Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
7th Report
(Study period: 7 - 11 March 2022)

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Main findings:

- Seventh national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 7-11 March 2022.
- Overall, 153 wastewater samples were collected from 17 Regions and 2 Autonomous Provinces (A.P.)
- Only mutations characteristic of the Omicron variant were detected, found in 15 Regions and two A.P (no sequences were obtained from the remaining two Regions);
- Aminoacid substitutions of sublineage BA.2 were detected in 64% of the sequences (11 Regions/Autonomous Provinces) by Sanger sequencing, while mutations of sublineages BA.1 and BA.1.1 were detected in 4% and 32% of the samples, respectively.
- 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 variants[1].

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 seventh national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 7-11 March 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.

Overall, 153 wastewater samples were collected between 7 and 11 March 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
SARI8385	Abruzzo	Pescara	08/03/2022	Villa Carmine	140.000
SARI8386	Abruzzo	Pescara	08/03/2022	Via Raiale	160.000
SARI8387	Abruzzo	Chieti	08/03/2022	S. Martino	114.500
SARI8388	Abruzzo	L'Aquila	10/03/2022	Pile	48.000
SARI8389	Abruzzo	Teramo	08/03/2022	Villa Pavone	41.824
SARI8440	Basilicata	Potenza	08/03/2022	Tiera di Vaglio	95.000
SARI8441	Basilicata	Matera	08/03/2022	Pantano	24.000
SARI8408	Campania	Avellino	10/03/2022	Manocalzati	140.000
SARI8409	Campania	Salerno	10/03/2022	Salerno	700.000
SARI8410	Campania	Salerno	10/03/2022	Nocera Sup	299.121
SARI8411	Campania	Napoli	10/03/2022	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI8413	Campania	Napoli	10/03/2022	Area Nolana	400.000
SARI8414	Campania	Napoli	10/03/2022	Napoli EST	1.750.000
SARI8420	Campania	Caserta	08/03/2022	Area Casertana	370.769
SARI8423	Campania	Napoli	08/03/2022	Napoli OVEST - Ingresso Principale	950.000
SARI8426	Campania	Caserta	08/03/2022	Villa Literno	631.714
SARI8427	Campania	Salerno	08/03/2022	Eboli	30.000
SARI8286	Emilia-Romagna	Ferrara	08/03/2022	Ferrara - Linea 1	120.000
SARI8287	Emilia-Romagna	Ferrara	08/03/2022	Ferrara - Linea 2	120.000
SARI8288	Emilia-Romagna	Modena	08/03/2022	Carpi	200.000
SARI8335	Emilia-Romagna	Ravenna - Forlì-Cesena	07/03/2022	Ravenna	240.000
SARI8349	Emilia-Romagna	Piacenza	08/03/2022	Borgoforte	163.333
SARI8351	Emilia-Romagna	Parma	09/03/2022	Parma ovest	168.000
SARI8352	Emilia-Romagna	Reggio Emilia	07/03/2022	Mancasale	280.000
SARI8361	Emilia-Romagna	Forlì-Cesena	08/03/2022	Cesena	197.500
SARI8362	Emilia-Romagna	Forlì-Cesena	08/03/2022	Forlì	250.000
SARI8363	Emilia-Romagna	Ravenna	08/03/2022	Faenza	100.000
SARI8364	Emilia-Romagna	Bologna	08/03/2022	Imola	75.000
SARI8393	Emilia-Romagna	Bologna	09/03/2022	IDAR	800.000
SARI8395	Emilia-Romagna	Rimini - Forlì-Cesena	09/03/2022	S. Giustina	560.000
SARI8397	Emilia-Romagna	Modena	09/03/2022	Naviglio	500.000
SARI8405	Friuli-Venezia Giulia	Udine	08/03/2022	Udine	200.000
SARI8406	Friuli-Venezia Giulia	Pordenone	08/03/2022	Cordenons	15.000
SARI8407	Friuli-Venezia Giulia	Trieste	09/03/2022	Servola	190.000
SARI8244	Lazio	Viterbo	07/03/2022	Viterbo - Strada Bagni	30.000
SARI8245	Lazio	Roma	07/03/2022	Anzio - Colle Cocchino	75.000
SARI8246	Lazio	Latina	07/03/2022	Aprilia (Via del Campo)	66.000
SARI8247	Lazio	Latina	07/03/2022	Latina Loc Latina Est	90.000
SARI8248	Lazio	Roma	07/03/2022	Guidonia - Ponte Lucano	50.000
SARI8249	Lazio	Roma	07/03/2022	Velletri (LA CHIUSA-SORBO)	36.700
SARI8250	Lazio	Roma	07/03/2022	Pomezia - Via Cincinnato	60.000
SARI8255	Lazio	Roma	08/03/2022	Civitavecchia Fiumaretta	86.400
SARI8365	Lazio	Roma	08/03/2022	Roma Est (linea 1 + linea 2)	900.000
SARI8366	Lazio	Roma	08/03/2022	Roma Nord	780.000

