Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
18th Report
(Study period: February 6th to February 10th, 2023)

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Main findings:
- During the week of February 6th to February 10th, 2023, 160 wastewater samples were collected from 18 Regions and 2 Autonomous Provinces.
- Mutations characteristic of the Omicron variant were detected in 11 Regions/Autonomous Provinces, while no sequences were obtained from the remaining regions.
- Sanger sequencing revealed that 39.4% of the obtained sequences showed amino acid substitutions of sublineage XBB.1.5, 27.3% of BQ.1.1, 9.1% of BA.4/5, 6.1% of both BN.1* and XBB.1, and 3.0% of both sublineages CH.1.1 and CM*.
- Next-generation sequencing (NGS) results confirmed the widespread presence of sublineages BQ.1.1 and XBB.1.5 across the majority of the Regions/Autonomous provinces and also confirmed the circulation of sublineages BA.4/5, BN.1*, CH.1.1 and XBB.1.

Introduction

On March 17th, 2021, the European Union Commission issued Recommendation 2021/472, encouraging Member States to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewater by October 1st, 2021. In response, the Istituto Superiore di Sanità (ISS) launched a series of "flash surveys", which are periodic, monthly sampling campaigns conducted at various locations throughout Italy over a short period of time. The purpose of these surveys is to provide additional information on SARS-CoV-2 variants in the population, supplementing information gathered through clinical surveillance. The aim of this report is to summarize the findings from the 18th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from February 6th to February 10th, 2023.

Methodology

During the period from February 6th to February 10th, 2023, the 18th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy was conducted. The survey included the collection of 160 sewage samples from 155 wastewater treatment plants (WTPs) located across 18 Regions and 2 Autonomous Provinces. Information on the WTPs participating in the SARS-CoV-2 surveillance in urban wastewater in Italy can be found on the ISS website[1]. The samples were processed and the viral concentration was determined by the SARI network laboratories using the protocol "Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3"[2]. The purified RNAs were then sent to ISS for variant detection using both Sanger sequencing and NGS methods.

A real-time RT-PCR assay was used to screen for the presence of the Omicron variant[3]. A long nested RT-PCR assay (~1600 bps, spanning amino acid residues 58 to 573 of the spike protein) was used to detect multiple nucleotide changes distinctive of Variants of Concern (VoCs) and Variants of Interest (VoIs) in the spike protein[4]. Amplicons from the long nested assay were

[1] 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)

[2] DOI 10.5281/zenodo.5758724.

[3] La Rosa G, Iaconelli M, Veneri C, Mancini P, Bonanno Ferraro G, Brandtner D, Lucentini L, Bonadonna L, Rossi M, Grigioni M; SARI network; Suffredini E. The rapid spread of SARS-COV-2 Omicron variant in Italy reflected early through wastewater surveillance. Sci Total Environ. 2022 Sep 1;837:155767. doi: 10.1016/j.scitotenv.2022.155767. Epub 2022 May 6. PMID: 35533857; PMCID: PMC9074219.

[4] 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

sequenced using both Sanger (single samples) and Next Generation Sequencing (NGS) (pools by Regions/AP) with the Oxford Nanopore Technology MinION platform. Bioinformatics analysis was carried out and variant calling was performed for recognized VoCs as previously described[5].

Results

A total of 153 out of 160 samples (95.6%) tested positive for SARS-CoV-2 using the real-time RT-qPCR method adopted for environmental surveillance (Table 1). The viral concentrations in the samples ranged from 2,5E+01 to 4,5E+06 genome copies (g.c.) per liter of sewage. Additionally, 124 out of the 160 (77.5%) samples tested positive for the Omicron variant through the RT-qPCR assay, with cycle threshold (CT) values ranging from 32.1 to 39.8.

Sanger Sequencing

Table 1 provides a summary of the results obtained from the real-time PCR assays, long nested PCR, and sequencing. A total of 34 samples from 11 Regions/Autonomous Provinces were successfully amplified using the long nested PCR assay. From these samples, high-quality sequences were obtained from 33 of them through Sanger sequencing. However, one sequence was unsuccessful due to high background noise. Sanger sequencing confirmed that all of them were characterized as the Omicron variant.

