



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
2nd Report
(Investigation period: 04 – 08 October 2021)

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Introduction

Several studies have shown that detecting SARS-CoV-2 in untreated wastewater can be a useful tool to follow outbreak trends, assess the prevalence of infections, and studying SARS-CoV-2 genetic diversity¹.

In Italy, SARS-CoV-2 RNA detection was first accomplished in areas of both high (Milan) and low (Rome) epidemic circulation between February and May 2020². In July 2020 a pilot study, the SARI project (Epidemiological Surveillance for SARS-CoV-2 in urban sewage), coordinated by Istituto Superiore di Sanità (ISS), was launched in Italy³ and a national network was built with the cooperation of Regions, Autonomous Provinces, wastewater service providers, regional environmental protection agencies, local health authorities, zooprophyllactic institutes, universities and research institutions. Over 100 sampling points were enrolled for environmental surveillance, common methods for sampling and testing were shared within the network and Geographic Information System (GIS) dashboards were developed by ISS for data collection and integration at national level.

On 17 March 2021, the European 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 1st October 2021, national wastewater surveillance systems targeted at data collection of SARS-CoV-2 and its variants⁴. Recommendation of the EU Commission was adopted at national level with Law Decree n° 73 of 25.05.2021.

Indeed, a number of studies have reported the importance of sequencing environmental SARS-CoV-2 as a tool to determine strains circulating in the community and to study SARS-CoV-2 diversity, and recently mutations characteristic of variants of concern (VOCs) were detected in sewage samples collected in Italy⁵.

Aim

The Commission Recommendation 2021/472 encouraged the establishment of surveillance systems for SARS-CoV-2 and its variants in wastewaters across the Union as a complementary data collection and management tool of the COVID-19 pandemic.

Responding to this, the ISS established “flash surveys”, i.e. periodic sampling campaigns to be held in different locations in Italy, aimed at assessing the quantity and diversity of SARS-CoV-2 in wastewater in the country. A first flash survey was held in July 2021.

¹Bonanno Ferraro G., Veneri C., Mancini P., Iaconelli M., Suffredini E., Bonadonna L., Lucentini L., Bowo-Ngandji A., Kengne-Nde C., Mbagha D.S., Mahamat G., Tazokong H.R., 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 and Environmental Virology Journal. 2021. <https://doi.org/10.1007/s12560-021-09498-6>

² La Rosa G, Iaconelli M, Mancini P, Bonanno Ferraro G, Veneri C, Bonadonna L, Lucentini L, Suffredini E. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci Total Environ. 2020 Sep 20;736:139652. doi: 10.1016/j.scitotenv.2020.139652.

³ [ISS, al via la rete ‘sentinella’ di sorveglianza epidemiologica del coronavirus nelle acque reflue \(salute.gov.it\)](https://www.salute.gov.it/ISS-al-via-la-rete-sentinella-di-sorveglianza-epidemiologica-del-coronavirus-nelle-acque-reflue)

⁴ 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. OJ L 98, 19.3.2021, p. 3–8 (<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472&qid=1628798981209>)

⁵ La Rosa G, Mancini P, Bonanno Ferraro G, Veneri C, Iaconelli M, Bonadonna L, Lucentini L, Suffredini E. SARS-CoV-2 has been circulating in northern Italy since December 2019: Evidence from environmental monitoring. Sci Total Environ. 2021 Jan 1;750:141711. doi: 10.1016/j.scitotenv.2020.141711.

Aim of this report is to summarize the results of the second national flash survey on SARS-CoV-2 variants in wastewater samples collected in the week between October 4 and October 8, 2021.

Methodology

The survey included sewage samples collected at wastewater treatment plants (WTPs) located in 14 Regions and 2 Autonomous Provinces (A.P.):

- North-West Italy: Liguria, Lombardia, Piemonte and Valle d'Aosta;
- North-East Italy: Emilia-Romagna, Friuli Venezia Giulia, 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, 92 wastewater samples were collected between October 04 and October 08, 2021 (**Table 1**), about threefold the samples collected in the previous flash survey conducted in July 2021.

