Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
1st Report
(Investigation period: 04 – 12 July 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 diversity.

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 2020 [1]. 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 Italy [2] and a national network was built on voluntary basis with the cooperation of Regions, Autonomous Provinces, wastewater service providers, regional environmental protection agencies, local health authorities, zooprophylactic 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 dashboards were developed by ISS for data collection and integration at national level.

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 targeted at data collection of SARS-CoV-2 and its variants [3]. 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 Italy [4].

Aim

The Commission Recommendation 2021/472 encouraged the implementation 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. Accordingly, a first national flash survey has been designed and implemented at national level to test the approach, organization and methods for SARS-CoV-2 variants surveillance in urban wastewater, as well as to gain preliminary data on VOCs in Italy based on wastewater monitoring.

This report summarizes the results of the first national flash survey on SARS-CoV-2 variants in wastewater sample collected in Italy in the week between July 4 and July 12, 2021.

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[1] 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.

[2] ISS, al via la rete 'sentinella' di sorveglianza epidemiologica del coronavirus nelle acque reflue (salute.gov.it)

[3] 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)

[4] 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.

Methodology

The survey was carried out in July 2021 and included sewage samples collected at wastewater treatment plants (WTPs) located in 14 Regions/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. Bolzano and A.P. Trento;
- Central Italy: Lazio and Toscana;
- Southern Italy and Islands: Campania, Puglia and Sicilia.

For each Region/A.P. a minimum of 2 sampling points were included.

Overall, 35 wastewater samples were collected between July 04 -12, 2021 (**Table 1**).

Table 1. Sampling sites and characteristics of the included WTPs

Sample ID	Region/A.P.	City	Sampling date	WTP	Population equivalent
FS-202107-14	Liguria	Lerici	07/07/2021	Camisano (Lerici)	500.000
FS-202107-15	Liguria	Genova	06/07/2021	Punta Vagno	220.000
FS-202107-16	Liguria	Genova	07/07/2021	Darsena	220.000
FS-202107-24	Lombardia	Milano	05/07/2021	Nosedo*	1.250.000
FS-202107-25	Lombardia	Varese	05/07/2021	Varese Olona	120.000
FS-202107-01	Piemonte	Torino	05/07/2021	Castiglione Torinese	1.934.000
FS-202107-02	Piemonte	Alessandria	05/07/2021	Orti	110.000
FS-202107-03	Valle d'Aosta	La Salle	04/07/2021	La Salle	60.000
FS-202107-04	Valle d'Aosta	Brissogne	04/07/2021	Brissogne	150.000
FS-202107-21	Emilia Romagna	Rimini	07/07/2021	S. Giustina (Rimini)	560.000
FS-202107-23	Emilia Romagna	Bologna	09/07/2021	IDAR (Bologna)	800.000
FS-202107-20	Friuli Venezia Giulia	Udine	06/07/2021	CAFC (Udine)	200.000
FS-202107-22	Friuli Venezia Giulia	Trieste	07/07/2021	Servola (Trieste)	190.000
FS-202107-18	Veneto	Verona	06/07/2021	Verona	410.000
FS-202107-19	Veneto	Venezia	06/07/2021	Fusina (Venezia)	400.000
FS-202107-28	A.P. Bolzano	Bolzano	05/07/2021	ARA Bozen / IDA Bolzano	450.000
FS-202107-29	A.P. Bolzano	Floronzo	05/07/2021	ARA Tobl / IDA Tobl	150.000
FS-202107-05	A.P. Trento	Trento	06/07/2021	Trento Nord	120.000
FS-202107-06	A.P. Trento	Trento	06/07/2021	Trento Sud	100.000
FS-202107-07	A.P. Trento	Rovereto	07/07/2021	Rovereto	95.000
FS-202107-08	Lazio	Roma	07/07/2021	Roma Est I Sez.	300000
FS-202107-09	Lazio	Roma	06/07/2021	Roma Est II Sez.	600.000
FS-202107-10	Lazio	Pomezia	07/07/2021	Crocetta I	60.000
FS-202107-11	Lazio	Roma	07/07/2021	Roma Ostia	350.000
FS-202107-12	Lazio	Roma	06/07/2021	Roma Nord	780.000
FS-202107-13	Lazio	Roma	07/07/2021	Roma Sud	1.100.000
FS-202107-17	Lazio	Roma	07/07/2021	Roma Est II Sez.	600.000