The analysis of wastewater samples revealed the presence of multiple SARS-CoV-2 variants. Among them, the most prevalent was XBB.1.5, which was found in 39.4% (13 samples) of the total samples. Following that, the Omicron BQ.1.1 variant was the next most common variant, identified in 27.3% (9 samples) of the samples. Omicron BA.4/5 and Omicron XBB.1 were detected in 6.1% (2 samples) and 9.1% (3 samples) respectively. The characteristic mutations of Omicron BN.1* were detected in two samples (6.1%). Furthemore, sublineages Omicron CM* and CH.1.1 were each found in one sample (3.0%). Discrimination between the Omicron CH.1.1 and XBB.1 variants was not possible in a single sample due to partial sequence acquisition.

Next Generation Sequencing

NGS results were successfully obtained for all regional pools that were tested, revealing the defining mutations of the Omicron variant (as shown in Table 1). To make it easier to understand, the mutations were grouped into panels or "mutation packages" as follows:

- **Package A** (Omicron BA.4/5) = DEL69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, **F486V**, Q498R, N501Y, Y505H
- **Package B** (Omicron BQ.1.1) = DEL69/70, G142D, V213G, G339D, **R346T**, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **K444T, L452R**, **N460K,** S477N, T478K, E484A, **F486V,** Q498R, N501Y, Y505H

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.

[5] La Rosa, G.; Brandtner, D.; Mancini, P.; Veneri, C.; Bonanno Ferraro, G.; Bonadonna, L.; Lucentini, L.; Suffredini, E. Key SARS-CoV-2 Mutations of Alpha, Gamma, and Eta Variants Detected in Urban Wastewaters in Italy by Long-Read Amplicon Sequencing Based on Nanopore Technology. *Water* **2021**, *13*, 2503. https://doi.org/10.3390/w13182503

- **Package C** (Omicron BN.1*)= G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, **R346T, K356T,** S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, **F490S,** Q498R, N501Y, Y505H
- **Package D** (Omicron CH.1.1)= G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, **R346T,** S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **K444T,** G446S, **L452R,** N460K, S477N, T478K, E484A, **F486S,** Q498R, N501Y, Y505H
- **Package E** (Omicron XBB.1.5)= V83A, G142D, DEL144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, **F486P,** F490S, Q498R, N501Y, Y505H
- **Package F** (Omicron XBB.1)= V83A, G142D, DEL144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, **F486S,** F490S, Q498R, N501Y, Y505H
- **Package G** (Omicron CM*)= G142D, M153T, N164K, V213G, H245N, G257D, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444R, N450D, L452M, N460K, S477N, T478K, E484R, F486S, Q498R, N501Y, Y505H

- The defining mutations of the Omicron variant (Table 1) were successfully detected in all tested Regional pools through NGS. For clarity, the mutations were grouped into seven panels, or "mutation packages." The findings are as follows:

- Package A (sublineage BA.4/5): Key mutations were identified in two regions, Liguria and Lombardia.

- Package B (sublineage BQ.1.1): Key mutations were detected in all tested regions and autonomous provinces.

- Package C (sublineage BN.1*): Amino acid substitutions characteristic of this sublineage were found in Lazio, Liguria, Puglia, and Sicilia.

- Package D (sublineage CH.1.1): Key mutations were observed in two regions (Puglia and Vneto) and the autonomous province of Trento.

- Package E (sublineage XBB.1.5): This sublineage was detected in most regions/autonomous provinces, with the exception of Emilia Romagna and A.P. Trento.

- Package F (sublineage XBB.1): This sublineage was detected in three regions: Friuli Venezia Giulia, Liguria, and Veneto.

