Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
3rd Report
(Study period: 01 – 05 November 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, Mirko Rossi (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- 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 in November 2021 in seven 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;
- Amino acid substitutions characteristic of the Alpha variant were detected in one sample collected in the region of Campania;
- There was no evidence of mutations associated the newly designated VoC Omicron;
- The Delta variant exhibited high genetic diversity, with 17 amino acid substitutions and 5 silent mutations found in the portion of the spike gene analysed. A total of 8 different amino acid combinations were detected;
- 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 variants[1].

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 diversity[2].

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

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)

2 Bonanno Ferraro G, Veneri C, Mancini P, Iaconelli M, Suffredini E, Bonadonna L, Lucentini L, Bowo-Ngandji A, Kengne-Nde C, Mbaga DS, Mahamat G, Tazokong HR, Ebogo-Belobo JT, Njouom R, Kenmoe S, La Rosa G. A State-of-the-Art Scoping Review on SARS-CoV-2 in Sewage Focusing on the Potential of Wastewater Surveillance for the Monitoring of the COVID-19 Pandemic. Food Environ Virol. 2021 Nov 2:1–40. doi: 10.1007/s12560-021-09498-6. Epub ahead of print. PMID: 34727334; PMCID: PMC8561373.

3 La Rosa G, Mancini P, Bonanno Ferraro G, Veneri C, Iaconelli M, Lucentini L, Bonadonna L, Brusaferro S, Brandtner D, Fasanella A, Pace L, Parisi A, Galante D, Suffredini E. 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 Res. 2021 Jun 1;197:117104. doi: 10.1016/j.watres.2021.117104.

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

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 third national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of November 1-5, 2021.

Methodology

The survey included sewage samples collected at wastewater treatment plants (WTPs) located in 13 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;
- Central Italy: Abruzzo, Lazio and Toscana;
- Southern Italy and Islands: Campania, Basilicata, Puglia and Sicilia.

Overall, 103 wastewater samples were collected. Of these, 98 were collected between 1 November and 5 November, 2021, while 5 – due to logistic constraints – were collected in the period 28-31 October (**Table 1**).

Table 1. Sampling sites and characteristics of the WTPs studied

Sample ID ISS	Region/A.P.	City	Sampling Date	WTP	Population equivalent[‡]
FS-202111-33	Abruzzo	Pescara	02/11/2021	Via Raiale	160.000
FS-202111-34		Montesilvano	02/11/2021	Villa Carmine	140.000
FS-202111-35		Chieti	02/11/2021	S. Martino	114.500
FS-202111-36		Teramo	02/11/2021	Villa Pavone	41.824
FS-202111-37		L'Aquila	02/11/2021	Pile	48.000
FS-202111-14	Basilicata	Potenza	03/11/2021	Tiera di Vaglio	95.000
FS-202111-15		Matera	03/11/2021	Pantano	24.000
FS-202111-75	Campania	area Nocera [a]	02/11/2021	Nocera Superiore	300.000
FS-202111-57			04/11/2021		
FS-202111-73		area Nolana [b]	02/11/2021	Depuratore (Area Nolana)	400.000
FS-202111-58			04/11/2021		
FS-202111-74		area Casertana [c]	02/11/2021	Depuratore (Area Casertana)	370.769
FS-202111-59			04/11/2021		
FS-202111-72		area Napoli [d]	02/11/2021	Impianto Napoli OVEST (Ingr. principale)	950.000
FS-202111-60			04/11/2021		
FS-202111-71		area Napoli Ovest [e]	02/11/2021	Impianto Napoli OVEST (Ingr. Camaldoli-Fogna NORD)	250.000
FS-202111-61			04/11/2021		
FS-202111-70		area Napoli Est [f]	02/11/2021	Napoli EST	1.750.000
FS-202111-62			04/11/2021		
FS-202111-63		Avellino	04/11/2021	Avellino (Monocalzati)	140.000
FS-202111-46	Emilia Romagna	Ferrara	02/11/2021	Gramicia	120.000
FS-202111-47		Bologna	02/11/2021	Idar	800.000
FS-202111-48		Forli	02/11/2021	Forlì	250.000
FS-202111-49		Ravenna	02/11/2021	Ravenna	240.000
FS-202111-50		Bologna	03/11/2021	Idar	800.000
FS-202111-51		Modena	03/11/2021	Naviglio	500.000

