



**Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
6th Report
(Study period: 7 - 11 February 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:

- Sixth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 7-11 February 2022.
- Overall, 153 wastewater samples were collected from 17 Regions and 2 Autonomous Provinces (A.P.)
- Characteristic mutations of the Omicron variant were detected in 14 Regions and two A.P located in the North, Centre, and South of Italy (no sequences were obtained from the remaining three Regions);
- The vast majority of the detected mutations belonged to sublineage BA.1, but aminoacid substitutions of sublineage BA.2 were also detected in the Regions of Basilicata, Friuli Venezia-Giulia, Liguria and Veneto.
- In four Regions (Liguria, Piemonte, Valle d'Aosta and Veneto) characteristic mutations of the Delta variant were also 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 sixth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 7-11 February 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: Campania, Basilicata, Molise, Puglia and Sicilia.

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>)

Overall, 153 wastewater samples were collected between 7 and 11 February 2022 (**Table 1**).

Table 1. Sampling sites and characteristics of the WTPs studied

Sample ID ISS	Region/A. P.	City	Sampling Date	WTP	Population equivalent
SARI7565	Abruzzo	Chieti	08/02/2022	S. Martino	114.500
SARI7566	Abruzzo	Pescara	08/02/2022	Via Raiale	160.000
SARI7567	Abruzzo	Pescara	08/02/2022	Villa Carmine	140.000
SARI7568	Abruzzo	L'Aquila	08/02/2022	Pile	48.000
SARI7569	Abruzzo	Teramo	08/02/2022	Villa Pavone	41.824
SARI7467	Basilicata	Potenza	08/02/2022	Tiera di Vaglio	95.000
SARI7468	Basilicata	Matera	08/02/2022	Pantano	24.000
SARI7491	Campania	Salerno	08/02/2022	Salerno	700.000
SARI7492	Campania	Salerno	08/02/2022	Nocera Sup	299.121
SARI7493	Campania	Avellino	08/02/2022	Manocalzati	140.000
SARI7496	Campania	Napoli	08/02/2022	Napoli EST	1.750.000
SARI7497	Campania	Napoli	08/02/2022	Area Nolana	400.000
SARI7723	Campania	Napoli	10/02/2022	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI7724	Campania	Napoli	10/02/2022	Napoli OVEST - Ingresso Principale	950.000
SARI7727	Campania	Caserta	10/02/2022	Villa Literno	631.714
SARI7728	Campania	Caserta	10/02/2022	Area Casertana	370.769
SARI7530	Emilia-Romagna	Reggio Emilia	07/02/2022	Mancasale	280.000
SARI7533	Emilia-Romagna	Parma	07/02/2022	Parma ovest	168.000
SARI7591	Emilia-Romagna	Bologna	07/02/2022	IDAR	800.000
SARI7592	Emilia-Romagna	Modena	07/02/2022	Naviglio	500.000
SARI7509	Emilia-Romagna	Ferrara	08/02/2022	Ferrara - Linea 1	120.000
SARI7511	Emilia-Romagna	Ferrara	08/02/2022	Ferrara - Linea 2	120.000
SARI7514	Emilia-Romagna	Modena	08/02/2022	Carpi	200.000
SARI7532	Emilia-Romagna	Piacenza	08/02/2022	Borgoforte	163.333
SARI7593	Emilia-Romagna	Forli-Cesena	08/02/2022	Cesena	197.500
SARI7594	Emilia-Romagna	Forli-Cesena	08/02/2022	Forlì	250.000
SARI7595	Emilia-Romagna	Ravenna	08/02/2022	Faenza	100.000
SARI7596	Emilia-Romagna	Bologna	08/02/2022	Imola	75.000
SARI7599	Emilia-Romagna	Ravenna - Forli-Cesena	09/02/2022	Ravenna	240.000
SARI7600	Emilia-Romagna	Rimini - Forli-Cesena	09/02/2022	S. Giustina	560.000
SARI7605	Friuli-Venezia Giulia	Udine	08/02/2022	Udine	200.000
SARI7606	Friuli-Venezia Giulia	Pordenone	08/02/2022	Cordenons	15.000
SARI7607	Friuli-Venezia Giulia	Trieste	09/02/2022	Servola	190.000
SARI7425	Lazio	Viterbo	07/02/2022	Viterbo - Strada Bagni	30.000
SARI7426	Lazio	Roma	07/02/2022	Ponte Lucano	50.000
SARI7427	Lazio	Roma	07/02/2022	Pomezia - Via Cincinnato	60.000