Table 1. PCR and sequencing results

	Sample ID	Region/A.P	City	WTP	RT-qPCR (c.g./L)	RT-qPCR Omicron-ID 999 (CT value)	Mutations found by Sanger sequencing (long PCR ID_980)	SARS-CoV-2 variant (Sanger sequencing)	Sequencing results (NGS)	SARS-CoV-2 variant (NGS)
1	18261		Pescara	Villa Carmine	8,26E+02	37.86				
2	18262		Pescara	Via Raiale	3,80E+02	ND				
3	18263	Abruzzo	Chieti	S. Martino	3,77E+02	ND				
4	18264		L'Aquila	Pile	<LOD	ND				
5	18265		Teramo	Villa Pavone	3,22E+02	ND				
6	18177	Basilicata	Potenza	Tiera di Vaglio	2,38E+03	ND				
7	18178		Matera	Pantano	2,64E+03	ND				
8	18306		Cosenza	Cosenza - Sant'Angelo	4,23E+02	37.33				
9	18307		Cosenza	Cosenza - Code di volpe	5,51E+02	39.79				
10	18308		Crotone	Crotone - località Papaniciaro	6,47E+02	ND				
11	18309	Calabria	Catanzaro	Catanzaro Lido - Loc. Verghello	1,88E+03	ND				
12	18332		Reggio Calabria	Ravagnese - località Aeroporto	7,90E+02	35.51				
13	18333		Catanzaro	Catanzaro - Zona industriale	3,79E+02	ND				
24	18179		Ferrara	Ferrara - Linea 1	3,13E+03	ND			• Package B	• Omicron BQ.1.1
25	18180		Ferrara	Ferrara - Linea 2	<LOD	ND				
26	18181		Modena	Carpi	1,97E+03	38.63				
27	18310		Bologna	IDAR	5,85E+04	38.61				
28	18312		Ravenna	Faenza	2,90E+04	37.21				
29	18313	Emilia-Romagna	Bologna	Imola	2,31E+04	ND				
30	18315		Ravenna - Forlì-Cesena	Ravenna	1,20E+04	ND				
31	18316		Modena	Naviglio	2,16E+04	39.26				
32	18319		Forlì-Cesena	Forlì	3,55E+04	38.69				
33	18320		Forlì-Cesena	Cesena	1,07E+05	35.62				

#	ID	Region	Province	Location	Copies	Ct	Package	Omicron	Summary Packages	Summary Omicron
34	18324		Rimini - Forlì-Cesena	S. Giustina	9,35E+03	36.21	Package B+del144	Omicron BQ.1.1+del144		
166	18151		Reggio Emilia	Mancasale	6,20E+03	33.02				
169	18148		Parma	Parma ovest	1,83E+04	35.66				
168	18147		Piacenza	Borgoforte	1,75E+03	34.08				
35	18325	Friuli-Venezia Giulia	Pordenone	Cordenons	9,93E+03	39.21			• Package B • Package B+del144 • Package E • Package F	• Omicron BQ.1.1 • Omicron BQ.1.1+del144 • Omicron XBB.1.5 • Omicron XBB.1
36	18326		Udine	Udine	2,36E+04	ND				
37	18327		Trieste	Servola	1,46E+04	ND	Package E	Omicron XBB.1.5		
38	18127	Lazio	Viterbo	Viterbo - Strada Bagni	8,90E+02	ND			• Package B • Package C • Package E	• Omicron BQ.1.1 • Omicron BN.1* • Omicron XBB.1.5
39	18128		Latina	Latina Loc Latina Est	<LOD	36.47				
40	18129		Latina	Aprilia (Via del Campo)	4,50E+03	37.52				
41	18130		Roma	Anzio - Colle Cocchino	<LOD	37.04				
42	18131		Roma	Velletri (LA CHIUSA-SORBO)	1,83E+04	37.02				
43	18132		Roma	Pomezia - Via Cincinnato	5,30E+03	37.53				
44	18133		Roma	Guidonia - Ponte Lucano	5,08E+03	37.77				
45	18155		Roma	Civitavecchia Fiumaretta	2,50E+01	ND				
46	18402		Roma	Roma Est (linea 1 + linea 2)	7,49E+04	33.62	Package C	Omicron BN.1*		
47	18403		Roma	Roma Nord	1,18E+05	36.16	Package E	Omicron XBB.1.5		
48	18404		Roma	Ostia	8,08E+04	35.29	Package E	Omicron XBB.1.5		
49	18405		Roma	Fregene	8,51E+04	35.68				
170	18406		Roma	Roma Sud	1,07E+05	32.12				
167	18144	Liguria	Genova	Punta Vagno Genova	1,51E+04	32.78			• Package A +Y145H+S255F+V445A • Package B • Package C • Package E • Package F	• Omicron BA.4/5+Y145H+S255F+V445A • Omicron BQ.1.1 • Omicron BN.1* • Omicron XBB.1.5 • Omicron XBB.1
50	18214		Genova	Pegli	2,53E+04	33.62				
51	18215		Genova	Voltri	3,08E+04	34.73				
52	18216		Genova	Quinto	2,15E+04	35.26				
53	18217		Genova	Rapallo	8,41E+04	33.32	Package A +Y145H+S255F+V445A	Omicron BA.4/5+Y145H+S255		