FS-202111-52		Faenza	03/11/2021	Formellino	1.000.000
FS-202111-53		Imola	03/11/2021	Santerno	75.000
FS-202111-54		Rimini	03/11/2021	S.Giustina	560.000
FS-202111-55		Ravenna	03/11/2021	Ravenna	240.000
FS-202111- 56		Cesena	02/11/2021	Cesena	197.500
FS-202111-76		Reggio Emilia	03/11/2021	Mancasale	280.000
FS-202111-77		Piacenza	03/11/2021	Borgoforte	163.333
FS-202111-78		Parma	03/11/2021	Parma Ovest	168.000
FS-202111-41	Lazio	Civitavecchia	02/11/2021	Fiumaretta	86.400
FS-202111-99		Roma	02/11/2021	Roma EST (linea 1- linea2)	900.000
FS-202111-100			02/11/2021	Roma Nord	780.000
FS-202111-101			03/11/2021	Roma Sud	1.100.000
FS-202111-102			03/11/2021	Ostia	350.000
FS-202111-103			03/11/2021	Fregene	76.000
FS-202111-32	Liguria	Genova	02/11/2021	Punta Vagno	220.000
FS-202111-80			02/11/2021	Pegli	40.000
FS-202111-81			02/11/2021	Prà-Voltri	62.000
FS-202111-82			02/11/2021	Quinto	60.000
FS-202111-84			02/11/2021	Sestri Ponente	130.000
FS-202111-85			02/11/2021	Sturla	60.000
FS-202111-91			03/11/2021	Darsena	220.000
FS-202111-92			03/11/2021	Valpolcevera	160.000
FS-202111-79		Sanremo	02/11/2021	Sanremo	75.000
FS-202111-83		Rapallo	02/11/2021	Rapallo	90.000
FS-202111-86		Borghetto S. Spirito	02/11/2021	Borghetto Santo Spirito	121.000
FS-202111-87		Savona	02/11/2021	Savona	280.000
FS-202111-88		La Spezia	02/11/2021	Stagnoni	82.000
FS-202111-89			02/11/2021	Camisano	40.000
FS-202111-90			02/11/2021	Silea	17.500
FS-202111-38	Lombardia	Monza	03/11/2021	Monza	600.000
FS-202111-39		Milano	03/11/2021	San Rocco	1.036.000
FS-202111-40			03/11/2021	Nosedo	1.250.000
FS-202111-97		Canegrate	02/11/2021	Canegrate	137.950
FS-202111-98		Varese Olona	02/11/2021	Varese Olona	74.402
FS-202111-68		Verziano	02/11/2021	Verziano	296.000
FS-202111-69			03/11/2021		
FS-202111-16	Piemonte	Torino	03/11/2021	Castiglione Torinese	1.934.099
FS-202111-17		Asti	03/11/2021	Asti	95.000
FS-202111-18		Alessandria	03/11/2021	Alessandria	110.000
FS-202111-19		Biella	03/11/2021	Biella Nord	120.000
FS-202111-20		Cuneo	03/11/2021	Cuneo	185.000
FS-202111-21		Novara	03/11/2021	Novara	184.000
FS-202111-93	Puglia	Bari	02/11/2021	Bari Est	389.000
FS-202111-94			02/11/2021	Bari Ovest	242.235
FS-202111-95		Taranto	03/11/2021	Taranto Gennarini	226.667
FS-202111-96			03/11/2021	Taranto Bellavista	116.723
FS-202111-22	Sicilia	Palermo	02/11/2021	Acqua dei Corsari	314.973
FS-202111-23			02/11/2021	Fondo Verde	53.886
FS-202111-24		Trapani	02/11/2021	Trapani	118.500
FS-202111-25		Mazara Del Vallo	02/11/2021	Mazara del Vallo	17.000
FS-202111-26		Marsala	02/11/2021	Marsala	40.000
FS-202111-27		Catania	04/11/2021	Pantano d'Arci	68.434
FS-202111-64	Toscana	Pisa	02/11/2021	S. Jacopo	52.000
FS-202111-65		Empoli	02/11/2021	Pagnana	88.670
FS-202111-66		Massa Carrara	03/11/2021	Lavello 2	21.678

FS-202111-67		Viareggio	03/11/2021	Viareggio	15.943
FS-202111-28	Valle D'Aosta	area La Salle [g]	01/11/2021	Depuratore di La Salle	60.000
FS-202111-30			03/11/2021		
FS-202111-29		area Aosta [h]	01/11/2021	Depuratore di Brissogne	150.000
FS-202111-31			03/11/2021		
FS-202111-4	Veneto	Venezia	02/11/2021	Venezia Fusina	400.000
FS-202111-5		Vicenza	02/11/2021	Vicenza Casale	92.000
FS-202111-6		Treviso	02/11/2021	Treviso	70.000
FS-202111-7		Verona	04/11/2021	Verona collettore 1M	82.000
FS-202111-8			04/11/2021	Verona collettore 3M	102.000
FS-202111-9			04/11/2021	Verona collettore 8M	226.000
FS-202111-10		Padova	02/11/2021	Padova Ca Nordio - Centro Storico	98.500
FS-202111-11			02/11/2021	Padova Ca Nordio - ZIP	98.500
FS-202111-12			02/11/2021	Padova Guizza	13.000
FS-202111-13		Abano Terme	02/11/2021	Abano	35.000
FS-202111-42	Provincia Autonoma di Bolzano	Bolzano	28/10/2021	ARA Bolzano	372.410
FS-202111-44			02/11/2021		
FS-202111-43		Merano	28/10/2021	ARA Merano	356.520
FS-202111-45			02/11/2021		
FS-202111-1	Provincia Autonoma di Trento	Trento	31/10/2021	Trento Nord	120.000
FS-202111-2		Trento	31/10/2021	Trento Sud	100.000
FS-202111-3		Rovereto	31/10/2021	Rovereto	95.000