SARI7428	Lazio	Roma	07/02/2022	Velletri (LA CHIUSA-SORBO)	36.700
SARI7429	Lazio	Roma	07/02/2022	Anzio - Colle Cocchino	75.000
SARI7430	Lazio	Latina	07/02/2022	Aprilia (Via del Campo)	66.000
SARI7431	Lazio	Latina	07/02/2022	Latina Loc Latina Est	90.000
SARI7500	Lazio	Roma	08/02/2022	Civitavecchia Fiumaretta	86.400
SARI7586	Lazio	Roma	08/02/2022	Roma Est (linea 1 + 2)	900.000
SARI7587	Lazio	Roma	08/02/2022	Roma Nord	780.000
SARI7588	Lazio	Roma	09/02/2022	Roma Sud	1.100.000
SARI7589	Lazio	Roma	09/02/2022	Ostia	350.000
SARI7590	Lazio	Roma	09/02/2022	Fiumicino	76.000
SARI7562	Liguria	Savona	07/02/2022	Savona	256.203
SARI7563	Liguria	Genova	08/02/2022	Pegli	20.507
SARI7564	Liguria	Genova	08/02/2022	Voltri	40.496
SARI7573	Liguria	Genova	08/02/2022	Quinto	48.748
SARI7574	Liguria	Genova	08/02/2022	Rapallo	90.000
SARI7575	Liguria	Genova	08/02/2022	Sestri P	51.368
SARI7576	Liguria	Genova	08/02/2022	Sturla	43.573
SARI7577	Liguria	Genova	08/02/2022	Rossiglione	15.000
SARI7578	Liguria	Savona	08/02/2022	Borghetto Santo Spirito	140.000
SARI7579	Liguria	La Spezia	08/02/2022	Camisano	40.840
SARI7580	Liguria	La Spezia	08/02/2022	Silea	17.500
SARI7581	Liguria	La Spezia	08/02/2022	La Spezia	82.000
SARI7582	Liguria	Imperia	08/02/2022	Sanremo - località Capo Verde	80.000
SARI7583	Liguria	Genova	09/02/2022	Darsena	118.276
SARI7584	Liguria	Genova	09/02/2022	Punta Vagno Genova	75.000
SARI7585	Liguria	Genova	09/02/2022	Valpolcevera	157.650
SARI7478	Lombardia	Milano - Varese	07/02/2022	Lonate Pozzolo	450.000
SARI7479	Lombardia	Milano - Varese	07/02/2022	Canegrate	137.950
SARI7480	Lombardia	Varese	07/02/2022	Varese	74.402
SARI7481	Lombardia	Milano - Monza e Brianza	07/02/2022	Peschiera Borromeo	566.000
SARI7482	Lombardia	Milano	07/02/2022	Bresso	220.000
SARI7549	Lombardia	Milano	07/02/2022	Milano Nosedo	1.250.000
SARI7551	Lombardia	Milano	07/02/2022	Milano San Rocco	1.036.000
SARI7554	Lombardia	Como	07/02/2022	Como	196.000
SARI7557	Lombardia	Pavia	07/02/2022	Pavia	132.912
SARI7617	Lombardia	Bergamo	07/02/2022	Bergamo	220.000
SARI7545	Lombardia	Como - Lecco - Milano - Monza e della Brianza	09/02/2022	Monza	600.000
SARI7546	Lombardia	Sondrio	09/02/2022	Sondrio	49.500
SARI7558	Lombardia	Pavia	09/02/2022	Vigevano	57.925
SARI7602	Lombardia	Cremona	09/02/2022	Citta di Cremona	180.000
SARI7604	Lombardia	Brescia	09/02/2022	Verziano	296.000
SARI7507	Marche	Pesaro-Urbino	08/02/2022	Borgheria	116.000
SARI7508	Marche	Pesaro-Urbino	08/02/2022	Ponte Metauro	60.000