Table 1. Sampling sites and characteristics of the included WTPs

Sample ID	Region/A.P.	City	Sampling date	WTP	Population equivalent ^a
FS-202110-46	Liguria	Imperia	05/10/2021	Imperia	116.000
FS-202110-47		Sanremo	05/10/2021	Sanremo	75.000
FS-202110-48		La Spezia	05/10/2021	Stagnoni	82.000
FS-202110-49		Lerici	05/10/2021	Camisano	40.000
FS-202110-50		Sarzana	05/10/2021	Silea	17.500
FS-202110-51		Savona	05/10/2021	Savona	280.000
FS-202110-52		Borghetto Santo Spirito	05/10/2021	Borghetto Santo Spirito	121.000
FS-202110-53		Genova	05/10/2021	Darsena	220.000
FS-202110-54		Genova	05/10/2021	Pegli	40.000
FS-202110-55		Genova	05/10/2021	Prà-Voltri	62.000
FS-202110-56		Genova	05/10/2021	Punta Vagno	250.000
FS-202110-57		Genova	05/10/2021	Quinto	60.000
FS-202110-58		Genova	05/10/2021	Sestri Ponente	130.000
FS-202110-59		Genova	05/10/2021	Sturla	60.000
FS-202110-60		Genova	05/10/2021	Valpolcevera	160.000
FS-202110-91		Genova	05/10/2021	Punta vagno	220.000
FS-202110-61	Abruzzo	Chieti	04/10/2021	S. Martino	114.500
FS-202110-62		Teramo	04/10/2021	Villa Pavone	41.824
FS-202110-63		Pescara	05/10/2021	Villa Raiale	160.000
FS-202110-64		Montesilvano	05/10/2021	Villa Carmine	140.000
FS-202110-65		Aquila	06/10/2021	Pile	48.000
FS-202110-27	Lombardia	Brescia	05/10/2021	Verziano	296.000

FS-202110-28		Brescia	06/10/2021	Verziano	296.000
FS-202110-78		Monza	06/10/2021	San Rocco	700.000
FS-202110-79		Monza	04/10/2021	San Rocco	700.000
FS-202110-80		Milano	04/10/2021	Milano Nosedo	1.250.000
FS-202110-81		Milano	04/10/2021	Milano S. Rocco	1.050.000
FS-202110-82		Milano	06/10/2021	Milano Nosedo	1.250.000
FS-202110-83		Milano	06/10/2021	Milano S. Rocco	1.050.000
FS-202110-15	Piemonte	Torino	06/10/2021	Castiglione Torinese	1.934.099
FS-202110-16		Asti	06/10/2021	Asti	95.000
FS-202110-17		Alessandria	06/10/2021	Alessandria	110.000
FS-202110-18		Biella	06/10/2021	Biella Nord	120.000
FS-202110-19		Cuneo	06/10/2021	Cuneo	185.000
FS-202110-20	Basilicata	Potenza	07/10/2021	Tiera di Viglio	95.000
FS-202110-74	Valle d'Aosta	La Salle	04/10/2021	Depuratore di La Salle	60.000
FS-202110-75		Brissogne	04/10/2021	Depuratore di Brissogne	150.000
FS-202110-76		La Salle	06/10/2021	Depuratore di La Salle	60.000
FS-202110-77		Brissogne	06/10/2021	Depuratore di Brissogne	150.000
FS-202110-84	Emilia Romagna	Modena	05/10/2021	Naviglio entrata	500.000
FS-202110-87		Rimini	06/10/2021	S.Giustina entrata	560.000
FS-202110-89		Bologna	07/10/2021	Idar entrata	800.000
FS-202110-90		Reggio Emilia	06/10/2021	Mancasale	280.000
FS-202110-92		Piacenza	05/10/2021	Borgoforte	163.333
FS-202110-93		Parma	06/10/2021	Parma ovest	168.000
FS-202110-88	Friuli Venezia Giulia	Trieste	06/10/2021	Servola entrata	190.000
FS-202110-85		Udine	05/10/2021	Livenza Tagliamento acque	15.000
FS-202110-86		Udine	05/10/2021	Udine Via Gonars	200.000
FS-202110-29	Veneto	Padova	04/10/2021	Padova Ca Nordio - Centro Storico	98.500
FS-202110-30		Padova	04/10/2021	Padova Ca Nordio - ZIP	98.500
FS-202110-31		Padova	04/10/2021	Padova Guizza	13.000
FS-202110-32		Abano Terme (PD)	04/10/2021	Abano	35.000
FS-202110-66		Venezia	05/10/2021	Venezia Fusina	400.000
FS-202110-67		Vicenza Casale	05/10/2021	Vicenza Casale	92.000
FS-202110-68		Treviso	05/10/2021	Treviso	70.000
FS-202110-69		Verona	07/10/2021	Verona collettore 1M	82.000
FS-202110-70		Verona	07/10/2021	Verona collettore 3M	102.000
FS-202110-71		Verona	07/10/2021	Verona collettore 8M	226.000
FS-202110-33	A.P. Bolzano	Bolzano	04/10/2021	Bolzano	450.000
FS-202110-34		Merano	04/10/2021	Merano	364.000
FS-202110-12	A.P. Trento	Trento	04/10/2021	Trento Nord	120.000
FS-202110-13		Trento	04/10/2021	Trento Sud	100.000
FS-202110-14		Rovereto	04/10/2021	Rovereto	95.000