SARI8367	Lazio	Roma	09/03/2022	Roma Sud	1.100.000
SARI8368	Lazio	Roma	09/03/2022	Ostia	350.000
SARI8369	Lazio	Roma	09/03/2022	Fregene	76.000
SARI8346	Liguria	Genova	08/03/2022	Punta Vagno Genova	75.000
SARI8370	Liguria	Savona	07/03/2022	Savona	256.203
SARI8371	Liguria	Savona	08/03/2022	Borghetto Santo Spirito	140.000
SARI8372	Liguria	Genova	08/03/2022	Pegli	20.507
SARI8373	Liguria	Genova	08/03/2022	Voltri	40.496
SARI8374	Liguria	Genova	08/03/2022	Quinto	48.748
SARI8375	Liguria	Genova	08/03/2022	Rapallo	90.000
SARI8376	Liguria	Genova	09/03/2022	Sestri P	51.368
SARI8377	Liguria	Genova	08/03/2022	Sturla	43.573
SARI8378	Liguria	Imperia	08/03/2022	Sanremo - località Capo Verde	80.000
SARI8379	Liguria	La Spezia	08/03/2022	Camisano	40.840
SARI8380	Liguria	La Spezia	08/03/2022	Silea	17.500
SARI8381	Liguria	La Spezia	08/03/2022	La Spezia	82.000
SARI8382	Liguria	Genova	09/03/2022	Darsena	118.276
SARI8383	Liguria	Genova	09/03/2022	Punta Vagno Genova	75.000
SARI8384	Liguria	Genova	09/03/2022	Valpolcevera	157.650
SARI8272	Lombardia	Milano	07/03/2022	Bresso	220.000
SARI8273	Lombardia	Milano - Monza e della Brianza	07/03/2022	Peschiera Borromeo	566.000
SARI8274	Lombardia	Milano - Varese	07/03/2022	Canegrate	137.950
SARI8275	Lombardia	Varese	07/03/2022	Varese	74.402
SARI8276	Lombardia	Milano - Varese	07/03/2022	Lonate Pozzolo	450.000
SARI8310	Lombardia	Milano	07/03/2022	Milano Nosedo	1.250.000
SARI8311	Lombardia	Milano	07/03/2022	Milano San Rocco	1.036.000
SARI8312	Lombardia	Como	07/03/2022	Como	196.000
SARI8313	Lombardia	Pavia	07/03/2022	Pavia	132.912
SARI8314	Lombardia	Como - Lecco - Milano - Monza e Brianza	07/03/2022	Monza	600.000
SARI8315	Lombardia	Pavia	09/03/2022	Vigevano	57.925
SARI8394	Lombardia	Bergamo	08/03/2022	Bergamo	220.000
SARI8396	Lombardia	Cremona	08/03/2022	Citta di Cremona	180.000
SARI8399	Lombardia	Brescia	08/03/2022	Verziano	296.000
SARI8552	Lombardia	Sondrio	09/03/2022	Sondrio	49.500
SARI8319	Marche	Pesaro-Urbino	08/03/2022	Borgheria	116.000
SARI8320	Marche	Pesaro-Urbino	08/03/2022	Ponte Metauro	60.000
SARI8321	Marche	Pesaro-Urbino	08/03/2022	Ponte Sasso	18.000
SARI8322	Marche	Ancona	08/03/2022	Zipa	100.000
SARI8323	Marche	Ancona	08/03/2022	Falconara	85.000
SARI8324	Marche	Ancona	08/03/2022	Camerano	33.000
SARI8390	Molise	Campobasso	08/03/2022	Termoli - località Porto	25.000
SARI8391	Molise	Campobasso	07/03/2022	Termoli - località Pantano Basso	25.000
SARI8392	Molise	Campobasso	07/03/2022	Campobasso - San Pietro	50.000
SARI8262	P.A. Bolzano	Bolzano	07/03/2022	IDA Bolzano	372.410
SARI8263	P.A. Bolzano	Bolzano	07/03/2022	IDA Merano	356.520
SARI8264	P.A. Bolzano	Bolzano	07/03/2022	IDA Termeno	68.945
SARI8256	P.A. Trento	Trento	07/03/2022	Trento nord	120.000
SARI8257	P.A. Trento	Trento	07/03/2022	Trento sud	100.000
SARI8258	P.A. Trento	Trento	07/03/2022	Rovereto	95.000
SARI8232	Piemonte	Biella	07/03/2022	Biella Nord	67.000
SARI8233	Piemonte	Biella	07/03/2022	Biella Sud	53.000