SARI7510	Marche	Pesaro-Urbino	08/02/2022	Ponte Sasso	18.000
SARI7512	Marche	Ancona	08/02/2022	Zipa	100.000
SARI7515	Marche	Ancona	08/02/2022	Falconara	85.000
SARI7516	Marche	Ancona	08/02/2022	Camerano	33.000
SARI7570	Molise	Campobasso	07/02/2022	Campobasso - San Pietro	50.000
SARI7571	Molise	Campobasso	07/02/2022	Termoli - località Porto	25.000
SARI7572	Molise	Campobasso	07/02/2022	Termoli - località Pantano Basso	25.000
SARI7542	P.A. Bolzano	Bolzano	07/02/2022	IDA Bolzano	372.410
SARI7543	P.A. Bolzano	Bolzano	07/02/2022	IDA Merano	356.520
SARI7544	P.A. Bolzano	Bolzano	07/02/2022	IDA Termeno	68.945
SARI7435	P.A. Trento	Trento	07/02/2022	Trento nord	120.000
SARI7436	P.A. Trento	Trento	07/02/2022	Trento sud	100.000
SARI7437	P.A. Trento	Trento	07/02/2022	Rovereto	95.000
SARI7409	Piemonte	Torino	07/02/2022	Castiglione Torinese	1.934.099
SARI7410	Piemonte	Biella	07/02/2022	Biella Nord	67.000
SARI7411	Piemonte	Biella	07/02/2022	Biella Sud	53.000
SARI7412	Piemonte	Novara	07/02/2022	Novara	184.000
SARI7475	Piemonte	Cuneo	09/02/2022	Cuneo	185.000
SARI7476	Piemonte	Asti	09/02/2022	Asti	95.000
SARI7477	Piemonte	Alessandria	09/02/2022	Alessandria	110.000
SARI7420	Puglia	Bari	07/02/2022	Altamura	95.414
SARI7421	Puglia	Brindisi	07/02/2022	Brindisi Fiume Grande	93.013
SARI7422	Puglia	Lecce	07/02/2022	Lecce	195.368
SARI7423	Puglia	Taranto	07/02/2022	Taranto Bellavista	116.723
SARI7424	Puglia	Taranto	07/02/2022	Taranto Gennarini	226.667
SARI7471	Puglia	Foggia	08/02/2022	Cerignola	56.355
SARI7472	Puglia	Foggia	08/02/2022	Foggia	208.000
SARI7473	Puglia	Foggia	08/02/2022	Manfredonia	89.724
SARI7483	Puglia	Bari	09/02/2022	Molfetta	84.803
SARI7484	Puglia	Barletta-Andria-Trani	09/02/2022	Andria	130.000
SARI7488	Puglia	Barletta-Andria-Trani	09/02/2022	Barletta	129.356
SARI7489	Puglia	Barletta-Andria-Trani	09/02/2022	Bisceglie	85.714
SARI7490	Puglia	Barletta-Andria-Trani	09/02/2022	Trani	83.667
SARI7547	Puglia	Bari	10/02/2022	Bari Ovest	360.000
SARI7548	Puglia	Bari	10/02/2022	Bari Est	389.000
SARI7553	Puglia	Bari	10/02/2022	Bitonto	79.332
SARI7461	Sicilia	Agrigento	07/02/2022	Agrigento	55.000
SARI7462	Sicilia	Enna	07/02/2022	Enna	34.000
SARI7432	Sicilia	Ragusa	08/02/2022	Modica	50.400
SARI7433	Sicilia	Ragusa	08/02/2022	Ragusa	98.000
SARI7434	Sicilia	Ragusa	08/02/2022	Vittoria	55.000
SARI7463	Sicilia	Palermo	08/02/2022	Bagheria	75.000

SARI7464	Sicilia	Caltanissetta	08/02/2022	Caltanissetta e San Cataldo	76.700
SARI7465	Sicilia	Palermo	08/02/2022	Acqua dei Corsari	314.973
SARI7466	Sicilia	Palermo	08/02/2022	Fondo Verde	53.886
SARI7469	Sicilia	Caltanissetta	08/02/2022	Gela Macchitella	12.000
SARI7470	Sicilia	Messina	08/02/2022	Mili Marina	227.000
SARI7485	Sicilia	Trapani	08/02/2022	Trapani	118.500
SARI7486	Sicilia	Trapani	08/02/2022	Mazzara del Vallo	17.000
SARI7487	Sicilia	Trapani	08/02/2022	Marsala	40.000
SARI7742	Sicilia	Catania	08/02/2022	Pantano d'Arci	68.434
SARI7743	Sicilia	Catania	08/02/2022	Giarre	47.600
SARI7744	Sicilia	Siracusa	08/02/2022	Siracusa	180.000
SARI7501	Toscana	Pisa	08/02/2022	Pisa Nord - S. Jacopo	52.000
SARI7502	Toscana	Firenze	08/02/2022	Empoli Pagnana	88.670
SARI7615	Toscana	Firenze	08/02/2022	San Colombano	600.000
SARI7504	Toscana	Massa	09/02/2022	Lavello 2	120.000
SARI7505	Toscana	Lucca	09/02/2022	Viareggio	93.000
SARI7506	Toscana	Massa	09/02/2022	Lavello 1	87.000
SARI7408	Umbria	Perugia	07/02/2022	Perugia - Pian della Genna	90.000
SARI7518	Umbria	Perugia	10/02/2022	Foligno Casone	90.000
SARI7519	Umbria	Terni	10/02/2022	Terni	150.000
SARI7537	Valle d'Aosta	Aosta	09/02/2022	La Salle	60.000
SARI7538	Valle d'Aosta	Aosta	09/02/2022	Brissogne	150.000
SARI7438	Veneto	Padova	08/02/2022	Padova Ca' Nordio - centro storico	98.500
SARI7439	Veneto	Padova	08/02/2022	Padova Ca' Nordio - zip	98.500
SARI7440	Veneto	Padova	08/02/2022	Padova Guizza	13.000
SARI7441	Veneto	Padova	08/02/2022	Abano Terme	35.000
SARI7458	Veneto	Treviso	08/02/2022	Treviso	70.000
SARI7459	Veneto	Venezia	08/02/2022	Venezia Fusina	400.000
SARI7460	Veneto	Vicenza	08/02/2022	Vicenza Casale	92.000
SARI7524	Veneto	Verona	10/02/2022	Verona_collettore 1M	82.000
SARI7525	Veneto	Verona	10/02/2022	Verona_collettore 3M	102.000
SARI7526	Veneto	Verona	10/02/2022	Verona_collettore 8M	226.000

