



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
8th Report
(Study period: 4 - 8 April 2022)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Marcello Iaconelli, Luca Lucentini, Lucia Bonadonna (Department of Environment and Health, Istituto Superiore di Sanità)
- David Brandtner (independent researcher)
- Mauro Grigioni (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Mirko Rossi (independent researcher)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità)

Main findings:

- Overall, 161 wastewater samples were collected in the week 4-8 April from 18 Regions and 2 Autonomous Provinces (A.P.).
- Only mutations characteristic of the Omicron variant were detected, found in 16 Regions and two A.P. (no sequences were obtained from the remaining two Regions).
- Aminoacid substitutions of sublineage BA.2 were detected in 97% of the sequences (17 Regions/A.P.) by Sanger sequencing, while mutations of sublineages BA.1.1 were detected in 3% of the samples.
- Sublineages BA.4/BA.5 were not detected.
- NGS results confirmed the predominance of sublineage BA.2, detected in all the NGS pools.

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 eighth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 4-8 April 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, 161 wastewater samples were collected between 4 and 8 April 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
SARI9170	Abruzzo	Chieti	05/04/2022	S. Martino	114500
SARI9171	Abruzzo	Pescara	05/04/2022	Via Raiale	160000
SARI9172	Abruzzo	Pescara	05/04/2022	Villa Carmine	140000
SARI9173	Abruzzo	L'Aquila	05/04/2022	Pile	48000
SARI9177	Abruzzo	Teramo	05/04/2022	Villa Pavone	41824
SARI9293	Basilicata	Potenza	05/04/2022	Tiera di Vaglio	95000
SARI9294	Basilicata	Matera	05/04/2022	Pantano	24000
SARI9267	Calabria	Cosenza	05/04/2022	Cosenza - Sant'Angelo	45000
SARI9268	Calabria	Cosenza	05/04/2022	Cosenza - Code di volpe	191000
SARI9269	Calabria	Crotone	05/04/2022	Crotone - località Papaniciaro	60000
SARI9270	Calabria	Catanzaro	05/04/2022	Catanzaro Lido - Loc. Verghello	90000
SARI9271	Calabria	Catanzaro	05/04/2022	Catanzaro - Zona industriale	120000
SARI9272	Calabria	Reggio Calabria	05/04/2022	Ravagnese - località Aeroporto	120000
SARI9251	Campania	Caserta	07/04/2022	Area Casertana	370769
SARI9266	Campania	Avellino	07/04/2022	Manocalzati	631714
SARI9254	Campania	Caserta	07/04/2022	Villa Literno	30000
SARI9342	Campania	Napoli	05/04/2022	Napoli OVEST - Ingresso Principale	400000
SARI9343	Campania	Napoli	05/04/2022	Napoli OVEST - ex ingresso Camaldoli	140000
SARI9255	Campania	Salerno	07/04/2022	Eboli	950000
SARI9346	Campania	Napoli	05/04/2022	Napoli EST	250000
SARI9350	Campania	Salerno	05/04/2022	Nocera Sup	1750000
SARI9352	Campania	Salerno	05/04/2022	Salerno	299121
SARI9256	Campania	Napoli	07/04/2022	Area Nolana	700000
SARI9142	Emilia-Romagna	Ferrara	05/04/2022	Ferrara - Linea 1	120000
SARI9144	Emilia-Romagna	Ferrara	05/04/2022	Ferrara - Linea 2	120000
SARI9146	Emilia-Romagna	Modena	05/04/2022	Carpi	200000
SARI9189	Emilia-Romagna	Piacenza	05/04/2022	Borgoforte	163333
SARI9190	Emilia-Romagna	Parma	04/04/2022	Parma ovest	168000
SARI9192	Emilia-Romagna	Reggio Emilia	04/04/2022	Mancasale	280000
SARI9195	Emilia-Romagna	Bologna	04/04/2022	IDAR	800000
SARI9196	Emilia-Romagna	Modena	04/04/2022	Naviglio	500000
SARI9197	Emilia-Romagna	Ravenna	04/04/2022	Faenza	100000
SARI9200	Emilia-Romagna	Bologna	04/04/2022	Imola	75000
SARI9201	Emilia-Romagna	Ravenna - Forli-Cesena	04/04/2022	Ravenna	240000
SARI9205	Emilia-Romagna	Forli-Cesena	05/04/2022	Cesena	197500
SARI9207	Emilia-Romagna	Forli-Cesena	05/04/2022	Forlì	250000
SARI9212	Emilia-Romagna	Rimini - Forli-Cesena	06/04/2022	S. Giustina	560000
SARI9223	Friuli-Venezia Giulia	Pordenone	05/04/2022	Cordenons	15000
SARI9224	Friuli-Venezia Giulia	Udine	05/04/2022	Udine	200000
SARI9225	Friuli-Venezia Giulia	Trieste	05/04/2022	Servola	190000
SARI8989	Lazio	Viterbo	04/04/2022	Viterbo - Strada Bagni	30000
SARI8990	Lazio	Latina	04/04/2022	Aprilia (Via del Campo)	66000
SARI8991	Lazio	Latina	04/04/2022	Latina Loc Latina Est	90000
SARI8992	Lazio	Roma	04/04/2022	Anzio - Colle Cocchino	75000
SARI8993	Lazio	Roma	04/04/2022	Guidonia - Ponte Lucano	50000
SARI8994	Lazio	Roma	04/04/2022	Velletri (LA CHIUSA-SORBO)	36700
SARI8995	Lazio	Roma	04/04/2022	Pomezia - Via Cincinnato	60000