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#	ID	Region	Province	Location	Conc.	Ct	Package	Variant	Package	Omicron
								F+V445A (Omicron BA.5.2.23)		
54	18218		Genova	Sestri P	5,64E+04	35.45	Package E	Omicron XBB.1.5		
55	18219		Genova	Sturla	3,66E+04	35.67				
56	18220		Savona	Savona	4,58E+04	36.82	Package B	Omicron BQ.1.1		
57	18221		Savona	Borghetto Santo Spirito	3,70E+04	34.27	Package F	Omicron XBB.1		
58	18222		La Spezia	Camisano	1,24E+05	37.68	Package E	Omicron XBB.1.5		
59	18223		La Spezia	Silea	2,42E+04	36.64				
60	18224		La Spezia	La Spezia	2,31E+04	34.27				
61	18225		Imperia	Imperia	9,02E+03	34.07				
62	18226		Imperia	Sanremo - località Capo Verde	6,29E+04	35.23	Package E	Omicron XBB.1.5		
63	18227		Genova	Darsena	1,01E+04	33.40	Package E	Omicron XBB.1.5		
64	18228		Genova	Punta Vagno Genova	2,65E+04	34.46				
65	18229		Genova	Valpolcevera	5,95E+04	33.01				
66	18172		Milano	Bresso	4,50E+06	36.01			• Package A+R346T • Package B • Package B+del144 • Package E	• Omicron BA.4/5+R346T • Omicron BQ.1.1 • Omicron BQ.1.1+del144 • Omicron XBB.1.5
67	18173		Milano - Monza e della Brianza	Peschiera Borromeo	4,55E+05	36.02	Package B	Omicron BQ.1.1		
68	18174		Milano - Varese	Canegrate	3,93E+05	37.24				
69	18175		Varese	Varese	4,15E+05	37.15	Package B+del144	Omicron BQ.1.1+del144		
70	18176		Milano - Varese	Lonate Pozzolo	4,40E+05	39.50	Package A+del144+R346T	Omicron BA.4/5+del144+R346T		
71	18230	Lombardia	Bergamo	Bergamo	7,75E+04	ND				
72	18231		Brescia	Verziano	5,19E+04	38.65				
73	18239		Cremona	Citta di Cremona	4,36E+04	38.52				
74	17378		Pavia	Vigevano	1,11E+04	ND				
75	18204		Milano	Milano Nosedo	0,00E+00	38.09				
76	18205		Milano	Milano San Rocco	1,00E+05	34.96	Package A+R346T	Omicron BA.4/5 + R346T		
77	18206		Como	Como	0,00E+00	38.51				