[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)

[a] area Nocera = Nocera Superiore, Castel San Giorgio, Siano, Roccapiemonte, Cava de' Tirreni

[b] area Nolana = Avella, Baiano, Brusciano, Camposano, Carbonara di Nola, Casamarciano, Castel Cisterna, Cicciano, Cimitile, Comiziano, Domicella, Lauro, Liveri, Mariglianella, Marigliano, Marzano di Nola, Moschiano, Mugnano del Cardinale, Nola, Pago del Vallo di Lauro, Palma Campania, Quadrelle, Quindici, Roccarainola, San Gennaro Vesuviano, San Paolo Belsito, San Vitaliano, Saviano, Scisciano, Sirignano, Sperone, Taurano, Tufino, Visciano, Zona ASI Nola-Marigliano

[c] area Casertana = Capodrise, Capua, Casagiove, Casapulla, Caserta, Curti, Macerata Campania, Maddaloni, Marcianise, Portico di Caserta, Recale, S. Marco Evangelista, S. Nicola La Strada, S. Prisco, S. Maria C.V., San Tammaro

[d] area Napoli = Napoli (zona centro-occidentale), Pozzuoli

[e] area Napoli Ovest = Calvizzano, Giugliano (quota parte), Marano di Napoli, Mugnano (quota parte), Qualiano, Quarto, Villaricca (quota parte)

[f] area Napoli Est = Portici, Cercola, Napoli Est, Pollena Trocchia, San Giorgio a Cremano, San Sebastiano al Vesuvio, Sant'Anastasia, Somma Vesuviana, Volla, Casoria, Casalnuovo di Napoli

[g] area La Salle = La Salle, Morgex, Pré Saint Didier e La Thuile

[h] area Aosta = Pollein, Charvensod, Gressan, Jovençan, Aymavilles, Villeneuve, Introd, Saint Nicolas, Saint Pierre, Sarre, Aosta, Saint Christophe

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 SARS-CoV-2 was quantified following the same protocol and positive samples were sequenced as previously described[5].

5 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.

For amplicon sequencing, a long nested RT-PCR assay (ID_980) (~1600 bps, spanning amino acid residues 58 to 573 of the spike protein) was designed 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, two additional short nested RT-PCRs (unpublished), designated as ID_987 and ID_991 (495 bp and 523 bp amplicons, respectively), were also used.

All positive samples underwent both conventional Sanger sequencing and Next Generation Sequencing (NGS) for a more in-depth analysis, using the Oxford Nanopore Technology MinION platform. Amplicons obtained from different samples collected in the same city were mixed in a single pool.

Bioinformatics analysis of NGS data was carried out as described in La Rosa et al., 2021[6]. Variant calling was performed for currently recognized VoCs (Beta, Gamma, Delta) and VoIs (Mu and Lambda). The presence of aminoacid substitutions associated with deescalated VoCs (i.e. Alpha) and the emerging variant lineage B.1.1.529 was also investigated. This variant, characterized by numerous S-gene mutations, was detected at the beginning of November 2021, and was designated as a VoC and assigned the label Omicron by the World Health Organization (WHO) and by ECDC on 26 November 2021[7;8]. The region amplified by the long PCR 980, covers a number of aminoacid substitutions of the Omicron variant (A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, 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.

Results

Overall, 68 out of 98 (69.4 %) samples tested positive for SARS-CoV-2 by real-time RT-qPCR (**Table 2),** viral concentrations ranged from 1.09E+03 to 5.12E+04 genome copies (g.c.)/L of sewage. The remaining samples were assumed below the detection limit of the analytical method.

Thirty-two 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 32 positive samples showed mutations characteristic of the Delta variant (lineage B.1.617.2). These samples were collected in 7 regions (Veneto, Liguria, Lombardia, Emilia Romagna, Lazio, Campania, and Sicilia) and the 2 autonomous provinces (Trento and Bolzano). The present survey detected no other VoCs (including Omicron) or VoIs besides Delta. One of the samples, collected in the province of Salerno, in the region of Campania, showed mutations characteristic of the Alpha variant (H69del, V70del, Y144del) which is considered a deescalated variant because of its drastically reduced circulation in the EU/EEA.

