



**Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
4th Report
(Study period: 30 November – 03 December 2021)**

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:

- Mutations characteristic of the Delta variant were detected in wastewater samples collected between 30 November and 3 December 2021 in thirteen regions and two autonomous provinces located in the North, Centre, and South of Italy;
- No amino acid substitutions associated to other VoCs/VoIs were detected;
- There was no evidence of mutations associated the newly designated VoC Omicron;
- The Delta variant exhibited high genetic diversity, with 25 amino acid substitutions and 1 silent mutations found in the portion of the spike gene analysed.
- Amino acid substitutions characteristic of sublineage AY.4.2 (“Delta Plus”) were 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.

Indeed, a number of studies have demonstrated the value of environmental SARS-CoV-2 sequencing as a tool to identify strains circulating in the community and to study SARS-CoV-2 diversity².

Recently, mutations characteristic of variants of concern (VoCs) were detected in sewage samples collected in Italy^{3,4}. 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.

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>)

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4 La Rosa, G.; Brandtner, D.; Mancini, P.; Veneri, C.; Bonanno Ferraro, G.; Bonadonna, L.; Lucentini, L.; Suffredini, E. 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*, 2503. <https://doi.org/10.3390/w13182503>

Aim

The aim of this report is to summarize the results of the third national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 30 November – 03 December, 2021.

Methodology

The survey included sewage samples collected at wastewater treatment plants (WTPs) located in 15 regions and 2 autonomous provinces (A.P.):

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

Overall, 123 wastewater samples were collected. Of these, 121 were collected between 30 November and 03 December, 2021, while two – due to logistic constraints – were collected on 29 November 2021 (**Table 1**).

Table 1. Sampling sites and characteristics of the WTPs studied

Sample ID ISS	Region/A. P.	City	Sampling Date	WTP	# Population equivalent
SARI5933	Abruzzo	Pescara	30/11/2021	Via Raiale	160.000
SARI5934	Abruzzo	Pescara	30/11/2021	Villa Carmine	140.000
SARI5935	Abruzzo	Chieti	30/11/2021	S. Martino	114.500
SARI5936	Abruzzo	L'Aquila	30/11/2021	Pile	48.000
SARI5937	Abruzzo	Teramo	30/11/2021	Villa Pavone	41.824
SARI5981	Basilicata	Potenza	02/12/2021	Pantano	24.000
SARI5983	Basilicata	Potenza	02/12/2021	Tiera di Vaglio	95.000
SARI5854	Campania	Napoli	30/11/2021	Area Nolana	400.000
SARI5855	Campania	Caserta	30/11/2021	Area Casertana	370.769
SARI5856	Campania	Salerno	30/11/2021	Nocera Sup	299.121
SARI5857	Campania	Napoli	30/11/2021	Napoli EST	1.750.000
SARI5858	Campania	Napoli	30/11/2021	Napoli OVEST - Ingresso Principale	950.000
SARI5859	Campania	Napoli	30/11/2021	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI5880	Campania	Napoli	02/12/2021	Napoli EST	1.750.000
SARI5881	Campania	Napoli	02/12/2021	Area Nolana	400.000
SARI5882	Campania	Caserta	02/12/2021	Area Casertana	370.769
SARI5883	Campania	Napoli	02/12/2021	Napoli OVEST - Ingresso Principale	950.000
SARI5884	Campania	Napoli	02/12/2021	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI5885	Campania	Salerno	02/12/2021	Nocera Sup	299.121
SARI5886	Campania	Avellino	02/12/2021	Manocalzati	140.000
SARI5887	Campania	Caserta	02/12/2021	Villa Literno	631.714
SARI5888	Campania	Salerno	02/12/2021	Salerno	700.000
SARI5861	Emilia-Romagna	Ferrara	30/11/2021	Ferrara L1	120.000