#	ID	Region	Province	Location	Copies	Ct	Package	Variant	Package	Variant
78	18207		Pavia	Pavia	5,42E+04	35.12				
79	18208		Como - Lecco - Milano - Monza e della Brianza	Monza	2,74E+04	35.45				
164	18209		Pavia	Vigevano	2,47E+04	38.74				
165	18348		Sondrio	Sondrio	1,84E+04	ND				
80	18198		Pesaro-Urbino	Borgheria	4,12E+03	35.45				
81	18199		Pesaro-Urbino	Ponte Metauro	1,21E+04	39.38				
82	18200		Pesaro-Urbino	Ponte Sasso	1,41E+03	34.41				
83	18201	Marche	Ancona	Zipa	1,41E+04	35.61				
84	18202		Ancona	Falconara	1,27E+04	33.92				
85	18203		Ancona	Camerano	4,08E+03	34.36				
86	18267		Campobasso	Campobasso - San Pietro	1,60E+02	33.40				
87	18269	Molise	Campobasso	Termoli - località Porto	1,45E+03	32.97				
88	18271		Campobasso	Termoli - località Pantano Basso	6,99E+02	ND				
89	18339	PA Bolzano	Bolzano	IDA Bolzano	4,18E+04	37.45			• Package B • Package E	Omicron BQ.1.1 Omicron XBB.1.5
90	18340		Bolzano	IDA Merano	8,52E+04	36.37	Package E/F (Partial sequence, From V83A to N440K)[a]	Omicron XBB.1.5/ XBB.1		
91	18341		Bolzano	IDA Termeno	4,24E+04	35.47	Package E	Omicron XBB.1.5		
92	18109	P.A. Trento	Trento	Trento nord	2,58E+05	37.41	Package D	Omicron CH.1.1	• Package B • Package D	• Omicron BQ.1.1 • Omicron CH.1.1
93	18110		Trento	Trento sud	2,76E+05	35.89				
94	18111		Trento	Rovereto	3,50E+05	37.45				
95	18076		Torino	Castiglione Torinese	3,15E+03	36.08			• Package B • Package E	• Omicron BQ.1.1 • Omicron XBB.1.5
96	18077		Biella	Biella Nord	2,32E+03	32.84				
97	18078	Piemonte	Biella	Biella Sud	2,42E+03	36.11				
98	18079		Novara	Novara	3,08E+03	35.53				
99	18157		Alessandria	Alessandria	4,05E+03	36.49				
100	18158		Asti	Asti	6,90E+03	35.62	Package E	Omicron XBB.1.5		

101	18159		Cuneo	Cuneo	1,25E+04	34.23					
102	18074		Bari	Bari Ovest	1,76E+03	34.05	Package B	Omicron BQ.1.1	• Package B	• Omicron BQ.1.1
103	18075		Bari	Bari Est	1,22E+04	35.10	Package G	Omicron CM*	• Package C	• Omicron BN.1*
104	18080		Taranto	Taranto Bellavista	3,56E+03	36.12			• Package E	• Omicron XBB.1.5
105	18081		Taranto	Taranto Gennarini	5,66E+03	ND				
106	18082		Brindisi	Brindisi Fiume Grande	1,85E+03	34.63				
107	18083		Lecce	Lecce	1,73E+03	34.58				
108	18085		Bari	Altamura	5,34E+03	33.13	Package B	Omicron BQ.1.1		
109	18116		Barletta-Andria-Trani	Andria	1,64E+03	34.74	Package E	Omicron XBB.1.5		
110	18118		Barletta-Andria-Trani	Barletta	1,21E+03	34.63				
111	18119	Puglia	Foggia	Foggia	4,99E+02	36.60				
112	18120		Foggia	Cerignola	<LOD	ND				
113	18121		Foggia	Manfredonia	9,54E+02	ND	Package D/F (Partial sequence, from G339H to Y505H)[a]	Omicron CH.1.1/XBB.1		
114	18160		Barletta-Andria-Trani	Bisceglie	6,16E+03	37.69				
115	18161		Bari	Bitonto	1,11E+04	ND	Package E	Omicron XBB.1.5		
116	18162		Bari	Molfetta	1,61E+03	37.94	Package B	Omicron BQ.1.1		
117	18163		Barletta-Andria-Trani	Trani	3,56E+03	36.32	Package E	Omicron XBB.1.5		
118	18134		Trapani	Trapani	7,66E+03	35.44			• Package B	• Omicron BQ.1.1
119	18135		Trapani	Mazara del Vallo	4,55E+03	38.50			• Package B+del144	• Omicron BQ.1.1+del144
120	18235		Ragusa	Modica	4,00E+02	37.85			• Package C	• Omicron BN.1*
121	18236	Sicilia	Ragusa	Vittoria	2,85E+03	ND			• Package E	• Omicron XBB.1.5
122	18237		Ragusa	Ragusa	4,18E+03	36.33				
123	18238		Caltanissetta	Gela Macchitella	4,48E+03	38.12	Package B+del144	Omicron BQ.1.1+del144		