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

7 WHO 2021. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern (who.int)

8 ECDC 2021. SARS-CoV-2 variants of concern as of 26 November 2021. SARS-CoV-2 variants of concern as of 26 November 2021 (europa.eu)

Sequence analysis showed a high degree of variability within the Delta variant sequences. Overall, in the ~1600 bps fragment, we detected 17 amino acid substitutions (W64C, T95I, G142D, Y145H, E156G, F157del, R158del, A222V, P251L, S255F, E281Q, L452R, T415N, F464L, T478K, A520S, S555F and five silent mutations (T85T, N344N, F401F, I410I, S444S), for a total of 8 different amino acid combinations. 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 found in sublineage AY.4.2 ("Delta plus") were also detected (Y145H, and A222V) in three of the northern regions (Veneto, Lombardia, and Emilia Romagna). These mutations are reported in 91% and 99.9%, 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 is currently the dominant SARS-CoV-2 variant circulating in the Italian population, as already shown in the previous survey conducted in October 2021. Results are in line with clinical surveillance data, reporting that 99,6% of sequences deposited in the Italian platform I-Co-Gen (Italian COVID-19 Genomic) between 9 October and 22 November, belong to the Delta variant[9]. Results also confirm a significant genetic diversity within lineage B.1.617.2 - as already found in the October survey - and the occurrence of aminoacid substitutions characteristic of sublineage AY.4.2 in three regions in northern Italy. Mutations characteristic of the Alpha variant were detected only in one of the 32 positive samples. The sample in question was collected in the region of Campania. This is in agreement with clinical data, showing that only 0.22% of the sequences deposited to the I-Co-Gen platform in Italy between 9 October and 22 November belong to the Alpha variant. The presence of the Alpha variant in Campania is also confirmed by clinical data: about 40 sequences characterized as Alpha variant have been submitted to GISAID from samples collected in Campania since October 2021.

9 Prevalenza e distribuzione delle varianti del virus SARS-CoV-2 di interesse per la sanità pubblica in Italia (iss.it)

Table 2. PCR and sequencing results

Sample ID (ISS)	Region/A.P.	City	Sampling Date	WTP	RT-qPCR (c.g./L)	RT-nested-PCR (long and/or short)	Sequencing results (Sanger + NGS)	SARS-CoV-2 Variant
FS-202111-33	Abruzzo	Pescara	02/11/2021	Via Raiale	<LOD	Neg	-	
FS-202111-34		Montesilvano	02/11/2021	Villa Carmine	1,01E+03		-	
FS-202111-35		Chieti	02/11/2021	S. Martino	4,36E-01	Neg	-	
FS-202111-36		Teramo	02/11/2021	Villa Pavone	2,13E+03	Neg	-	
FS-202111-37		L'aquila	02/11/2021	Pile	<LOD	Neg	-	
FS-202111-14	Basilicata	Potenza	03/11/2021	Tiera di Vaglio	<LOD	Neg	-	
FS-202111-15		Matera	03/11/2021	Pantano	<LOD	Neg	-	
FS-202111-75	Campania	area Nocera [a]	02/11/2021	Nocera Superiore	8,31E+03	Neg	-	
FS-202111-57			04/11/2021		3,35E+03	**Pos**	○ H69del, V70del, Y144del ○ L452R, T478K	Alpha + Delta
FS-202111-73		area Nolana [b]	02/11/2021	Depuratore (Area Nolana)	8,70E+02	Neg	-	
FS-202111-58			04/11/2021		<LOD	Neg	-	
FS-202111-74		area Casertana [c]	02/11/2021	Depuratore (Area Casertana)	<LOD	Neg	-	
FS-202111-59			04/11/2021		<LOD	Neg	-	
FS-202111-72		area Napoli [d]	02/11/2021	Impianto Napoli OVEST (Ingr. principale)	n.d. [†]	Neg	-	
FS-202111-60			04/11/2021		<LOD	Neg	-	
FS-202111-71		area Napoli Ovest [e]	02/11/2021	Impianto Napoli OVEST (Ingr. Camaldoli-Fogna NORD)	9,50E+02	Neg	-	
FS-202111-61			04/11/2021		<LOD	Neg	-	
FS-202111-70		area Napoli Est [f]	02/11/2021	Napoli EST	1,47E+03	**Pos**	○ L452R, T478K	Delta
FS-202111-62			04/11/2021		3,00E+03	Neg	-	
FS-202111-63		Avellino	04/11/2021	Avellino (Monocalzati)	2,06E+03	Neg	-	
FS-202111-46	Emilia Romagna	Ferrara	02/11/2021	Gramicia	9,45E+02	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-47		Bologna	02/11/2021	Idar	9,41E+03	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, Y145H, E156G, F157del, R158del, A222V, L452R, T478K	Delta (Y145H + A222V suggesting lineage AY.4.2)