‡ 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.

2 G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferrò, D. Brandtner, A. Fasanella, 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>.

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 (VoIs). To increase the probability of amplification and characterization, one additional short nested RT-PCR (unpublished), designed within the long fragment, was also used to analyse samples tested negative by the long PCR. This short nested PCR is designated as ID_987/994 and generates PCR amplicons of 478 to 495 bp depending on the variant.

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. Positive PCR products generated by the short nested PCR assay underwent only conventional Sanger sequencing.

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 VoIs (Mu and Lambda) and for the de-escalated variant Alpha.

Results

The data on viral concentration were produced by the SARI network laboratories. Overall, 144 of the 153 samples (94%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 surveillance (**Table 2**), with viral concentrations ranging from 2.5 E+01 to 4.8 E+06 genome copies (g.c.)/L of sewage. Overall, 106 samples were amplified by the long ($n = 37$) or the short ($n = 69$) PCR assay. Real-time PCR, nested PCR, and sequencing results are summarized in **Table 2**. Partial sequencing of the Spike region does not allow conclusive assignment 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.

Sanger Sequencing

Good sequencing results were obtained for 92 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained for 14 samples. Characteristic mutations of the Omicron variant were detected in 90 samples (98%). Key mutations of the omicron variant were detected in 16 Regions/AA.PP. (Abruzzo, Basilicata, Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Piemonte, Puglia, Sicilia, Umbria, Valle d’Aosta, Veneto, A.P. Trento and A.P. Bolzano), two of which (Basilicata and Liguria), showed amino acid substitutions of sublineage Omicron BA.2. Mutations of the Delta variant were also detected in two Regions (Piemonte and Valle d’Aosta).

Next Generation Sequencing

NGS results could be successfully obtained for 12 Regions. Characteristic mutations of the Omicron variant were detected in all the NGS pools. Mutations associated to Omicron sublineage BA.1 were detected in 12 Regions/AA.PP (Basilicata, Emilia-Romagna, Friuli-Venezia Giulia, Liguria, Lombardia,

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>

Piemonte, Puglia, Sicilia, Umbria, Veneto, A.P. Trento and A.P. Bolzano). Samples collected in Basilicata, Friuli Venezia-Giulia, Liguria and Veneto also showed characteristic mutations of Omicron sublineage BA.2, such as “V213G, T376A, D405N, R408S”. Overall, 15 different mutation combinations were detected within the omicron variant (the vast majority within BA.1 sequences), suggesting a considerable genetic variability of this variant. Overall, compared to the Wuhan reference sequence, 42 mutations (aminoacid substitution or deletions) were detected by NGS in association with the Omicron variant in the ~1600 bps fragment amplified by the long PCR: 34 amino acid substitutions (A67V, T95I, V127F, G142D, E154K, K187N, V213G, A222V, L242F, D253Y, S255F, E324K, G339D, R346K, S371F, S371L, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460N, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K), 1 silent mutation (N460N), 6 deletions (H69del, V70del, V143del, Y144del, Y145del, N211del), and the unique insertion ins214EPE.

Moreover, characteristic amino acid substitutions of the Delta variant (panel “G142D, E156G, 157-158DEL, L452R, T484K”) were detected in the Regions of Liguria and Veneto.