124	18241		Agrigento	Agrigento	4,54E+04	ND		
125	18242		Enna	Enna	1,05E+04	38.72	M.E.	
126	18243		Palermo	Bagheria	1,15E+04	33.83		
127	18244		Palermo	Acqua dei Corsari	1,80E+04	ND		
128	18245		Palermo	Fondo Verde	1,82E+04	ND		
129	18246		Caltanissetta	Caltanissetta e San Cataldo	1,52E+04	ND		
130	18328		Catania	Pantano d'Arci	2,13E+04	38.30	Package C	Omicron BN.1*
131	18330		Catania	Giarre	2,14E+04	ND		
132	18331		Siracusa	Siracusa	2,33E+04	39.83		
133	18253		Pisa	Pisa Nord - S. Jacopo	4,57E+05	ND		
134	18254		Firenze	Empoli Pagnana	8,65E+03	38.16		
135	18255		Massa	Lavello 2	3,52E+04	37.51		
136	18256		Lucca	Viareggio	9,75E+04	ND		
137	18257		Massa	Lavello 1	1,26E+04	ND		
138	18258		Lucca	Pontetetto	<LOD	36.81		
139	18259	Toscana	Livorno	Rivellino	2,98E+04	39.69		
140	18260		Livorno	Rivellino	1,90E+04	ND		
141	18275		Firenze	San Colombano	4,50E+03	39.21		
142	18276		Firenze	San Colombano	3,23E+03	34.11		
143	18277		Prato	Baciacavallo	7,50E+02	36.15		
144	18278		Prato	Baciacavallo	3,85E+03	34.08		
145	18280		Arezzo	Casolino - San Leo	1,15E+03	38.40		
146	18281		Grosseto	San Giovanni - Pianetto	3,00E+04	36.85		
147	18282		Pistoia	Centrale Pistoia	1,05E+04	37.16		
148	18283		Siena	Ponte a Tressa	1,21E+04	36.70		
149	18108		Perugia	Perugia - Pian della Genna	6,75E+04	36.93		
150	18196	Umbria	Perugia	Foligno Casone	2,26E+04	38.01		
151	18197		Terni	Terni	6,80E+04	34.91		
152	18233	Valle d'Aosta	Aosta	La Salle	1,72E+02	32.62		

No	Code	Region	Province	Location	Conc	Ct	Package	Variant		
153	18234		Aosta	Brissogne	<LOD	32.49				
154	18104		Padova	Padova Ca' Nordio - centro storico	5,30E+04	34.10			• Package B • Package B+del144	• Omicron BQ.1.1 • Omicron BQ.1.1+del144
155	18105		Padova	Padova Ca' Nordio - zip	3,88E+04	33.32	Package F	Omicron XBB.1	• Package D	• Omicron CH.1.1
156	18106		Padova	Padova Guizza	4,23E+04	33.02			• Package E	• Omicron XBB.1.5
157	18107		Padova	Abano Terme	2,92E+04	37.47			• Package F	• Omicron XBB.1
158	18149	Veneto	Vicenza	Vicenza Casale	2,36E+04	37.50				
159	18153		Treviso	Treviso	9,08E+04	39.53	Package B+del144	Omicron BQ.1.1+del144		
160	18154		Venezia	Venezia Fusina	1,28E+04	37.37	Package E	Omicron XBB.1.5		
161	18211		Verona	Verona_collettore 1M	1,57E+04	ND				
162	18212		Verona	Verona_collettore 3M	8,45E+03	35.57				
163	18213		Verona	Verona_collettore 8M	1,05E+04	34.22				