SARI5984	Emilia-Romagna	Ferrara	30/11/2021	Ferrara L2	120.000
SARI5890	Emilia-Romagna	Modena	30/11/2021	Naviglio	500.000
SARI5891	Emilia-Romagna	Forlì-Cesena	30/11/2021	Forlì	250.000
SARI5892	Emilia-Romagna	Forlì-Cesena	30/11/2021	Cesena	197.500
SARI5893	Emilia-Romagna	Ravenna	30/11/2021	Faenza	100.000
SARI5922	Emilia-Romagna	Bologna	01/12/2021	IDAR	800.000
SARI5923	Emilia-Romagna	Modena	01/12/2021	Naviglio	500.000
SARI5925	Emilia-Romagna	Ravenna - Forlì-Cesena	01/12/2021	Ravenna	240.000
SARI5926	Emilia-Romagna	Rimini - Forlì-Cesena	01/12/2021	S. Giustina	560.000
SARI5941	Emilia-Romagna	Reggio Emilia	01/12/2021	Mancasale	280.000
SARI5942	Emilia-Romagna	Piacenza	30/11/2021	Borgoforte	163.333
SARI5943	Emilia-Romagna	Parma	01/12/2021	Parma ovest	168.000
SARI6070	Emilia-Romagna	Bologna	02/12/2021	Santerno entrata	75.000
SARI6059	Friuli-Venezia Giulia	Pordenone	30/11/2021	Cordenons	15.000
SARI6063	Friuli-Venezia Giulia	Udine	30/11/2021	Udine	200.000
SARI6068	Friuli-Venezia Giulia	Trieste	01/12/2021	Servola	190.000
SARI5862	Lazio	Viterbo	01/12/2021	Viterbo - Strada Bagni	30.000
SARI5863	Lazio	Latina	01/12/2021	Aprilia (Via del Campo)	66.000
SARI5894	Lazio	Roma	01/12/2021	Anzio - Colle Cocchino	75.000
SARI5895	Lazio	Latina	01/12/2021	Latina Loc Latina Est	90.000
SARI5896	Lazio	Roma	01/12/2021	Velletri (LA CHIUSA-SORBO)	36.700
SARI5897	Lazio	Roma	01/12/2021	Via Cincinnato	60.000
SARI5898	Lazio	Roma	01/12/2021	Ponte Lucano	50.000
SARI5915	Lazio	Roma	30/11/2021	Civitavecchia Fiumaretta	86.400
SARI5927	Lazio	Roma	30/11/2021	Roma Est (linea 1 + linea 2)	900.000
SARI5928	Lazio	Roma	30/11/2021	Roma Nord	780.000
SARI5929	Lazio	Roma	01/12/2021	Roma Sud	1.100.000
SARI5930	Lazio	Roma	01/12/2021	Ostia	350.000
SARI5931	Lazio	Roma	01/12/2021	Fiumicino	76.000
SARI5864	Liguria	Genova	30/11/2021	Darsena	118.276
SARI5865	Liguria	Genova	30/11/2021	Pegli	20.507
SARI5866	Liguria	Genova	30/11/2021	Voltri	40.496
SARI5867	Liguria	Genova	30/11/2021	Quinto	48.748
SARI5868	Liguria	Genova	30/11/2021	Rapallo	90.000
SARI5869	Liguria	Genova	30/11/2021	Sestri P	51.368
SARI5870	Liguria	Genova	30/11/2021	Sturla	43.573
SARI5871	Liguria	Imperia	30/11/2021	Sanremo - località Capo Verde	80.000
SARI5872	Liguria	Savona	30/11/2021	Savona	256.203
SARI5873	Liguria	Savona	30/11/2021	Borghetto Santo Spirito	140.000
SARI5874	Liguria	La Spezia	30/11/2021	La Spezia	82.000
SARI5875	Liguria	La Spezia	30/11/2021	Camisano	40.840
SARI5876	Liguria	La Spezia	30/11/2021	Silea	17.500
SARI5877	Liguria	Genova	01/12/2021	Darsena	118.276
SARI5878	Liguria	Genova	01/12/2021	Punta Vagno Genova	75.000