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

- Package A (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
- Package B (Omicron BA.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 A/B (Omicron, short fragment from PCR ID_987/994): A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del
- Package C (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 D (Delta, long fragment from PCR ID_980) = T95I, G142D, E156G, F157del, R158del, L452R, T478K
- Package E (Delta, short fragment from PCR ID_987/994) = G142D, E156G, F157del, R158del

Mutation packages derived from the long PCR fragments are suggestive of the Omicron sublineages BA.1 or BA.2, while mutation packages derived from the short fragment are not discriminative enough and are generically assigned to the Omicron variant.

Table 2. PCR and sequencing results

Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_980)	Mutations found by Sanger sequencing (short PCR ID_987/994)	SARS-CoV-2 variant †	Mutations found by NGS (long PCR ID_980 in pools)	SARS-CoV-2 variant †
1	7565	Abruzzo	Chieti	S. Martino	<LOD	-	M.E. ^a		
2	7566		Pescara	Via Raiale	<LOD	-	M.E. ^a		
3	7567		Pescara	Villa Carmine	6,25E+02	-	M.E. ^a	-	
4	7568		L'Aquila	Pile	6,75E+02	-	Package A/B	Omicron	
5	7569		Teramo	Villa Pavone	<LOD	-	-		
6	7467	Basilicata	Potenza	Tiera di Vaglio	8,63E+03	Package B	Omicron BA.1	Package A	
7	7468		Matera	Pantano	5,95E+04	Package C + T547K (partial, from V213G to Y505H) ^c	Omicron BA.2	Package B Package C Package C + T547K	Omicron BA.1 Omicron BA.2
8	7491	Campania	Salerno	Salerno	1,00E+04	-	-		
9	7492		Salerno	Nocera Sup	1,45E+04	-	Package A/B + F135S	Omicron	
10	7493		Avellino	Manocalzati	1,74E+04	-	-		
11	7723		Napoli	Napoli OVEST - ex ingresso Camaldoli	<LOD	-	M.E. ^a		
12	7724		Napoli	Napoli OVEST - Ingresso Principale	<LOD	-	Package A/B	Omicron	-
13	7496		Napoli	Napoli EST	4,09E+03	-	-		
14	7497		Napoli	Area Nolana	1,46E+04	-	-		
15	7727		Caserta	Villa Literno	2,78E+03	-	-		
16	7728	Caserta	Area Casertana	<LOD	-	-			

17 ^b	7530		Reggio Emilia	Mancasale	1,42E+04	-	-			
18 ^b	7533		Parma	Parma ovest	2,52E+03	-	-			
19	7591		Bologna	IDAR	1,24E+05	Package B			Omicron BA.1	
20	7592		Modena	Naviglio	2,07E+05	Package A			Omicron BA.1	
21	7509		Ferrara	Ferrara - Linea 1	2,19E+03	-	-			
22	7511		Ferrara	Ferrara - Linea 2	<LOD	-	-			
23	7514	Emilia-Romagna	Modena	Carpi	7,20E+03	-	Package A/B	Omicron	Package A Package A + L242F	
24 ^b	7532		Piacenza	Borgoforte	<LOD	-	-		Package B	Omicron BA.1
25	7593		Forlì-Cesena	Cesena	1,82E+05	Package B			Package B + S255F	
26	7594		Forlì-Cesena	Forlì	1,30E+05	Package A				Omicron BA.1
27	7595		Ravenna	Faenza	2,25E+05	-	Package A/B	Omicron		
28	7596		Bologna	Imola	2,28E+05	Package A				Omicron BA.1
29	7599		Ravenna - Forlì-Cesena	Ravenna	9,45E+04	Package B + S255F			Omicron BA.1	
30	7600		Rimini - Forlì-Cesena	S. Giustina	1,10E+05	Package B			Omicron BA.1	
31	7605	Friuli-Venezia Giulia	Udine	Udine	1,46E+05	Package A			Package A Package A + E324K	
32	7606		Pordenone	Cordenons	1,63E+05	Package A			Package B	Omicron BA.1
33	7607		Trieste	Servola	1,57E+05	-	Package A/B	Omicron	Package C	Omicron BA.2
34	7425		Viterbo	Viterbo - Strada Bagni	8,50E+03	-	Package A/B	Omicron		
35	7426		Roma	Ponte Lucano	1,82E+04	-	-	Omicron		
36	7427		Roma	Via Cincinnato	1,89E+04	-	Package A/B	Omicron		
37	7428	Lazio	Roma	Velletri (LA CHIUSA-SORBO)	1,33E+04	-	Package A/B	Omicron		
38	7429		Roma	Anzio - Colle Cocchino	9,98E+03	-	-			
39	7430		Latina	Aprilia (Via del Campo)	1,86E+04	-	Package A/B	Omicron		
40	7431		Latina	Latina Loc Latina Est	1,67E+04	-	Package A/B (partial, from T95I to Y145del) ^c	Omicron		