SARI9127	Lazio	Roma	05/04/2022	Civitavecchia Fiumaretta	86400
SARI9143	Lazio	Roma	05/04/2022	Roma Est (linea 1 + linea 2)	900000
SARI9145	Lazio	Roma	05/04/2022	Roma Nord	780000
SARI9147	Lazio	Roma	06/04/2022	Roma Sud	1100000
SARI9148	Lazio	Roma	06/04/2022	Ostia	350000
SARI9149	Lazio	Roma	06/04/2022	Fregene	76000
SARI9112	Liguria	Genova	05/04/2022	Pegli	20507
SARI9113	Liguria	Genova	05/04/2022	Voltri	40496
SARI9114	Liguria	Genova	05/04/2022	Quinto	48748
SARI9115	Liguria	Genova	05/04/2022	Rapallo	90000
SARI9116	Liguria	Genova	05/04/2022	Sestri P	51368
SARI9117	Liguria	Genova	05/04/2022	Sturla	43573
SARI9118	Liguria	Savona	05/04/2022	Borghetto Santo Spirito	140000
SARI9119	Liguria	Imperia	05/04/2022	Sanremo - località Capo Verde	80000
SARI9120	Liguria	Savona	05/04/2022	Savona	256203
SARI9121	Liguria	La Spezia	05/04/2022	Camisano	40840
SARI9122	Liguria	La Spezia	05/04/2022	Silea	17500
SARI9123	Liguria	La Spezia	05/04/2022	La Spezia	82000
SARI9124	Liguria	Genova	06/04/2022	Darsena	118276
SARI9125	Liguria	Genova	06/04/2022	Punta Vagno Genova	75000
SARI9126	Liguria	Genova	06/04/2022	Valpolcevera	157650
SARI9186	Liguria	Genova	05/04/2022	Punta Vagno Genova	75000
SARI9015	Lombardia	Milano	04/04/2022	Bresso	220000
SARI9016	Lombardia	Milano - Monza e della Brianza	04/04/2022	Peschiera Borromeo	566000
SARI9017	Lombardia	Milano - Varese	04/04/2022	Canegrate	137950
SARI9018	Lombardia	Varese	04/04/2022	Varese	74402
SARI9019	Lombardia	Milano - Varese	04/04/2022	Lonate Pozzolo	450000
SARI9072	Lombardia	Milano	04/04/2022	Milano Nosedo	1250000
SARI9073	Lombardia	Milano	04/04/2022	Milano San Rocco	1036000
SARI9074	Lombardia	Como	04/04/2022	Como	196000
SARI9075	Lombardia	Pavia	04/04/2022	Pavia	132912
SARI9076	Lombardia	Como - Lecco - Milano - Monza e della Brianza	04/04/2022	Monza	600000
SARI9077	Lombardia	Pavia	06/04/2022	Vigevano	57925
SARI9198	Lombardia	Bergamo	05/04/2022	Bergamo	220000
SARI9202	Lombardia	Cremona	05/04/2022	Citta di Cremona	180000
SARI9203	Lombardia	Sondrio	06/04/2022	Sondrio	49500
SARI9206	Lombardia	Brescia	05/04/2022	Verziano	296000
SARI9152	Marche	Pesaro-Urbino	05/04/2022	Borgheria	116000
SARI9153	Marche	Pesaro-Urbino	05/04/2022	Ponte Metauro	60000
SARI9154	Marche	Pesaro-Urbino	05/04/2022	Ponte Sasso	18000
SARI9155	Marche	Ancona	05/04/2022	Zipa	100000
SARI9156	Marche	Ancona	05/04/2022	Falconara	85000
SARI9157	Marche	Ancona	05/04/2022	Camerano	33000
SARI9174	Molise	Campobasso	04/04/2022	Campobasso - San Pietro	50000
SARI9175	Molise	Campobasso	04/04/2022	Termoli - località Porto	25000
SARI9176	Molise	Campobasso	04/04/2022	Termoli - località Pantano Basso	25000
SARI9012	P.A. Bolzano	Bolzano	04/04/2022	IDA Bolzano	372410
SARI9013	P.A. Bolzano	Bolzano	04/04/2022	IDA Merano	356520
SARI9014	P.A. Bolzano	Bolzano	04/04/2022	IDA Termeno	68945
SARI8996	P.A. Trento	Trento	04/04/2022	Trento nord	120000
SARI8997	P.A. Trento	Trento	04/04/2022	Trento sud	100000