SARI5879	Liguria	Genova	01/12/2021	Valpolcevera	157.650
SARI5944	Liguria	Genova	30/11/2021	Punta Vagno Genova	75.000
SARI6012	Liguria	Genova	02/12/2021	Rapallo	90.000
SARI5914	Lombardia	Como - Lecco - Milano - Monza e della Brianza	01/12/2021	Monza	600.000
SARI5916	Lombardia	Brescia	01/12/2021	Verziano	296.000
SARI5917	Lombardia	Brescia	30/11/2021	Verziano	296.000
SARI5920	Lombardia	Milano - Varese	01/12/2021	Canegrate	137.950
SARI5921	Lombardia	Varese	01/12/2021	Varese	74.402
N.A.	Lombardia	Milano	30/11/2021	Nosedo	1.250.000
N.A.	Lombardia	Milano	30/11/2021	San Rocco	1.036.000
SARI5938	Molise	Campobasso	30/11/2021	Campobasso - San Pietro	50.000
SARI5939	Molise	Campobasso	30/11/2021	Termoli - località Porto	25.000
SARI5940	Molise	Campobasso	30/11/2021	Termoli - località Pantano Basso	25.000
SARI5952	P.A. Bolzano	Bolzano	02/12/2021	IDA Bolzano	372.410
SARI5953	P.A. Bolzano	Bolzano	02/12/2021	IDA Merano	356.520
SARI5824	P.A. Trento	Trento	01/12/2021	Trento nord	120.000
SARI5825	P.A. Trento	Trento	01/12/2021	Trento sud	100.000
SARI5826	P.A. Trento	Trento	01/12/2021	Rovereto	95.000
SARI5839	Piemonte	Cuneo	01/12/2021	Cuneo	185.000
SARI5840	Piemonte	Asti	01/12/2021	Asti	95.000
SARI5841	Piemonte	Alessandria	01/12/2021	Alessandria	110.000
SARI5842	Piemonte	Torino	01/12/2021	Castiglione Torinese	1.934.099
SARI5843	Piemonte	Novara	01/12/2021	Novara	184.000
SARI5844	Piemonte	Biella	01/12/2021	Biella Nord	67.000
SARI6419	Puglia	Taranto	01/12/2021	Taranto Bellavista	116.723
SARI6418	Puglia	Taranto	01/12/2021	Taranto Gennarini	226.667
SARI6416	Puglia	Bari	01/12/2021	Bari EST	389.000
SARI6417	Puglia	Bari	01/12/2021	Bari OVEST	242.235
SARI5829	Sicilia	Ragusa	30/11/2021	Modica	50.400
SARI5830	Sicilia	Ragusa	30/11/2021	Ragusa	98.000
SARI5833	Sicilia	Palermo	30/11/2021	Acqua dei Corsari	314.973
SARI5834	Sicilia	Palermo	30/11/2021	Fondo Verde	53.886
SARI5835	Sicilia	Caltanissetta	30/11/2021	Caltanissetta e San Cataldo	76.700
SARI5906	Sicilia	Trapani	30/11/2021	Marsala	40.000
SARI5907	Sicilia	Trapani	30/11/2021	Trapani	118.500
SARI5908	Sicilia	Ragusa	30/11/2021	Vittoria	55.000
SARI5932	Sicilia	Caltanissetta	30/11/2021	Gela Macchitella	12.000
SARI5954	Sicilia	Catania	01/12/2021	Pantano d'Arci	68.434
SARI5831	Sicilia	Agrigento	29/11/2021	Agrigento	55.000
SARI5947	Toscana	Pisa	29/11/2021	Pisa Nord - S. Jacopo	52.000
SARI5948	Toscana	Firenze	30/11/2021	Empoli Pagnana	88.670
SARI5949	Toscana	Massa	01/12/2021	Lavello 2	120.000
SARI5950	Toscana	Lucca	01/12/2021	Viareggio	93.000
SARI5918	Valle d'Aosta	Aosta	01/12/2021	La Salle	60.000

SARI5919	Valle d'Aosta	Aosta	01/12/2021	Brissogne	150.000
SARI5836	Veneto	Vicenza	30/11/2021	Vicenza Casale	92.000
SARI5837	Veneto	Treviso	30/11/2021	Treviso	70.000
SARI5838	Veneto	Venezia	30/11/2021	Venezia Fusina	400.000
SARI5845	Veneto	Padova	30/11/2021	Padova Ca' Nordio - centro storico	98.500
SARI5846	Veneto	Padova	30/11/2021	Padova Ca' Nordio - zip	98.500
SARI5847	Veneto	Padova	30/11/2021	Padova Guizza	13.000
SARI5848	Veneto	Padova	30/11/2021	Abano Terme	35.000
SARI5909	Veneto	Venezia	02/12/2021	Venezia Fusina	400.000
SARI5910	Veneto	Verona	02/12/2021	Verona_collettore 1M	82.000
SARI5911	Veneto	Verona	02/12/2021	Verona_collettore 3M	102.000
SARI5912	Veneto	Verona	02/12/2021	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". RNAs were shipped in dry ice to ISS, where samples were sequenced as previously described⁵.