41	7500	Roma	Civitavecchia Fiumaretta	2,50E+01	-	-		
42	7586	Roma	Roma Est (linea 1 + linea 2)	1,99E+05	-	Package A/B	Omicron	
43	7587	Roma	Roma Nord	2,75E+05	-	Package A/B	Omicron	
44	7588	Roma	Roma Sud	1,55E+05	-	Package A/B	Omicron	
45	7589	Roma	Ostia	5,16E+05	-	Package A/B	Omicron	
46	7590	Roma	Fiumicino	2,56E+05	-	Package A/B	Omicron	
47	7562	Savona	Savona	2,71E+04	-	Package A/B	Omicron	
49	7563	Genova	Pegli	2,15E+04	-	Package A/B	Omicron	
50	7564	Genova	Voltri	4,55E+04	Package C		Omicron BA.2	
51	7573	Genova	Quinto	4,36E+04	Package B		Omicron BA.1	
52	7574	Genova	Rapallo	1,48E+05	-	M.E. ^a		
53	7575	Genova	Sestri P	3,28E+04	-	Package A/B	Omicron	
54	7576	Genova	Sturla	8,17E+04	-	Package A/B	Omicron	
55	7577	Genova	Rossiglione	2,89E+04	-	Package A/B	Omicron	Package A
56	7578	Savona	Borghetto Santo Spirito	6,45E+03	-	Package A/B	Omicron	Package B
57	7579	La Spezia	Camisano	3,02E+04	-	Package A/B	Omicron	Package B + V127F
58	7580	La Spezia	Silea	7,96E+04	Package B		Omicron BA.1	Package B + K187N
59	7581	La Spezia	La Spezia	3,12E+04	Package A (partial, from G339D to T547K) ^c		Omicron BA.1	Package C
60	7582	Imperia	Sanremo - località Capo Verde	1,22E+05	Package A (partial, from G339D to T547K) ^c		Omicron BA.1	Package D
61	7583	Genova	Darsena	6,04E+04	-	Package A/B	Omicron	
151	7584	Genova	Punta Vagno Genova	7,95E+04	-	-		
62	7585	Genova	Valpolcevera	8,95E+03	-	Package A/B	Omicron	

Liguria

Omicron BA.1
Omicron BA.2
Delta

63	7478		Milano - Varese	Lonate Pozzolo	3,50E+04	-	Package A/B	Omicron	
64	7479		Milano - Varese	Canegrate	4,98E+04	-	Package A/B	Omicron	
65	7480		Varese	Varese	4,03E+04	Package B		Omicron BA.1	
66	7481		Milano - Monza e della Brianza	Peschiera Borromeo	3,60E+04	-	Package A/B	Omicron	
67	7482		Milano	Bresso	4,65E+04	-	Package A/B	Omicron	
68	7549		Milano	Milano Nosedo	3,63E+06	Package B		Omicron BA.1	Package A
69	7551		Milano	Milano San Rocco	4,80E+06	Package A		Omicron BA.1	Package A + E154K
70	7554	Lombardia	Como	Como	3,78E+06	Package A		Omicron BA.1	Package A + N460N
72	7557		Pavia	Pavia	3,68E+06	-	Package A/B	Omicron	Package B
73	7617		Bergamo	Bergamo	5,28E+03	-	-		
158	7545		Como - Lecco - Milano - Monza e della Brianza	Monza	6,19E+03	Package B (partial, from G142D to S375F and from S477N to T547K) ^c		Omicron BA.1	
74	7546		Sondrio	Sondrio	2,08E+03	-	Package A/B	Omicron	
75	7558		Pavia	Vigevano	3,30E+06	Package A + E154K		Omicron BA.1	
76	7602		Cremona	Citta di Cremona	1,87E+04	-	Package A/B	Omicron	
77	7604		Brescia	Verziano	1,76E+04	-	Package A/B	Omicron	
78^b	7507		Pesaro-Urbino	Borgheria	2,28E+04	-	-		
79^b	7508		Pesaro-Urbino	Ponte Metauro	1,78E+04	-	-		
80^b	7510	Marche	Pesaro-Urbino	Ponte Sasso	1,46E+04	-	-		-
81^b	7512		Ancona	Zipa	2,68E+04	-	-		
82^b	7515		Ancona	Falconara	1,31E+04	-	-		
83^b	7516		Ancona	Camerano	4,75E+03	-	-		

