



**Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
17th Report
(Study period: January 9th to January 13th, 2023)**

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Main findings:

- During the week of January 9th to January 13th, 2023, 168 wastewater samples were collected from 18 Regions and 2 Autonomous Provinces (A.P.).
- Mutations characteristic of the Omicron variant were detected in 13 Regions/Autonomous Provinces, while no sequences were obtained from the remaining regions.
- Of the obtained Sanger sequencing, 42.4% showed amino acid substitutions of sublineage BQ.1.1, 15.1% of both BA.4/5 + R346T and BQ.1, 9.1% showed substitutions of BA.4/5, and 6.1% showed substitutions of both sublineages BA.2.75 and BN.1*.
- Next-generation sequencing (NGS) results confirmed the widespread presence of sublineages BQ.1.1 across the majority of the Regions/A.P. and also confirmed the circulation of sublineages BA.4/5, BA.4/5 + R346T, BQ.1, BN.1* and BA.2.75.

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 17th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from January 9th to January 13th, 2023.

Methodology

During the period from January 9th to January 13th, 2023, the 17th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy was conducted. The survey included the collection of 168 sewage samples from 165 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 website¹. 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 variant³. 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 (Vols) in the spike protein⁴. 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 \(iss.it\)](https://www.iss.it/sites/default/files/8e5e2edb-bae0-f1b0-ee6e-08255c76484f_iss.it)

² DOI [10.5281/zenodo.5758724](https://doi.org/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. Brusaferrero, 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 described⁵.

Results

A total of 153 out of 168 samples (91.1%) 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 2,50E+02 to 4,48E+06 genome copies (g.c.) per liter of sewage. Additionally, 145 out of the 168 (86.3%) samples tested positive for the Omicron variant through the RT-qPCR assay, with cycle threshold (CT) values ranging from 28.2 to 39.9.

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 33 samples from 12 Regions/Autonomous Provinces were amplified by the long nested PCR assay. Sanger sequencing confirmed that all of them were characterized as the Omicron variant.

The survey of wastewater samples identified several SARS-CoV-2 variants. The most common variant detected was BQ.1.1, which was found in 42.4% (14 samples) of the total samples. The next most common variant was Omicron BA.4/5 + R346T mutation, which was identified in 15.1% (5 samples) of the samples. Another 15.1% (5 samples) were identified as BQ.1. The Omicron BA.4/5 sublineage was detected in 9.1% (3 samples). The mutations characteristic of Omicron BN.1 and Omicron BA.2.75 were each detected in two samples. Additionally, in two samples (6.1%), the presence of double peaks at the spike mutation site 1038 (corresponding to the amino acids R346) suggested the simultaneous presence of more than one sublineage in different combinations (BQ.1 or BQ.1.1).

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 BA.4/5 + R346T) = DEL69/70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, 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 BQ.1)** = DEL69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H
- **Package D (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
- **Package E (Omicron BA.2.75)** = G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H
- **Package F (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

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 six panels, or "mutation packages." The key mutations of sublineage BA.4/5 (Package A) were found in five regions: Calabria, Lazio, Liguria, Puglia, and Umbria. The key mutations of sublineage BA.4/5 + R346T (Package B) were detected in six regions/AP: Emilia Romagna, Puglia, Sicilia, Umbria, Veneto, and A.P. of Trento. The amino acid substitutions of sublineage BQ.1 (Package C) were found in Emilia Romagna, Lombardia, Puglia, Sicilia, Umbria, Valle d'Aosta, and Veneto. The sublineage BQ.1.1 (Package D) was detected in most regions/AP, except for Lazio, Liguria, and P.A. Trento. Finally, sublineages BA.2.75 (Package E) and BN.1* (Package F) were detected in two Regions (Puglia and Sicilia) and four Regions (Calabria, Lombardia, Sicilia, and Veneto), respectively.