FS-202110-01	Lazio	Civitavecchia	04/10/2021	Fiumaretta	86.400
FS-202110-35		Velletri	04/10/2021	La Chiusa	35.000
FS-202110-36		Viterbo	04/10/2021	Strada Bagni	35.000
FS-202110-37		Pomezia	04/10/2021	Capoluogo	60.000
FS-202110-38		Guidonia	04/10/2021	Ponte Lucano	60.775
FS-202110-39		Latina	04/10/2021	Latina Est	60.000
FS-202110-40		Anzio	04/10/2021	Colle Cocchino	75.000
FS-202110-41		Aprilia	04/10/2021	Via del Capo	66.000
FS-202110-42		Roma	05/10/2021	Roma Est (linea 1+ linea 2)	900.000
FS-202110-43		Fiumicino	06/10/2021	Fregene	76.000
FS-202110-44		Roma	06/10/2021	Roma Sud	1.100.000
FS-202110-08	Toscana	Pisa	05/10/2021	Depuratore Pisa Nord S. Jacopo	52.000
FS-202110-09		Empoli	05/10/2021	Depuratore Empoli Pagnana	88.670
FS-202110-10		Massa Carrara	06/10/2021	Lavello 2	120.000
FS-202110-11		Viareggio	06/10/2021	Depuratore Viareggio	93.000
FS-202110-02	Campania	Napoli	05/10/2021	Napoli Est	1.750.000
FS-202110-03		Marigliano	05/10/2021	Area Nolana	400.000
FS-202110-04		Marcianise	05/10/2021	Area Casertana	370.769
FS-202110-05		Napoli	05/10/2021	Napoli Ovest (Ingresso Calmaldoli, NORD)	1.200.000
FS-202110-06		Napoli	05/10/2021	Napoli Ovest (Ingresso Principale)	1.200.000
FS-202110-07		Nocera	05/10/2021	Nocera	299.121
FS-202110-22	Puglia	Brindisi	04/10/2021	Bari Est	389.000
FS-202110-23		Foggia	04/10/2021	Bari Ovest	242.235
FS-202110-24		Taranto	04/10/2021	Taranto Gennarini	226.667
FS-202110-25		Taranto	04/10/2021	Taranto Bellavista	116.723
FS-202110-21	Sicilia	Messina	04/10/2021	Mili Marina	227.000
FS-202110-26		Palermo	06/10/2021	Acqua dei Corsari	314.973
FS-202110-72		Catania	06/10/2021	Depuratore Municipale Pantano d'Archi	68.434

^a 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 Acknowledgement section) and viral concentration and nucleic acid extraction was 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 Istituto Superiore di Sanità (ISS), which provided to quantify SARS-CoV-2 according to the a protocol and sequence positive samples described elsewhere⁶.