SARI8234	Piemonte	Novara	07/03/22	Novara	184.000
SARI8235	Piemonte	Torino	07/03/22	Castiglione Torinese	1.934.099
SARI8283	Piemonte	Alessandria	09/03/22	Alessandria	110.000
SARI8284	Piemonte	Asti	09/03/22	Asti	95.000
SARI8285	Piemonte	Cuneo	09/03/22	Cuneo	185.000
SARI8236	Puglia	Bari	07/03/22	Bari Est	389.000
SARI8237	Puglia	Bari	07/03/22	Bari Ovest	360.000
SARI8238	Puglia	Taranto	07/03/22	Taranto Gennarini	226.667
SARI8239	Puglia	Taranto	07/03/22	Taranto Bellavista	116.723
SARI8240	Puglia	Brindisi	07/03/22	Brindisi Fiume Grande	93.013
SARI8241	Puglia	Lecce	07/03/22	Lecce	195.368
SARI8265	Puglia	Foggia	08/03/22	Cerignola	56.355
SARI8266	Puglia	Foggia	08/03/22	Foggia	208.000
SARI8267	Puglia	Foggia	08/03/22	Manfredonia	77.000
SARI8268	Puglia	Barletta-Andria-Trani	08/03/22	Andria	130.000
SARI8289	Puglia	Barletta-Andria-Trani	08/03/22	Barletta	129.356
SARI8290	Puglia	Barletta-Andria-Trani	09/03/22	Bisceglie	85.714
SARI8291	Puglia	Barletta-Andria-Trani	09/03/22	Trani	83.667
SARI8292	Puglia	Bari	09/03/22	Molfetta	84.803
SARI8293	Puglia	Bari	09/03/22	Bitonto	79.332
SARI8342	Puglia	Bari	10/03/22	Altamura	95.414
SARI8277	Sicilia	Ragusa	08/03/22	Ragusa	98.000
SARI8278	Sicilia	Ragusa	08/03/22	Modica	50.400
SARI8279	Sicilia	Ragusa	08/03/22	Vittoria	55.000
SARI8280	Sicilia	Caltanissetta	08/03/22	Gela Macchitella	12.000
SARI8281	Sicilia	Messina	08/03/22	Mili Marina	227.000
SARI8336	Sicilia	Agrigento	07/03/22	Agrigento	55.000
SARI8337	Sicilia	Enna	07/03/22	Enna	34.000
SARI8338	Sicilia	Palermo	08/03/22	Bagheria	75.000
SARI8339	Sicilia	Palermo	08/03/22	Acqua dei Corsari	3.149.73
SARI8340	Sicilia	Palermo	08/03/22	Fondo Verde	53.886
SARI8341	Sicilia	Caltanissetta	08/03/22	Caltanissetta e San Cataldo	76.700
SARI8354	Sicilia	Trapani	08/03/22	Mazara del Vallo	17.000
SARI8355	Sicilia	Trapani	08/03/22	Marsala	40.000
SARI8356	Sicilia	Trapani	08/03/22	Trapani	1.18.500
SARI8434	Sicilia	Catania	08/03/22	Pantano d'Arci	68.434
SARI8435	Sicilia	Catania	08/03/22	Giarre	47.600
SARI8436	Sicilia	Siracusa	08/03/22	Siracusa	180.000
SARI8328	Toscana	Pisa	07/03/22	Pisa Nord - S. Jacopo	52.000
SARI8329	Toscana	Firenze	08/03/22	Empoli Pagnana	88.670
SARI8330	Toscana	Massa	09/03/22	Lavello 2	120.000
SARI8331	Toscana	Lucca	09/03/22	Viareggio	93.000
SARI8332	Toscana	Massa	09/03/22	Lavello 1	87.000
SARI8231	Umbria	Perugia	07/03/22	Perugia - Pian della Genna	90.000
SARI8317	Umbria	Perugia	10/03/22	Foligno Casone	90.000
SARI8318	Umbria	Terni	10/03/22	Terni	150.000
SARI8403	Valle d'Aosta	Aosta	09/03/22	La Salle	60.000
SARI8404	Valle d'Aosta	Aosta	09/03/22	Brissogne	150.000
SARI8251	Veneto	Padova	08/03/22	Padova Ca' Nordio - centro storico	98.500
SARI8252	Veneto	Padova	08/03/22	Padova Ca' Nordio - zip	98.500
SARI8253	Veneto	Padova	08/03/22	Padova Guizza	13.000

SARI8254	Veneto	Padova	08/03/22	Abano Terme	35.000
SARI8269	Veneto	Treviso	08/03/22	Treviso	70.000
SARI8270	Veneto	Vicenza	08/03/22	Vicenza Casale	92.000
SARI8271	Veneto	Venezia	08/03/22	Venezia Fusina	400.000
SARI8358	Veneto	Verona	10/03/22	Verona_collettore 1M	82.000
SARI8359	Veneto	Verona	10/03/22	Verona_collettore 3M	102.000
SARI8360	Veneto	Verona	10/03/22	Verona_collettore 8M	226.000

ǂ 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 described[2] 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 (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_995/996 and generates PCR amplicons of 577 to 693 bp depending on the variant. However, we found out that this short nested PCR is not able to detect the BA.2 variant due to mutations occurring in the region of primer annealing. Therefore, the short PCR can only detect sublineages BA.1 or BA.1.1.