SARI8998	P.A. Trento	Trento	04/04/2022	Rovereto	95000
SARI8983	Piemonte	Biella	04/04/2022	Biella Nord	67000
SARI8984	Piemonte	Torino	04/04/2022	Castiglione Torinese	1934099
SARI8985	Piemonte	Novara	04/04/2022	Novara	184000
SARI9042	Piemonte	Alessandria	06/04/2022	Alessandria	110000
SARI9043	Piemonte	Asti	06/04/2022	Asti	95000
SARI9044	Piemonte	Cuneo	06/04/2022	Cuneo	185000
SARI8986	Puglia	Bari	04/04/2022	Altamura	95414
SARI8987	Puglia	Bari	04/04/2022	Bari Est	389000
SARI8988	Puglia	Bari	04/04/2022	Bari Ovest	360000
SARI9036	Puglia	Brindisi	04/04/2022	Brindisi Fiume Grande	93013
SARI9037	Puglia	Lecce	04/04/2022	Lecce	195368
SARI9038	Puglia	Foggia	05/04/2022	Foggia	208000
SARI9039	Puglia	Foggia	05/04/2022	Cerignola	56355
SARI9040	Puglia	Foggia	05/04/2022	Manfredonia	77000
SARI9047	Puglia	Barletta-Andria-Trani	06/04/2022	Bisceglie	85714
SARI9048	Puglia	Barletta-Andria-Trani	06/04/2022	Trani	83667
SARI9049	Puglia	Bari	06/04/2022	Molfetta	84803
SARI9050	Puglia	Bari	06/04/2022	Bitonto	79332
SARI9150	Puglia	Taranto	07/04/2022	Taranto Bellavista	116723
SARI9151	Puglia	Taranto	07/04/2022	Taranto Gennarini	226667
SARI9182	Puglia	Barletta-Andria-Trani	08/04/2022	Andria	130000
SARI9183	Puglia	Barletta-Andria-Trani	08/04/2022	Barletta	129356
SARI9051	Sicilia	Agrigento	04/04/2022	Agrigento	55000
SARI9052	Sicilia	Enna	04/04/2022	Enna	34000
SARI9053	Sicilia	Palermo	05/04/2022	Bagheria	75000
SARI9054	Sicilia	Palermo	05/04/2022	Acqua dei Corsari	314973
SARI9055	Sicilia	Palermo	05/04/2022	Fondo Verde	53886
SARI9056	Sicilia	Caltanissetta	05/04/2022	Caltanissetta e San Cataldo	76700
SARI9059	Sicilia	Trapani	05/04/2022	Trapani	118500
SARI9060	Sicilia	Trapani	05/04/2022	Mazara del Vallo	17000
SARI9061	Sicilia	Trapani	05/04/2022	Marsala	40000
SARI9078	Sicilia	Caltanissetta	05/04/2022	Gela Macchitella	12000
SARI9079	Sicilia	Messina	05/04/2022	Mili Marina	227000
SARI9080	Sicilia	Ragusa	05/04/2022	Vittoria	55000
SARI9081	Sicilia	Ragusa	05/04/2022	Modica	50400
SARI9082	Sicilia	Ragusa	05/04/2022	Ragusa	98000
SARI9227	Sicilia	Catania	05/04/2022	Pantano d'Arce	68434
SARI9228	Sicilia	Catania	05/04/2022	Giarre	47600
SARI9229	Sicilia	Siracusa	05/04/2022	Siracusa	180000
SARI9162	Toscana	Pisa	05/04/2022	Pisa Nord - S. Jacopo	52000
SARI9163	Toscana	Firenze	05/04/2022	Empoli Pagnana	88670
SARI9164	Toscana	Massa	05/04/2022	Lavello 2	120000
SARI9165	Toscana	Lucca	05/04/2022	Viareggio	93000
SARI9166	Toscana	Massa	05/04/2022	Lavello 1	87000
SARI9167	Toscana	Lucca	05/04/2022	Pontetetto	95000
SARI9168	Toscana	Livorno	05/04/2022	Rivellino	21000
SARI9169	Toscana	Livorno	06/04/2022	Rivellino	21000
SARI8999	Umbria	Perugia	05/04/2022	Perugia - Pian della Genna	90000
SARI9132	Umbria	Perugia	07/04/2022	Foligno Casone	90000
SARI9133	Umbria	Terni	07/04/2022	Terni	150000
SARI9180	Valle d'Aosta	Aosta	06/04/2022	La Salle	60000