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

ND not detected

Table 2. Sanger sequencing results

	DEL69/70	V83A	G142D	DEL144/144	H146Q	K147E	W152R	M153T	F157L	N164K	Q183E	I210V	V213G	V213E	H245N	G252V	S255F	G257D	G257S	G339D	G339H	R346T	K356T	L368I	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	K444T	K444R	V445P	G446S	N450D	L452R	L452M	N460K	S477N	T478K	E484A	E484R	F486V	F486S	F486P	F490S	Q498R	N501Y	Y505H	VARIANTS
53, 70, 76	■		■										■							■					■	■	■	■	■	■	■	■						■			■	■	■		■				■	■	■	Package A (Omicron BA.4/5)
34, 56, 67, 69, 102, 108, 116, 123, 159	■		■										■							■		■			■	■	■	■	■	■	■	■	■					■		■	■	■	■		■				■	■	■	Package B (Omicron BQ.1.1)
46, 130			■			■	■		■			■							■		■	■	■		■	■	■	■	■	■	■	■				■				■	■	■	■					■	■	■	■	Package C (Omicron BN.1*)
92, 156			■			■	■		■			■							■		■	■	■		■	■	■	■	■	■	■	■	■			■		■		■	■	■	■			■		■	■	■	■	Package D (Omicron CH.1.1)
37, 47, 48, 54, 58, 62, 63, 91, 100, 109, 115, 117, 160		■	■	■	■						■			■		■					■	■		■	■	■	■	■	■	■	■	■			■	■				■	■	■	■				■	■	■	■	■	Package E (Omicron XBB.1.5)
57, 155		■	■	■	■						■			■		■					■	■		■	■	■	■	■	■	■	■	■			■	■				■	■	■	■			■		■	■	■	■	Package F (Omicron XBB.1)
103			■					■		■		■		■			■		■		■			■	■	■	■	■	■	■	■	■		■				■		■	■	■		■					■	■	■	Package G (Omicron CM*)

Limitations of the study

The geographical and population coverage of this flash survey was incomplete, as it covered 20 out of 21 of the Italian regions/Autonomous Provinces.

The molecular analytical methods used for complex environmental matrices, such as wastewater, can be hindered by low viral concentrations, poor analyte recovery, and/or PCR amplification inhibition. As a result, both detection/quantification and PCR amplification for sequencing may produce false negatives, making it difficult to achieve molecular characterization and variant detection for all samples.

Partial sequencing of the Spike region does not provide conclusive results for sublineage assignment. However, the detection of defined mutation panels that are characteristic of certain lineages/sublineages, through Sanger or NGS sequencing, should be considered as a presumptive detection.

Conclusions and final considerations

This report is part of a monthly series on SARS-CoV-2 and its variants in wastewaters in Italy, as established by the EU Commission Recommendation 2021/472. The aim is to provide additional information on SARS-CoV-2 variants in the population, supplementing information gathered through clinical surveillance. The results indicate that the Omicron variant is the sole presence of SARS-CoV-2 in Italy, with the sublineages XBB.1.5 and BQ.1.1 being the most prevalent. However, mutations characteristic of sublineages Omicron BA.4/5, BN.1*, CH.1.1, CM* and XBB.1 were also detected.

The sequencing of SARS-CoV-2 in wastewater provides additional information to the sequencing of clinical cases, allowing for a more accurate description of the circulating variants in the country.