FS-202111-48		Forli	02/11/2021	Forlì	3,39E+03	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-49		Ravenna	02/11/2021	Ravenna	<LOD	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-50		Bologna	03/11/2021	Idar	2,54E+03	Neg	-	
FS-202111-51		Modena	03/11/2021	Naviglio	<LOD	Neg	-	
FS-202111-52		Faenza	03/11/2021	Formellino	2,92E+03	Neg	-	
FS-202111-53		Imola	03/11/2021	Santerno	<LOD	Neg	-	
FS-202111-54		Rimini	03/11/2021	S.Giustina	2,66E+03	**Pos**	○ T95I, G142D, Y145H, E156G, F157del, R158del ○ L452R, T478K	Delta (Y145H suggesting lineage AY.4.2)
FS-202111-55		Ravenna	03/11/2021	Ravenna	<LOD	Neg	-	
FS-202111- 56		Cesena	02/11/2021	Cesena	4,84E+03	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-76		Reggio Emilia	03/11/2021	Mancasale	1,20E+03	Neg	-	
FS-202111-77		Piacenza	03/11/2021	Borgoforte	8,51E+02	Neg	-	
FS-202111-78		Parma	03/11/2021	Parma Ovest	<LOD	Neg	-	
FS-202111-41	Lazio	Civitavecchia	02/11/2021	Fiumaretta	<LOD	Neg	-	
FS-202111-99		Roma	02/11/2021	Roma EST	3,61E+04	**Pos**	○ L452R, T478K	Delta
FS-202111-100		Roma	02/11/2021	Roma Nord	4,38E+03	Neg	-	
FS-202111-101		Roma	03/11/2021	Roma Sud	6,08E+03	Neg	-	
FS-202111-102		Roma	03/11/2021	Ostia	3,07E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del, L452R, T478K	Delta
FS-202111-103		Roma	03/11/2021	Fregene	4,28E+04	Neg	-	
FS-202111-32	Liguria	Genova	02/11/2021	Punta Vagno	4,81E+03	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del, L452R, T478K	Delta
FS-202111-79		Sanremo	02/11/2021	Sanremo	6,62E+03	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-80		Genova	02/11/2021	Pegli	8,57E+02	Neg	-	
FS-202111-81		Genova	02/11/2021	Prà-Voltri	2,47E+03	**Pos**	○ L452R, T478K	Delta
FS-202111-82		Genova	02/11/2021	Quinto	6,00E+03	Neg	-	
FS-202111-83		Rapallo	02/11/2021	Rapallo	7,66E+03	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-84		Genova	02/11/2021	Sestri Ponente	3,18E+03	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-85		Genova	02/11/2021	Sturla	9,79E+02	Neg	-	
FS-202111-86		Borghetto Santo Spirito	02/11/2021	Borghetto S. S.	2,21E+03	Neg	-	
FS-202111-87		Savona	02/11/2021	Savona	1,59E+04	Neg	-	
FS-202111-88		La Spezia	02/11/2021	Stagnoni	2,46E+04	**Pos**	○ G142D, E156G, F157del, R158del	Delta
FS-202111-89		La Spezia	02/11/2021	Camisano	<LOD	Neg	-	
FS-202111-90		La Spezia	02/11/2021	Silea	1,02E+03	Neg	-	