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., 2021[3]. 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, 143 of the 153 samples (93%) 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 2.8 E+06 genome copies (g.c.)/L of sewage.

2 G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferro, 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.

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

Sanger Sequencing

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 2 and 3.** Overall, 56 samples from 16 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, Basilicata, and Toscana. All amplicons were characterized as Omicron variant by Sanger sequencing. Specifically, amino acid substitutions of the sublineage Omicron **BA.1** were detected in two samples from 2 Regions/A.P., **BA.1.1** in 18 samples from 13 Regions/A.P., and **BA.2** in 36 samples from 11 Regions/A.P. Overall, 64% of the sequences showed key mutations of sublineage Omicron BA.2, while mutations of BA1.1 and BA.1 were found in 32% and 4% of the sequences, respectively.

Other 24 positive samples were detected with the short PCR assay (**Table 4**). However, this assay is not able to detect the sublineage BA.2, therefore sequencing of PCR amplicons showed only mutations of BA.1 (9 samples) or BA.1.1 (16 samples). The simultaneous presence of Omicron BA.2 in these samples cannot be excluded.

Next Generation Sequencing

NGS results could be successfully obtained for 16 Regions. Characteristic mutations of the Omicron variant were detected in all the NGS pools.

Mutations associated to Omicron sublineage BA.1 (Package A) were detected in 7 Regions/AA.PP (Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Puglia, Valle d'Aosta, and A.P. Trento,). Mutations associated to Omicron sublineage BA.1.1 were dected in 14 Regions/AA.PP (Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Marche, Molise, Piemonte, Puglia, Sicilia, Valle d'Aosta, Veneto, and A.P. of Trento and Bolzano). Finally, mutations of sublineage BA.2 were detected in 15 Regions/A.P. (Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Marche, Molise, Puglia, Sicilia, Umbria, Valle d'Aosta, Veneto, and A.P. of Trento and Bolzano). Overall, 196.236 reads (48.6%) were assigned to Omicron BA.2, 183.884 reads to BA.1.1 (45.5%), and 23.326 to BA.1 (5.7%).

Key mutations of the Omicron sublineages BA.1, BA1.1, and BA.2 were detected simultaneously in seven Regions (Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Puglia, Valle d'Aosta and A.P. Trento). Mutations of both sublineages BA.1.1 and BA.2 were present in six Regions (Liguria, Marche, Molise, Sicilia, Veneto and A.P. Bolzano). In three Regions, only one Omicron sublineage was identified (BA.2 in Lombardia and Umbria or BA.1.1 in Piemonte).

Mutations associated with other VOCs (es. Delta) or VOIs were not detected.

Sequencing results are summarized in Tables 2-4. 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 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.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** (Omicron BA.1, short fragment from PCR ID_995/996) = G339D; S371L; S373P; S375F
- **Package E** (Omicron BA1.1, short fragment from PCR ID_995/996) = G339D; R346K; S371L; S373P; S375F

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	SARS-CoV-2 variant by Sanger sequencing	Mutations found by NGS	SARS-CoV-2 variant by NGS
1	8385	Abruzzo	Pescara	Villa Carmine	1,46E+04	-			
2	8386		Pescara	Via Raiale	<LOD	-			
3	8387		Chieti	S. Martino	<LOD	-			
4	8388		L'Aquila	Pile	1,93E+04	-			
5	8389		Teramo	Villa Pavone	1,93E+04	-			
153	8440	Basilicata	Potenza	Tiera di Vaglio	4,27E+03	-			
154	8441		Matera	Pantano	1,50E+04	-			
6	8408	Campania (Pool 1)	Avellino	Manocalzati	2,87E+04	-		• Package A	• **Omicron BA.1**
7	8409		Salerno	Salerno	2,29E+04	Package B	**Omicron BA.1.1**	• Package B	• **Omicron BA.1.1**
8	8410		Salerno	Nocera Sup	2,52E+04	-		• Package C	• **Omicron BA.2**
9	8413		Napoli	Area Nolana	1,00E+04	-			
10	8414		Napoli	Napoli EST	5,06E+03	-			
11	8420		Caserta	Area Casertana	1,64E+04	-			
14	8423		Napoli	Napoli OVEST - Ingresso Principale	1,24E+04	-			
17	8426		Caserta	Villa Literno	2,53E+04	-			
18	8427		Salerno	Eboli	1,61E+04	Package B	**Omicron BA1.1**		
151	8411		Napoli	Napoli OVEST - ex ingresso Camaldoli	9,99E+03	-			
19	8286	Emilia-Romagna (Pool 2)	Ferrara	Ferrara - Linea 1	1,60E+03	-		• Package A	• **Omicron BA.1**
20	8287		Ferrara	Ferrara - Linea 2	<LOD	-		• Package B	• **Omicron BA.1.1**
21	8288		Modena	Carpi	<LOD	-		• Package C	• **Omicron BA.2**
22	8335		Ravenna - Forlì-Cesena	Ravenna	6,90E+04	-			
23	8349		Piacenza	Borgoforte	<LOD	-			
24	8351		Parma	Parma ovest	1,31E+04	-			
25	8352		Reggio Emilia	Mancasale	1,74E+04	-			
26	8361		Forlì-Cesena	Cesena	1,32E+05	Package C	**Omicron BA.2**		
27	8362		Forlì-Cesena	Forlì	1,37E+05	Package B	**Omicron BA.1.1**		
28	8363		Ravenna	Faenza	7,58E+04	Package C	**Omicron BA.2**		