SARI9181	Valle d'Aosta	Aosta	06/04/2022	Brissogne	150000
SARI9002	Veneto	Padova	05/04/2022	Padova Ca' Nordio - centro storico	98500
SARI9003	Veneto	Padova	05/04/2022	Padova Ca' Nordio - zip	98500
SARI9004	Veneto	Padova	05/04/2022	Padova Guizza	13000
SARI9005	Veneto	Padova	05/04/2022	Abano Terme	35000
SARI9030	Veneto	Treviso	05/04/2022	Treviso	70000
SARI9031	Veneto	Vicenza	05/04/2022	Vicenza Casale	92000
SARI9032	Veneto	Venezia	05/04/2022	Venezia Fusina	400000
SARI9159	Veneto	Verona	07/04/2022	Verona_collettore 1M	82000
SARI9160	Veneto	Verona	07/04/2022	Verona_collettore 3M	102000
SARI9161	Veneto	Verona	07/04/2022	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)⁵ 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 (Vols).

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 currently recognized VoCs (Beta, Gamma, Delta and Omicron) and Vols (Mu and Lambda) and for the de-escalated variant Alpha. For the Omicron variant, analyses were run for all the four lineages (BA.1, BA.2, BA.4, BA.5) currently recognized as VoCs⁴.

Results

Overall, 148 of the 161 samples (92%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 surveillance (**Table 2**), viral concentrations ranging from 2.5 E+01 to 7.3 E+06 genome copies (g.c.)/L of sewage.

Sanger Sequencing

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 12 May 2022. [SARS-CoV-2 variants of concern as of 12 May 2022 \(europa.eu\)](https://ec.europa.eu/eurosurv/infodiv/ncov/ncov-variants-of-concern/)

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 2-3**. Overall, 70 samples from 18 Regions/A.P. were amplified by the long PCR assay. Long amplicons could not be obtained from samples collected in the Regions/A.P. of Abruzzo and Campania. Sanger sequences were obtained for 67 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained for three samples, which could not therefore be assigned. All amplicons were characterized as Omicron variant by Sanger sequencing. Specifically, amino acid substitutions of the sublineage Omicron **BA.1.1** were detected in two samples (collected in Piemonte and P.A. Trento), and **BA.2** in 65 samples from 17 Regions/A.P. Overall, 97% of the sequences showed key mutations of sublineage Omicron BA.2, while mutations of BA1.1 were found in 3% of the sequences.

Next Generation Sequencing

NGS results could be successfully obtained for 18 Regions. Characteristic mutations of the Omicron variant were detected in all the NGS pools.

Mutations associated to Omicron sublineage BA.1 (Package C) were detected only in the A.P. Trento. Mutations associated to Omicron sublineage BA.1.1 (Package B) were detected in three Regions/AA.PP. (Molise, Piemonte and A.P. Trento). Finally, mutations of sublineage BA.2 were detected in 18 Regions/A.P. (Basilicata, Calabria, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Marche, Molise, Piemonte, Puglia, Sicilia, Toscana, Umbria, Valle d'Aosta, Veneto, and A.P. of Trento and Bolzano). Overall, 468.659 reads (93.74%) were assigned to Omicron BA.2, 30.623 reads to BA.1.1 (6.12%), and 687 to BA.1 (0.14%).

Key mutations of the Omicron sublineages BA.1, BA1.1, and BA.2 were detected simultaneously in one Regions/A.P (A.P. Trento). Mutations of both sublineages BA.1.1 and BA.2 were present in three Regions (Molise, Piemonte and A.P. Trento). Characteristic mutations of BA.2 were detected in all the NGS pools.