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	17491	Pescara	Via Raiale	7,73E+02	35.44				
2	17492	Pescara	Villa Carmine	1,22E+03	36.80				
3	17493	Chieti	S. Martino	4,32E+02	38.48				
4	17494	L'Aquila	Pile	4,51E+02	36.66				
5	17495	Teramo	Villa Pavone	4,29E+02	36.30				
6	17509	Potenza	Tiera di Vaglio	3,13E+03	38.90				
7	17510	Matera	Pantano	9,78E+03	37.09				
8	17476	Cosenza	Cosenza - Sant'Angelo	9,24E+03	35.23			• Package A	• Omicron BA.4/5 +K182E+R346I
9	17477	Cosenza	Cosenza - Code di volpe	1,62E+04	33.25	Package D	Omicron BQ.1.1	• Package D	• Omicron BQ.1.1
10	17478	Catanzaro	Catanzaro Lido - Loc. Verghello	6,14E+03	34.81	Package A	Omicron BA.4/5 +K182E+R346I	• Package D +K147E	• Omicron BQ.1.1 +K147E
11	17479	Crotone	Crotone - località Papaniciaro	1,25E+04	35.27	Package F	Omicron BN.1*	• Package D + Y144del	• Omicron BQ.1.1 + Y144del
12	17480	Reggio Calabria	Ravagnese - località Aeroporto	7,71E+03	35.17	Package D	Omicron BQ.1.1	• Package F	• Omicron BN.1*
13	17481	Catanzaro	Catanzaro - Zona industriale	4,38E+03	36.31				
14	17775	Salerno	Salerno	<LOD	ND				
15	17776	Salerno	Nocera Sup	<LOD	39.99				
16	17777	Avellino	Manocalzati	<LOD	36.36				
17	17778	Napoli	Napoli OVEST (ingresso principale)	<LOD	37.04				
18	17779	Napoli	Napoli OVEST (ingresso camaldoli)	2,25E+03	38.31				
19	17780	Napoli	Area Nolana	<LOD	37.60				

20	17781		Napoli	Napoli EST	<LOD	ND				
21	17782		Caserta	Area Casertana	<LOD	39.90				
166	17774		Salerno	Eboli	<LOD	36.18				
167	17783		Caserta	Villa Literno	<LOD	37.29				
22	17359	Emilia-Romagna	Ferrara	Ferrara - Linea 1	1,60E+03	ND		• Package B	• Omicron BA.4/5	
23	17360		Ferrara	Ferrara - Linea 2	<LOD	ND		• Package C	• +R346T	
24	17361		Modena	Carpi	7,50E+03	38.62		• Package D	• Omicron BQ.1	
25	17422		Bologna	IDAR	4,95E+04	33.07	Package D	Omicron BQ.1.1	• Omicron BQ.1.1	
26	17423		Ravenna - Forlì-Cesena	Ravenna	1,93E+04	ND		+ Y144del	• Omicron BQ.1.1	
27	17424		Bologna	Imola	2,70E+04	35.25	Package D	Omicron BQ.1.1	• + Y144del	
28	17425		Ravenna	Faenza	1,15E+04	36.41				
29	17426		Modena	Naviglio	1,13E+04	37.09	Package D	Omicron BQ.1.1		
30	17427		Forlì-Cesena	Cesena	4,20E+04	34.78	Package C	Omicron BQ.1		
31	17428		Forlì-Cesena	Forlì	3,65E+04	36.15	Package D	Omicron BQ.1.1		
32	17429		Rimini - Forlì-Cesena	S. Giustina	5,90E+04	34.54	Package D	Omicron BQ.1.1		
33	17438		Reggio Emilia	Mancasale	4,85E+04	36.76				
168	17435		Piacenza	Borgoforte	2,60E+04	36.87				
169	17437		Parma	Parma ovest	5,68E+04	34.36				
34	17466		Friuli-Venezia Giulia	Udine	Udine	3,93E+03	37.60			
35	17507			Trieste	Servola	6,43E+04	37.26			
36	17508			Pordenone	Cordenons	1,58E+05	38.40			
37	17296		Lazio	Roma	Civitavecchia Fiumaretta	2,50E+02	37.76		• Package A	• Omicron BA.4/5
38	17330			Viterbo	Viterbo - Strada Bagni	2,88E+03	37.61		• Package A	• Omicron BA.4/5
39	17331	Roma		Anzio - Colle Cocchino	5,15E+03	36.01		+ Y144del	+ Y144del	
40	17332	Latina		Aprilia (Via del Campo)	2,40E+03	38.80				
41	17333	Latina		Latina Loc Latina Est	1,32E+04	34.05				
42	17334	Roma		Pomezia - Via Cincinnato	3,65E+03	36.01				