For amplicon sequencing, 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 resulting in protein mutations (deletion and/or amino acid substitutions) distinctive of the major known circulating SARS-CoV-2 variants, including the Variants of Concern (VoCs) and Variants of Interest (VoIs). To increase the probability of amplification and characterization, three additional short nested RT-PCRs (unpublished), designated as ID_994, ID_996 and ID_991, generating PCR amplicons ranging from 478 to 523 bps, were also used.

Positive PCR products generated by the short assays underwent conventional Sanger sequencing, while the amplicons from the long nested assay, were also sequenced by Next Generation Sequencing (NGS), using the Oxford Nanopore Technology MinION platform, for a more in-depth analysis. For NGS analysis, 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., 2021⁶. Variant calling was performed for currently recognized VoCs (Beta, Gamma, Delta and Omicron) and VoIs (Mu and Lambda). The presence of aminoacid substitutions associated with deescalated VoCs (i.e. Alpha) were also investigated. As regard to the emerging variant lineage B.1.1.529 (Omicron variant), the region amplified by the long PCR ID_980, covers a number of aminoacid substitutions: of the novel VoC (A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I,

5 G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusafarro, 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>.

6 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>

ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K), which can therefore be easily identified by amplicon sequencing. The regions amplified by the short PCRs also cover a number of aminoacid substitutions: A67V, H69del, V70del, T95I, G142T, V143del, Y144del, Y145del, N211del, L212I in PCR ID_994; G339D, S371L, S373P, S375F in PCR ID_996; S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K in PCR ID_991.

Results

Viral concentration data were provided by the laboratories of the SARI network. Overall, 86 of 123 (69.9%) samples tested positive for SARS-CoV-2 by real-time RT-qPCR (**Table 2**), viral concentrations ranged from 3,40E+02 to 3,31E+05 genome copies (g.c.)/L of sewage. For nine samples SARS-CoV-2 concentration was not available. The remaining samples were assumed below the detection limit of the analytical method.

Sixty-six samples were successfully amplified by the long and/or the short PCR assays. Nested PCR, Real-Time PCR, and sequencing results are shown in **Table 2**.

All of the 66 positive samples showed mutations characteristic of the Delta variant (lineage B.1.617.2). These samples were collected in 13 regions (Basilicata, Campania, Emilia Romagna, Friuli Venezia Giulia, Lazio, Liguria, Lombardia, Molise, Piemonte, Puglia, Sicilia, Valle d'Aosta, Veneto) and the 2 autonomous provinces (Trento and Bolzano). The present survey detected no other VoCs (including Omicron) or Vols besides Delta.

Sequence analysis showed a high degree of variability within the Delta variant sequences. Overall, in the ~1600 bps fragment, we detected 25 amino acid substitutions (V70D, K77T, T95I, S98F, G142D, Y145H, M153I, E156G, F157del, R158del, S162I, Y170C, G181V, G184V, A222V, I235T, T240I, P251L, D253A, S255F, G257S, E281Q, S366A, L452R, and T478K) and one silent mutation (T274T). Some of the amino acid substitutions found were very frequent among sequences belonging to the Delta variant, while others were extremely rare (less than 1%). Key mutations (Y145H and A222V) found in sublineage AY.4.2 ("Delta plus") were also detected in four regions (Emilia Romagna, Friuli Venezia Giulia, Lazio and Molise). These mutations are reported in 99% and 99.6% respectively of sequences belonging to sublineage AY.4.2, while they sporadically occur in other sublineages.

The results of this flash survey confirm that the Delta variant was the dominant SARS-CoV-2 variant circulating in the Italian population at the beginning of December, and that the Omicron variant had not yet significantly spread in the population at the same date. Results also confirm a significant genetic diversity within lineage B.1.617.2 - as already found in the [October](#) and [November](#) surveys - and the occurrence of aminoacid substitutions characteristic of sublineage AY.4.2 in several regions.