29	8364		Bologna	Imola	4,45E+04	-			
30	8393		Bologna	IDAR	1,40E+05	Package C	**Omicron BA.2**		
31	8395		Rimini - Forlì-Cesena	S. Giustina	3,48E+04	Package C	**Omicron BA.2**		
32	8397		Modena	Naviglio	2,80E+04	Package C + S494P	**Omicron BA.2**		
33	8405	Friuli-Venezia Giulia (Pool 3)	Udine	Udine	5,98E+04	Package B	**Omicron BA.1.1**	• Package A • Package B • Package C	• **Omicron BA.1** • **Omicron BA.1.1** • **Omicron BA.2**
34	8406		Pordenone	Cordenons	1,11E+05	Package C	**Omicron BA.2**		
35	8407		Trieste	Servola	1,11E+05	Package C	**Omicron BA.2**		
36	8244	Lazio (Pool 4)	Viterbo	Viterbo - Strada Bagni	1,76E+03	Package B	**Omicron BA.1.1**	• Package A • Package B • Package C	• **Omicron BA.1** • **Omicron BA.1.1** • **Omicron BA.2**
37	8245		Roma	Anzio - Colle Cocchino	1,14E+03	-			
38	8246		Latina	Aprilia (Via del Campo)	6,30E+02	-			
39	8247		Latina	Latina Loc Latina Est	3,88E+02	-			
40	8248		Roma	Guidonia - Ponte Lucano	6,35E+02	-			
41	8249		Roma	Velletri (LA CHIUSA-SORBO)	2,78E+03	-			
42	8250		Roma	Pomezia - Via Cincinnato	4,08E+02	Package B	**Omicron BA.1.1**		
43	8255		Roma	Civitavecchia Fiumaretta	2,50E+01	-			
44	8365		Roma	Roma Est (linea 1 + linea 2)	4,98E+04	Package C + D215E	**Omicron BA.2**		
45	8366		Roma	Roma Nord	8,17E+04	-			
46	8367		Roma	Roma Sud	5,27E+04	-			
47	8368		Roma	Ostia	2,23E+04	-			
48	8369		Roma	Fregene	7,00E+04	-			
49	8346	Liguria (Pool 5)	Genova	Punta Vagno Genova	7,34E+04	Package C	**Omicron BA.2**	• Package B • Package C	• **Omicron BA.1.1** • **Omicron BA.2**
50	8370		Savona	Savona	1,99E+03	-			
51	8371		Savona	Borghetto Santo Spirito	6,69E+04	Package C	**Omicron BA.2**		
52	8372		Genova	Pegli	1,45E+04	-			
53	8373		Genova	Voltri	3,11E+04	Package C	**Omicron BA.2**		
54	8374		Genova	Quinto	6,49E+04	Package C	**Omicron BA.2**		
55	8375		Genova	Rapallo	8,41E+04	Package C	**Omicron BA.2**		
56	8376		Genova	Sestri P	7,65E+04	Package C	**Omicron BA.2**		
57	8377		Genova	Sturla	7,00E+04	Package C	**Omicron BA.2**		
58	8378		Imperia	Sanremo - località Capo Verde	4,27E+04	-			
59	8379		La Spezia	Camisano	3,29E+04	-			
60	8380		La Spezia	Silea	1,20E+05	-			
61	8381		La Spezia	La Spezia	9,88E+04	-			

