	Napoli	05/05/2022	Napoli EST	1750000
SARI10060	Campania	Napoli	05/05/2022	Napoli OVEST - ex ingresso Camaldoli	250000
SARI10061	Campania	Caserta	05/05/2022	Area Casertana	370769
SARI10064	Campania	Salerno	03/05/2022	Eboli	30000
SARI10085	Campania	Napoli	03/05/2022	Area Nolana	400000
SARI9889	Emilia-Romagna	Ferrara	03/05/2022	Ferrara - Linea 1	120000
SARI9890	Emilia-Romagna	Ferrara	03/05/2022	Ferrara - Linea 2	120000
SARI9891	Emilia-Romagna	Modena	03/05/2022	Carpi	200000
SARI9895	Emilia-Romagna	Piacenza	03/05/2022	Borgoforte	163333
SARI9896	Emilia-Romagna	Parma	02/05/2022	Parma ovest	168000
SARI9898	Emilia-Romagna	Reggio Emilia	02/05/2022	Mancasale	280000
SARI9907	Emilia-Romagna	Bologna	02/05/2022	IDAR	800000
SARI9908	Emilia-Romagna	Ravenna	02/05/2022	Faenza	100000
SARI9910	Emilia-Romagna	Modena	02/05/2022	Naviglio	500000
SARI9913	Emilia-Romagna	Bologna	02/05/2022	Imola	75000
SARI9948	Emilia-Romagna	Ravenna - Forli-Cesena	02/05/2022	Ravenna	240000
SARI9983	Emilia-Romagna	Forli-Cesena	03/05/2022	Cesena	197500
SARI9984	Emilia-Romagna	Forli-Cesena	03/05/2022	Forli	250000
SARI9986	Emilia-Romagna	Rimini - Forli-Cesena	04/05/2022	S. Giustina	560000
SARI10078	Friuli-Venezia Giulia	Udine	03/05/2022	Udine	200000
SARI10015	Friuli-Venezia Giulia	Pordenone	03/05/2022	Cordenons	15000
SARI10016	Friuli-Venezia Giulia	Trieste	03/05/2022	Servola	190000
SARI9832	Lazio	Roma	03/05/2022	Civitavecchia Fiumaretta	86400
SARI9838	Lazio	Viterbo	02/05/2022	Viterbo - Strada Bagni	30000
SARI9839	Lazio	Roma	02/05/2022	Velletri (LA CHIUSA-SORBO)	36700
SARI9840	Lazio	Roma	02/05/2022	Pomezia - Via Cincinnato	60000
SARI9841	Lazio	Roma	02/05/2022	Guidonia - Ponte Lucano	50000
SARI9842	Lazio	Roma	02/05/2022	Anzio - Colle Cocchino	75000
SARI9843	Lazio	Latina	02/05/2022	Aprilia (Via del Campo)	66000