Table 2. PCR and sequencing results

	Sample ID	Region/A. P.	City	WTP	RT-qPCR (c.g./L)	Mutation found by Sanger sequencing	SARS-CoV-2 variant	Mutation found by NGS (samples pooled by Region/A.P.)	SARS-CoV-2 variant
1	SARI5933	Abruzzo	Pescara	Via Raiale	<LOD	-		-	-
2	SARI5934		Pescara	Villa Carmine	<LOD	-			
3	SARI5935		Chieti	S. Martino	<LOD	-			
4	SARI5936		L'Aquila	Pile	<LOD	-			
5	SARI5937		Teramo	Villa Pavone	<LOD	-			
6	SARI5981	Basilicata	Potenza	Pantano	n.a. ^a	o L452R, T478K	Delta ^b	-	-
7	SARI5983		Potenza	Tiera di Vaglio	n.a. ^a	-			
8	SARI5854	Campania	Napoli	Area Nolana	7,51E+03	-		o G142D, E156G, 157-158del, L452R, T478K	Delta
9	SARI5855		Caserta	Area Casertana	<LOD	-			
10	SARI5856		Salerno	Nocera Sup	9,52E+02	o G142D, E156G, 157-158del	Delta		
11	SARI5857		Napoli	Napoli EST	1,40E+03	-			
12	SARI5858		Napoli	Napoli OVEST - Ingresso Principale	2,16E+03	o G142D, E156G, 157-158del, L452R, T478K	Delta		
13	SARI5859		Napoli	Napoli OVEST - ex ingresso Camaldoli	4,81E+03	-			
14	SARI5880		Napoli	Napoli EST	2,65E+03	-			
15	SARI5881		Napoli	Area Nolana	1,13E+03	-			
16	SARI5882		Caserta	Area Casertana	<LOD	-			
17	SARI5883		Napoli	Napoli OVEST - Ingresso Principale	2,98E+03	-			
18	SARI5884	Napoli	Napoli OVEST - ex	2,66E+03	-				

				ingresso Camaldoli						
19	SARI5885		Salerno	Nocera Sup	4,77E+03	o G142D, E156G, 157-158del		Delta		
20	SARI5886		Avellino	Manocalzati	6,98E+02	-				
21	SARI5887		Caserta	Villa Literno	1,67E+03	-				
22	SARI5888		Salerno	Salerno	5,38E+03	o G142D, E156G, 157-158del		Delta		
23	SARI5861	Emilia- Romagna	Ferrara	Ferrara L1	6,53E+03	-				
124	n.a. ^a		Ferrara	Ferrara L2	n.a. ^a	o G142D, E156G, 157-158del		Delta		
24	SARI5890		Modena	Naviglio	3,32E+04	o L452R, T478K		Delta ^b		
25	SARI5891		Forlì-Cesena	Forlì	1,68E+05	o G142D, E156G, 157-158del, L452R, T478K		Delta		
26	SARI5892		Forlì-Cesena	Cesena	1,50E+05	o T95I, G142D, E156G, 157-158del, L452R, T478K o G142D, E156G, 157-158del		Delta		
27	SARI5893		Ravenna	Faenza	3,40E+04	-				
28	SARI5922		Bologna	IDAR	3,13E+05	o T95I, G142D, E156G, 157-158del, L452R, T478K o G142D, E156G, 157-158del		Delta	o G142D, E156G, 157-158del, L452R, T478K o T95I, G142D E156G, 157- 158del, S255F, E281Q, L452R, T478K o T95I, G142D, Y145H, E156G, 157-158del, A222V, L452R, T478K	
29	SARI5923		Modena	Naviglio	8,45E+04	o T95I, G142D, E156G, 157-158del, L452R, T478K		Delta		
30	SARI5925		Ravenna - Forlì-Cesena	Ravenna	4,80E+04	o G142D, E156G, 157-158del, L452R, T478K		Delta		
31	SARI5926		Rimini - Forlì-Cesena	S. Giustina	1,34E+05	o G142D, E156G, 157-158del, L452R, T478K		Delta		
32	SARI5941		Reggio Emilia	Mancasale	7,18E+03	o T95I, G142D, Y145H, E156G, 157-158del, A222V, L452R, T478K		Delta (presumptive lineage AY.4.2) ^c		
33	SARI5942		Piacenza	Borgoforte	<LOD	-				
34	SARI5943		Parma	Parma ovest	<LOD	-				
130	SARI6070		Bologna	Santerno	1,25E+03	o T95I, G142D, E156G, 157-158del, L452R, T478K		Delta		
127	SARI6059	Friuli-Venezia Giulia	Pordenone	Cordenons	3,31E+05	o T95I, G142D, Y145H, E156G, 157-158del, A222V, L452R, T478K o G142D, E156G, 157-158del, G181V		Delta (including also presumptive lineage AY.4.2) ^c		Delta (including also presumptive lineage AY.4.2) ^c
128	SARI6063		Udine	Udine	5,73E+04	o G142D, E156G, 157-158del, L452R, T478K		Delta		
129	SARI6068		Trieste	Servola	2,65E+05	o G142D, E156G, 157-158del, L452R, T478K		Delta		
35	SARI5862	Lazio	Viterbo	Viterbo - Strada Bagni	<LOD	o T95I, G142D, E156G, 157-158del		Delta		Delta