FS-202107-26	Toscana	Pisa	06/07/2021	Pisa	52.000
FS-202107-27	Toscana	Viareggio	07/07/2021	Viareggio	93.000
FS-202107-34	Campania	Napoli	12/07/2021	Napoli Ovest (Cuma)	1.200.000
FS-202107-35	Campania	Napoli	12/07/2021	Napoli Est	1.750.000
FS-202107-36	Puglia	Brindisi	07/07/2021	Fiume Grande (Brindisi)	93.000
FS-202107-37	Puglia	Foggia	06/07/2021	Foggia	208.000
FS-202107-33	Sicilia	Catania	09/07/2021	Pantano d'Arci (Catania)	68.000
FS-202107-38	Sicilia	Palermo	05/07/2021	Acqua dei Corsari (Palermo)	440.000

* 50% Nosedo - 50% Ampliamento est

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.2" (April 27, 2021). RNAs were shipped in dry ice to Istituto Superiore di Sanità (ISS), which provided to detect and quantify SARS-CoV-2 RNA (protocol "Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3" (July, 25, 2021)) and sequence positive samples as described elsewhere [5].

For 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, two short nested RT-PCRs, designated as ID_973 and ID_975 (~300 bps, spanning amino acid residues 58 to 151 and 480 to 573 of the spike protein, respectively), were also used to increase probability of amplification.

All positive samples were subjected to conventional Sanger sequencing. Samples that tested positive by the long nested RT-PCR underwent also Next Generation Sequencing (NGS) using the Oxford Nanopore Technology MinION platform. Bioinformatics analysis for NGS results was performed as described in La Rosa et al., 2021 (under preparation)[6]. To ensure quality of the NGS analysis, two *ad hoc* control samples were assembled using selected SARS-CoV-2 VOCs: i) sample NGSQC1 – mix of PCR amplicons (long nested RT-PCR ID_980) of Alpha, Beta, Gamma and Delta variants (25% each); ii) sample NGSQC2 – mix of the RNA of SARS-CoV-2 variants Alpha, Beta, Gamma and Delta (25% each) submitted to long nested RT-PCR ID_980. Sample NSGCQ1 was meant as a control for library preparation, NGS and bioinformatics analysis. Sample NSGCQ2 was meant as a control for the whole process of variant detection, taking into account influence of reverse transcription and PCR amplification on complex RNA mixtures.

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[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.

[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. (*under preparation*)

Results

Three of the 35 collected samples (FS-202107-20, FS-202107-34, and FS-202107-35) were excluded from the analysis having failed to meet the quality assurance criteria due to high inhibition of PCR reaction. Three additional samples (FS-202107-21, FS-202107-22, and FS-202107-23) displayed inhibition values significantly higher than the average, but were still within the acceptability criteria.

Overall, 13 out of 32 (40.6%) samples tested positive for SARS-CoV-2 by real-time RT-qPCR (**Table 2).** The viral concentration in sewage ranged from 1.2×10^3 to 2.7×10^4 genome copies (g.c.)/L. The remaining negative samples were assumed below the detection limit of the analytical method. The relatively low number of positive samples may be due to the low circulation of the virus during the sampling period, when the total number of active cases in Italy reached the minimum for year 2021 on the last day of this survey (July 12, 2021: 40.426 cases [7]).

Of the 13 positive samples, two were successfully amplified by the long PCR assay ID_980, and additional three samples were amplified by the short PCR assay ID_973. These five samples were characterized by sequencing (**Table 3**):

a) Long PCR assay

Sanger sequencing: Samples IDs FS-202107-29 and FS-202107-06, collected in Floronzo (BZ) and Trento, respectively, displayed the mutations characteristic of the Delta variant (B.1.617.2) such as E156G, F157del, R158del, L452R, and T478K, plus some less frequently detected mutations (K77T, G142D, and W258L).

NGS by nanopore technology: NGS analysis confirmed the detection in linkage of key mutations characteristic of the Delta variant in both samples but further showed the presence of a panel of mutations of the Beta variant (B.1.351) (D80A, D215G, L241-, L242-, L243-, K417N, E484K, N501Y) in sample ID FS-202107-29.

b) Short PCR assay

Mutations characteristic of the Alpha variant (B.1.1.7) were detected in one sample (ID FS-202107-23, Bologna) by Sanger sequencing. One mutation, G142D - present in more than 50% of GISAID sequences belonging the Delta variant (B.1.617.2) - was detected in samples collected in Rome (FS-202107-09) and in Verona (ID FS-202107-18). Single mutations, however, are not discriminative enough to achieve variant identification and the G142D mutation can also be found in sequences of the Kappa variant [B.1.617.1]. Therefore, its detection should only be considered as indicative of the presence of these two variants.