⁶ G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferrero, D. Brandtner, A. Fasanella, L. Pace, A. Parisi, D. Galante, E. Suffredini. Rapid screening for SARS-CoV-2 variants of concern

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). Furthermore, three newly designed shorter nested RT-PCRs (unpublished), designated as ID_987, ID_991, and ID_993 (producing amplicons from 495 bp to 601 bp, covering combined the entire 1600 bp fragment of the long PCR ID_980), were also used to increase probability of amplification and characterization.

All positive samples were subjected to conventional Sanger sequencing. Samples that tested positive by nested RT-PCR underwent also Next Generation Sequencing (NGS) 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 for NGS results was performed as described in La Rosa et al., 2021⁷.

Results

All of the 92 collected samples were included in the analysis met the quality assurance criteria defined in the protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3”. Overall, 55 out of 92 (60 %) samples tested positive for SARS-CoV-2 by real-time RT-qPCR (**Table 2**), viral concentration ranging from 1,1E+03 to 2,7E+05 genome copies (g.c.)/L of sewage. The remaining samples were negative, and assumed to be below the detection limit of the analytical method.

Twenty-three samples were successfully amplified by the long and/or the short PCR assays for variant analysis. Nineteen of these samples showed mutations characteristic of the Delta variant (lineage B.1.617.2), while the remaining samples could be amplified only by one of the short assays which was not discriminative enough to achieve variant identification (lack of mutations characteristic of any VoC or Vol in the amplified fragments).

Twelve samples - collected in Rovereto (TN), Verona, Padova, Abano Terme (PD), Genova, Bologna, Anzio (RM), Bari, and Messina - showed the full set of spike mutations characteristic of lineage B.1.617.2 (E156G, FR157-158del⁸, L452R, T478K), while in the remaining samples - collected in Milano, Bolzano, Brissogne (AO) and Trieste - only a subset of mutations could be detected due to lack of amplification of the entire long fragment.

NGS analysis showed a high degree of sequence variability within the Delta variant sequences. In total, 15 amino acid substitutions were detected in the ~1600 bps fragment; 5 silent mutations were also detected, generating a total of 14 different amino acid combinations.

Besides the key mutations associated with the Delta variant, other mutations frequently reported in GISAID were detected, such as T95I - found in Rovereto (TN), Verona, Padova, Abano Terme (PD), Genova, Bologna, and Bari - and G142D - found in Rovereto (TN), Verona, Padova, Abano Terme (PD), Genova, Bologna, Anzio (RM), Bari and Messina.

Mutations found in specific sublineages of the Delta variant were also detected, and in detail:

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>.

⁷ 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>

⁸ F157del, R158del

- i) Y145H (Verona), almost exclusively found in sublineage AY.4.2;
- ii) A222V (Trieste and Rovereto), present with a prevalence >90% in Delta sublineage AY.4.2, as well as in AY.2, AY.9, AY.26 and AY.27;

Other rare amino acid substitutions, usually detected in less than 1% of the Delta sequences available in GISAID, were detected in some samples: H69Y in Abano Terme (PD), H146Y in Rovereto (TN), T323K and W353S in Bolzano, and K535N in Bari.

Silent mutations, including P230P, I410I, Y508Y, S477S, and P561P, were also occasionally detected.

The results of this flash survey is in line with data from clinical surveillance, reporting that 99,9% of sequences deposited in the Italian platform I-Co-Gen (Italian COVID-19 Genomic) in the period September 11 – October 25 2021 belong to the Delta variant. Interestingly, the results of this survey also highlight a significant genetic diversity within lineage B.1.617.2.