36	SARI5863		Latina	Aprilia (Via del Campo)	n.a. ^a	○ G142D, E156G, 157-158del	Delta	○ G142D, E156G, 157-158del, P251L, L452R, T478K				
37	SARI5894		Roma	Anzio - Colle Cocchino	<LOD	○ T95I, G142D, Y145H, E156G, 157-158del, A222V ○ G142D, E156G, 157-158del	Delta (presumptive lineage AY.4.2)					
38	SARI5895		Latina	Latina Loc Latina Est	<LOD	-						
39	SARI5896		Roma	Velletri (LA CHIUSA-SORBO)	<LOD	○ T95I, G142D, E156G, 157-158del	Delta					
40	SARI5897		Roma	Via Cincinnato	<LOD	○ T95I, G142D, Y145H, E156G, 157-158del, A222V ○ G142D, E156G, 157-158del	Delta (including also presumptive lineage AY.4.2) ^c					
41	SARI5898		Roma	Ponte Lucano	<LOD	-						
42	SARI5915		Roma	Civitavecchia Fiumaretta	<LOD	-						
43	SARI5927		Roma	Roma Est (linea 1 + linea 2)	1,69E+04	○ T95I, G142D, E156G, 157-158del	Delta					
44	SARI5928		Roma	Roma Nord	<LOD	-						
45	SARI5929		Roma	Roma Sud	3,40E+02	-						
46	SARI5930		Roma	Ostia	3,09E+04	○ T95I, G142D, E156G, 157-158del	Delta					
47	SARI5931		Roma	Fiumicino	9,77E+03	○ G142D, M153I, E156G, 157-158del ○ S255F	Delta					
48	SARI5864		Liguria	Genova	Darsena	2,73E+04	○ G142D, E156G, 157-158del			Delta	○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, Y170C, L452R, T478K	Delta
49	SARI5865			Genova	Pegli	7,29E+02	○ T95I, G142D, E156G, 157-158del			Delta		
50	SARI5866	Genova		Voltri	<LOD	○ T95I, G142D, E156G, 157-158del	Delta					
51	SARI5867	Genova		Quinto	1,43E+04	○ T95I, G142D, E156G, 157-158del	Delta					
52	SARI5868	Genova		Rapallo	8,50E+04	-						
53	SARI5869	Genova		Sestri P	<LOD	○ T95I, G142D, E156G, 157-158del	Delta					
54	SARI5870	Genova		Sturla	3,57E+04	○ T95I, G142D, E156G, 157-158del, L452R, T478K	Delta					
55	SARI5871	Imperia		Sanremo - Capo Verde	2,08E+05	○ T95I, G142D, E156G, 157-158del, Y170C, L452R, T478K	Delta					