43	17335	Roma	Velletri (LA CHIUSA-SORBO)	2,85E+03	37.15		
44	17336	Roma	Guidonia - Ponte Lucano	7,28E+03	34.77		
45	17911	Roma	Roma Est (linea 1 + linea 2)	3,68E+03	35.49	Package A +Y144del	Omicron BA.4/5 +Y144del
46	17912	Roma	Roma Nord	1,34E+05	35.15		
47	17913	Roma	Roma Sud	4,22E+03	34.14		
48	17914	Roma	Ostia	4,66E+03	37.46		
170	17915	Roma	Fregene	5,58E+04	33.44		
49	17385	Savona	Savona	7,78E+04	34.82		• Package A • Omicron BA.4/5
50	17386	Savona	Borghetto Santo Spirito	6,37E+04	34.56		
51	17387	Genova	Pegli	3,13E+04	38.18	Package A (Partial, from G339D to Y505H) ^a	Omicron BA.4/5
52	17388	Genova	Voltri	8,38E+04	34.40		
53	17389	Genova	Quinto	1,15E+05	35.09		
54	17390	Genova	Rapallo	1,20E+04	36.58		
55	17391	Genova	Sestri P	2,33E+04	37.64		
56	17392	Genova	Sturla	4,61E+04	34.89		
57	17393	La Spezia	Camisano	1,06E+04	ND		
58	17394	La Spezia	Silea	3,64E+03	37.74		
59	17395	La Spezia	La Spezia	2,45E+04	36.18		
60	17396	Imperia	Imperia	1,26E+04	37.13		
61	17397	Imperia	Sanremo - località Capo Verde	4,84E+04	35.22		
62	17398	Genova	Darsena	6,45E+03	38.57		
63	17399	Genova	Punta Vagno Genova	6,97E+04	34.58		
64	17400	Genova	Valpolcevera	3,26E+04	37.19		
65	17432	Genova	Punta Vagno Genova	5,45E+04	36.76		

66	17345	Lombardia	Milano	Bresso	5,60E+05	36.28	Double peaks in specific positions ^b	Omicron BQ.1/BQ.1.1 ^b	<ul style="list-style-type: none"> • Package C • Package D • Package F 	<ul style="list-style-type: none"> • Omicron BQ.1 • Omicron BQ.1.1 • Omicron BN.1
67	17346		Milano - Monza e della Brianza	Peschiera Borromeo	3,03E+05	ND				
68	17347		Milano - Varese	Canegrate	2,45E+05	36.35	Double peaks in specific positions ^b	Omicron BQ.1/BQ.1.1 ^b		
69	17348		Varese	Varese	1,37E+05	36.67				
70	17349		Milano - Varese	Lonate Pozzolo	1,04E+06	35.64				
71	17405		Bergamo	Bergamo	8,89E+03	37.52				
72	17406		Cremona	Citta di Cremona	2,09E+04	36.14				
73	17408		Brescia	Verziano	2,75E+04	36.78				
74	n.a		Sondrio	Sondrio	n.a	38.62				
75	17373		Milano	Milano Nosedo	1,68E+06	39.26				
76	17374		Milano	Milano San Rocco	3,75E+05	38.77				
77	17375		Como	Como	4,20E+05	37.49				
78	17376		Como - Lecco - Milano - Monza e della Brianza	Monza	<LOD	37.81				
79	17377		Pavia	Pavia	<LOD	39.66				
81	17235		Marche	Pesaro-Urbino	Borgheria	3,14E+04	35.04			
82	17236			Pesaro-Urbino	Ponte Metauro	2,42E+04	35.39			
83	17237			Pesaro-Urbino	Ponte Sasso	1,20E+04	36.55			
84	17238			Ancona	Zipa	4,94E+04	35.29			
85	17239			Ancona	Falconara	3,38E+04	35.29			
86	17240	Ancona		Camerano	1,94E+04	35.55				
87	17497	Molise	Campobasso	Termoli - località Pantano Basso	1,91E+03	35.29	Package C+Y144del	Omicron BQ.1+Y144del	<ul style="list-style-type: none"> • Package D • Package D + Y144del 	<ul style="list-style-type: none"> • Omicron BQ.1.1 • Omicron BQ.1.1 + Y144del
88	17498		Campobasso	Termoli - località Porto	2,35E+03	34.06				
89	17496		Campobasso	Campobasso - San Pietro	3,21E+03	34.39				