SARI9844	Lazio	Latina	02/05/2022	Latina Loc Latina Est	90000
SARI9992	Lazio	Roma	03/05/2022	Roma Est (linea 1 + linea 2)	900000
SARI9993	Lazio	Roma	03/05/2022	Roma Nord	780000
SARI9996	Lazio	Roma	04/05/2022	Roma Sud	1100000
SARI9999	Lazio	Roma	04/05/2022	Ostia	350000
SARI10009	Lazio	Roma	04/05/2022	Fregene	76000
SARI9892	Liguria	Genova	03/05/2022	Punta Vagno Genova	75000
SARI9966	Liguria	Genova	03/05/2022	Voltri	40496
SARI9967	Liguria	Genova	03/05/2022	Quinto	48748
SARI9969	Liguria	Genova	03/05/2022	Rapallo	90000
SARI9971	Liguria	Genova	03/05/2022	Sestri P	51368
SARI9973	Liguria	Genova	03/05/2022	Sturla	43573
SARI9974	Liguria	La Spezia	03/05/2022	Camisano	40840
SARI9975	Liguria	La Spezia	03/05/2022	Silea	17500
SARI9976	Liguria	La Spezia	03/05/2022	La Spezia	82000
SARI9977	Liguria	Imperia	03/05/2022	Sanremo - località Capo Verde	80000
SARI9978	Liguria	Savona	03/05/2022	Borghetto Santo Spirito	140000
SARI9979	Liguria	Savona	03/05/2022	Savona	256203
SARI9980	Liguria	Genova	04/05/2022	Darsena	118276
SARI9981	Liguria	Genova	04/05/2022	Punta Vagno Genova	75000
SARI9982	Liguria	Genova	04/05/2022	Valpolcevera	157650
SARI9878	Lombardia	Milano	02/05/2022	Bresso	220000
SARI9879	Lombardia	Milano - Monza e della Brianza	02/05/2022	Peschiera Borromeo	566000
SARI9880	Lombardia	Milano - Varese	02/05/2022	Canegrate	137950
SARI9881	Lombardia	Varese	02/05/2022	Varese	74402
SARI9882	Lombardia	Milano - Varese	02/05/2022	Lonate Pozzolo	450000
SARI9942	Lombardia	Milano	02/05/2022	Milano Nosedo	1250000
SARI9943	Lombardia	Milano	02/05/2022	Milano San Rocco	1036000
SARI9944	Lombardia	Como	02/05/2022	Como	196000
SARI9945	Lombardia	Pavia	02/05/2022	Pavia	132912
SARI9946	Lombardia	Como - Lecco - Milano - Monza e della Brianza	02/05/2022	Monza	600000
SARI9947	Lombardia	Pavia	04/05/2022	Vigevano	57925
SARI9972	Lombardia	Sondrio	04/05/2022	Sondrio	49500
SARI10010	Lombardia	Bergamo	03/05/2022	Bergamo	220000
SARI10011	Lombardia	Cremona	03/05/2022	Citta di Cremona	180000
SARI10013	Lombardia	Brescia	03/05/2022	Verziano	296000
SARI9914	Marche	Pesaro-Urbino	03/05/2022	Borgheria	116000
SARI9915	Marche	Pesaro-Urbino	03/05/2022	Ponte Metauro	60000
SARI9916	Marche	Pesaro-Urbino	03/05/2022	Ponte Sasso	18000
SARI9917	Marche	Ancona	03/05/2022	Zipa	100000
SARI9918	Marche	Ancona	03/05/2022	Falconara	85000
SARI9919	Marche	Ancona	03/05/2022	Camerano	33000
SARI10001	Molise	Campobasso	03/05/2022	Termoli - località Porto	25000
SARI10002	Molise	Campobasso	03/05/2022	Termoli - località Pantano Basso	25000
SARI9858	P.A. Bolzano	Bolzano	02/05/2022	IDA Bolzano	372410
SARI9860	P.A. Bolzano	Bolzano	02/05/2022	IDA Merano	356520
SARI9862	P.A. Bolzano	Bolzano	02/05/2022	IDA Termeno	68945
SARI9835	P.A. Trento	Trento	02/05/2022	Trento nord	120000
SARI9836	P.A. Trento	Trento	02/05/2022	Trento sud	100000
SARI9837	P.A. Trento	Trento	02/05/2022	Rovereto	95000
SARI9829	Piemonte	Biella	02/05/2022	Biella Nord	67000