Table 2. PCR and sequencing results of the tested samples

Sample ID	Region / A.P.	City	Sampling date	WTP	QA criteria	RT-qPCR (g.c./L)	RT-nested-PCR long and/or short	Combination of Detected Mutations	SARS-CoV-2 Variant
FS-202110-46	Liguria	Imperia	05/10/2021	Imperia	pass	<LOD	Neg		
FS-202110-47		Sanremo	05/10/2021	Sanremo	pass	3,8E+03	Neg		
FS-202110-48		La Spezia	05/10/2021	Stagnoni	pass	2,1E+03	Neg		
FS-202110-49		Lerici	05/10/2021	Camisano	pass	<LOD	Neg		
FS-202110-50		Sarzana	05/10/2021	Silea	pass	1,1E+03	Neg		
FS-202110-51		Savona	05/10/2021	Savona	pass	<LOD	Neg		
FS-202110-52		Borghetto Santo Spirito	05/10/2021	Borghetto Santo Spirito	pass	<LOD	Neg		
FS-202110-53		Genova	05/10/2021	Darsena	pass	5,2E+03	Neg		
FS-202110-54		Genova	05/10/2021	Pegli	pass	9,7E+03	Neg		
FS-202110-55		Genova	05/10/2021	Prà-Voltri	pass	2,1E+04	Neg		
FS-202110-56		Genova	05/10/2021	Punta Vagno	pass	9,0E+03	Neg		
FS-202110-57		Genova	05/10/2021	Quinto	pass	1,0E+03	Neg		
FS-202110-58		Genova	05/10/2021	Sestri Ponente	pass	1,9E+03	Neg		
FS-202110-59		Genova	05/10/2021	Sturla	pass	1,8E+03	Pos	○ T95I, G142D, E156G, FR157-158del	Delta
FS-202110-60		Genova	05/10/2021	Valpolcevera	pass	4,4E+03	Pos	○ L452R, T478K	
FS-202110-91		Genova	05/10/2021	Punta vagno	pass	3,4E+03	Neg		
FS-202110-61	Abruzzo	Chieti	04/10/2021	S. Martino	pass	<LOD	Neg		
FS-202110-62		Teramo	04/10/2021	Villa Pavone	pass	7,9E+03	Neg		
FS-202110-63		Pescara	05/10/2021	Villa Raiale	pass	1,2E+03	Neg		
FS-202110-64		Montesilvano	05/10/2021	Villa Carmine	pass	<LOD	Neg		
FS-202110-65		Aquila	06/10/2021	Pile	pass	<LOD	Neg		
FS-202110-27	Lombardia	Brescia	05/10/2021	Verziano	pass	1,3E+03	Neg		
FS-202110-28		Brescia	06/10/2021	Verziano	pass	<LOD	Neg		
FS-202110-78		Monza	06/10/2021	San Rocco	pass	<LOD	Neg		
FS-202110-79		Monza	04/10/2021	San Rocco	pass	2,3E+03	Neg		
FS-202110-80		Milano	04/10/2021	Milano Nosedo	pass	<LOD	Neg		
FS-202110-81		Milano	04/10/2021	Milano S. Rocco	pass	1,6E+03	Pos	○ L452R, T478K	Delta
FS-202110-82		Milano	06/10/2021	Milano Nosedo	pass	<LOD	Pos		
FS-202110-83		Milano	06/10/2021	Milano S. Rocco	pass	5,0E+03	Neg		
FS-202110-15	Piemonte	Torino	06/10/2021	Castiglione Torinese	pass	<LOD	Neg		
FS-202110-16		Asti	06/10/2021	Asti	pass	<LOD	Neg		
FS-202110-17		Alessandria	06/10/2021	Alessandria	pass	<LOD	Neg		