62	8382		Genova	Darsena	6,91E+04	Package C + E154K	**Omicron BA.2**		
63	8383		Genova	Punta Vagno Genova	6,37E+04	-			
64	8384		Genova	Valpolcevera	2,66E+04	-			
65	8272	Lombardia (Pool 6)	Milano	Bresso	3,95E+03	-		• Package C	• **Omicron BA.2**
66	8273		Milano - Monza e della Brianza	Peschiera Borromeo	8,95E+03	Package C	**Omicron BA.2**		
67	8274		Milano - Varese	Canegrate	6,18E+03	-			
68	8275		Varese	Varese	3,55E+03	-			
69	8276		Milano - Varese	Lonate Pozzolo	1,16E+04	Package C	**Omicron BA.2**		
70	8310		Milano	Milano Nosedo	2,76E+06	-			
71	8311		Milano	Milano San Rocco	1,77E+06	-			
72	8312		Como	Como	1,21E+06	-			
73	8313		Pavia	Pavia	1,67E+06	-			
74	8314		Como - Lecco - Milano - Monza e della Brianza	Monza	<LOD	-			
75	8315		Pavia	Vigevano	1,01E+06	Package C (partial, from G339D to Y505H)[a]	**Omicron BA.2**		
76	8394		Bergamo	Bergamo	8,45E+03	-			
77	8396		Cremona	Città di Cremona	5,15E+03	-			
78	8399		Brescia	Verziano	1,16E+04	-			
152	8552		Sondrio	Sondrio	7,88E+03	-			
79	8319	Marche (Pool 7)	Pesaro-Urbino	Borgheria	1,36E+04	-		• Package B	• **Omicron BA.1.1**
80	8320		Pesaro-Urbino	Ponte Metauro	9,77E+03	-		• Package C	• **Omicron BA.2**
81	8321		Pesaro-Urbino	Ponte Sasso	4,29E+03	-			
82	8322		Ancona	Zipa	3,45E+04	Package B	**Omicron BA.1.1**		
83	8323		Ancona	Falconara	2,52E+04	-			
84	8324		Ancona	Camerano	3,77E+03	-			
85	8390	Molise (Pool 8)	Campobasso	Termoli - località Porto	<LOD	Package B	**Omicron BA.1.1**	• Package B	• **Omicron BA.1.1**
86	8391		Campobasso	Termoli - località Pantano Basso	2,03E+04	Package A	**Omicron BA.1**	• Package C	• **Omicron BA.2**
87	8392		Campobasso	Campobasso - San Pietro	<LOD	-			
88	8262	P.A. Bolzano	Bolzano	IDA Bolzano	5,67E+03	-			

89	8263	(Pool 9)	Bolzano	IDA Merano	1,10E+04	Package C	**Omicron BA.2**	• Package B • Package C	• **Omicron BA.1.1** • **Omicron BA.2**
90	8264		Bolzano	IDA Termeno	1,18E+04	Package B (partial, from G339D to T547K)[a]	**Omicron BA.1.1**		
91	8256	P.A. Trento (Pool 10)	Trento	Trento nord	6,71E+04	-		• Package A	• **Omicron BA.1**
92	8257		Trento	Trento sud	1,31E+05	Package C	**Omicron BA.2**	• Package B	• **Omicron BA.1.1**
93	8258		Trento	Rovereto	1,78E+05	Package C	**Omicron BA.2**	• Package C	• **Omicron BA.2**
94	8232	Piemonte (Pool 11)	Biella	Biella Nord	4,23E+03	-		• Package B	• **Omicron BA.1.1**
95	8233		Biella	Biella Sud	9,98E+03	Package B + G446I	**Omicron BA.1.1**		
96	8234		Novara	Novara	1,28E+03	-			
97	8235		Torino	Castiglione Torinese	6,88E+03	-			
98	8283		Alessandria	Alessandria	<LOD	-			
99	8284		Asti	Asti	4,45E+02	-			
100	8285		Cuneo	Cuneo	7,53E+02	-			
101	8236	Puglia (Pool 12)	Bari	Bari Est	2,20E+06	Package B	**Omicron BA.1.1**	• Package A	• **Omicron BA.1**
102	8237		Bari	Bari Ovest	2,61E+06	Package B	**Omicron BA.1.1**	• Package B	• **Omicron BA.1.1**
103	8238		Taranto	Taranto Gennarini	3,74E+05	-		• Package C	• **Omicron BA.2**
104	8239		Taranto	Taranto Bellavista	2,83E+06	-			
105	8240		Brindisi	Brindisi Fiume Grande	2,48E+06	-			
106	8241		Lecce	Lecce	8,19E+06	Package C + V539A	**Omicron BA.2**		
107	8265		Foggia	Cerignola		-			
108	8266		Foggia	Foggia	4,35E+06	Package C	**Omicron BA.2**		
109	8267		Foggia	Manfredonia	1,55E+05	-			
110	8268		Barletta-Andria-Trani	Andria	1,21E+06	Package C + L296P	**Omicron BA.2**		
111	8289		Barletta-Andria-Trani	Barletta	2,92E+06	-			
112	8290		Barletta-Andria-Trani	Bisceglie	3,96E+05	-			
113	8291		Barletta-Andria-Trani	Trani	4,58E+05	Package A (partial, from G339D to T547K)[a]	**Omicron BA.1**		
114	8292		Bari	Molfetta	1,30E+06	-			
115	8293		Bari	Bitonto	1,29E+05	-			
116	8342		Bari	Altamura	1,06E+06	Package B	**Omicron BA.1.1**		