Mutations associated with other VOCs (included BA.4 and BA.5) or VOIs 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
- **Package B** (Omicron BA1.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- **Package C** (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K

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	Chieti	S. Martino	1,50E+02	-			
2		Pescara	Via Raiale	<LOD	-			
3		Pescara	Villa Carmine	9,25E+02	-			
4		L'Aquila	Pile	<LOD	-			
5		Teramo	Villa Pavone	1,08E+03	-			
6	Basilicata	Potenza	Tiera di Vaglio	1,60E+03	Package A	Omicron BA.2	• Package A	• Omicron BA.2
7		Matera	Pantano	1,72E+04	-			
157	Calabria	Cosenza	Cosenza - Sant'Angelo	2,59E+03	-		• Package A	• Omicron BA.2
158		Cosenza	Cosenza - Code di volpe	1,93E+03	-			
159		Crotone	Crotone - località Papaniciaro	1,02E+03	-			
160		Catanzaro	Catanzaro Lido - Loc. Verghello	5,83E+02	-			
161		Catanzaro	Catanzaro - Zona industriale	2,25E+03	Package A	Omicron BA.2		
162		Reggio Calabria	Ravagnese - località Aeroporto	2,04E+03	-			
8 ^b	Campania	Caserta	Area Casertana	7,11E+04	-			
9 ^b		Avellino	Manocalzati	6,95E+04	-			
10 ^b		Caserta	Villa Literno	6,06E+04	-			
11		Napoli	Napoli OVEST - Ingresso Principale	8,55E+03	-			
12		Napoli	Napoli OVEST - ex ingresso Camaldoli	<LOD	-			
13 ^b		Salerno	Eboli	9,07E+04	-			
14		Napoli	Napoli EST	4,53E+03	-			

15	9350		Salerno	Nocera Sup	1,48E+04	-		
16	9352		Salerno	Salerno	8,25E+03	-		
17 ^b	9256		Napoli	Area Nolana	4,06E+04	-		
18	9142	Emilia-Romagna	Ferrara	Ferrara - Linea 1	2,60E+03	-	• Package A	• Omicron BA.2
19	9144		Ferrara	Ferrara - Linea 2	<LOD	-		
20	9146		Modena	Carpi	<LOD	-		
21	9189		Piacenza	Borgoforte	3,70E+03	-		
22	9190		Parma	Parma ovest	2,73E+04	-		
23	9192		Reggio Emilia	Mancasale	4,44E+04	Package A	Omicron BA.2	
24	9195		Bologna	IDAR	3,95E+05	-		
25	9196		Modena	Naviglio	7,93E+04	Package A	Omicron BA.2	
26	9197		Ravenna	Faenza	1,20E+05	Package A	Omicron BA.2	
27	9200		Bologna	Imola	1,63E+05	Package A	Omicron BA.2	
28	9201		Ravenna - Forlì- Cesena	Ravenna	3,80E+04	-		
29	9205		Forlì-Cesena	Cesena	1,81E+05	Package A	Omicron BA.2	
30	9207		Forlì-Cesena	Forlì	9,45E+04	Package A	Omicron BA.2	
31	9212		Rimini - Forlì-Cesena	S. Giustina	7,43E+04	Package A (partial, from G339D to Y505H)	Omicron BA.2	
32	9223		Friuli-Venezia Giulia	Pordenone	Cordenons	7,45E+03	-	• Package A
33	9224	Udine		Udine	2,22E+04	Package A	Omicron BA.2	
34	9225	Trieste		Servola	2,04E+05	Package A	Omicron BA.2	
35	8989	Lazio	Viterbo	Viterbo - Strada Bagni	3,50E+04	-	• Package A	• Omicron BA.2
36	8990		Latina	Aprilia (Via del Campo)	1,09E+04	-		
37	8991		Latina	Latina Loc Latina Est	7,30E+04	-		
38	8992		Roma	Anzio - Colle Cocchino	1,14E+04	-		
39	8993		Roma	Guidonia - Ponte Lucano	4,10E+04	-		
40	8994		Roma	Velletri (LA CHIUSA-SORBO)	3,23E+04	Package A	Omicron BA.2	
41	8995		Roma	Pomezia - Via Cincinnato	1,32E+05	Package A + D215E	Omicron BA.2	
42	9127		Roma	Civitavecchia Fiumaretta	2,50E+01	-		