90	17516	P.A. Bolzano	Bolzano	IDA Bolzano	3,96E+04	35.62	Package D +K147I+W152L	Omicron BQ.1.1 + K147I+W152L	• Package D +K147I+W152L	• Omicron BQ.1.1 + K147I+W152L
91	17517		Bolzano	IDA Merano	4,48E+04	37.31				
92	17518		Bolzano	IDA Termeno	6,93E+04	33.50				
93	17302	P.A. Trento	Trento	Trento nord	2,30E+05	37.57			• Package B	• Omicron BA.4/5 + R346T
94	17303		Trento	Trento sud	1,89E+05	36.05				
95	17304		Trento	Rovereto	2,67E+05	32.71	Package B	Omicron BA.4/5 + R346T		
96	17256	Piemonte	Torino	Castiglione Torinese	1,72E+03	35.78				
97	17257		Biella	Biella Nord	2,71E+03	35.41				
98	17258		Biella	Biella Sud	8,18E+03	35.87				
99	17259		Novara	Novara	4,00E+03	37.25				
100	17353		Alessandria	Alessandria	3,30E+03	ND				
101	17354		Asti	Asti	2,50E+03	ND				
102	17355	Cuneo	Cuneo	1,75E+03	36.06					
103	17249	Puglia	Bari	Bari Est	1,72E+04	32.30	Package B	Omicron BA.4/5 + R346T	• Package A • Package B	• Omicron BA.4/5 • Omicron BA.4/5 + R346T
104	17250		Bari	Bari Ovest	1,16E+05	34.49			• Package C	
105	17254		Bari	Bitonto	4,95E+03	37.38			• Package D	• Omicron BQ.1
106	17255		Bari	Molfetta	3,41E+04	35.56	Package C	Omicron BQ.1	• Package D + Y144del	• Omicron BQ.1.1
107	17272		Brindisi	Brindisi Fiume Grande	6,04E+03	39.42			• Package E	• Omicron BQ.1.1 + Y144del
108	17277		Lecce	Lecce	1,74E+05	36.21				• Omicron BA.2.75
109	17278		Taranto	Taranto Bellavista	6,71E+04	38.15				
110	17279		Taranto	Taranto Gennarini	7,11E+03	35.85				
111	17301		Bari	Altamura	3,45E+04	29.55				
112	17317		Barletta-Andria- Trani	Barletta	1,85E+04	30.15	Package B	Omicron BA.4/5 + R346T		
113	17318		Barletta-Andria- Trani	Andria	2,91E+04	28.20	Package D	Omicron BQ.1.1		
114	17319		Foggia	Cerignola	2,62E+03	ND				
115	17320		Foggia	Foggia	1,31E+04	35.16	Package E	Omicron BA.2.75		

116	17321		Foggia	Manfredonia	6,33E+03	ND		
117	17350		Barletta-Andria-Trani	Bisceglie	9,41E+03	39.05	Package E	Omicron BA.2.75
118	17351		Barletta-Andria-Trani	Trani	5,63E+03	ND		
119	17328		Trapani	Trapani	5,67E+03	35.32		• Package B
120	17329		Trapani	Mazara del Vallo	5,73E+03	32.22		• Package C
121	17410		Agrigento	Agrigento	1,80E+04	32.45		• Package C
122	17411		Enna	Enna	1,66E+04	34.33		• + Y144del
123	17412		Palermo	Bagheria	2,80E+04	36.61		• Package D
124	17413		Palermo	Acqua dei Corsari	2,34E+04	35.78		• Package E
125	17414		Palermo	Fondo Verde	4,99E+04	33.97		• Package F
126	17415		Caltanissetta	Caltanissetta e San Cataldo	5,05E+04	33.27		• Omicron BA.4/5 + R346T
127	17440	Sicilia	Catania	Pantano d'Archi	6,54E+02	32.84	Package B	Omicron BA.4/5 + R346T
128	17442		Catania	Giarre	5,86E+02	32.59		
129	17443		Siracusa	Siracusa	9,06E+02	32.16	Package D	Omicron BQ.1.1
130	17401		Ragusa	Modica	5,20E+03	37.46	Package F	Omicron BN.1*
131	17402		Ragusa	Vittoria	7,08E+03	37.82	Package C	Omicron BQ.1
132	17403		Ragusa	Ragusa	1,24E+04	ND		
133	17404		Caltanissetta	Gela Macchitella	6,33E+03	ND		
135	17356		Palermo	Bagheria	6,43E+04	36.16		
136	17357		Palermo	Acqua dei Corsari	2,04E+05	36.23		
137	17358		Palermo	Fondo Verde	1,25E+05	34.58		
138	17459		Lucca	Viareggio	1,59E+05	34.95		
139	17460		Massa	Lavello 1	1,01E+04	ND		
140	17461	Toscana	Lucca	Pontetetto	8,36E+04	38.50		
141	17462		Livorno	Rivellino	1,70E+05	37.23		
142	17463		Livorno	Rivellino	4,43E+04	39.90		
143	17467		Firenze	San Colombano	2,58E+04	ND		