FS-202111-91		Genova	03/11/2021	Darsena	9,52E+03	Neg	-	
FS-202111-92		Genova	03/11/2021	Valpolcevera	5,32E+03	Neg	-	
FS-202111-38	Lombardia	Monza	03/11/2021	Monza	1,24E+03	Neg	-	
FS-202111-39		Milano	03/11/2021	San Rocco	1,10E+04	**Pos**	○ T95I, G142D, E156G, F157del, R158del	Delta
FS-202111-40		Milano	03/11/2021	Nosedo	3,17E+03	**Pos**	○ T95I, G142D, Y145H, E156G, F157del, R158del ○ L452R, T478K	Delta (Y145H suggesting lineage AY.4.2)
FS-202111-97		Canegrate	02/11/2021	Canegrate	2,87E+03	Neg	-	
FS-202111-98		Varese Olona	02/11/2021	Varese Olona	4,65E+03	Neg	-	
FS-202111-68		Verziano	02/11/2021	Verziano	3,65E+03	**Pos**	○ G142D, E156G, F157del, R158del ○ L452R, T478K	Delta
FS-202111-69		Verziano	03/11/2021	Verziano	<LOD	Neg	-	
FS-202111-16	Piemonte	Torino	03/11/2021	Castiglione Torinese	2,08E+03	Neg	-	
FS-202111-17		Asti	03/11/2021	Asti	2,11E+03	Neg	-	
FS-202111-18		Alessandria	03/11/2021	Alessandria	<LOD	Neg	-	
FS-202111-19		Biella	03/11/2021	Biella Nord	2,05E+03	Neg	-	
FS-202111-20		Cuneo	03/11/2021	Cuneo	<LOD	Neg	-	
FS-202111-21		Novara	03/11/2021	Novara	6,84E+03	Neg	-	
FS-202111-93	Puglia	Bari	02/11/2021	Bari Est	2,14E+03	Neg	-	
FS-202111-94		Bari	02/11/2021	Bari Ovest	6,44E+03	Neg	-	
FS-202111-95		Taranto	03/11/2021	Taranto Gennarini	<LOD	Neg	-	
FS-202111-96		Taranto	03/11/2021	Taranto Bellavista	<LOD	Neg	-	
FS-202111-22	Sicilia	Palermo	02/11/2021	Acqua dei Corsari	4,47E+03	Neg	-	
FS-202111-23		Palermo	02/11/2021	Fondo Verde	<LOD	Neg	-	
FS-202111-24		Trapani	02/11/2021	Trapani	1,65E+03	Neg	-	
FS-202111-25		Mazara Del Vallo	02/11/2021	Mazara del Vallo	<LOD	Neg	-	
FS-202111-26		Marsala	02/11/2021	Marsala	<LOD	**Pos**	○ T95I, G142D, E156G, F157del, R158del, L452R, T478K	Delta
FS-202111-27		Catania	04/11/2021	Pantano d'Arci	<LOD	Neg	-	
FS-202111-64	Toscana	Pisa	02/11/2021	S. Jacopo	2,02E+03	Neg	-	
FS-202111-65		Empoli	02/11/2021	Pagnana	<LOD	Neg	-	

FS-202111-66		Massa Carrara	03/11/2021	Lavello 2	<LOD	Neg	-	
FS-202111-67		Viareggio	03/11/2021	Viareggio	6,27E+02	Neg	-	
FS-202111-28	Valle D'Aosta	area La Salle [g]	01/11/2021	Depuratore di La Salle	<LOD	Neg	-	
FS-202111-30			03/11/2021		3,65E+03	Neg	-	
FS-202111-29		area Aosta [h]	01/11/2021	Depuratore di Brissogne	<LOD	Neg	-	
FS-202111-31			03/11/2021		<LOD	Neg	-	
FS-202111-4	Veneto	Venezia	02/11/2021	Venezia Fusina	9,88E+03	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del, S255F, E281Q, L452R, T478K ○ (T85T), T95I, G142D, E156G, F157del, R158del, S255F, E281Q, L452R, T478K	Delta
FS-202111-5		Vicenza	02/11/2021	Vicenza Casale	6,41E+03	**Pos**	○ T95I, G142D, E156G, F157del, R158del ○ L452R, T478K	Delta
FS-202111-6		Treviso	02/11/2021	Treviso	1,05E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, Y145H, E156G, F157del, R158del, A222V, L452R, T478K	Delta (Y145H + A222V suggesting lineage AY.4.2)
FS-202111-7		Verona	04/11/2021	Verona collettore 1M	3,36E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, Y145H, E156G, F157del, R158del, A222V, L452R, T478K ○ W64C, G142D, E156G, F157del, R158del, P251L, L452R, T478K ○ W64C, G142D, E156G, F157del, R158del, P251L, T415N, (S444S), L452R, T478K ○ G142D, E156G, F157del, R158del, (F401F), (I410I), L452R, T478K	Delta (Y145H + A222V suggesting lineage AY.4.2)
FS-202111-8		Verona	04/11/2021	Verona collettore 3M	2,34E+04	**Pos**		
FS-202111-9		Verona	04/11/2021	Verona collettore 8M	1,54E+04	**Pos**		
FS-202111-10		Padova	02/11/2021	Padova Ca Nordio - Centro Storico	n.d.[†]	Neg	-	
FS-202111-11		Padova	02/11/2021	Padova Ca Nordio - ZIP	n.d.[†]	Neg	-	
FS-202111-12		Padova	02/11/2021	Padova Guizza	n.d.[†]	Neg	-	
FS-202111-13		Abano Terme	02/11/2021	Abano	n.d.[†]	Neg	-	
FS-202111-42	Provincia Autonoma di Bolzano	Bolzano	28/10/2021	ARA Bolzano	1,82E+04	**Pos**	○ L452R, F464L, T478K, A520S, S555F	Delta
FS-202111-43		Merano	28/10/2021	ARA Merano	1,96E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K	Delta
FS-202111-45		Merano	02/11/2021	ARA Merano	5,12E+04	**Pos**	○ T95I, G142D, E156G, F157del, R158del, L452R, T478K ○ (T85T), G142D, E156G, F157del, R158del, L452R, T478K	
FS-202111-44		Bolzano	02/11/2021	ARA Bolzano	2,36E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K	Delta