SARI9830	Piemonte	Novara	02/05/2022	Novara	184000
SARI9831	Piemonte	Torino	02/05/2022	Castiglione Torinese	1934099
SARI9887	Piemonte	Alessandria	04/05/2022	Alessandria	110000
SARI9888	Piemonte	Cuneo	04/05/2022	Cuneo	185000
SARI9821	Puglia	Bari	02/05/2022	Bari Ovest	360000
SARI9822	Puglia	Bari	02/05/2022	Bari Est	389000
SARI9823	Puglia	Bari	02/05/2022	Altamura	95414
SARI9824	Puglia	Lecce	02/05/2022	Lecce	195368
SARI9825	Puglia	Brindisi	02/05/2022	Brindisi Fiume Grande	93013
SARI9826	Puglia	Taranto	02/05/2022	Taranto Bellavista	116723
SARI9827	Puglia	Taranto	02/05/2022	Taranto Gennarini	226667
SARI9845	Puglia	Barletta-Andria-Trani	03/05/2022	Andria	130000
SARI9846	Puglia	Barletta-Andria-Trani	03/05/2022	Barletta	129356
SARI9847	Puglia	Foggia	03/05/2022	Foggia	208000
SARI9848	Puglia	Foggia	03/05/2022	Manfredonia	77000
SARI9849	Puglia	Foggia	03/05/2022	Cerignola	56355
SARI9873	Puglia	Bari	04/05/2022	Molfetta	84803
SARI9874	Puglia	Bari	04/05/2022	Bitonto	79332
SARI9875	Puglia	Barletta-Andria-Trani	04/05/2022	Trani	83667
SARI9877	Puglia	Barletta-Andria-Trani	04/05/2022	Bisceglie	85714
SARI9883	Sicilia	Trapani	03/05/2022	Trapani	118500
SARI9884	Sicilia	Trapani	03/05/2022	Marsala	40000
SARI9885	Sicilia	Trapani	03/05/2022	Mazara del Vallo	17000
SARI9920	Sicilia	Agrigento	02/05/2022	Agrigento	55000
SARI9921	Sicilia	Enna	02/05/2022	Enna	34000
SARI9922	Sicilia	Palermo	03/05/2022	Bagheria	75000
SARI9923	Sicilia	Palermo	03/05/2022	Acqua dei Corsari	314973
SARI9924	Sicilia	Palermo	03/05/2022	Fondo Verde	53886
SARI9925	Sicilia	Caltanissetta	03/05/2022	Caltanissetta e San Cataldo	76700
SARI10004	Sicilia	Ragusa	05/05/2022	Modica	50400
SARI10005	Sicilia	Ragusa	03/05/2022	Vittoria	55000
SARI10006	Sicilia	Ragusa	05/05/2022	Ragusa	98000
SARI10007	Sicilia	Caltanissetta	03/05/2022	Gela Macchitella	12000
SARI10008	Sicilia	Messina	03/05/2022	Mili Marina	227000
SARI10030	Sicilia	Catania	03/05/2022	Pantano d'Arci	68434
SARI10032	Sicilia	Catania	05/05/2022	Giarre	47600
SARI10033	Sicilia	Siracusa	05/05/2022	Siracusa	180000
SARI9926	Toscana	Pisa	03/05/2022	Pisa Nord - S. Jacopo	52000
SARI9927	Toscana	Firenze	03/05/2022	Empoli Pagnana	88670
SARI9928	Toscana	Massa	03/05/2022	Lavello 2	120000
SARI9929	Toscana	Lucca	03/05/2022	Viareggio	93000
SARI9930	Toscana	Massa	03/05/2022	Lavello 1	87000
SARI9931	Toscana	Lucca	03/05/2022	Pontetetto	95000
SARI9932	Toscana	Livorno	03/05/2022	Rivellino	21000
SARI9933	Toscana	Livorno	04/05/2022	Rivellino	21000
SARI9828	Umbria	Perugia	02/05/2022	Perugia - Pian della Genna	90000
SARI9911	Umbria	Perugia	05/05/2022	Foligno Casone	90000
SARI9912	Umbria	Terni	05/05/2022	Terni	150000
SARI9990	Valle d'Aosta	Aosta	04/05/2022	La Salle	60000
SARI9991	Valle d'Aosta	Aosta	04/05/2022	Brissogne	150000
SARI9850	Veneto	Padova	03/05/2022	Padova Ca' Nordio - centro storico	98500

SARI9851	Veneto	Padova	03/05/2022	Padova Ca' Nordio - zip	98500
SARI9852	Veneto	Padova	03/05/2022	Padova Guizza	13000
SARI9853	Veneto	Padova	03/05/2022	Abano Terme	35000
SARI9868	Veneto	Treviso	03/05/2022	Treviso	70000
SARI9869	Veneto	Vicenza	03/05/2022	Vicenza Casale	92000
SARI9870	Veneto	Venezia	03/05/2022	Venezia Fusina	400000
SARI9904	Veneto	Verona	05/05/2022	Verona_collettore 1M	82000
SARI9905	Veneto	Verona	05/05/2022	Verona_collettore 3M	102000
SARI9906	Veneto	Verona	05/05/2022	Verona_collettore 8M	226000