117	8354	Sicilia (Pool 13)	Trapani	Mazara del Vallo	3,03E+04	-		• Package B • Package C	• **Omicron BA.1.1** • **Omicron BA.2**
118	8277		Ragusa	Ragusa	8,74E+04	-			
119	8278		Ragusa	Modica	1,58E+05	-			
120	8279		Ragusa	Vittoria	2,22E+05	-			
121	8280		Caltanissetta	Gela Macchitella	7,53E+05	-			
122	8281		Messina	Mili Marina	3,04E+05	-			
123	8336		Agrigento	Agrigento	2,50E+04	Package C	**Omicron BA.2**		
124	8337		Enna	Enna	2,98E+04	-			
125	8338		Palermo	Bagheria	3,73E+04	Package C	**Omicron BA.2**		
126	8339		Palermo	Acqua dei Corsari	4,58E+04	Package B (partial, from G339D to T547K)[a]	**Omicron BA.1.1**		
127	8340		Palermo	Fondo Verde	3,31E+04	-			
128	8341		Caltanissetta	Caltanissetta e San Cataldo	3,66E+04	Package C	**Omicron BA.2**		
129	8355		Trapani	Marsala	7,07E+03	-			
130	8356		Trapani	Trapani	5,79E+03	-			
155	8434		Catania	Pantano d'Arci	<LOD	-			
156	8435		Catania	Giarre	4,80E+04	Package B (partial, from A67V to ins214EPE)[a]	**Omicron BA.1.1**		
157	8436		Siracusa	Siracusa	1,86E+04	Package C	**Omicron BA.2**		
131	8328	Toscana	Pisa	Pisa Nord - S. Jacopo	2,10E+04	-			
132	8329		Firenze	Empoli Pagnana	1,01E+04	-			
133	8330		Massa	Lavello 2	5,70E+03	-			
134	8331		Lucca	Viareggio	2,70E+04	-			
135	8332		Massa	Lavello 1	7,05E+03	-			
136	8231	Umbria (Pool 14)	Perugia	Perugia - Pian della Genna	6,00E+04	-		• Package C	• **Omicron BA.2**
137	8317		Perugia	Foligno Casone	1,41E+05	Package C	**Omicron BA.2**		
138	8318		Terni	Terni	1,33E+05	Package B	**Omicron BA.1.1**		
139	8403	Valle d'Aosta (Pool 15)	Aosta	La Salle	4,87E+03	Package B	**Omicron BA.1.1**	• Package A • Package B • Package C	• **Omicron BA.1** • **Omicron BA.1.1** • **Omicron BA.2**
140	8404		Aosta	Brissogne	7,85E+03	-			
141	8251	Veneto (Pool 16)	Padova	Padova Ca' Nordio - centro storico	4,57E+04	-		• Package B • Package C	• **Omicron BA.1.1** • **Omicron BA.2**
142	8252		Padova	Padova Ca' Nordio - zip	1,68E+04	-			
143	8253		Padova	Padova Guizza	1,69E+04	-			

144	8254	Padova	Abano Terme	5,16E+03	Package B	**Omicron BA.1.1**
145	8269	Treviso	Treviso	1,54E+04	Package C	**Omicron BA.2**
146	8270	Vicenza	Vicenza Casale	1,31E+04	Package C (partial, from G339D to Y505H)[a]	**Omicron BA.2**
147	8271	Venezia	Venezia Fusina	2,99E+04	Package C	**Omicron BA.2**
148	8358	Verona	Verona_collettore 1M	2,67E+04	Package C	**Omicron BA.2**
149	8359	Verona	Verona_collettore 3M	4,77E+04	Package C	**Omicron BA.2**
150	8360	Verona	Verona_collettore 8M	5,03E+04	Package C	**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.