84	7570		Campobasso	Campobasso - San Pietro	7,00E+02	-	-		
85	7571	Molise	Campobasso	Termoli - località Porto	1,35E+03	-	-	-	
86	7572		Campobasso	Termoli - località Pantano Basso	7,00E+02	-	-		
87	7542		Bolzano	IDA Bolzano	1,39E+05	Package B		Omicron BA.1	
88	7543	P.A. Bolzano	Bolzano	IDA Merano	7,04E+04	-	-	Package B	Omicron BA.1
89	7544		Bolzano	IDA Termeno	8,01E+04	Package B		Omicron BA.1	
90	7435		Trento	Trento nord	2,46E+05	Package A		Omicron BA.1	Package A
91	7436	P.A. Trento	Trento	Trento sud	1,90E+05	-	Package A/B	Omicron	Package B
92	7437		Trento	Rovereto	3,23E+05	-	Package A/B	Omicron	Package B + D253Y
93	7409		Torino	Castiglione Torinese	1,16E+03	-	-		
94	7410		Biella	Biella Nord	5,63E+02	-	M.E. ^a		
95	7411		Biella	Biella Sud	1,08E+03	Package B + D253Y		Omicron BA.1	Package A
96	7412	Piemonte	Novara	Novara	8,55E+01	-	-		Package A + D253Y
97	7475		Cuneo	Cuneo	3,78E+03	-	-		Package B
98	7476		Asti	Asti	6,30E+02	-	Package E	Delta	Package B + D253Y
99	7477		Alessandria	Alessandria	8,58E+02	-	-		
100	7420		Bari	Altamura	1,30E+04	Package B		Omicron BA.1	
101	7421		Brindisi	Brindisi Fiume Grande	6,36E+03	-	-		
102	7422		Lecce	Lecce	1,27E+04	Package B		Omicron BA.1	
103	7423		Taranto	Taranto Bellavista	1,66E+03	Package B		Omicron BA.1	
104	7424		Taranto	Taranto Gennarini	2,12E+03	-	Package A/B	Omicron	Package A
105	7471	Puglia	Foggia	Cerignola	4,81E+02	-	-		Package B
106	7472		Foggia	Foggia	1,19E+04	-	Package A/B	Omicron	
107	7473		Foggia	Manfredonia	5,81E+02	-	M.E. ^a		
108	7483		Bari	Molfetta	1,03E+04	-	Package A/B	Omicron	
109	7484		Barletta-Andria-Trani	Andria	3,74E+03	-	-		

110	7488		Barletta-Andria-Trani	Barletta	1,42E+03	-	-		
111	7489		Barletta-Andria-Trani	Bisceglie	4,79E+03	-	-		
112	7490		Barletta-Andria-Trani	Trani	3,41E+03	Package A			Omicron BA.1
113	7547		Bari	Bari Ovest	5,99E+03	-	Package A/B		Omicron
114	7548		Bari	Bari Est	9,48E+03	-	Package A/B		Omicron
115	7553		Bari	Bitonto	9,96E+03	-	Package A/B		Omicron
116	7461		Agrigento	Agrigento	3,40E+04	-	-		
117	7462		Enna	Enna	2,08E+04	-	Package A/B		Omicron
118	7432		Ragusa	Modica	6,78E+04	-	Package A/B		Omicron
119	7433		Ragusa	Ragusa	5,60E+04	-	-		
120	7434		Ragusa	Vittoria	1,78E+05	-	M.E. ^a		
121	7463		Palermo	Bagheria	4,09E+04	-	Package A/B		Omicron
122	7464		Caltanissetta	Caltanissetta e San Cataldo	4,44E+04	-	Package A/B		Omicron
123	7465	Sicilia	Palermo	Acqua dei Corsari	2,66E+04	-	Package A/B	Package A	Omicron BA.1
124	7466		Palermo	Fondo Verde	4,21E+04	-	Package A/B	Package B	
125	7469		Caltanissetta	Gela Macchitella	8,59E+04	-	M.E. ^a		
126	7470		Messina	Mili Marina	1,93E+05	Package A			
127	7485		Trapani	Trapani	7,53E+03	-	-		
128	7486		Trapani	Mazzara del Vallo	3,47E+04	-	-		
129	7487	Trapani	Marsala	1,38E+04	-	Package A/B		Omicron	
152	7743		Catania	Giarre	1,09E+04	-	M.E. ^a		
155	7742		Catania	Pantano d'Arce	<LOD	-	-		
156	7744		Siracusa	Siracusa	7,08E+03	-	Package A/B		Omicron
130	7501	Toscana	Pisa	Pisa Nord - S. Jacopo	5,40E+04	-	-		
131	7502		Firenze	Empoli Pagnana	1,29E+04	-	-		
132	7504		Massa	Lavello 2	2,34E+04	-	-		
133	7505		Lucca	Viareggio	2,63E+04	-	-		