‡ Parameter describing the design treatment capacity of WTPs. It is a measure of total organic biodegradable load in a WTP, including industrial, commercial and domestic organic load, converted to the equivalent number of population (population equivalents)

Samples were processed by the laboratories of the SARI network (see Acknowledgements). Viral concentration measurements and nucleic acid extraction were performed according to the protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3”. Purified RNAs were shipped in dry ice to ISS, where samples were sequenced as previously described² with some modifications.

A long nested RT-PCR assay (ID_980, ~1600 bps, spanning amino acid residues 58 to 573 of the spike protein) was used to detect multiple key nucleotide changes (deletion and/or amino acid substitutions) distinctive of the Variants of Concern (VoCs) and Variants of Interest (VoIs).

The amplicons from the long nested assay were sequenced by both Sanger and Next Generation Sequencing (NGS), using the Oxford Nanopore Technology MinION platform, for a more in-depth analysis. For NGS, the amplicons obtained from different samples collected in the same Region were mixed in a single pool.

Bioinformatics analysis of NGS data was carried out as described in La Rosa et al., 2021³. Variant calling was performed for the currently recognized VoCs (Omicron, lineages BA.1, BA.2, BA.4, and BA.5) and for the de-escalated variants (Alpha, Beta, Gamma, Delta, Lambda and Mu)⁴.

Results

Overall, 149 of the 158 samples (94%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 environmental surveillance (**Table 2**), viral concentrations ranging from 2.7 E+01 to 4.6 E+05 genome copies (g.c.)/L of sewage.

Sanger Sequencing

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 2-3**. Overall, 55 samples from 17 Regions/A.P. were amplified by the long PCR assay. Long amplicons could not be obtained from samples collected in the Regions of Abruzzo, Campania and Molise. Sanger sequences were obtained for 54 samples, whereas – due to the simultaneous presence of more

2 G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferrero, D. Brandtner, A. Fasanello, L. Pace, A. Parisi, D. Galante, E. Suffredini. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein, *Water Research*, 2021, Volume 197, 1 June 2021, 117104. <https://doi.org/10.1016/j.watres.2021.117104>.

3 G La Rosa, D. Brandtner, P. Mancini, C. Veneri, G. Bonanno Ferraro, L. Bonadonna, L. Lucentini, E. Suffredini. Key SARS-CoV-2 mutations of Alpha, Gamma and Eta variants detected in urban wastewaters in Italy by long-read amplicon sequencing based on nanopore technology. *Water* 2021, 13(18), 2503; <https://doi.org/10.3390/w13182503>

4 SARS-CoV-2 variants of concern as of 9 June 2022. [SARS-CoV-2 variants of concern as of 9 June 2022 \(europa.eu\)](https://ec.europa.eu/eurosurv/infodashboard/sars-cov-2-variants)

than one strain – mixed electropherograms were obtained for one sample, which could not therefore be assigned. Key mutations of the Omicron BA.2 subvariant were detected in all the samples.

Next Generation Sequencing

NGS results could be successfully obtained for 17 Regions, all of which showing characteristic mutations of the Omicron variant sublineage BA.2. Mutations associated with other VOCs (included BA.4 and BA.5) or with de-escalated variants were not detected.