FS-202110-18		Biella	06/10/2021	Biella Nord	pass	<LOD	Neg		
FS-202110-19		Cuneo	06/10/2021	Cuneo	pass	1,2E+03	Neg		
FS-202110-20	Basilicata	Potenza	07/10/2021	Tiera di Viglio	pass	<LOD	Neg		
FS-202110-74	Valle d'Aosta	La Salle	04/10/2021	La Salle	pass	1,2E+04	Neg		
FS-202110-75		Brissogne	04/10/2021	Brissogne	pass	2,4E+04	Pos	○ L452R, T478K	Delta
FS-202110-77		Brissogne	06/10/2021	Brissogne	pass	4,2E+03	Pos		
FS-202110-76		La Salle	06/10/2021	La Salle	pass	<LOD	Neg		
FS-202110-84	Emilia Romagna	Modena	05/10/2021	Naviglio entrata	pass	<LOD	Neg		
FS-202110-87		Rimini	06/10/2021	S.Giustina entrata	pass	<LOD	Neg		
FS-202110-89		Bologna	07/10/2021	Idar entrata	pass	2,4E+03	Pos	○ T95I, G142D, E156G, FR157-158del, L452R, T478K	Delta
FS-202110-90		Reggio Emilia	06/10/2021	Mancasale	pass	5,7E+03	Neg		
FS-202110-92		Piacenza	05/10/2021	Borgoforte	pass	<LOD	neg		
FS-202110-93		Parma	06/10/2021	Parma ovest	pass	4,6E+03	Neg		
FS-202110-85	Friuli Venezia Giulia	Udine	05/10/2021	Livenza Tagliamento acque	pass	<LOD			
FS-202110-86		Udine	05/10/2021	CAFC	pass	4,6E+03	Neg		
FS-202110-88		Trieste	06/10/2021	Servola entrata	pass	<LOD	Pos	○ A222V ○ L452R, T478K	Delta
FS-202110-29	Veneto	Padova	04/10/2021	Padova Ca Nordio - Centro Storico	pass	1,2E+03	Pos	○ L452R, T478K	Delta
FS-202110-30		Padova	04/10/2021	Padova Ca Nordio - ZIP	pass	6,8E+02	Neg		
FS-202110-31		Padova	04/10/2021	Padova Guizza	pass	5,0E+03	Pos	○ T95I, G142D, E156G, FR157-158del	Delta
FS-202110-32		Abano Terme (PD)	04/10/2021	Abano	pass	<LOD	Pos	○ H69Y, G142D, E156G, FR157-158del, L452R, T478K ○ G142D, E156G, FR157-158del, L452R, T478K ○ T95I, N137N, G142D, E156G FR157-158del ○ L452R, T478K, Y508Y	Delta
FS-202110-66		Venezia	05/10/2021	Venezia Fusina	pass	8,5E+03	Pos	○ None*	Unassigned
FS-202110-67		Vicenza Casale	05/10/2021	Vicenza Casale	pass	4,1E+03	Neg		
FS-202110-68		Treviso	05/10/2021	Treviso	pass	<LOD	Neg		
FS-202110-69		Verona	07/10/2021	Verona collettore 1M	pass	5,4E+03	Pos		
FS-202110-70		Verona	07/10/2021	Verona collettore 3M	pass	1,1E+04	Pos	○ G142D, E156G, FR157-158del, L452R, T478K ○ T95I, G142D, Y145H, E156G, FR157-158del ○ T95I, G142D, E156G, FR157-158del	Delta (Y145H suggesting lineage AY.4.2)
FS-202110-71		Verona	07/10/2021	Verona collettore 8M	pass	3,0E+03	Pos		
FS-202110-33	A.P. Bolzano	Bolzano	04/10/2021	Bolzano	pass	2,4E+03	Pos	○ P230P, T323K, W353S ○ L452R, T478K	Delta
FS-202110-34		Merano	04/10/2021	Merano	pass	8,7E+03	Neg		
FS-202110-12	A.P. Trento	Trento	04/10/2021	Trento Nord	pass	6,0E+03	Neg		