43	9143		Roma	Roma Est (linea 1 + linea 2)	9,74E+04	Package A + T307P	Omicron BA.2		
44	9145		Roma	Roma Nord	<LOD	-			
45	9147		Roma	Roma Sud	5,86E+04	-			
46	9148		Roma	Ostia	1,66E+05	Package A	Omicron BA.2		
47	9149		Roma	Fregene	6,03E+04	-			
48	9112	Liguria	Genova	Pegli	1,51E+04	Package A	Omicron BA.2	• Package A	• Omicron BA.2
49	9113		Genova	Voltri	2,64E+04	Package A (partial, from G142D to N440K)	Omicron BA.2		
50	9114		Genova	Quinto	5,44E+04	Package A	Omicron BA.2		
51	9115		Genova	Rapallo	4,47E+04	Package A	Omicron BA.2		
52	9116		Genova	Sestri P	8,12E+04	-			
53	9117		Genova	Sturla	1,46E+05	Package A	Omicron BA.2		
54	9118		Savona	Borghetto Santo Spirito	7,05E+04	Package A + A352V	Omicron BA.2		
55	9119		Imperia	Sanremo - località Capo Verde	6,42E+04	Package A + N87Y	Omicron BA.2		
56	9120		Savona	Savona	8,35E+04	Package A	Omicron BA.2		
57	9121		La Spezia	Camisano	6,71E+04	Package A	Omicron BA.2		
58	9122		La Spezia	Silea	6,83E+04	Package A	Omicron BA.2		
59	9123		La Spezia	La Spezia	7,96E+04	Package A	Omicron BA.2		
60	9124	Genova	Darsena	6,29E+04	-				
61	9125	Genova	Punta Vagno Genova	1,38E+05	-				
62	9126	Genova	Valpolcevera	2,89E+04	Package A	Omicron BA.2			
63	9186	Genova	Punta Vagno Genova	9,12E+04	Package A	Omicron BA.2			
64	9015	Lombardia	Milano	Bresso	4,05E+04	-		• Package A	• Omicron BA.2
65	9016		Milano - Monza e della Brianza	Peschiera Borromeo	1,13E+05	-			
66	9017		Milano - Varese	Canegrate	7,50E+04	-			
67	9018		Varese	Varese	7,28E+04	Package A	Omicron BA.2		
68	9019		Milano - Varese	Lonate Pozzolo	8,23E+04	Package A	Omicron BA.2		
69	9072		Milano	Milano Nosedo	6,40E+06	Package A	Omicron BA.2		

70	9073		Milano	Milano San Rocco	7,30E+06	Package A	Omicron BA.2	
71	9074		Como	Como	4,57E+06	Package A	Omicron BA.2	
72	9075		Pavia	Pavia	4,71E+06	Package A + F186S + A520S	Omicron BA.2	
73	9076		Como - Lecco - Milano - Monza e della Brianza	Monza	2,49E+04	-		
74	9077		Pavia	Vigevano	1,45E+06	Package A	Omicron BA.2	
75	9198		Bergamo	Bergamo	5,93E+03	-		
76	9202		Cremona	Città di Cremona	1,48E+04	-		
77	9203		Sondrio	Sondrio	7,25E+04	-		
78	9206		Brescia	Verziano	6,50E+03	-		
79	9152	Marche	Pesaro-Urbino	Borgheria	4,72E+04	-		• Package A • Omicron BA.2
80	9153		Pesaro-Urbino	Ponte Metauro	2,01E+04	-		
81	9154		Pesaro-Urbino	Ponte Sasso	1,73E+04	-		
82	9155		Ancona	Zipa	4,53E+04	-		
83	9156		Ancona	Falconara	1,17E+04	-		
84	9157		Ancona	Camerano	1,74E+03	M.E.		
85	9174	Molise	Campobasso	Campobasso - San Pietro	<LOD	-		• Package A • Omicron BA.2
86	9175		Campobasso	Termoli - località Porto	1,95E+03	M.E.		• Package B • Omicron BA.1.1
87	9176		Campobasso	Termoli - località Pantano Basso	1,25E+02	Package A	Omicron BA.2	
88	9012	P.A. Bolzano	Bolzano	IDA Bolzano	2,27E+04	-		• Package A • Omicron BA.2
89	9013		Bolzano	IDA Merano	2,47E+04	Package A	Omicron BA.2	
90	9014		Bolzano	IDA Termeno	3,40E+04	Package A	Omicron BA.2	
91	8996	P.A. Trento	Trento	Trento nord	3,10E+05	Package A	Omicron BA.2	• Package A • Omicron BA.2
92	8997		Trento	Trento sud	1,61E+05	-		• Package B • Omicron BA.1.1
93	8998		Trento	Rovereto	3,31E+05	Package B	Omicron BA.1.1	• Package C • Omicron BA.1
94	8983	Piemonte	Biella	Biella Nord	1,04E+03	-		• Package A • Omicron BA.2
95	8984		Torino	Castiglione Torinese	1,72E+04	-		• Package B • Omicron BA.1.1
96	8985		Novara	Novara	1,58E+03	Package B	Omicron BA.1.1	