Sequencing results are summarized in Tables 2. To improve readability of the table, mutations were combined into panels ('mutation packages') as follow:

- **Package A** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H

Table 2. PCR and sequencing results (long PCR ID 980)

Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_980)	SARS-CoV-2 variant (sanger sequencing)	Sequencing results (NGS)	SARS-CoV-2 variant (NGS)
1	Abruzzo	Pescara	Via Raiale	1,00E+02	-			
2		Pescara	Villa Carmine	1,00E+02	-			
3		Chieti	S. Martino	<LOD	-			
4		L'Aquila	Pile	<LOD	-			
5		Teramo	Villa Pavone	1,25E+02	-			
6	Basilicata	Potenza	Tiera di Vaglio	1,60E+04	Package A + F490V	Omicron BA.2	• Package A	• Omicron BA.2
7		Matera	Pantano	1,60E+04	-			
8	Calabria	Cosenza	Cosenza - Code di volpe	7,71E+01	-		• Package A	• Omicron BA.2
9		Cosenza	Cosenza - Sant'Angelo	1,61E+02	-			
10		Reggio Calabria	Ravagnese - località Aeroporto	1,91E+02	Package A	Omicron BA.2		
11		Catanzaro	Catanzaro - Zona industriale	2,72E+01	-			
12		Crotone	Crotone - località Papaniciaro	6,72E+01	-			
13		Catanzaro	Catanzaro Lido - Loc. Verghello	1,01E+02	-			
14		Campania	Napoli	Napoli OVEST - Ingresso Principale	1,31E+05	-		
15	Salerno		Salerno	3,85E+04	-			
16	Caserta		Villa Literno	5,33E+04	-			
17	Avellino		Manocalzati	1,91E+04	-			
18	Salerno		Nocera Sup	5,84E+04	-			
19	Napoli		Napoli EST	2,27E+04	-			
20	Napoli		Napoli OVEST - ex ingresso Camaldoli	2,52E+04	-			

21	10061		Caserta	Area Casertana	1,46E+04	-		
158	10064		Salerno	Eboli	7,53E+04	-		
159	10085		Napoli	Area Nolana	4,90E+04	-		
22	9889		Ferrara	Ferrara - Linea 1	1,68E+03	-	• Package A	• Omicron BA.2
23	9890		Ferrara	Ferrara - Linea 2	<LOD	Package A		Omicron BA.2
24	9891		Modena	Carpi	1,10E+03	-		
25	9895		Piacenza	Borgoforte	<LOD	Package A		Omicron BA.2
26	9896		Parma	Parma ovest	6,75E+04	M.E. ^b		
27	9898		Reggio Emilia	Mancasale	4,33E+04	Package A		Omicron BA.2
28	9907		Bologna	IDAR	3,38E+05	Package A		Omicron BA.2
29	9908	Emilia-Romagna	Ravenna	Faenza	1,02E+05	-		
30	9910		Modena	Naviglio	1,57E+05	Package A		Omicron BA.2
31	9913		Bologna	Imola	4,13E+05	-		
32	9948		Ravenna - Forlì-Cesena	Ravenna	6,33E+04	-		
155	9983		Forlì-Cesena	Cesena	1,87E+05	-		
156	9984		Forlì-Cesena	Forlì	5,51E+04	-		
157	9986		Rimini - Forlì-Cesena	S. Giustina	5,95E+04	-		
33	10078	Friuli-Venezia Giulia	Udine	Udine	2,19E+05	Package A	Package A	Omicron BA.2 • Omicron BA.2
34	10015		Pordenone	Cordenons	3,53E+04	-		
35	10016		Trieste	Servola	1,49E+04	Package A		Omicron BA.2
36	9832		Roma	Civitavecchia Fiumaretta	2,50E+04	-	• Package A	• Omicron BA.2
37	9838		Viterbo	Viterbo - Strada Bagni	9,88E+03	-		
38	9839	Lazio	Roma	Velletri (LA CHIUSA-SORBO)	9,45E+03	-		
39	9840		Roma	Pomezia - Via Cincinnato	2,01E+04	-		
40	9841		Roma	Guidonia - Ponte Lucano	1,52E+04	-		
41	9842		Roma	Anzio - Colle Cocchino	3,03E+03	-		