FS-202110-13		Trento	04/10/2021	Trento Sud	pass	3,6E+03	Neg		
FS-202110-14		Rovereto	04/10/2021	Rovereto	pass	2,7E+05	Pos	○ G142D, H146Y, E156G, FR157-158del, A222V, I410I, L452R, T478K ○ G142D, E156G, FR157-158del, L452R, T478K ○ T95I, G142D, H146Y, E156G, FR157-158del	Delta
FS-202110-01	Lazio	Civitavecchia	04/10/2021	Fiumaretta	pass	<LOD	neg		
FS-202110-35		Velletri	04/10/2021	La Chiusa	pass	5,1E+03	neg		
FS-202110-36		Viterbo	04/10/2021	Strada Bagni	pass	1,5E+04	Pos	○ None*	Unassigned
FS-202110-37		Pomezia	04/10/2021	Capoluogo	pass	7,1E+03	Pos	○ None*	Unassigned
FS-202110-38		Guidonia	04/10/2021	Ponte Lucano	pass	1,4E+03	neg		
FS-202110-39		Latina	04/10/2021	Latina Est	pass	1,1E+03	neg		
FS-202110-40		Anzio	04/10/2021	Colle Cocchino	pass	2,0E+04	Pos	○ G142D, E156G, FR157-158del ○ L452R, T478K	Delta
FS-202110-41		Aprilia	04/10/2021	Via del Capo	pass	2,5E+03	neg		
FS-202110-42		Roma	05/10/2021	Roma Est (linea 1+ linea 2)	pass	1,6E+04	neg		
FS-202110-44		Roma	06/10/2021	Roma Sud	pass	3,9E+03	neg		
FS-202110-43		Fiumicino	06/10/2021	Fregene	pass	5,0E+03	neg		
FS-202110-08	Toscana	Pisa	05/10/2021	Depuratore Pisa Nord S. Jacopo	pass	<LOD	neg		
FS-202110-09		Empoli	05/10/2021	Depuratore Empoli Pagnana	pass	<LOD	neg		
FS-202110-10		Massa Carrara	06/10/2021	Lavello 2	pass	<LOD	neg		
FS-202110-11		Viareggio	06/10/2021	Depuratore Viareggio	pass	1,2E+03	neg		
FS-202110-03	Campania	Marigliano	05/10/2021	Area Nolana	pass	1,6E+03	neg		
FS-202110-04		Marcianise	05/10/2021	Area Casertana	pass	<LOD	neg		
FS-202110-02		Napoli	05/10/2021	Napoli Est	pass	<LOD	neg		
FS-202110-05		Napoli	05/10/2021	Napoli Ovest (Ingresso Calmaldoli, NORD)	pass	1,4E+03	neg		
FS-202110-06		Napoli	05/10/2021	Napoli Ovest (Ingresso Principale)	pass	2,5E+03	neg		
FS-202110-07		Nocera	05/10/2021	Nocera	pass	<LOD	neg		
	Puglia							○ T95I, G142D, E156G, FR157-158del, L452R, T478K, K535N ○ T95I, G142D, E156G, FR157-158del, L452R, T478K ○ G142D, E156G, FR157-158del, L452R, T478K, K535N	Delta
FS-202110-22		Bari	04/10/2021	Bari Est	pass	<LOD	Pos		
FS-202110-23		Bari	04/10/2021	Bari Ovest	pass	<LOD	Neg		
FS-202110-24		Taranto	04/10/2021	Taranto Gennarini	pass	<LOD	Neg		

FS-202110-25		Taranto	04/10/2021	Taranto Bellavista	pass	<LOD	Neg		
	Sicilia							○ G142D, E156G, FR157-158del, L452R, T478K	
FS-202110-21		Messina	04/10/2021	Mili Marina	pass	6,5E+03	Pos	○ G142D, E156G, FR157-158del, L452R, S477S, T478K, P561P	Delta
FS-202110-26		Palermo	06/10/2021	Acqua dei Corsari	pass	2,6E+03	neg		
				Depuratore					
FS-202110-72		Catania	06/10/2021	Municipale Pantano d'Arci	pass	<LOD	neg		

* None: amplification of a short fragment lacking mutations characteristic of any VoC or Vol

Main findings:

- Only the delta variant was detected in wastewater samples collected the first week of October 2021;
- A wide genetic diversity was observed within the delta variant, with 15 amino acid substitutions and 5 silent mutations found in the portion of the spike gene analysed; a total of 14 different amino acid combinations were detected;
- Amino acid substitutions characteristic of sublineage AY.4.2 were detected.

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 for all Italian Regions. Therefore, geographical and population coverage of the flash survey is not complete, even if it has improved compared to the first flash survey.
- 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. As a consequence, molecular characterization and variant detection may not be achieved for all samples.

Conclusions and final considerations

This is the first of a series of reports on SARS-CoV-2 and its variants in wastewaters that will be periodically issued as part of the surveillance established in Italy following EU Commission Recommendation 2021/472, to provide information on SARS-CoV-2 variants in the population complementary to data acquired through other surveillance programmes.

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 October 2021, in Italy, in line with results obtained in clinical setting.

Compared with the previous flash survey, performed in July 2021, which detected the presence of Alpha and Beta variants along with Delta, the present survey detected no other VoCs or Vols besides Delta, demonstrating that this variant has by now taken over as the dominant SARS-CoV-2 strains circulating in the Italian population.

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