56	SARI5872		Savona	Savona	8,35E+04	o G142D, E156G, 157-158del, L452R, T478K	Delta		
57	SARI5873		Savona	Borghetto Santo Spirito	4,74E+04	-			
58	SARI5874		La Spezia	La Spezia	2,94E+04	o T95I, G142D, E156G, 157-158del	Delta		
59	SARI5875		La Spezia	Camisano	8,39E+03	o T95I, G142D, E156G, 157-158del	Delta		
60	SARI5876		La Spezia	Silea	<LOD	-			
61	SARI5877		Genova	Darsena	<LOD	-			
62	SARI5878		Genova	Punta Vagno Genova	8,99E+03	-			
63	SARI5879		Genova	Valpolcevera	<LOD	-			
126	SARI5944		Genova	Punta Vagno Genova	3,57E+04	o T95I, G142D, E156G, 157-158del	Delta		
125	SARI6012		Genova	Rapallo	7,42E+04	o T95I, G142D, E156G, 157-158del	Delta		
67	SARI5914	Lombardia	Como - Lecco - Milano - Monza e della Brianza	Monza	5,25E+03	o G142D, E156G, 157-158del	Delta	o G142D, E156G, 157-158del, L452R, T478K	Delta
68	SARI5916		Brescia	Verziano	1,66E+03	-			
69	SARI5917		Brescia	Verziano	1,24E+03	-			
70	SARI5920		Milano - Varese	Canegrate	5,98E+04	o G142D, E156G, 157-158del, L452R, T478K	Delta		
71	SARI5921		Varese	Varese	1,28E+04	o L452R, T478K	Delta ^b		
72	n.a. ^a		Milano	Nosedo	n.a. ^a	o T95I, G142D, E156G, 157-158del	Delta		
73	n.a. ^a		Milano	San Rocco	n.a. ^a	o G142D, E156G, 157-158del, L452R, T478K	Delta		
80	SARI5938	Molise	Campobasso	Campobasso - San Pietro	<LOD	o T95I, G142D, Y145H, E156G, 157-158del	Delta (presumptive lineage AY.4.2) ^c		
81	SARI5939		Campobasso	Termoli - Porto	<LOD	-		-	-
82	SARI5940		Campobasso	Termoli - Pantano Basso	<LOD	-			
83	SARI5952	P.A. Bolzano	Bolzano	IDA Bolzano	2,30E+04	o T95I, G142D, E156G, 157-158del	Delta	-	Delta
84	SARI5953		Bolzano	IDA Merano	2,97E+04	o T95I, G142D, E156G, 157-158del	Delta		

85	SARI5824	P.A. Trento	Trento	Trento nord	7,97E+04	○ T95I, G142D, E156G, 157-158del, L452R, T478K	Delta	○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, I235T, L452R, T478K ○ G142D, E156G, 157-158del, T240I, L452R, T478K ○ G142D, E156G, 157-158del, P251L, L452R, T478K ○ G142D, E156G, 157-158del, S366A, L452R, T478K	Delta
86	SARI5825		Trento	Trento sud	9,85E+04	○ G142D, E156G, 157-158del, L452R, T478K	Delta		
87	SARI5826		Trento	Rovereto	1,71E+05	○ G142D, E156G, 157-158del, L452R, T478K	Delta		
88	SARI5839	Piemonte	Cuneo	Cuneo	1,57E+03	-		○ G142D, E156G, 157-158del, L452R, T478K ○ G142D, E156G, 157-158del, L452R, T478K	Delta
89	SARI5840		Asti	Asti	5,00E+03	○ G142D, E156G, 157-158del, L452R, T478K ○ V70D, G142D, E156G, 157-158del	Delta		
90	SARI5841		Alessandria	Alessandria	5,28E+03	○ G142D, E156G, 157-158del, S162I	Delta		
91	SARI5842		Torino	Castiglione Torinese	8,70E+03	-			
92	SARI5843		Novara	Novara	1,68E+03	-			
93	SARI5844		Biella	Biella Nord	6,28E+03	-			
94	SARI6419	Puglia	Taranto	Taranto Bellavista	n.a. ^a	-		-	Delta
95	SARI6418		Taranto	Taranto Gennarini	n.a. ^a	-			
96	SARI6416		Bari	Bari EST	n.a. ^a	○ K77T, G142D, E156G, 157-158del	Delta		
97	SARI6417		Bari	Bari Ovest	n.a. ^a	-			
98	SARI5829	Sicilia	Ragusa	Modica	<LOD	-		○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, L452R, T478K	Delta
99	SARI5830		Ragusa	Ragusa	2,70E+03	-			
100	SARI5833		Palermo	Acqua dei Corsari	5,98E+03	-			
101	SARI5834		Palermo	Fondo Verde	5,90E+03	-			