42	9843		Latina	Aprilia (Via del Campo)	5,55E+03	-		
43	9844		Latina	Latina Loc Latina Est	4,08E+03	-		
44	9992		Roma	Roma Est (linea 1 + linea 2)	4,60E+05	-		
45	9993		Roma	Roma Nord	3,04E+05	Package A		Omicron BA.2
46	9996		Roma	Roma Sud	1,83E+05	Package A		Omicron BA.2
47	9999		Roma	Ostia	1,96E+05	-		
48	10009		Roma	Fregene	3,60E+05	-		
49	9892		Genova	Punta Vagno Genova	4,63E+04	-	• Package A	• Omicron BA.2
50	9966		Genova	Voltri	4,71E+04	Package A		Omicron BA.2
51	9967		Genova	Quinto	1,11E+05	Package A		Omicron BA.2
52	9969		Genova	Rapallo	1,05E+05	-		
53	9971		Genova	Sestri P	1,29E+05	-		
54	9973		Genova	Sturla	7,11E+04	-		
55	9974		La Spezia	Camisano	9,03E+03	-		
56	9975		La Spezia	Silea	1,61E+05	Package A		Omicron BA.2
57	9976	Liguria	La Spezia	La Spezia	1,56E+05	Package A		Omicron BA.2
58	9977		Imperia	Sanremo - località Capo Verde	1,41E+05	-		
59	9978		Savona	Borghetto Santo Spirito	7,23E+04	Package A		Omicron BA.2
60	9979		Savona	Savona	1,20E+05	Package A		Omicron BA.2
61	9980		Genova	Darsena	7,79E+04	Package A		Omicron BA.2
62	9981		Genova	Punta Vagno Genova	1,88E+05	-		
63	9982		Genova	Valpolcevera	4,73E+04	-		
64	9878		Milano	Bresso	1,37E+04	-	• Package A	• Omicron BA.2
65	9879		Milano - Monza e della Brianza	Peschiera Borromeo	2,31E+04	-		
66	9880	Lombardia	Milano - Varese	Canegrate	4,70E+03	-		
67	9881		Varese	Varese	7,03E+03	-		
68	9882		Milano - Varese	Lonate Pozzolo	2,75E+04	-		

69	9942		Milano	Milano Nosedo	1,11E+03	-		
70	9943		Milano	Milano San Rocco	2,77E+03	-		
71	9944		Como	Como	<LOD	-		
72	9945		Pavia	Pavia	8,32E+02	-		
73	9946		Como - Lecco - Milano - Monza e della Brianza	Monza	2,41E+03	-		
74	9947		Pavia	Vigevano	1,39E+03	-		
75	9972		Sondrio	Sondrio	2,30E+04	-		
76	10010		Bergamo	Bergamo	1,85E+04	Package A	Omicron BA.2	
77	10011		Cremona	Citta di Cremona	1,41E+04	-		
78	10013		Brescia	Verziano	3,00E+04	Package A	Omicron BA.2	
79	9914	Marche	Pesaro-Urbino	Borgheria	2,56E+04	-	• Package A	• Omicron BA.2
80	9915		Pesaro-Urbino	Ponte Metauro	1,31E+04	Package A	Omicron BA.2	
81	9916		Pesaro-Urbino	Ponte Sasso	1,10E+04	-		
82	9917		Ancona	Zipa	1,79E+04	-		
83	9918		Ancona	Falconara	2,85E+03	-		
84	9919		Ancona	Camerano	1,60E+03	-		
85	10001	Molise	Campobasso	Termoli - località Porto	1,00E+02	-		
86	10002		Campobasso	Termoli - località Pantano Basso	1,00E+02	-		
87	9858	P.A. Bolzano	Bolzano	IDA Bolzano	3,36E+04	-	• Package A	• Omicron BA.2
88	9860		Bolzano	IDA Merano	2,77E+04	Package A	Omicron BA.2	
89	9862		Bolzano	IDA Termeno	3,51E+04	Package A	Omicron BA.2	
90	9835	P.A. Trento	Trento	Trento nord	1,07E+05	Package A	Omicron BA.2	• Package A • Omicron BA.2
91	9836		Trento	Trento sud	3,09E+04	-		
92	9837		Trento	Rovereto	5,82E+04	-		
93	9829	Piemonte	Biella	Biella Nord	3,10E+03	Package A	Omicron BA.2	• Package A • Omicron BA.2
94	9830		Novara	Novara	2,40E+03	-		
95	9831		Torino	Castiglione Torinese	6,98E+03	-		
96	9887		Alessandria	Alessandria	1,46E+03	-		