- **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 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.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)

	A67V	H69del	V70del	T95I	G142D	V143del	Y144-145del	E154K	N211del	L212I	V213G	ins214EPE	D215E	L296P	G339D	R346K	S371F	S371L	S373P	S375F	T376A	D405N	R408S	K417N	N440K	G446I	G446S	S477N	T478K	E484A	Q493R	S494P	G496S	Q498R	N501Y	Y505H	V539A	T547K	
86, 113*																																							**Package A** (Omicron BA.1)
7, 18, 27, 33, 36, 42, 82, 85, 90*, 101, 102, 116, 126*, 138, 139, 144, 156*																																							**Package B** (Omicron BA.1.1)
95																																							**Package B + G446I** (Omicron BA.1.1)
26, 28, 30, 31, 34, 35, 49, 51, 53, 54, 55, 56, 57, 66, 69, 75*, 89, 92, 93, 108, 123, 125, 128, 137, 145, 146*, 147, 148, 149, 150, 157																																							**Package C** (Omicron BA.2)
62																																							**Package C + E154K** (Omicron BA.2)
44																																							**Package C + D215E** (Omicron BA.2)
110																																							**Package C + L296P** (Omicron BA.2)
32																																							**Package C + S494P** (Omicron BA.2)
106																																							**Package C + V539A** (Omicron BA.2)

* Partial sequence

Table 4. Sanger sequencing results in samples providing amplification with the short PCR ID 996*

	Sample ID	Region/A.P.	City	WTP	Mutations found by Sanger sequencing (short PCR ID_996)	SARS-CoV-2 variant†
153	8440	Basilicata	Potenza	Tiera di Vaglio	Package E	**Omicron BA1.1**
154	8441		Matera	Pantano	Package E	**Omicron BA1.1**
22	8335	Emilia Romagna	Ravenna - Forlì-Cesena	Ravenna	Package D	**Omicron BA.1**
38	8246	Lazio	Latina	Aprilia (Via del Campo)	Package E	**Omicron BA1.1**
39	8247		Latina	Latina Loc Latina Est	Package D	**Omicron BA.1**
45	8366		Roma	Roma Nord	Package E	**Omicron BA1.1**
46	8367		Roma	Roma Sud	Package D	**Omicron BA.1**
60	8380	Liguria	La Spezia	Silea	Package D	**Omicron BA.1**
61	8381		La Spezia	La Spezia	Package D	**Omicron BA.1**
63	8383		Genova	Punta Vagno Genova	Package D	**Omicron BA.1**
64	8384		Genova	Valpolcevera	Package E	**Omicron BA1.1**
72	8312	Lombardia	Como	Como	Package E	**Omicron BA1.1**
105	8240	Puglia	Brindisi	Brindisi Fiume Grande	Package E	**Omicron BA1.1**
109	8267		Foggia	Manfredonia	Package E	**Omicron BA1.1**
114	8292		Bari	Molfetta	Package E	**Omicron BA1.1**
117	8354	Sicilia	Trapani	Mazara del Vallo	Package D	**Omicron BA.1**
120	8279		Ragusa	Vittoria	Package D	**Omicron BA.1**
127	8340		Palermo	Fondo Verde	Package E	**Omicron BA1.1**
155	8434		Catania	Pantano d'Arci	Package E	**Omicron BA1.1**
136	8231	Umbria	Perugia	Perugia - Pian della Genna	Package E	**Omicron BA1.1**
140	8404	Valle d'Aosta	Aosta	Brissogne	Package E	**Omicron BA1.1**
141	8251	Veneto	Padova	Padova Ca' Nordio – c. storico	Package E	**Omicron BA1.1**
142	8252		Padova	Padova Ca' Nordio - zip	Package D	**Omicron BA.1**
143	8253		Padova	Padova Guizza	Package E	**Omicron BA1.1**

* The short PCR ID 996 is not able to detect Omicron sublineage BA.2

- **Package D** (Omicron BA.1, short fragment from PCR ID_995/996) = G339D, S371L, S373P, S375F
- **Package E** (Omicron BA1.1, short fragment from PCR ID_995/996) = G339D, R346K, S371L, S373P, S375F

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.

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.

The short nested PCR ID_996 was not able to detect the characteristic mutations of the Omicron BA.2 variant; specific primers for BA.2 Omicron variant are currently under development.

Conclusions and final considerations

This is the seventh 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 March 2022 in Italy, in line with clinical results. Characteristic mutations of lineage BA.2 were predominant, but while characteristic mutations of sublineage BA.1 and BA.1.1 were detected also 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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- **Friuli Venezia Giulia:** Marika Mariuz, Gabriella Trani (Direzione Centrale Salute FVG); Anna Pariani (LABORATORIO HERAtech di Sasso Marconi –BO);
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- **Piemonte:** Bartolomeo Griglio, Renza Berruti, Mauro Cravero, Angela Costa (Regione Piemonte); Manila Bianchi, Lucia Decastelli; Angelo Romano; Clara Tramuta (IZSTO - Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta SC Sicurezza e Qualità degli Alimenti); Elisabetta Carraro, Cristina Pignata (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino), Silvia Bonetta, Lisa Richiardi (Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino);
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