The results of SARS-CoV-2 surveillance in wastewaters is consistent with the data of the integrated surveillance for SARS-CoV-2 in Italy, reporting the predominance of the Delta variant (B.1.617.2) in the first half of July 2021, with an estimated prevalence of 94.8% according to the flash survey on cases notified on July 20, 2021[8]. Interestingly, deep sequencing of one sewage sample showed the presence of the Beta variant (B.1.351) in a wastewater treatment plant (150.000 population

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[7] https://github.com/pcm-dpc/COVID-19/blob/master/schede-riepilogative/regioni/dpc-covid19-ita-scheda-regioni-20210712.pdf

[8] Istituto Superiore di Sanità, Fondazione Bruno Kessler, Ministero della Salute – Stima della prevalenza delle varianti VOC (Variants of Concern) in Italia: B.1.1.7, B.1.351, P.1 e B.1.617.2, e altre varianti di SARS-CoV-2 (Indagine del 20/7/2021) (https://www.iss.it/documents/20126/0/FLASH+SURVEY+Varianti_SARS-CoV-2_30luglio.pdf/6c1c9969-e62c-cf19-6d1e-d9679e21692a?t=1627647977533)

equivalent) in province of Bolzano, undetected by the integrated surveillance. This may be related to a small cluster or sporadic cases of the Beta variant occurring in the catchment area of the WTP during the sampling time, gone unnoticed due to low sample size, asymptomatic infection or transit of the infected subjects in the area.

Table 2. Epidemiological data at the time of collection and PCR results of the tested samples

Sample ID	Region/A.P.	Cases reported in the Region/A.P. in the weeks 28 June – 11 July		N° of active cases in the Region/A.P. on 4 July 2021	City	Sampling date	WTP	QA criteria [d]	RT-qPCR	RT-nested-PCR		
		[a]	[b]	[c]					(g.c./L)	Long 980	Short 973	Short 975
FS-202107-14	Liguria	80	119	125	Lerici	07/07/21	Camisano (Lerici)	pass	1.6×10^3	-	-	-
FS-202107-15					Genova	06/07/21	Punta Vagno	pass	-	-	-	-
FS-202107-16					Genova	07/07/21	Darsena	pass	-	-	-	-
FS-202107-24	Lombardia	903	1361	8976	Milano	05/07/21	Nosedo*	pass	-	-	-	-
FS-202107-25					Varese	05/07/21	Varese Olona	pass	-	-	-	-
FS-202107-01	Piemonte	175	234	777	Torino	05/07/21	Castiglione Torinese	pass	-	-	-	-
FS-202107-02					Alessandria	05/07/21	Orti	pass	2.7×10^3	-	-	-
FS-202107-03	Valle d'Aosta	9	6	32	La Salle	04/07/21	La Salle	pass	-	-	-	-
FS-202107-04					Brissogne	04/07/21	Brissogne	pass	-	-	-	-
FS-202107-21	Emilia Romagna	347	604	2593	Rimini	07/07/21	S. Giustina (Rimini)	pass	1.2×10^3	-	-	-
FS-202107-23					Bologna	09/07/21	IDAR (Bologna)	pass	3.1×10^3	-	**+**	-
FS-202107-20	Friuli Venezia Giulia	80	102	186	Udine	06/07/21	CAFC (Udine)	fail	-	-	-	-
FS-202107-22					Trieste	07/07/21	Servola (Trieste)	pass	2.1×10^3	-	-	-
FS-202107-18	Veneto	370	876	4570	Verona	06/07/21	Verona	pass	2.7×10^4	-	**+**	-
FS-202107-19					Venezia	06/07/21	Fusina (Venezia)	pass	-	-	-	-
FS-202107-28	A.P. Bolzano	41	61	118	Bolzano	05/07/21	ARA Bozen / IDA Bolzano	pass	1.5×10^3	-	-	-
FS-202107-29					Floronzo	05/07/21	ARA Tobl / IDA Tobl	pass	1.9×10^3	**+**	-	-
FS-202107-05	A.P. Trento	32	28	56	Trento	06/07/21	Trento Nord	pass	-	-	-	-
FS-202107-06					Trento	06/07/21	Trento Sud	pass	5.6×10^3	**+**	**+**	-
FS-202107-07					Rovereto	07/07/21	Rovereto	pass	-	-	-	-
FS-202107-08	Lazio	485	966	2773	Roma	07/07/21	Roma Est I Sez.	pass	2.9×10^3	-	-	-
FS-202107-09					Roma	06/07/21	Roma Est II Sez.	pass	1.7×10^3	-	**+**	-
FS-202107-10					Pomezia	07/07/21	Crocetta I	pass	-	-	-	-
FS-202107-11					Roma	07/07/21	Roma Ostia	pass	1.6×10^3	-	-	-
FS-202107-12					Roma	06/07/21	Roma Nord	pass	-	-	-	-
FS-202107-13					Roma	07/07/21	Roma Sud	pass	3.9×10^3	-	-	-
FS-202107-17					Roma	07/07/21	Roma Est II Sez.	pass	-	-	-	-
FS-202107-26	Toscana	320	485	1567	Pisa	06/07/21	Pisa	pass	-	-	-	-
FS-202107-27					Viareggio	07/07/21	Viareggio	pass	-	-	-	-
FS-202107-34	Campania	732	1071	7583	Napoli	12/07/21	Napoli Ovest (Cuma)	fail	-	-	-	-
FS-202107-35					Napoli	12/07/21	Napoli Est	fail	-	-	-	-
FS-202107-36	Puglia	236	283	2660	Brindisi	07/07/21	Fiume Grande (Brindisi)	pass	-	-	-	-

FS-202107-37					Foggia	06/07/21	Foggia	pass	-	-	-	-
FS-202107-33	Sicilia	811	1224	3563	Catania	09/07/21	Pantano d’Arci (Catania)	pass	-	-	-	-
FS-202107-38					Palermo	05/07/21	Acqua dei Corsari (Palermo)	pass	-	-	-	-

[a] Ministero della Salute, Istituto Superiore di Sanità – Cabina di Regia ai sensi del DM Salute 30 aprile 2020 “Monitoraggio Fase 2 - Report settimanale - Report 60, Sintesi nazionale” https://www.salute.gov.it/imgs/C_17_monitoraggi_69_0_fileNazionale.pdf

[b] Ministero della Salute, Istituto Superiore di Sanità – Cabina di Regia ai sensi del DM Salute 30 aprile 2020 “Monitoraggio Fase 2 - Report settimanale - Report 61, Sintesi nazionale” https://www.salute.gov.it/imgs/C_17_monitoraggi_70_0_fileNazionale.pdf

[c] 04 luglio 2021 - Aggiornamento casi Covid-19 - Dati aggregati quotidiani Regioni/PPAA - Ministero della Salute - Istituto Superiore di Sanità https://github.com/pcm-dpc/COVID-19/blob/master/schede-riepilogative/regioni/dpc-covid19-ita-scheda-regioni-20210704.pdf

[d] Due to the progressive implementation of the national surveillance, QA criteria for the present flash survey were limited to the assessment of PCR inhibition. QA criteria related to sample extraction efficiency will be included before the start of the surveillance according to Rec. 2021/472 on 1st October 2021.

Table 3. SARS-CoV-2 variants and mutations detected in the samples

Sample ID	**City**	**SARS-CoV-2 variants in wastewaters**			**SARS-CoV-2 variants detected by the integrated surveillance**	
		Long RT-nested-PCR + NGS	**Long/short RT-nested-PCR + Sanger seq**			
		Variants detected	**Mutations detected**	**Plausibly associated variants**	**Period 05.06.21 – 19.07.21 Data by province** [a]	**Flash survey 20.07.21 Data by Region/A.P.** [b]
FS-202107-23	Bologna	-	H69-V70del, Y144del	Alpha [B.1.1.7]	B.1.1.7, P.1, B.1.617.2, B.1.617.1/2	B.1.1.7 (n=9), B.1.617.2 (n=118)
FS-202107-18	Verona	-	G142D	Delta [B.1.617.2] / Kappa [B.1.617.1] [c]	B.1.1.7+E484K, P.1, B.1.617.2	B.1.1.7 (n=3), B.1.617.2 (n=140), other (n=1)
FS-202107-29	Floronzo (BZ)	Delta [B.1.617.2] (++) Beta [B.1.351] (+)	K77T, G142D, E156G, F157-R158del, W258L, L452R, T478K	Delta [B.1.617.2]	B.1.617.2	B.1.617.2 (n=10)
FS-202107-06	Trento	Delta [B.1.617.2] (++)	G142D, E156G, F157-R158del, L452R, T478K	Delta [B.1.617.2]	B.1.617.2, B.1.525	B.1.1.7 (n=1), B.1.617.2 (n=10)
FS-202107-09	Roma	-	G142D	Delta [B.1.617.2] / Kappa [B.1.617.1] [c]	B.1.1.7, P.1, B.1.617.2	B.1.1.7 (n=5), P.1 (n=2), B.1.617.2 (n=125)
NGSQC1-control	Alpha [B.1.1.7] (+++)	Beta [B.1.351] (+++)	Gamma [P.1] (+++)	Delta [B.1.617.2] (+++)		
NGSQC2-control	Alpha [B.1.1.7] (++)	Beta [B.1.351] (++)	Gamma [P.1] (++)	Delta [B.1.617.2] (+++)		

Plus signs represent the coverage obtained for each detected variant: (+++) = high coverage (≥ 10^5 reads); (++) = intermediate coverage (10^3 - 10^5 reads); (+) = detection level (10^2 - 10^3 reads). Sequences with coverage <10^2 were not considered. ***Note:*** higher sequence coverage for specific variants does not necessary imply higher presence of such variants in the initial wastewater sample (see section 'Limitations of the study')

[a] Istituto Superiore di Sanità – Prevalenza e distribuzione delle varianti di SARS-CoV-2 di interesse per la sanità pubblica in Italia - Rapporto n. 5 del 23 luglio 2021 (https://www.iss.it/documents/20126/0/versione+h++17+BOLLETTINO+VARIANTI+n.5+23+luglio.pdf/e3a41bf9-0913-a01b-6b5d-5b55c249e0d7?t=1627050956399)

[b] Istituto Superiore di Sanità, Fondazione Bruno Kessler, Ministero della Salute – Stima della prevalenza delle varianti VOC (Variants of Concern) in Italia: B.1.1.7, B.1.351, P.1 e B.1.617.2, e altre varianti di SARS-CoV-2 (Indagine del 20/7/2021) (https://www.iss.it/documents/20126/0/FLASH+SURVEY+Varianti_SARS-CoV-2_30luglio.pdf/6c1c9969-e62c-cf19-6d1e-d9679e21692a?t=1627647977533)

[c] Single mutations are not informative enough to allow variant discrimination and, according to EU Commission Recommendation 2021/472, a minimum of three genetic markers per variant should be reported. Therefore, single mutations can only be considered as indicative of the presence of variants.

Limitations of the study

- Given the ongoing implementation of the national surveillance for SARS-CoV-2 in wastewaters, sample collection/processing was not feasible for all Italian Regions and was restricted to two urban centres per Region/Autonomous Province. Therefore, geographical and population coverage of the flash survey herein described has limitations, and an increase of representativeness should be achieved in the next surveys. Furthermore, while the overall results of this survey confirmed that the analytical approach is fit-for-purpose for surveillance of SARS-CoV-2 variants in wastewaters, a reduction of the analytical turnaround time for samples submitted to deep sequencing should be accomplished to meet the requirements for a systematic surveillance.

- Molecular analytical methods applied to complex environmental matrices as wastewaters may be hampered by low target concentration, poor recovery of the analyte, and/or inhibiting effect of substances on PCR amplification. Therefore, both detection/quantification and PCR amplification for sequencing purposes may provide negative results in presence of low concentrations of SARS-CoV-2 in wastewaters. As a consequence, molecular characterization and variant detection may not be achieved for all samples.

- Quality controls of the NGS applied in the flash survey herein described (NGSQC1, NGSQC2) indicates that identification of SARS-CoV-2 variants in samples containing a mixture of them is achieved under different conditions. However, reverse transcription and PCR amplification processes in complex matrices display variable degrees of efficiency on different molecular targets and may significantly affect the final proportions of the amplified sequences. Therefore, amplicons coverage in sequenced samples do not necessary represent variants proportions either in wastewater samples or in the population and sequencing results should not be considered in a quantitative manner.

Conclusions and final considerations

The results of SARS-CoV-2 surveillance in wastewaters confirm the predominance of the Delta variant (B.1.617.2) in the first half of July 2021, in Italy. The Alpha (B.1.1.7) and Beta (B.1.351) variants were also detected in two samples.

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

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