97	9888		Cuneo	Cuneo	5,30E+03	Package A	Omicron BA.2		
98	9821		Bari	Bari Ovest	1,98E+04	Package A	Omicron BA.2	• Package A	• Omicron BA.2
99	9822		Bari	Bari Est	2,36E+04	Package A	Omicron BA.2		
100	9823		Bari	Altamura	1,31E+04	Package A	Omicron BA.2		
101	9824		Lecce	Lecce	1,56E+04	Package A	Omicron BA.2		
102	9825		Brindisi	Brindisi Fiume Grande	2,70E+03	Package A	Omicron BA.2		
103	9826		Taranto	Taranto Bellavista	1,29E+04	Package A	Omicron BA.2		
104	9827		Taranto	Taranto Gennarini	2,12E+04	Package A	Omicron BA.2		
105	9845	Puglia	Barletta-Andria-Trani	Andria	1,16E+04	Package A	Omicron BA.2		
106	9846		Barletta-Andria-Trani	Barletta	3,34E+03	Package A	Omicron BA.2		
107	9847		Foggia	Foggia	4,13E+03	Package A	Omicron BA.2		
108	9848		Foggia	Manfredonia	2,12E+03	-			
109	9849		Foggia	Cerignola	4,45E+03	Package A + K444N	Omicron BA.2		
110	9873		Bari	Molfetta	4,67E+03	Package A + S469P	Omicron BA.2		
111	9874		Bari	Bitonto	4,96E+03	Package A + T76I	Omicron BA.2		
112	9875		Barletta-Andria-Trani	Trani	1,51E+03	Package A	Omicron BA.2		
114	9877		Barletta-Andria-Trani	Bisceglie	5,25E+03	Package A	Omicron BA.2		
115	9883			Trapani	Trapani	1,24E+04	-		• Package A
116	9884		Trapani	Marsala	9,01E+03	-			
117	9885		Trapani	Mazara del Vallo	7,13E+03	-			
118	9920		Agrigento	Agrigento	8,83E+04	-			
119	9921	Sicilia	Enna	Enna	8,73E+04	Package A	Omicron BA.2		
120	9922		Palermo	Bagheria	6,04E+04	-			
121	9923		Palermo	Acqua dei Corsari	1,04E+05	-			
122	9924		Palermo	Fondo Verde	7,24E+04	-			
123	9925		Caltanissetta	Caltanissetta e San Cataldo	2,03E+05	Package A	Omicron BA.2		
124	10004		Ragusa	Modica	7,25E+03	-			

125	10005		Ragusa	Vittoria	2,26E+04	-		
126	10006		Ragusa	Ragusa	6,85E+03	-		
127	10007		Caltanissetta	Gela Macchitella	6,50E+03	-		
128	10008		Messina	Mili Marina	2,80E+03	-		
129	10030		Catania	Pantano d'Arci	1,64E+05	-		
130	10032		Catania	Giarre	2,83E+05	-		
131	10033		Siracusa	Siracusa	9,67E+04	-		
132	9926	Toscana	Pisa	Pisa Nord - S. Jacopo	5,62E+04	-	• Package A	• Omicron BA.2
133	9927		Firenze	Empoli Pagnana	4,74E+03	-		
134	9928		Massa	Lavello 2	4,13E+04	-		
135	9929		Lucca	Viareggio	<LOD	-		
136	9930		Massa	Lavello 1	<LOD	-		
137	9931		Lucca	Pontetetto	6,83E+03	-		
138	9932		Livorno	Rivellino	<LOD	Package A + Y200F	Omicron BA.2	
139	9933		Livorno	Rivellino	<LOD	-		
140	9828		Umbria	Perugia	Perugia - Pian della Genna	1,59E+05	Package A + G504C	Omicron BA.2
141	9911	Perugia		Foligno Casone	8,80E+04	Package A	Omicron BA.2	
142	9912	Terni		Terni	1,05E+05	Package A	Omicron BA.2	
143	9990	Valle d'Aosta	Aosta	La Salle	1,33E+04	Package A	Omicron BA.2	• Package A • Omicron BA.2
144	9991		Aosta	Brissogne	1,04E+04	-		
145	9850	Veneto	Padova	Padova Ca' Nordio - centro storico	1,46E+05	Package A	Omicron BA.2	• Package A • Omicron BA.2
146	9851		Padova	Padova Ca' Nordio - zip	9,99E+04	-		
147	9852		Padova	Padova Guizza	8,83E+04	Package A (Partial, from G142D to V213G) ^a Package A (Partial, from G339D to Y505H)	Omicron BA.2	
148	9853		Padova	Abano Terme	1,97E+04	-		
149	9868		Treviso	Treviso	1,03E+04	Package A	Omicron BA.2	