102	SARI5835		Caltanissetta	Caltanissetta e San Cataldo	5,16E+03	-			
103	SARI5906		Trapani	Marsala	1,86E+03	-			
104	SARI5907		Trapani	Trapani	1,52E+03	-			
105	SARI5908		Ragusa	Vittoria	1,40E+03	-			
106	SARI5932		Caltanissetta	Gela Macchitella	5,93E+03	-			
131	SARI5954		Catania	Pantano d'Archi	5,23E+02	-			
132	SARI5831		Agrigento	Agrigento	4,86E+03	Week positive, unsuitable for Sanger sequencing			
107	SARI5947	Toscana	Pisa	Pisa Nord – S. Jacopo	4,95E+03	-			
108	SARI5948		Firenze	Empoli Pagnana	<LOD	-			-
109	SARI5949		Massa	Lavello 2	7,53E+03	-			
110	SARI5950		Lucca	Viareggio	<LOD	-			
111	SARI5918	Valle d'Aosta	Aosta	La Salle	2,06E+03	○ T95I, G142D, E156G, 157-158del, L452R, T478K ○ G142D, E156G, 157-158del	Delta	○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del, L452R, T478K	Delta
112	SARI5919		Aosta	Brissogne	1,45E+05	○ G142D, E156G, 157-158del, L452R, T478K	Delta	○ S98F, G142D, E156G, 157-158del, L452R, T478K	
113	SARI5836	Veneto	Vicenza	Vicenza Casale	2,05E+03	○ G142D, E156G, 157-158del, L452R, T478K	Delta	○ G142D, E156G, 157-158del, L452R, T478K	Delta
114	SARI5837		Treviso	Treviso	9,23E+03	○ G142D, E156G, 157-158del, L452R, T478K	Delta		
115	SARI5838		Venezia	Venezia Fusina	8,25E+03	○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del	Delta		
116	SARI5845		Padova	Padova Ca' Nordio - centro storico	1,85E+03	-			

117	SARI5846		Padova	Padova Ca' Nordio - zip	1,88E+03	○ G142D, E156G, 157-158del	Delta		
118	SARI5847		Padova	Padova Guizza	6,58E+02	○ G142D, E156G, 157-158del, L452R, T478K	Delta		
119	SARI5848		Padova	Abano Terme	1,66E+03	○ T95I, G142D, E156G, 157-158del, L452R, T478K ○ G142D, E156G, 157-158del ○ P251L	Delta		
120	SARI5909		Venezia	Venezia Fusina	3,57E+04	○ G142D, E156G, 157-158del, L452R, T478K ○ P251L	Delta		
121	SARI5910		Verona	Verona_collettore 1M	3,71E+04	○ G142D, E156G, 157-158del, G257S, L452R, T478K ○ T95I, G142D, E156G, 157-158del	Delta		
122	SARI5911		Verona	Verona_collettore 3M	2,13E+04	○ T95I, G142D, E156G, 157-158del ○ L452R, T478K	Delta		
123	SARI5912		Verona	Verona_collettore 8M	1,96E+04	○ G142D, E156G, 157-158del, L452R, T478K ○ T95I, G142D, E156G, 157-158del ○ D253A	Delta		

^a n.a. = not available

^b The mutation combination Spike_L452R, Spike_T478k is present in 96.9% of delta and 1.7% of the omicron sequences as on 24/12/21.

^c Suggested by the presence of mutation Spike_Y145H or by the combination of mutations Spike_Y145H + Spike_A222V

Limitations of the study

- Given the progressive implementation of the national surveillance for SARS-CoV-2 in wastewaters, sample collection/processing is not yet feasible in all Italian regions. Therefore, this flash survey's geographical and population coverage was incomplete, as it covered 17/21 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 fourth 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 Delta variant (B.1.617.2) in late November/early December 2021 in Italy, in line with clinical results. No other VoCs (including Omicron) or VoIs were detected in this survey.

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