144	17468		Firenze	San Colombano	4,28E+04	37.74					
145	17469		Prato	Baciacavallo	3,48E+04	36.01					
146	17470		Prato	Baciacavallo	<LOD	36.35					
147	17471		Arezzo	Casolino - San Leo	6,35E+04	ND					
148	17472		Grosseto	San Giovanni - Pianetto	1,36E+06	ND					
149	17473		Pistoia	Centrale Pistoia	<LOD	ND					
150	17474		Siena	Ponte a Tressa	4,48E+06	37.88					
151	17253		Perugia	Perugia - Pian della Genna	8,89E+04	33.92	Package B	Omicron BA.4/5 + R346T	• Package A	• Omicron BA.4/5	
152	17371	Umbria	Perugia	Foligno Casone	4,89E+04	34.71			• Package B	• Omicron BA.4/5 + R346T	
153	17372		Terni	Terni	7,21E+04	33.28			• Package C	• Omicron BQ.1	
154	17420		Aosta	La Salle	7,87E+02	35.35	Package C	Omicron BQ.1	• Package D	• Omicron BQ.1.1	
155	17421	Valle d'Aosta	Aosta	Brissogne	2,14E+03	35.66	Package D	Omicron BQ.1.1	• Package C	• Omicron BQ.1	
156	17291		Padova	Padova Ca' Nordio - centro storico	3,49E+04	ND	Package D	Omicron BQ.1.1	• Package D	• Omicron BQ.1.1	
157	17292		Padova	Padova Ca' Nordio - zip	7,62E+04	ND	Package D	Omicron BQ.1.1	• Package B	• Omicron BA.4/5 + R346T	
158	17293		Padova	Padova Guizza	3,87E+04	ND	Package D	Omicron BQ.1.1	• Package B	• Omicron BA.4/5 + R346T	
159	17294		Padova	Abano Terme	2,69E+04	ND			• Package C	• Omicron BQ.1	
160	17325	Veneto	Treviso	Treviso	1,71E+04	31.98			• Package C	• Omicron BQ.1	
161	17326		Vicenza	Vicenza Casale	1,71E+04	31.67			+ Y144del	+ Y144del	
162	17327		Venezia	Venezia Fusina	1,28E+04	32.18			• Package D	• Omicron BQ.1.1	
163	17363		Verona	Verona_collettore 1M	2,43E+04	35.05			• Package D	• Omicron BQ.1.1	
164	17364		Verona	Verona_collettore 3M	9,18E+03	38.00			+ Y144del	+ Y144del	
165	17365		Verona	Verona_collettore 8M	8,95E+03	36.03			• Package F	• Omicron BN.1	

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

^b Double peaks in one positions: R346

ND not detected

Table 2. Sanger sequencing results

ID SAMPLES	DEL69/70	G142D	K147E	W152R	F157L	I210V	V213G	G257S	G339D	G339H	R346T	K356T	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	K444T	G446S	L452R	N460K	S477N	T478K	E484A	F486V	F490S	Q498R	N501Y	Y505H	VARIANTS	
10, 45, 51																																	Package A (Omicron BA.4/5)	
95, 103, 112, 127, 151																																		Package B (Omicron BA.4/5+R346T)
30, 87, 106, 131, 154																																		Package C (Omicron BQ.1)
9, 12, 25, 27, 29, 31, 32, 90, 113, 129, 155, 156, 157, 158																																		Package D (Omicron BQ.1.1)
115, 117																																		Package E (Omicron BA.2.75)
11, 130																																		Package F (Omicron BN.1*)

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 sublineage BQ.1.1 being the most prevalent. However, mutations characteristic of sublineages Omicron BA.4/5, BA.4/5 + R346T, BQ.1, BN.1* and BA.2.75 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.

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