				(Partial, from G142D to V213G) ^a Package A (Partial, from G339D to Y505H)	
150	9869	Vicenza	Vicenza Casale	1,15E+03	-
151	9870	Venezia	Venezia Fusina	1,01E+04	Package A Omicron BA.2
152	9904	Verona	Verona_collettore 1M	2,21E+04	-
153	9905	Verona	Verona_collettore 3M	2,82E+04	Package A Omicron BA.2
154	9906	Verona	Verona_collettore 8M	2,31E+04	Package A (Partial, from G339D to Y505H) ^a Omicron BA.2

^a Partial sequence due to mixed electropherograms and/or high signal noise; within brackets the region for which a sequence was provided;

^b M.E. mixed electropherograms

- **Package A** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H

Table 3. Sanger sequencing results (long PCR ID980)

	T76I	G142D	Y200F	V213G	G339D	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	K444N	S469P	S477N	T478K	E484A	F490V	Q493R	G496S	Q498R	N501Y	Y505H	G504C	
10, 23, 25, 27, 28, 30, 33, 35, 45, 46, 50, 51, 56, 57, 59, 60, 61, 76, 78, 80, 88, 89, 90, 93, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 112, 114, 119, 123, 141, 142, 143, 145, 147*, 149*, 151, 153, 154*																										Package A (Omicron BA.2)
111																										Package A + T76I (Omicron BA.2)
138																										Package A + Y200F (Omicron BA.2)
109																										Package A + K444N (Omicron BA.2)
110																										Package A + S469P (Omicron BA.2)
6																										Package A + F490V (Omicron BA.2)
140																										Package A + G504C (Omicron BA.2)

* Partial sequence

Limitations of the study

This flash survey's geographical and population coverage was incomplete, as it covered 20/21 of the Italian regions/Autonomous Provinces.

Molecular analytical methods applied to complex environmental matrices as wastewaters may be hampered by low viral concentration, poor recovery of the analyte, and/or inhibition of PCR amplification. Therefore, both detection/quantification and PCR amplification for sequencing purposes may produce false negatives. Consequently, molecular characterization and variant detection may not be achieved for all samples.

Partial sequencing of the Spike region does not allow conclusive assignation of sublineages. However, the detection within the Spike region of multiple, linked mutations associated to specific lineages/sublineages is strongly suggestive of their presence. Therefore, the detection, either by Sanger or NGS sequencing, of defined mutation panels characteristic of certain lineages/sublineages should be considered as a presumptive detection.

Conclusions and final considerations

This is the ninth of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters that will continue to be issued as a part of the surveillance established in Italy under EU Commission Recommendation 2021/472, with the aim of providing information on SARS-CoV-2 variants in the population to supplement information acquired through the clinical surveillance.

The results of SARS-CoV-2 surveillance in wastewaters show the exclusive presence of the Omicron variant, lineage BA.2. Mutations of sublineages BA.1 and BA.1.1, still present in April 2022 have therefore disappeared in May. Sublineages BA.4/BA.5 were not detected, as well as key mutations of other de-escalated VOCs.

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