134	7506		Massa	Lavello 1	3,85E+04	-	M.E. ^a			
160	7615		Firenze	San Colombano	1,78E+04	-	M.E. ^a			
135	7408	Umbria	Perugia	Perugia - Pian della Genna	5,95E+04	-	Package A/B	Omicron	Package A	Omicron BA.1
136	7518		Perugia	Foligno Casone	2,09E+04	-	M.E. ^a		Package B	
137	7519		Terni	Terni	7,84E+04	Package A			Package A + A222V	
138	7537	Valle	Aosta	La Salle	2,00E+03	-	Package E	Delta		
139	7538	d'Aosta	Aosta	Brissogne	4,58E+03	-	Package A/B	Omicron		
140	7438		Padova	Padova Ca' Nordio - centro storico	2,38E+04	Package A		Omicron BA.1		
141	7439		Padova	Padova Ca' Nordio - zip	1,95E+04	-	-			
142	7440		Padova	Padova Guizza	1,40E+03	-	-			
143	7441		Padova	Abano Terme	1,46E+04	Package A		Omicron BA.1	Package A	
144	7458	Veneto	Treviso	Treviso	1,22E+04	Package A (partial, from G339D to T547K) ^c		Omicron BA.1	Package B	Omicron BA.1
145	7459		Venezia	Venezia Fusina	2,06E+04	-	M.E. ^a		Package C	Omicron BA.2
146	7460		Vicenza	Vicenza Casale	4,95E+03	-	Package A/B	Omicron	Package D	Delta
147	7524		Verona	Verona_collettore 1M	3,23E+04	-	Package A/B	Omicron		
148	7525		Verona	Verona_collettore 3M	3,31E+04	Package B		Omicron BA.1		
149	7526		Verona	Verona_collettore 8M	4,55E+04	Package A		Omicron BA.1		

[†] Mutation packages derived from the long PCR fragments are suggestive of the Omicron sublineages BA.1 or BA.2, while mutation packages derived from the short fragment are not discriminative enough and are generically assigned to the Omicron variant.

^a M.E. mixed electropherograms; ^b Due to shipment problems, samples were thawed at delivery; ^c Partial sequence due to mixed electropherograms and/or high signal noise; within brackets the region for which a sequence was provided

- Package A (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
- Package B (Omicron BA.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 A/B (Omicron, short fragment from PCR ID_987/994): A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del
- Package C (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 D (Delta, long fragment from PCR ID_980) = T95I, G142D, E156G, F157del, R158del, L452R, T478K
- Package E (Delta, short fragment from PCR ID_987/994) = G142D, E156G, F157del, R158del

Limitations of the study

This flash survey's geographical and population coverage was incomplete, as it covered 19/21 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.

Conclusions and final considerations

This is the sixth of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters that will continue to be issued as 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 February 2022 in Italy, in line with clinical results. Characteristic mutations of lineage BA.1 were predominant while characteristic mutations of sublineage BA.2 were detected in four regions. Mutations of the Delta variant were detected in only four Regions/A.P.

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

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 - **Sicilia:** Mario Palermo (Regione Sicilia); Carmelo Massimo Maida, Walter Mazzucco (Università degli Studi di Palermo-Dipartimento PROMISE - sezione di Igiene); Simona De Grazia, Giovanni Giammanco (Centro di Riferimento Regionale per la Sorveglianza delle Paralisi Flaccide Acute (PFA) e ambientale della circolazione di poliovirus in Sicilia - AOUP Palermo); Giuseppa Purpari (IZS - Istituto Zooprofilattico Sperimentale della Sicilia); Margherita Ferrante; Antonella Agodi, Martina Barchitta (Università degli Studi di Catania - Dipartimento "G. F. Ingrassia");
 - **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, 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 Amoroso (Università di Padova);
 - **P.A. Bolzano:** Lorella Zago (P.A. Bolzano), Alberta Stenico, Anna-Maria Prast (Laboratorio biologico - Agenzia provinciale per l'ambiente e la tutela del clima (A.P.P.A.);
 - **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).