Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
11th report
(Study period: 04 – 08 July 2022)

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Main findings:

- Overall, 165 wastewater samples were collected in the week of 4-8 July 2022 from 18 Regions and 2 Autonomous Provinces (A.P.).
- Mutations characteristic of the SARS-CoV-2 Omicron variant were detected in 60 samples from 18 Regions/A.P.; no sequences were obtained from the remaining Regions.
- Sublineages Omicron BA.4/BA.5 were detected in 58 (97%) samples by Sanger sequencing, the remaining 3% being characterized as Omicron BA.2.
- Sequencing of the M gene allowed us to assign the vast majority of the BA.4/5 sequences to BA.5 (95%).
- NGS results confirmed the presence of sublineages BA.4/BA.5 in all the Regions/A.P. (18) for which sequences were obtained.

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 the Member States to put in place no later than 1 October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants[1].

Following the above EU Recommendation, the Istituto Superiore di Sanità (ISS) instituted "flash surveys", i.e., periodic (monthly) sampling campaigns to be held in different locations in Italy throughout a brief period, aimed at assessing the diversity of SARS-CoV-2 in wastewater in the country.

This report aims to summarize the results of the eleventh national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week 4-8 July 2022.

Methodology

The survey included 165 sewage samples collected between 4 and 8 July 2022 at 165 wastewater treatment plants (WTPs) located in 18 regions and 2 autonomous provinces (A.P.). Details on WTPs enrolled in the environmental surveillance of SARS-CoV-2 in Italy can be found on the ISS website[2]. Samples were processed by the laboratories of the SARI network (see Acknowledgements). Viral concentration measurements and nucleic acid extraction were performed according to the protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3”. Purified RNAs were shipped in dry ice to ISS, where samples were sequenced as previously described[3] with some modifications.

A long nested RT-PCR assay (ID_980, ~1600 bps, spanning amino acid residues 58 to 573 of the spike protein) was used to detect multiple key nucleotide changes (deletion and/or amino acid substitutions) distinctive of the Variants of Concern (VoCs).

[1] Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish 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] Surveillance of SARS-CoV-2 in urban wastewater in Italy 1° Report (Study period: 01 October 2021 - 31 March 2022) 8e5e2edb-bae0-f1b0-ee6e-08255c76484f (iss.it)

[3] 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.

The amplicons from the long nested assay were sequenced by both Sanger and Next Generation Sequencing (NGS), using the Oxford Nanopore Technology MinION platform. For NGS, the 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 previously described[4]. Variant calling was performed for the currently recognized VoCs[5].

The Omicron sublineages BA.4 and BA.5 have identical spike proteins in the sequenced region. Therefore, a new nested PCR was designed to detect a mutation differentiating the two sublineages in the M gene, specifically D3N, which is specific to BA.5.

Results

Overall, 161 of the 165 samples (97.5%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 environmental surveillance (**Table 1**), viral concentrations ranging from 4.4 E+01 to 4.5 E+07 genome copies (g.c.)/L of wastewater.

Sanger Sequencing

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 1-2**. Overall, 65 samples from 18 Regions/A.P. were amplified by the long PCR assay. Long amplicons could not be achieved from samples collected in Molise and the A.P. of Bolzano. High-quality sequences were obtained for 60 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained in one sample, and low-quality sequences were obtained for four samples and could not be assigned. All amplicons were characterized as belonging to the Omicron variant. Specifically, amino acid substitutions of the sublineages Omicron **BA.4/BA.5** (characterized by the amino-acid substitutions L452R and F486V in addition to those typical of BA.2) were detected in 58/60 samples (97%) from 16 Regions/A.P (Basilicata, Calabria, Emilia Romagna, Friuli Venezia Giulia, Lazio, Liguria, Lombardia, Marche, Piemonte, Puglia, Sicilia, Toscana, Umbria, Val d'Aosta, Veneto, A.P. of Trento). Of these, the vast majority (55 samples) were assigned to Omicron BA.5 using the assay targeting the M gene (presence of mutation D3N), two to BA.4 (absence of mutation D3N). One showed the co-presence of the two subvariants (double A/G peak in correspondence to the mutation site). Mutations of the Omicron sublineage **BA.2** were detected in only two samples from the Regions of Sicilia and Veneto.

[4] 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

[5] SARS-CoV-2 variants of concern as of 9 June 2022. SARS-CoV-2 variants of concern as of 9 June 2022 (europa.eu)

Next Generation Sequencing

NGS results could be successfully obtained for the 18 tested pools, all showing the characteristic mutations of the Omicron sub-variants BA.4/5. Amino-acid substitutions of sublineage BA.2 could not be detected by NGS, even in the two pools containing amplicons characterized as BA.2 by Sanger sequencing. This could be due to variable depth of coverage among pools subjected to NGS and/or to the variability of read quality (errors introduced by the MinION Nanopore sequencing platform), which may have reduced the detection of less abundant variants.

Sequencing results are summarized in **Table 1**. To improve the readability of the table, panels of mutations ('mutation packages') were defined, as follow:

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = **DEL69/70**, G142D, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, **F486V**, Q498R, N501Y, Y505H)
- **Package B** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)

Table 1. PCR and sequencing results (long PCR ID 980)

	Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_980)	SARS-CoV-2 variant (Sanger sequencing)	Sequencing results (NGS)	SARS-CoV-2 variant (NGS)
1	11993	Abruzzo	Chieti	S. Martino	<LOD	-		**Package A**	Omicron BA.4/5
2	11998		Pescara	Via Raiale	2,89E+02	-			
3	11999		Pescara	Villa Carmine	5,36E+02	-			
4	12000		L'Aquila	Pile	2,98E+02	LQ [b]	-		
5	12001		Teramo	Villa Pavone	5,71E+02	-			
6	12012	Basilicata	Potenza	Tiera di Vaglio	7,84E+03	-		**Package A**	Omicron BA.4/5
7	12013		Matera	Pantano	1,95E+04	**Package A**	Omicron BA4/5 [d]		
8	11906	Calabria	Cosenza	Cosenza - Sant'Angelo	3,02E+02	**Package A**	Omicron BA.4/5 [d]	**Package A**	Omicron BA.4/5
9	11907		Cosenza	Cosenza - Code di volpe	9,57E+01	**Package A**	Omicron BA.4/5 [d]		
10	11908		Reggio Calabria	Ravagnese - località Aeroporto	5,63E+01	-			
11	11909		Catanzaro	Catanzaro - Zona industriale	4,41E+01	-			
12	11910		Crotone	Crotone - località Papaniciaro	1,04E+02	-			
13	11911		Catanzaro	Catanzaro Lido - Loc. Verghello	1,47E+02	-			
14	12143	Campania	Salerno	Salerno	6,50E+03	-		**Package A**	Omicron BA.4/5
15	12144		Salerno	Nocera Sup	4,08E+04	-			
16	12145		Salerno	Eboli	1,00E+04	-			
17	12146		Napoli	Napoli OVEST - Ingresso Principale	6,50E+05	-			
18	12147		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,04E+06	LQ [b]	-		
19	12148		Napoli	Area Nolana	1,62E+05	-			
20	12149		Napoli	Napoli EST	1,72E+06	-			

21	12150		Caserta	Area Casertana	3,11E+05	-			
22	12151		Caserta	Villa Literno	5,30E+04	-			
23	11870	Emilia-Romagna	Modena	Carpi	<LOD	-		**Package A**	Omicron BA.4/5
24	11929		Ravenna	Faenza	5,23E+05	**Package A**	Omicron BA.4/5 [d]		
25	11930		Bologna	Imola	9,85E+03	-			
26	11931		Modena	Naviglio	3,13E+05	-			
27	11932		Forlì-Cesena	Cesena	1,07E+06	**Package A**	Omicron BA.4/5 [d]		
28	11933		Bologna	IDAR	1,10E+06	**Package A**	Omicron BA.4/5 [d]		
29	11935		Rimini - Forlì-Cesena	S. Giustina	7,70E+05	-			
30	11936		Ravenna - Forlì-Cesena	Ravenna	7,40E+05	-			
31	11937		Forlì-Cesena	Forlì	2,86E+05	**Package A**	Omicron BA.4/5 [c]		
32	11960		Piacenza	Borgoforte	5,00E+02	-			
33	11961		Parma	Parma ovest	1,20E+05	-			
34	11963		Reggio Emilia	Mancasale	1,13E+05	-			
35	12002	Friuli-Venezia Giulia	Pordenone	Cordenons	1,01E+06	**Package A**	Omicron BA.4/5 [d]	**Package A**	Omicron BA.4/5
36	12003		Udine	Udine	4,47E+05	**Package A**	Omicron BA.4/5 [d]		
37	12004		Trieste	Servola	3,75E+05	**Package A**	Omicron BA.4/5 [d]		
38	11828	Lazio	Roma	Civitavecchia Fiumaretta	2,50E+02	-		**Package A**	Omicron BA.4/5
39	11841		Viterbo	Viterbo - Strada Bagni	1,43E+04	-			
40	11844		Roma	Anzio - Colle Cocchino	1,43E+04	**Package A**	Omicron BA.4/5 [d]		
41	11848		Latina	Aprilia (Via del Campo)	1,52E+04	-			
42	11850		Latina	Latina Loc Latina Est	2,53E+04	**Package A**	Omicron BA.4/5 [d]		
43	11851		Roma	Velletri (LA CHIUSA-SORBO)	8,68E+03	-			
44	11852		Roma	Pomezia - Via Cincinnato	3,20E+04	-			
45	11853		Roma	Guidonia - Ponte Lucano	2,40E+04	-			

46	11938		Roma	Roma Est (linea 1 + linea 2)	1,03E+05	-			
47	11939		Roma	Roma Nord	3,33E+04	ME [a]			
48	11940		Roma	Roma Sud	1,21E+05	-			
49	11941		Roma	Ostia	4,53E+05	-			
50	11942		Roma	Fregene	3,18E+05	**Package A**	Omicron BA.4/5 [d]		
51	11886	Liguria	Genova	Pegli	6,88E+05	-		**Package A**	Omicron BA.4/5
52	11887		Genova	Voltri	5,55E+05	**Package A**	Omicron BA.4/5 [d]		
53	11888		Genova	Quinto	7,05E+05	**Package A**	Omicron BA.4/5 [d]		
54	11890		Genova	Rapallo	7,03E+05	-			
55	11895		Genova	Sturla	1,41E+06	-			
56	11897		Savona	Savona	2,38E+05	**Package A**	Omicron BA.4/5 [d]		
57	11898		Savona	Borghetto Santo Spirito	9,43E+05	-			
58	11899		Imperia	Imperia	4,57E+05	-			
59	11900		Imperia	Sanremo - località Capo Verde	4,27E+05	-			
60	11901		Genova	Darsena	6,75E+05	-			
61	11902		Genova	Punta Vagno Genova	1,14E+06	**Package A**	Omicron BA.4/5 [d]		
62	11903		Genova	Valpolcevera	7,45E+05	**Package A**	Omicron BA.4/5 [d]		
63	11904		Genova	Sestri P	1,35E+06	**Package A**	Omicron BA.4/5 [d]		
64	11957		Genova	Punta Vagno Genova	1,60E+05	**Package A**	Omicron BA.4/5 [d]		
66	12032	Lombardia	Sondrio	Sondrio	8,35E+04	**Package A**	Omicron BA.4/5 [c]	**Package A**	Omicron BA.4/5
67	11863		Milano	Bresso	1,17E+05	-			
68	11865		Milano - Monza e della Brianza	Peschiera Borromeo	1,02E+05	-			
69	11867		Milano - Varese	Canegrate	1,03E+02	-			
70	11868		Varese	Varese	7,33E+05	-			
71	11869		Milano - Varese	Lonate Pozzolo	1,65E+05	-			
72	11872		Milano	Milano Nosedo	3,45E+04	-			
73	11873		Milano	Milano San Rocco	3,68E+04	-			

74	11874		Como	Como	3,66E+04	-		
75	11875		Pavia	Pavia	3,50E+04	**Package A**	Omicron BA.4/5[d]	
76	11876		Como - Lecco - Milano - Monza e della Brianza	Monza	3,64E+04	-		
77	11877		Pavia	Vigevano	4,53E+04	-		
78	11924		Bergamo	Bergamo	2,39E+04	-		
79	11925		Cremona	Citta di Cremona	9,46E+03	-		
80	11927		Brescia	Verziano	1,54E+04	-		
81	11914	Marche	Pesaro-Urbino	Borgheria	2,21E+04	LQ [b]	-	**Package A** Omicron BA.4/5
82	11915		Pesaro-Urbino	Ponte Metauro	2,19E+04	-		
83	11916		Pesaro-Urbino	Ponte Sasso	3,12E+04	-		
84	11917		Ancona	Zipa	3,62E+04	-		
85	11918		Ancona	Falconara	3,06E+04	LQ [b]	-	
86	11919		Ancona	Camerano	1,14E+04	-		
87	11990	Molise	Campobasso	Campobasso - San Pietro	5,95E+02	-		**Package A** Omicron BA.4/5
88	11991		Campobasso	Termoli - località Porto	3,92E+02	-		
89	11992		Campobasso	Termoli - località Pantano Basso	2,09E+03	-		
90	11835	A.P. Bolzano	Bolzano	IDA Bolzano	5,61E+04	-		
91	11836		Bolzano	IDA Merano	1,16E+05	-		
92	11837		Bolzano	IDA Termeno	7,93E+04	-		
93	11878	A.P. Trento	Trento	Trento nord	2,77E+05	-		**Package A** Omicron BA.4/5
94	11879		Trento	Trento sud	2,11E+05	-		
95	11880		Trento	Rovereto	3,46E+05	**Package A**	Omicron BA.4/5 [d]	
96	11787	Piemonte	Torino	Castiglione Torinese	2,50E+04	-		**Package A** Omicron BA.4/5
97	11788		Biella	Biella Nord	2,22E+04	**Package A**	Omicron BA.4/5 [d]	
98	11789		Biella	Biella Sud	1,17E+04	**Package A**	Omicron BA.4/5 [e]	
99	11790		Novara	Novara	2,50E+04	-		
100	11858		Alessandria	Alessandria	2,21E+03	-		

101	11860		Asti	Asti	4,43E+03	-			
102	11862		Cuneo	Cuneo	1,13E+04	-			
103	11779		Bari	Bari Ovest	1,01E+04	**Package A**	Omicron BA.4/5 [d]	**Package A**	Omicron BA.4/5
104	11780		Bari	Bari Est	1,58E+03	**Package A**	Omicron BA.4/5 [d]		
105	11796		Lecce	Lecce	1,73E+04	**Package A**	Omicron BA.4/5 [d]		
106	11798		Brindisi	Brindisi Fiume Grande	6,56E+03	-			
107	11800		Taranto	Taranto Bellavista	7,06E+03	**Package A**	Omicron BA.4/5 [d]		
108	11802		Taranto	Taranto Gennarini	1,48E+04	**Package A**	Omicron BA.4/5 [d]		
109	11829		Foggia	Cerignola	8,00E+02	-			
110	11830		Foggia	Manfredonia	7,97E+03	**Package A**	Omicron BA.4/5 [d]		
111	11831	Puglia	Foggia	Foggia	5,64E+03	**Package A**	Omicron BA.4/5 [d]		
112	11854		Bari	Molfetta	1,24E+04	**Package A**	Omicron BA.4/5 [d]		
113	11855		Barletta-Andria-Trani	Bisceglie	1,47E+04	**Package A**	Omicron BA.4/5 [d]		
114	11856		Barletta-Andria-Trani	Trani	2,52E+04	**Package A**	Omicron BA.4/5 [d]		
115	11859		Barletta-Andria-Trani	Andria	1,38E+03	-			
116	11864		Barletta-Andria-Trani	Barletta	9,86E+04	**Package A**	Omicron BA.4/5 [d]		
117	11866		Bari	Bitonto	3,95E+04	**Package A**	Omicron BA.4/5 [d]		
118	11871		Bari	Altamura	1,95E+05	**Package A**	Omicron BA.4/5 [d]		
119	11823		Trapani	Trapani	1,27E+04	-		**Package A**	Omicron BA.4/5
120	11824		Trapani	Mazara del Vallo	3,45E+03	-			
121	11825		Trapani	Marsala	4,79E+03	-			
122	11842	Sicilia	Agrigento	Agrigento	1,66E+05	-			
123	11843		Enna	Enna	4,28E+05	-			
124	11845		Palermo	Bagheria	1,01E+05	-			
125	11846		Palermo	Acqua dei Corsari	1,19E+05	**Package A**	Omicron BA.4/5 [d]		
126	11847		Palermo	Fondo Verde	2,84E+05	**Package A**	Omicron BA.4/5 [d]		

127	11849		Caltanissetta	Caltanissetta e San Cataldo	1,97E+05	**Package A**	Omicron BA.4/5 [d]		
128	11943		Ragusa	Modica	2,19E+05	**Package A**	Omicron BA.4/5 [d]		
129	11944		Ragusa	Vittoria	2,71E+05	**Package A**	Omicron BA.4/5 [d]		
130	11945		Ragusa	Ragusa	1,89E+05	**Package B**	Omicron BA.2		
131	11946		Caltanissetta	Gela Macchitella	4,48E+07	**Package A**	Omicron BA.4/5 [d]		
132	11947		Messina	Mili Marina	9,81E+04	**Package A**	Omicron BA.4/5 [d]		
133	11949		Catania	Pantano d'Arci	3,00E+04	**Package A**	Omicron BA.4/5 [d]		
134	11951		Catania	Giarre	3,20E+04	**Package A**	Omicron BA.4/5 [d]		
135	11952		Siracusa	Siracusa	4,47E+04	-			
136	11948		Messina	Mili Marina	6,54E+04	**Package A**	Omicron BA.4/5 [d]		
137	11965	Toscana	Firenze	San Colombano	5,58E+04	-		**Package A**	Omicron BA.4/5
138	11966		Firenze	San Colombano	5,33E+04	-			
139	11967		Prato	Baciacavallo	1,68E+04	-			
140	11968		Prato	Baciacavallo	2,78E+04	-			
141	11969		Arezzo	Casolino - San Leo	6,58E+03	-			
142	11970		Grosseto	San Giovanni - Pianetto	8,15E+04	-			
143	11971		Siena	Ponte a Tressa	2,31E+05	**Package A**	Omicron BA.4/5 [d]		
144	11972		Pisa	Pisa Nord - S. Jacopo	9,20E+04	-			
145	11973		Firenze	Empoli Pagnana	<LOD	-			
146	11974		Massa	Lavello 2	6,81E+04	-			
147	11975		Lucca	Viareggio	6,23E+04	-			
148	11976		Massa	Lavello 1	3,12E+04	-			
149	11977		Lucca	Pontetetto	6,68E+04	-			
150	11978		Livorno	Rivellino	4,11E+04	-			
151	11979		Livorno	Rivellino	5,04E+04	-			
152	11791	Umbria	Perugia	Perugia - Pian della Genna	7,69E+05	**Package A**	Omicron BA.4/5 [d]	**Package A**	Omicron BA.4/5
153	11882		Perugia	Foligno Casone	7,82E+04	-			

154	11883		Terni	Terni	4,40E+05	**Package A**	Omicron BA.4/5 [d]		
155	11922	Valle d'Aosta	Aosta	La Salle	<LOD	-		**Package A**	Omicron BA.4/5
156	11923		Aosta	Brissogne	2,24E+04	**Package A**	Omicron BA.4/5 [d]		
157	11817	Veneto	Padova	Padova Ca' Nordio - centro storico	1,57E+05	-		**Package A**	Omicron BA.4/5
158	11818		Padova	Padova Ca' Nordio - zip	1,26E+05	-			
159	11819		Padova	Padova Guizza	1,04E+05	-			
160	11820		Padova	Abano Terme	3,20E+05	**Package A**	Omicron BA.4/5[d]		
161	11838		Treviso	Treviso	3,29E+04	**Package A**	Omicron BA.4/5 [d]		
162	11839		Venezia	Venezia Fusina	1,98E+04	**Package B**	Omicron BA.2		
163	11840		Vicenza	Vicenza Casale	3,07E+04	**Package A**	Omicron BA.4/5 [d]		
164	11891		Verona	Verona_collettore 1M	2,53E+04	**Package A**	Omicron BA.4/5 [d]		
165	11892		Verona	Verona_collettore 3M	2,77E+04	**Package A**	Omicron BA.4/5 [d]		
166	11893		Verona	Verona_collettore 8M	2,03E+04	**Package A**	Omicron BA.4/5 [d]		

[a] M.E. mixed electropherograms
[b] LQ (low-quality sequences)
[c] the absence of mutation D3N in the M gene is suggestive of the presence of BA.4
[d] the presence of mutation D3N in the M gene is suggestive of the presence of BA.5
[e] the presence of a dual A/G peak in correspondence to the mutation site (corresponding to the presence/absence of mutation D3N in the M gene) is suggestive of the simultaneous presence of BA.4 and BA.5

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = DEL69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H)
- **Package B** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)

Table 3. Sanger sequencing results (long PCR ID980)

	CHARACTERISTIC MUTATIONS																				
ID SAMPLES	**DEL69/70**	**G142D**	**V213G**	**G339D**	**S371F**	**S373P**	**S375F**	**T376A**	**D405N**	**R408S**	**K417N**	**N440K**	**L452R**	**S477N**	**T478K**	**E484A**	**F486V**	**Q493R**	**Q498R**	**N501Y**	**Y505H**	**VARIANTS**
7-8-9-24-27-28-31-35-36-37-40-42-50-52-53-56-61-62-63-64-66-75-95-97-98-103-104-105-107-108-110-111-112-113-114-116-117-118-125-126-127-128-129-131-132-133-134-136-143-152-154-156-160-161-163-164-165-166																						**Package A*** (Omicron BA.4/5; 58 samples)
132-162																						**Package B** (Omicron BA.2; two samples)

* Upon sequencing the M gene, 55/58 of the Ba.4/BA.5 sequences were assigned to Omicron BA.5, two to BA.4, and one showed the presence of both sublineages.

Limitations of the study

This flash survey's geographical and population coverage was incomplete, as it covered 20/21 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, detection/quantification and sequencing may produce false negatives, and variant detection may not be achieved for all samples.
Partial sequencing of the Spike region does not allow conclusive assignation of variants/sublineages. However, the detection within the Spike region of multiple, linked mutations associated with specific variants/sublineages is strongly suggestive of their presence.

Conclusions and final considerations

The present report is the eleventh of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters, delivered as a part of the surveillance established in Italy under EU Commission Recommendation 2021/472 to complement information acquired through clinical surveillance. This survey showed that the SARS-CoV-2 Omicron sublineage BA.5 is the dominant variant in the country as of July 2022.
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