							○ T95I, G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del, (N344N), L452R, T478K	
FS-202111-1	Provincia Autonoma di Trento	Trento	31/10/2021	Trento Nord	1,23E+04	Neg	-	
FS-202111-2		Trento	31/10/2021	Trento Sud	1,93E+04	**Pos**	○ T95I, G142D, E156G, F157del, R158del	Delta
FS-202111-3		Rovereto	31/10/2021	Rovereto	2,30E+04	**Pos**	○ G142D, E156G, F157del, R158del, L452R, T478K ○ T95I, G142D, E156G, F157del, R158del	Delta

Silent mutations are reported in brackets

[†] n.d. = not done (insufficient RNA)

[a] area Nocera = Nocera Superiore, Castel San Giorgio, Siano, Roccapiemonte, Cava de' Tirreni

[b] area Nolana = Avella, Baiano, Brusciano, Camposano, Carbonara di Nola, Casamarciano, Castel Cisterna, Cicciano, Cimitile, Comiziano, Domicella, Lauro, Liveri, Mariglianella, Marigliano, Marzano di Nola, Moschiano, Mugnano del Cardinale, Nola, Pago del Vallo di Lauro, Palma Campania, Quadrelle, Quindici, Roccarainola, San Gennaro Vesuviano, San Paolo Belsito, San Vitaliano, Saviano, Scisciano, Sirignano, Sperone, Taurano, Tufino, Visciano, Zona ASI Nola-Marigliano

[c] area Casertana = Capodrise, Capua, Casagiove, Casapulla, Caserta, Curti, Macerata Campania, Maddaloni, Marcianise, Portico di Caserta, Recale, S. Marco Evangelista, S. Nicola La Strada, S. Prisco, S. Maria C.V., San Tammaro

[d] area Napoli = Napoli (zona centro-occidentale), Pozzuoli

[e] area Napoli Ovest = Calvizzano, Giugliano (quota parte), Marano di Napoli, Mugnano (quota parte), Qualiano, Quarto, Villaricca (quota parte)

[f] area Napoli Est = Portici, Cercola, Napoli Est, Pollena Trocchia, San Giorgio a Cremano, San Sebastiano al Vesuvio, Sant'Anastasia, Somma Vesuviana, Volla, Casoria, Casalnuovo di Napoli

[g] area La Salle = La Salle, Morgex, Pré Saint Didier e La Thuile

[h] area Aosta = Pollein, Charvensod, Gressan, Jovençan, Aymavilles, Villeneuve, Introd, Saint Nicolas, Saint Pierre, Sarre, Aosta, Saint Christophe

Limitations of the study

- Given the early stages of 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 15/21 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 third 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 the first week of November 2021 in Italy, in line with clinical results. No other VoCs (including Omicron) or VoIs were detected in this survey, but mutations of the Alpha variant were found in one sample.

Acknowledgements

We thank all of the members of the SARI network ("Sorveglianza Ambientale di SARS-CoV-2 attraverso i Reflui urbani in Italia") for the cooperation in sample collection and processing, data gathering and management, organization and logistic support. The SARI network includes:

- Abruzzo: Giuseppe Bucciarelli, Paolo Torlontano (Regione Abruzzo), Giuseppe Aprea (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale")
- Basilicata: Michele La Bianca (AREA PREVENZIONE E SANITA' PUBBLICA-Regione Basilicata), Rosa Anna Cifarelli (Servizio Tecnico Ambiente e Salute-ARPAB), Achille Palma (DTS-ARPAB), Rosanna Brienza e Patrizia Motenegro (Acquedotto Lucano).
- Calabria: Eduardo Malacaria (Regione Calabria), Giuseppe Folino (Arpacal) Campania: Angelo D'Argenzio (Regione Campania), Luigi Cossentino, Renato Olivares (Arpac - Agenzia Regionale per la Protezione Ambientale in Campania), Antonio Pizzolante, Giovanna Fusco (Istituto Zooprofilattico Sperimentale del Mezzogiorno), Vincenzo Belgiorno (C.U.G.RI Consorzio inter-Universitario per la previsione e la prevenzione dei Grandi Rischi), Alessandra Tosco, Amalia Porta (Università degli Studi di Salerno), Francesca Pennino (Università degli Studi di Napoli "Federico II")
- Emilia Romagna: Paola Angelini (Regione Emilia – Romagna), Laura De Lellis, Daniele Nasci (HERATech), Giovanni Alborali; Nicoletta Formenti (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna), Nadia Fontani, Franca Palumbo (IREN), Lisa Gentili (Arpae Emilia- Romagna)
- Friuli Venezia Giulia: Marika Mariuz, Gabriella Trani, (Direzione Centrale Salute FVG), Anna Pariani (LABORATORIO HERAtech di Sasso Marconi –BO)
- Lazio: Carla Ancona (DEPLAZIO - Dipartimento di Epidemiologia del Servizio Sanitario Regionale - Regione Lazio), Doriana Antonella Giorgi, Irene Ferrante (ARPA Lazio - Agenzia Regionale per la Protezione Ambientale del Lazio), Maria Teresa Scicluna, Antonella Cersini (IZSLT - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana), Maria Concetta Arizzi, Giancarlo Cecchini, Claudio Ottaviano (Acea Elabori)
- Liguria: Elena Nicosia, Daniele Colobraro, Quinto Sturla (Regione Liguria settore tutela della salute negli ambienti di vita e di lavoro), Giulia Nani, Franca Palumbo, Gianluca Borlone (Iren), Elena Grasselli; Alberto Izzotti (UNIGE - DISTAV; UNIGE – DIMES), Rosa Maria Bertolotto, Elena Nicosia (ARPAL), Micaela Tiso (MICAMO srl)
- Lombardia: Emanuela Ammoni, Danilo Cereda (Regione Lombardia), Barbara Bertasi (IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia), Andrea Aliscioni, Desdemona Oliva (CAP Holding), Sara Castiglioni; Silvia Schiarea, Ettore Zuccato (Istituto Mario Negri IRCCS), Manuela Antonelli, Arianna Azzellino, Francesca Malpei, Andrea Turolla (POLIMI), Sandro Binda; Valeria Primache (Università di Milano), Clementina Cocuzza, Andrea Franzetti, Rosario Musumeci e Marianna Martinelli (Università di Milano-Bicocca), Giorgio Bertanza (Università di Brescia), Maria Luisa Callegari (Università Cattolica del Sacro Cuore)
- Marche: Luigi Bolognini, Fabio Filippetti (Regione Marche), Marta Paniccia' (IZSUM - Istituto Zooprofilattico Sperimentale Umbria Marche), Silvia Magi (ARPAM)
- Molise: Michele Colitti, Angela Ciccaglione (Regione Molise), Giuseppe Aprea (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"), Maria Grazia Cerroni, Bernandino Principi (Arpa Molise)
- Piemonte: Bartolomeo Griglio (Regione Piemonte), Renza Berruti, Mauro Cravero, Angela Costa (Regione Piemonte), Manila Bianchi, Lucia Decastelli; Angelo Romano; Fabio Zuccon (IZSTO - Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta SC

Sicurezza e Qualità degli Alimenti), Elisabetta Carraro, Cristina Pignata; Silvia Bonetta (DSSPP Dipartimento di Scienze della Sanita' Pubblica e Pediatriche - Universita' di Torino)

- Puglia: Giuseppe Di Vittorio, Onofrio Mongelli (Regione Puglia); Osvalda De Giglio, Francesca Apollonio Francesco Triggiano, Maria Teresa Montagna (Università degli Studi di Bari Aldo Moro - Dipartimento Scienze Biomediche e Oncologia Umana), Nicola Ungaro (ARPA Puglia)
- Sicilia: Mario Palermo, Pietro Schembri (Regione Sicilia), Carmelo Massimo Maida (Universita' degli Studi di Palermo-Dipartimento PROMISE - sezione di Igiene), 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"), Walter Mazzucco (Università degli Studi di Palermo)
- Toscana: Piergiuseppe Cala' (Regione Toscana), Annalaura Carducci, Marco Verani (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Universita' di Pisa), Matteo Ramazzotti, Alberto Antonelli (SOD microbiologia e virologia, azienda ospedaliera universitaria Careggi, Firenze)
- Umbria: Enrica Ricci, Giovanni Santoro (Regione Umbria), Mencacci Antonella (Laboratorio Microbiologia Azienda ospedaliero universitaria di Perugia)
- Valle D'Aosta: Mauro Ruffier (Regione Valle d'Aosta), Francesca Borney (Laboratorio chimico biologico microbiologico Arpa Valle d'Aosta), Eric Grange
- Veneto: Francesca Russo; Gisella Pitter; Vanessa Groppi (Regione Veneto), Franco Rigoli (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto) Tatjana Baldovin, Irene Amoruso (Universita' di Padova), Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto)
- P. A. Bolzano: Lorella Zago (P.A. Bolzano), Alberta Stenico (Ufficio tutela acque - Agenzia provinciale per l'ambiente e la tutela del clima (A.P.P.A.)
- P.A. Trento: Elena Mengon (P.A. Trento) Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Francesco Pizzo; Alessandra Schiavuzzi (P. A. Trento), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento).