



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
10th Report
(Study period: 6 – 10 June 2022)

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Main findings:

- Overall, 167 wastewater samples were collected in the week 6-10 June from 18 Regions and 2 Autonomous Provinces (A.P.).
- Mutations characteristic of the Omicron variant were detected in 13 Regions and two A.P. while no sequences were obtained from the remaining Regions.
- Sublineages BA.4/BA.5 were detected in 54% of the samples by Sanger sequencing with a slight prevalence of BA.5.
- Aminoacid substitutions of sublineages BA.2 were detected in 43% of the sequences.
- NGS results confirmed the co-occurrence of sublineages BA.4/BA.5 and BA.2 in the vast majority of the Regions/A.P.

Introduction

On 17 March 2021, the “EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU”, strongly encouraged Member States to put in place, no later than 1 October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants¹.

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

The aim of this report is to summarize the results of the tenth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 6-10 June 2022.

Methodology

The survey included 167 sewage samples collected between 6 and 10 June 2022 at 167 wastewater treatment plants (WTPs) located in 17 regions and 2 autonomous provinces (A.P.). Details on WTPs enrolled in the Surveillance of SARS-CoV-2 in urban wastewater in Italy can be found at the ISS website². 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 described³ with some modifications.

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 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 \(iss.it\)](https://www.iss.it/sites/default/files/2022/06/8e5e2edb-bae0-f1b0-ee6e-08255c76484f_iss.it)

³ G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferrò, 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>.

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) and Variants of Interest (VoIs).

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 described in La Rosa et al., 2021⁴. Variant calling was performed for the currently recognized VoCs⁵.

Since the Omicron sublineages BA.4 and BA.5 have identical spike proteins in the sequenced region, a new nested PCR was designed able to detect a mutation differentiating the Omicron sublineages BA.4 and BA.5, specifically D3N located in the M gene, in order to discriminate between the two subvariants.

Results

Overall, 146 of the 167 samples (87%) 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 2.6 E+01 to 3.0 E+05 genome copies (g.c.)/L of sewage.

Sanger Sequencing

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 1-2**. Overall, 38 samples from 15 Regions/A.P. were amplified by the long PCR assay. Long amplicons could not be obtained from samples collected in the Regions of Abruzzo, Campania, Marche, Molise and Valle d'Aosta. Sanger sequences were obtained for 35 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained for three samples which, therefore, could not be assigned. All amplicons were characterized as Omicron variant by Sanger sequencing. Specifically, amino acid substitutions of the sublineage Omicron **BA.4/5** (characterized by the amino-acid substitutions L452R and F486V in addition to those typical of BA.2) were detected in 19 samples (54%) from 8 Regions/A.P. (Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Puglia, Veneto and A.P. of Bolzano). Using the newly designed assay targeting the M gene, 8 of these 19 samples were assigned to Omicron BA.5 (presence of mutation D3N in the M gene), 7 to BA.4, and 4 showed the presence of both subvariants (presence of a double A/G peak in correspondence to the mutation site). Mutation of the Omicron sublineage **BA.2** were detected in 15 samples (43%) from 9 Regions/A.P. (Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Sicilia, Umbria and A.P. of Bolzano and Trento). Moreover, one sample, collected in the Region of Calabria, showed the presence of the characteristic mutation of BA.2, plus L452R (but not F486V) and could not be assigned since this panel of mutations is shared by BA.2.11, BA.2.35, and BA.2.28.

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 \(europa.eu\)](https://ec.europa.eu/eurosurv/infodiv/ncov/ncov-variants-of-concern/)

Next Generation Sequencing

NGS results could be successfully obtained for all the 15 tested pools, all of which showing characteristic mutations of the Omicron variant. Overall, 57.9% of the reads were assigned to Omicron BA.4/5, and 42.1% to BA.2.

Key mutations of the Omicron sublineages BA.4/5 and BA.2 were detected simultaneously in 13 Regions (Calabria, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Piemonte, Puglia, Sicilia, Toscana, Umbria, Veneto and A.P. of Trento and Bolzano). In two Regions, only one Omicron sublineage was identified (BA.4/5 in Lombardia and BA.2 in Basilicata).

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

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = 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)
- **Package C** (Unassigned, shared among Omicron BA.2.11/BA.2.35/ BA.2.28, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, 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	10988	Abruzzo	Chieti	S. Martino	<LOD	-		
2	10992		Pescara	Via Raiale	<LOD	-		
3	10994		Pescara	Villa Carmine	1,25E+02	-		
4	10996		L'Aquila	Pile	<LOD	-		
5	10997		Teramo	Villa Pavone	<LOD	-		
6	11132	Basilicata	Potenza	Tiera di Vaglio	1,31E+03	M.E. ^a	• Package B	• Omicron BA.2
7	11133		Matera	Pantano	<LOD	-		
9	11178	Calabria	Cosenza	Cosenza - Sant'Angelo	5,82E+01	-	• Package A	• Omicron BA.4/5
10	11179		Crotone	Crotone - località Papaniciaro	4,61E+01	-	• Package B	• Omicron BA.2
11	11180		Catanzaro	Catanzaro Lido - Loc. Verghello	2,93E+01	-		
159	11181		Catanzaro	Catanzaro - Zona industriale	2,98E+01	-		
160	11182		Reggio Calabria	Ravagnese - località Aeroporto	2,61E+01	Package C	unassigned ^b	
13	11149	Campania	Salerno	Salerno	2,88E+03	-		
14	11150		Salerno	Nocera Sup	4,39E+04	-		
15	11151		Avellino	Manocalzati	<LOD	-		
16	11152		Napoli	Napoli OVEST - Ingresso Principale	3,22E+04	-		
17	11153		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,51E+04	-		
18	11155		Napoli	Area Nolana	3,37E+04	-		
19	11156		Napoli	Napoli EST	4,88E+03	-		
20	11161		Caserta	Area Casertana	1,14E+04	-		

21	11162		Caserta	Villa Literno	3,91E+04	-		
22	11030	Emilia-Romagna	Ferrara	Ferrara - Linea 1	<LOD	-	• Package A	• Omicron BA.4/5
23	11031		Ferrara	Ferrara - Linea 2	<LOD	-	• Package B	• Omicron BA.2
24	11032		Modena	Carpi	<LOD	-		
25	11117		Parma	Parma ovest	4,73E+04	-		
26	11119		Reggio Emilia	Mancasale	1,71E+04	-		
27	11121		Piacenza	Borgoforte	3,10E+03	-		
28	11093		Bologna	IDAR	2,38E+04	-		
29	11094		Ravenna	Faenza	1,40E+04	-		
30	11095		Bologna	Imola	2,98E+04	-		
31	11097		Ravenna - Forlì-Cesena	Ravenna	1,33E+04	-		
32	11103		Forlì-Cesena	Cesena	2,20E+04	-		
33	11104		Forlì-Cesena	Forlì	1,34E+04	Package B	Omicron BA.2	
34	11106		Modena	Naviglio	2,07E+04	-		
35	11107		Rimini - Forlì-Cesena	S. Giustina	2,55E+04	Package A	Omicron BA.4/5 ^c	
36	11136	Friuli-Venezia Giulia	Pordenone	Cordenons	1,15E+05	Package A	Omicron BA.4/5 ^d	• Package A
37	11137		Udine	Udine	1,17E+04	-	• Package B	• Omicron BA.2
38	11138		Trieste	Servola	3,23E+04	Package B	Omicron BA.2	
39	10937	Lazio	Viterbo	Viterbo - Strada Bagni	4,88E+03	Package A	Omicron BA.4/5 ^c	• Package A
40	10938		Roma	Anzio - Colle Cocchino	6,20E+02	-	• Package B	• Omicron BA.2
41	10939		Latina	Aprilia (Via del Campo)	7,90E+03	-		
42	10940		Latina	Latina Loc Latina Est	1,93E+03	-		
43	10941		Roma	Guidonia - Ponte Lucano	7,70E+03	-		
44	10942		Roma	Velletri (LA CHIUSA-SORBO)	4,98E+03	Package A	Omicron BA.4/5 ^e	
45	10943		Roma	Pomezia - Via Cincinnato	2,70E+03	-		
46	11057		Roma	Civitavecchia Fiumaretta	2,50E+02	-		

47	11109		Roma	Roma Est (linea 1 + linea 2)	5,26E+04	Package B	Omicron BA.2		
48	11110		Roma	Roma Nord	1,45E+04	-			
49	11111		Roma	Roma Sud	4,10E+03	-			
50	11112		Roma	Ostia	7,33E+03	-			
51	11113		Roma	Fregene	3,23E+03	-			
52	11033	Liguria	Savona	Savona	3,46E+04	-		• Package A	• Omicron BA.4/5
53	11034		Genova	Pegli	2,73E+04	Package B	Omicron BA.2	• Package B	• Omicron BA.2
54	11035		Genova	Voltri	7,81E+03	-			
55	11036		Genova	Quinto	1,37E+05	Package B	Omicron BA.2		
56	11037		Genova	Rapallo	5,09E+04	-			
57	11038		Genova	Sestri P	6,84E+04	-			
58	11039		Genova	Sturla	5,73E+04	Package B	Omicron BA.2		
59	11040		La Spezia	Camisano	1,13E+04	-			
60	11041		La Spezia	Silea	1,19E+05	Package B	Omicron BA.2		
61	11042		La Spezia	La Spezia	2,74E+04	-			
62	11043		Imperia	Sanremo - località Capo Verde	2,08E+04	-			
63	11044		Savona	Borghetto Santo Spirito	3,54E+03	-			
64	11045		Genova	Darsena	1,03E+04	-			
65	11046		Genova	Punta Vagno Genova	6,80E+04	Package A	Omicron BA.4/5 ^e		
66	11047	Genova	Valpolcevera	2,75E+04	Package A	Omicron BA.4/5 ^d			
158	11114	Genova	Punta Vagno Genova	3,08E+05	Package A (Partial, from G339D to Y505H) ^f	Omicron BA.4/5 ^d			
67	10962	Lombardia	Milano	Milano Nosedo	1,17E+04	Package A	Omicron BA.4/5 ^d	• Package A	• Omicron BA.4/5
68	10963		Milano	Milano San Rocco	7,35E+03	-			
69	10964		Como	Como	2,10E+04	-			
70	10965		Pavia	Pavia	5,46E+03	-			

71	10966		Como - Lecco - Milano - Monza e della Brianza	Monza	3,56E+04	-			
73	10976		Milano	Bresso	3,68E+04	-			
74	10977		Milano - Monza e della Brianza	Peschiera Borromeo	2,55E+04	-			
75	10978		Milano - Varese	Canegrate	8,68E+04	-			
76	10979		Varese	Varese	3,00E+04	-			
77	10980		Milano - Varese	Lonate Pozzolo	3,50E+04	-			
78	11091		Sondrio	Sondrio	7,13E+04	-			
79	11098		Bergamo	Bergamo	1,27E+04	-			
80	11099		Cremona	Citta di Cremona	<LOD	-			
81	11101		Brescia	Verziano	4,21E+03	-			
82	11048		Pesaro-Urbino	Borgheria	1,81E+04	-			
83	11049		Pesaro-Urbino	Ponte Metauro	2,80E+03	-			
84	11050	Marche	Pesaro-Urbino	Ponte Sasso	7,86E+03	-			
85	11051		Ancona	Zipa	1,88E+04	-			
86	11052		Ancona	Falconara	7,31E+03	-			
87	11053		Ancona	Camerano	3,54E+03	-			
88	10982		Campobasso	Termoli - località Porto	<LOD	-			
89	10984	Molise	Campobasso	Termoli - località Pantano Basso	1,25E+02	-			
90	10973	A.P. Bolzano	Bolzano	IDA Bolzano	3,20E+04	Package A	Omicron BA.4/5 ^e	• Package A	• Omicron BA.4/5
91	10974		Bolzano	IDA Merano	1,61E+04	Package B	Omicron BA.2	• Package B	• Omicron BA.2
92	10975		Bolzano	IDA Termeno	4,48E+04	Package A	Omicron BA.4/5 ^d		
93	10949	A.P. Trento	Trento	Trento nord	4,16E+04	-		• Package A	• Omicron BA.4/5
94	10950		Trento	Trento sud	7,24E+04	-		• Package B	• Omicron BA.2
95	10951		Trento	Rovereto	3,47E+04	Package B	Omicron BA.2		
96	10900	Piemonte	Torino	Castiglione Torinese	7,50E+02	M.E. ^a		• Package A	• Omicron BA.4/5
97	10901		Biella	Biella Nord	3,08E+02	-		• Package B	• Omicron BA.2
98	10902		Biella	Biella Sud	5,00E+01	-			

99	10903		Novara	Novara	1,50E+02	-			
100	10987		Alessandria	Alessandria	1,32E+03	-			
101	10989		Asti	Asti	7,60E+02	-			
102	10990		Cuneo	Cuneo	4,83E+03	-			
103	10871	Puglia	Bari	Bari Est	3,62E+03	Package A	Omicron BA.4/5 ^c	• Package A	• Omicron BA.4/5
104	10872		Bari	Bari Ovest	1,91E+03	-		• Package B	• Omicron BA.2
105	10904		Brindisi	Brindisi Fiume Grande	3,93E+03	-			
106	10905		Lecce	Lecce	2,71E+03	-			
107	10906		Taranto	Taranto Bellavista	9,10E+02	-			
108	10907		Taranto	Taranto Gennarini	3,34E+03	-			
109	10952		Foggia	Foggia	3,39E+03	Package A (Partial, from G339D to Y505H) ^f	Omicron BA.4/5 ^e		
110	10953		Foggia	Manfredonia	8,34E+03	-			
111	10954		Foggia	Cerignola	7,65E+03	Package A	Omicron BA.4/5 ^c		
112	10983		Barletta-Andria- Trani	Andria	1,98E+03	-			
113	10985		Barletta-Andria- Trani	Barletta	1,46E+04	-			
114	10991		Barletta-Andria- Trani	Trani	1,19E+05	-			
115	10993		Bari	Molfetta	1,86E+04	-			
116	10995		Barletta-Andria- Trani	Bisceglie	2,89E+04	Package A	Omicron BA.4/5 ^d		
117	11007	Bari	Bitonto	2,20E+04	-				
118	11092	Bari	Altamura	5,60E+03	Package A	Omicron BA.4/5 ^d			
119	11022	Sicilia	Agrigento	Agrigento	4,14E+04	-		• Package A	• Omicron BA.4/5
120	11023		Enna	Enna	6,26E+04	-		• Package B	• Omicron BA.2
121	11025		Palermo	Acqua dei Corsari	3,11E+04	-			
122	11026		Palermo	Fondo Verde	5,16E+04	-			
123	11027		Caltanissetta	Caltanissetta e San Cataldo	6,21E+04	Package B	Omicron BA.2		

124	11054		Trapani	Trapani	7,31E+03	-		
125	11055		Trapani	Mazara del Vallo	1,72E+03	-		
126	11056		Trapani	Marsala	4,60E+03	-		
127	11058		Ragusa	Modica	3,98E+04	-		
128	11059		Ragusa	Vittoria	8,65E+03	-		
129	11060		Ragusa	Ragusa	3,04E+04	Package B		Omicron BA.2
130	11061		Caltanissetta	Gela Macchitella	1,81E+04	-		
131	11062		Messina	Mili Marina	1,29E+04	-		
132	11167		Catania	Pantano d'Arci	1,68E+04	-		
133	11169		Catania	Giarre	3,92E+04	-		
134	11170		Siracusa	Siracusa	2,50E+04	-		
170	11024		Palermo	Bagheria	3,11E+04	-		
171	11063		Messina	Mili Marina	3,90E+03	-		
135	11064		Pisa	Pisa Nord - S. Jacopo	2,92E+04	-	• Package A	• Omicron BA.4/5
136	11065		Firenze	Empoli Pagnana	<LOD	M.E. ^a	• Package B	• Omicron BA.2
137	11066		Massa	Lavello 2	<LOD	-		
138	11067		Lucca	Viareggio	<LOD	-		
139	11068		Massa	Lavello 1	<LOD	-		
140	11069		Lucca	Pontetetto	<LOD	-		
141	11070		Livorno	Rivellino	<LOD	-		
142	11071		Livorno	Rivellino	<LOD	-		
161	11124	Toscana	Firenze	San Colombano	<LOD	-		
162	11125		Firenze	San Colombano	3,30E+03	-		
163	11126		Prato	Baciacavallo	<LOD	-		
164	11127		Prato	Baciacavallo	<LOD	-		
165	11128		Arezzo	Casolino - San Leo	6,35E+03	-		
166	11129		Grosseto	San Giovanni - Pianetto	1,09E+04	-		
167	11130		Pistoia	Centrale Pistoia	9,73E+03	-		
168	11131		Siena	Ponte a Tressa	1,55E+04	-		

143	10899	Umbria	Perugia	Perugia - Pian della Genna	1,21E+05	Package B	Omicron BA.2	• Package A • Package B	• Omicron BA.4/5 • Omicron BA.2
144	11011		Perugia	Foligno Casone	5,26E+04	-			
145	11012		Terni	Terni	1,02E+05	-			
146	11087	Valle d'Aosta	Aosta	La Salle	6,87E+03	-			
147	11089		Aosta	Brissogne	1,09E+04	-			
148	10945	Veneto	Padova	Padova Ca' Nordio - centro storico	2,06E+04	-		• Package A • Package B	• Omicron BA.4/5 • Omicron BA.2
149	10946		Padova	Padova Ca' Nordio - zip	3,76E+04	Package A	Omicron BA.4/5 ^c		
150	10947		Padova	Padova Guizza	3,35E+04	-			
151	10948		Padova	Abano Terme	2,20E+04	-			
152	10998		Treviso	Treviso	1,11E+04	Package A (Partial, from G339D to Y505H) ^f	Omicron BA.4/5 ^c		
153	10999		Vicenza	Vicenza Casale	2,03E+03	Package B	Omicron BA.2		
154	11000		Venezia	Venezia Fusina	6,35E+03	Package B	Omicron BA.2		
155	11073	Verona	Verona_collettore 1M	9,08E+03	Package A	Omicron BA.4/5 ^c			
156	11074	Verona	Verona_collettore 3M	1,44E+04	Package A	Omicron BA.4/5 ^d			
157	11075	Verona	Verona_collettore 8M	2,27E+04	Package B	Omicron BA.2			

^a M.E. mixed electropherograms

^b Panel C, containing mutation L452R but not F486V is shared by Omicron BA.2.11/BA.2.35/BA.2.28

^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 double A/G peak in correspondence to the mutation site (corresponding to presence/absence of mutation D3N in the M gene) is suggestive of the simultaneous presence of BA.4 and BA.5

^f Partial sequence due to mixed electropherograms and/or high signal noise; within brackets the region for which a sequence was provided.

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = 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)
- **Package C** (Unassigned, shared among Omicron BA.2.11/BA.2.35/ BA.2.28, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)

Table 3. Sanger sequencing results (long PCR ID980)

	G142D	V213G	G339D	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	L452R	S477N	T478K	E484A	F486V	Q493R	Q498R	N501Y	Y505H	
35, 36, 39, 44, 65, 66, 67, 90, 92, 103, 109*, 111, 116, 118, 149, 152*, 155, 156, 158*																					Package A** (Omicron BA.4/5)
33, 38, 47, 53, 55, 58, 60, 91, 95, 123, 129, 143, 153, 154, 157																					Package B (Omicron BA.2)
160																					Package C (unassigned)

* Partial sequence

** Upon testing for the newly designed assay targeting the M gene, of the 19 BA.4/BA.5 sample 8 were assigned to Omicron BA.5, 7 to BA.4, and 4 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, 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.

Partial sequencing of the Spike region does not allow conclusive assignation of sublineages. However, the detection within the Spike region of multiple, linked mutations associated to specific lineages/sublineages is strongly suggestive of their presence. Therefore, the detection, either by Sanger or NGS sequencing, of defined mutation panels characteristic of certain lineages/sublineages should be considered as a presumptive detection.

Conclusions and final considerations

This is the tenth of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters that will continue to be issued as a 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 showed the presence of characteristic mutations of the Omicron variant, sublineages BA.2 and BA.4/BA.5 (with BA.5 slightly prevalent compared to BA.4).

Results suggest that we are now in a replacement phase of the Omicron subvariants, with BA.4/BA.5 expected to replace the BA.2 variant as the dominant strains in the country.

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, Silvia Scattolini, Daniela D’Angelantonio, Giacomo Migliorati (Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale”);
- **Basilicata:** Michele La Bianca (Regione Basilicata); Rosa Anna Cifarelli, Achille Palma, Giovanna La Vecchia e Giuseppe Lauria (Agenzia Regionale per la Protezione dell’Ambiente Basilicata – ARPAB); Rosanna Brienza e Patrizia Montenegro (Acquedotto Lucano-AQL);
- **Calabria:** Eduardo Malacaria (Regione Calabria), Giuseppe Folino, Michelangelo Iannone, Filomena Casaburi, Giorgia Bulotta, Emanuela Barillari, Melania Dragone, Iolanda Sacco, Carmine Tomaino, Cristina Felicetta, Adelaide Calabria, Ottavia Varcasia, Francesca Stefanizzi, Concetta Vizza (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); Alessandra Tosco, Amalia Porta (Università degli Studi di Salerno); Francesca Pennino, Triassi Maria (Università degli Studi di Napoli “Federico II”);
- **Emilia Romagna:** Paola Angelini, Lisa Gentili (Regione Emilia – Romagna); Laura De Lellis, Daniele Nasci (HERATech); Giovanni Alborali; Nicoletta Formenti, Flavia Guarneri (Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia-Romagna); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (IREN);
- **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); Alessandra Barca, Flavia Serio (Regione Lazio); Doriana Antonella Giorgi, Irene Ferrante, Monica Monfrinotti, Silvia Riosa, Valeria Capparuccini (ARPA Lazio - Agenzia Regionale per la Protezione Ambientale del Lazio); Maria Teresa Scicluna, Antonella Cersini (IZSLT - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana); Mariaconcetta Arizzi, Giancarlo Cecchini, Claudio Ottaviano (Acea Elaborasi);
- **Liguria:** Elena Nicosia (Regione Liguria settore tutela della salute negli ambienti di vita e di lavoro); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (Iren); Elena Grasselli; Giorgia Allaria, Lorenzo Dondero, Francesca Rispo (UNIGE - DISTAV); Alberto Izzotti (UNIGE – DIMES); Rosa Maria Bertolotto, Elena Nicosia, Stefano Rosatto, Marta Bellisomi, Irene Tomesani (ARPAL); Micaela Tiso (MICAMO srl);
- **Lombardia:** Emanuela Ammoni, Danilo Cereda (Regione Lombardia); Marina Nadia Losio, Barbara Bertasi (IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia); Desdemona Oliva, Maria Giovanna Guiso, Fabio Ferrari, Maria Mundo ed Antonino Martines (CAP Holding); Sara Castiglioni, Silvia Schiarea, Giulia Salmoiraghi (Istituto Mario Negri IRCCS); Manuela Antonelli, Arianna Azzellino, Francesca Malpei, Andrea Turolla (POLIMI); Sandro Binda, Pellegrinelli Laura, Valeria Primache (Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute), 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', Francesca Ciuti, Sara Briscolini (IZSUM - Istituto Zooprofilattico Sperimentale Umbria Marche); Silvia Magi (ARPAM);
- **Molise:** Michele Colitti (Regione Molise); Carmen Montanaro (ASReM); Giuseppe Aprea, Silvia Scattolini, Daniela D’Angelantonio, Giacomo Migliorati (Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale”); Maria Grazia Cerroni (Arpa Molise);

- **Piemonte:** Bartolomeo Griglio, Renza Berruti, Mauro Cravero, Angela Costa (Regione Piemonte); Manila Bianchi, Lucia Decastelli; Angelo Romano; Clara Tramuta (IZSTO - Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta SC Sicurezza e Qualità degli Alimenti); Elisabetta Carraro, Cristina Pignata (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino), Silvia Bonetta (Dipartimento di Scienze della Vita e Biologia dei Sistemi), Lisa Richiardi (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino);
- **Puglia:** Giuseppe Di Vittorio, Onofrio Mongelli (Regione Puglia); Osvolda De Giglio, Francesca Apollonio, Francesco Triggiano, Maria Teresa Montagna (Università degli Studi di Bari Aldo Moro - Dipartimento Interdisciplinare di Medicina); Nicola Ungaro (ARPA Puglia);
- **Sicilia:** Mario Palermo (Regione Sicilia); Carmelo Massimo Maida, Walter Mazzucco (Università degli Studi di Palermo-Dipartimento PROMISE - sezione di Igiene); Simona De Grazia, 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");
- **Toscana:** Piergiuseppe Cala' (Regione Toscana); Annalaura Carducci, Marco Verani, Ileana Federigi, Giulia Lauretani, Sara Muzio (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Università di Pisa); Matteo Ramazzotti (Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi di Firenze), Alberto Antonelli (SOD microbiologia e virologia, azienda ospedaliera universitaria Careggi, Firenze);
- **Umbria:** Giovanni Santoro (Regione Umbria), Ermanno Federici, Maya Petricciuolo, Sofia Barigelli (Laboratorio Microbiologia Applicata e Ambientale, DCBB Università di Perugia);
- **Valle D'Aosta:** Mauro Ruffier (Regione Valle d'Aosta); Francesca Borney, Eric Grange, Florida Damasco (Laboratorio chimico biologico microbiologico Arpa Valle d'Aosta);
- **Veneto:** Francesca Russo, Gisella Pitter, Vanessa Groppi (Regione Veneto); Franco Rigoli, Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto); Tatjana Baldovin, Irene Amoruso (Università di Padova);
- **P.A. Bolzano:** Lorella Zago (P.A. Bolzano); Alberta Stenico, Anna-Maria Prast (A.P.P.A. Agenzia provinciale per l'ambiente e la tutela del clima, Laboratorio biologico)
- **P.A. Trento:** Francesco Pizzo; Alessandra Schiavuzzi, Elena Mengon (P. A. Trento) (P.A. Trento); Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento).

We also thank Lidia Orlandi, Claudia Del Giudice, Simona Di Pasquale (ISS) for technical and logistical support.