97	9042	Alessandria	Alessandria	4,98E+04	-		
98	9043	Asti	Asti	2,48E+04	Package A	Omicron BA.2	
99	9044	Cuneo	Cuneo	1,53E+04	-		
101	8986	Bari	Altamura	2,55E+03	Package A	Omicron BA.2	• Package A • Omicron BA.2
102	8987	Bari	Bari Est	2,59E+03	Package A	Omicron BA.2	
103	8988	Bari	Bari Ovest	2,52E+03	M.E.		
104	9036	Brindisi	Brindisi Fiume Grande	1,62E+04	Package A	Omicron BA.2	
105	9037	Lecce	Lecce	4,85E+04	Package A	Omicron BA.2	
106	9038	Foggia	Foggia	1,47E+04	-		
107	9039	Foggia	Cerignola	1,34E+04	Package A	Omicron BA.2	
108	9040	Foggia	Manfredonia	2,11E+04	Package A	Omicron BA.2	
109	9047	Barletta-Andria-Trani	Bisceglie	4,20E+03	Package A	Omicron BA.2	
110	9048	Barletta-Andria-Trani	Trani	6,86E+03	Package A	Omicron BA.2	
111	9049	Bari	Molfetta	1,59E+04	Package A	Omicron BA.2	
112	9050	Bari	Bitonto	1,45E+04	Package A	Omicron BA.2	
113	9150	Taranto	Taranto Bellavista	1,62E+03	Package A	Omicron BA.2	
114	9151	Taranto	Taranto Gennarini	2,32E+03	Package A	Omicron BA.2	
115	9182	Barletta-Andria-Trani	Andria	2,91E+03	Package A	Omicron BA.2	
116	9183	Barletta-Andria-Trani	Barletta	3,07E+03	Package A	Omicron BA.2	
117	9051	Agrigento	Agrigento	2,25E+04	-		• Package A • Omicron BA.2
118	9052	Enna	Enna	1,37E+04	-		
119	9053	Palermo	Bagheria	2,29E+03	-		
120	9054	Palermo	Acqua dei Corsari	4,63E+04	-		
121	9055	Palermo	Fondo Verde	3,96E+04	-		
122	9056	Caltanissetta	Caltanissetta e San Cataldo	8,11E+03	-		
123	9059	Trapani	Trapani	3,73E+03	-		
124	9060	Trapani	Mazara del Vallo	8,36E+03	-		
125	9061	Trapani	Marsala	2,84E+04	-		
126	9078	Caltanissetta	Gela Macchitella	6,33E+03	-		
127	9079	Messina	Mili Marina	1,59E+04	-		

128	9080		Ragusa	Vittoria	5,70E+03	-		
129	9081		Ragusa	Modica	4,70E+03	-		
130	9082		Ragusa	Ragusa	6,58E+03	-		
131	9227		Catania	Pantano d'Arce	5,39E+03	-		
132	9228		Catania	Giarre	4,31E+03	-		
133	9229		Siracusa	Siracusa	8,81E+03	Package A	Omicron BA.2	
134	9162	Toscana	Pisa	Pisa Nord - S. Jacopo	4,49E+04	Package A	Omicron BA.2	• Package A • Omicron BA.2
135	9163		Firenze	Empoli Pagnana	<LOD	-		
136	9164		Massa	Lavello 2	<LOD	-		
137	9165		Lucca	Viareggio	<LOD	-		
138	9166		Massa	Lavello 1	<LOD	-		
139	9167		Lucca	Pontetetto	4,98E+03	-		
140	9168		Livorno	Rivellino	<LOD	-		
141	9169		Livorno	Rivellino	<LOD	-		
142	8999		Umbria	Perugia	Perugia - Pian della Genna	1,29E+05	-	
143	9132	Perugia		Foligno Casone	1,35E+05	-		
144	9133	Terni		Terni	1,32E+05	Package A	Omicron BA.2	
145	9180	Valle d'Aosta	Aosta	La Salle	2,16E+03	-		• Package A • Omicron BA.2
146	9181		Aosta	Brissogne	2,92E+04	Package A	Omicron BA.2	
147	9002	Veneto	Padova	Padova Ca' Nordio - centro storico	1,17E+05	Package A	Omicron BA.2	• Package A • Omicron BA.2
148	9003		Padova	Padova Ca' Nordio - zip	1,71E+05	-		
149	9004		Padova	Padova Guizza	4,80E+04	-		
150	9005		Padova	Abano Terme	8,39E+04	-		
151	9030		Treviso	Treviso	4,68E+03	Package A	Omicron BA.2	
152	9031		Vicenza	Vicenza Casale	3,78E+03	Package A	Omicron BA.2	
153	9032		Venezia	Venezia Fusina	9,63E+03	Package A	Omicron BA.2	
154	9159		Verona	Verona_collettore 1M	1,70E+04	Package A	Omicron BA.2	
155	9160		Verona	Verona_collettore 3M	6,88E+03	Package A	Omicron BA.2	
156	9161	Verona	Verona_collettore 8M	1,76E+04	Package 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 Due to shipment problems, samples were thawed at delivery;

^c 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
- **Package B** (Omicron BA.1.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- **Package C** (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K

Table 3. Sanger sequencing results (long PCR ID980)

	A67V	H69del	V70del	N87Y	T95I	G142D	V143del	Y144-145del	F186S	N211del	L212I	V213G	ins214EPE	T307P	G339D	R346K	A352V	S371F	S371L	S373P	S375F	T376A	D405N	R408S	K417N	N440K	G446S	S477N	T478K	E484A	Q493R	G496S	Q498R	N501Y	Y505H	A520S	T547K		
6, 23, 25, 26, 27, 29, 30, 31*, 33, 34, 40, 41, 46, 48, 49*, 50, 51,53, 56, 57, 58, 59, 62, 63, 67, 68, 69, 70, 71, 74, 87, 89, 90, 91, 98, 101, 102, 104, 105, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 133, 134, 144, 146, 147, 151, 152, 153, 154, 155, 156, 161						█						█			█			█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█				Package A (Omicron BA.2)
43						█						█		█	█			█	█	█	█	█	█	█	█	█	█											Package A + T307P (Omicron BA.2)	
54						█						█			█		█		█	█	█	█	█	█	█	█	█											Package A + A352V (Omicron BA.2)	
55				█		█						█			█			█	█	█	█	█	█	█	█	█	█											Package A + N87Y (Omicron BA.2)	
72						█		█				█			█			█	█	█	█	█	█	█	█	█	█											Package A + F186S + A520S (Omicron BA.2)	
93, 96	█	█	█		█	█	█		█	█	█	█	█		█	█		█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	Package B (Omicron BA.1.1)	

* 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 eighth 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 confirm the predominance and significant variability of the Omicron variant in April 2022 in Italy, in line with clinical results. Characteristic mutations of lineage BA.2 were predominant, but characteristic mutations of sublineage BA.1 and BA.1.1 were also detected. Sublineages BA.4/BA.5 were not detected. Key mutations of variant other than Omicron were not detected.

Results of the present survey, confirm that, since December 2021, Omicron has taken over in the whole country.

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- **Toscana:** Piergiuseppe Cala' (Regione Toscana); Annalaura Carducci, Marco Verani, Ileana Federigi, Giulia Lauretani, Sara Muzio (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Università di Pisa); Matteo Ramazzotti (Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi di Firenze), Alberto Antonelli (SOD microbiologia e virologia, azienda ospedaliera universitaria Careggi, Firenze);
- **Umbria:** Giovanni Santoro (Regione Umbria), Ermanno Federici, Maya Petricciuolo, Sofia Barigelli (Laboratorio Microbiologia Applicata e Ambientale, DCBB Università di Perugia);
- **Valle D'Aosta:** Mauro Ruffier (Regione Valle d'Aosta); Francesca Borney, Eric Grange, Florida Damasco (Laboratorio chimico biologico microbiologico Arpa Valle d'Aosta);
- **Veneto:** Francesca Russo, Gisella Pitter, Vanessa Groppi (Regione Veneto); Franco Rigoli, Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto); Tatjana Baldovin, Irene Amoruso (Università di Padova);
- **P.A. Bolzano:** Lorella Zago (P.A. Bolzano); Alberta Stenico, Anna-Maria Prast (A.P.P.A. Agenzia provinciale per l'ambiente e la tutela del clima, Laboratorio biologico)
- **P.A. Trento:** Francesco Pizzo; Alessandra Schiavuzzi, Elena Mengon (P. A. Trento) (P.A. Trento); Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento).

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