Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
15th Report
(Study period: November 7th to 11th, 2022)

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> **Main findings:**
> - Overall, 168 wastewater samples were collected between November 7th and 11th from 18 Regions and 2 Autonomous Provinces.
> - Mutations associated with the Omicron variant were identified in 14 Regions and Autonomous provinces, while no sequences were obtained from the remaining regions.
> - Using Sanger sequencing, 33.3% of the sequences showed amino acid substitutions of sublineages BA.5, 18% of BA.4/5 + R346T, 25.6% of BQ.1, and 18% of BQ.1.1. Mutations of Omicron XBB.1 and BA.2.75 were detected in two samples.
> - NGS results further confirmed the heterogeneous presence of sublineages, including BA.4/BA.5, BA.4/5 + R346T, BQ.1, BQ.1.1, XBB.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 to this recommendation, the Istituto Superiore di Sanità (ISS) initiated "flash surveys", which are periodic, monthly sampling campaigns conducted at various locations throughout Italy over a short period of time. This report summarizes the results of the fifteenth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy during the week of November 7th to 11th, 2022.

Methodology

The survey included 168 wastewater samples collected from 161 wastewater treatment plants (WTPs) located in 18 regions and 2 autonomous provinces (AP) between November 7th and 11th, 2022. 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 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

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]. To differentiate between the Omicron sublineages BA.4 and BA.5, which have identical spike proteins in the sequenced region, a nested PCR was carried out to detect a mutation in the M gene at position D3 (BA.5 has the D3N mutation).

Results

Out of the 168 samples tested,91.6% (154 samples) tested positive for SARS-CoV-2 using the real-time RT-qPCR method adopted by the SARI network laboratories (Table 1). The viral concentration ranged from 4,39E+02 to 5,83E+05 genome copies per liter of sewage. Furthermore, 143 of the 168 samples were found to be positive for the Omicron variant using the RT-qPCR assay, with CT values ranging from 30.25 to 39.55.

Sanger Sequencing
The results of the real-time PCR assays, long nested PCR, and sequencing are summarized in **Table 1**. A total of 40 samples from 14 Regions/AP were amplified using the long PCR assay, but long amplicons could not be obtained from samples collected in Abruzzo, Basilicata, Campania, Molise, Piemonte, and Valle d'Aosta. High-quality Sanger sequences were successfully obtained from 39 samples. However, one sample showed a mixed electropherogram due to the presence of multiple strains. All amplicons were determined to belong to the Omicron variant through Sanger sequencing. Specifically, 33.3% of the samples (13 samples from 6 regions: Calabria, Friuli-Venezia Giulia, Lazio, Liguria, Puglia, Veneto) were found to have the amino-acid substitutions characteristic of the Omicron BA.4/5 sublineage (L452R and F486V in addition to those typical of BA.2). An additional 18% of the samples (7 samples) were identified as Omicron BA.4/5 + R346T mutation. Moreover, 25.6% of the samples (10 samples) were identified as BQ.1 (with K444T and N460K substitutions in addition to those typical of BA.4/5), and 18% (7 samples) were identified as BQ.1.1 (with R346T in addition to those typical of BQ.1). The mutations characteristic of Omicron XBB.1 and Omicron BA.2.75 were detected in two samples.
The assay targeting the M gene assigned all the samples to Omicron BA.5 (due to the presence of the D3N mutation).

Next Generation Sequencing

NGS results were successfully obtained for all tested Regional pools, which showed the defining mutations of the Omicron variant (Table 1).
For ease of reading, the mutations were grouped into panels ('mutation packages') as follow:

- **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

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[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 B** (<u>Omicron BA.4/5 + R346T</u>), long fragment from PCR ID_980) = DEL69/70, G142D, V213G, G339D, **R346T**, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, **F486V**, Q498R, N501Y, Y505H
- **Package C** (<u>Omicron BQ.1</u>, long fragment from PCR ID_980) = 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** (<u>Omicron BQ.1.1</u>, long fragment from PCR ID_980) = 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** (<u>Omicron XBB.1,</u> long fragment from PCR ID_980) = **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 F** (<u>Omicron BA.2.75</u>, long fragment from PCR ID_980) = G142D, **K147E**, **W152R**, **F157L**, **I210V**, V213G, **G257S**, **G339H**, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **G446S**, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H

The mutations of the Omicron sublineages BA.4/5 (Package A) were found in 11 Regions/AP (Calabria, Emilia Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Marche, Puglia, Toscana, Veneto, A.P. of Bolzano and Trento). The key mutations of sublineage Omicron BA.4/5 + R346T (Package B) were found in 11 Regions/AP: Calabria, Emilia Romagna, Friuli-Venezia Giulia, Liguria, Lombardia, Marche, Puglia, Toscana, Umbria, Veneto, and A.P. of Bolzano. The amino-acid substitutions of sublineage BQ.1 (Package C) were found in 10 Regions/AP: Calabria, Emilia Romagna, Friuli-Venezia Giulia, Liguria, Lombardia, Marche, Puglia, Toscana, and Veneto, and A.P. of Bolzano. The amino-acid substitutions of sublineage BQ.1.1 (Package D) were found in 8 Regions/AP: Friuli-Venezia Giulia, Liguria, Puglia, Sicilia, Toscana, Umbria, Veneto, and A.P. of Trento. Finally, Omicron XBB.1 (Package E) and BA.2.75 (Package F) were detected in Toscana and Veneto respectively, by both Sanger and Next Generation Sequencing. The sequencing results are summarized in Table 1.

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 package)	SARS-CoV-2 variant (NGS)
1	15643		Chieti	S. Martino	4,39E+02	39.20				
2	15644		Pescara	Villa Carmine	1,21E+04	35.21				
3	15645	Abruzzo	Pescara	Via Raiale	7,05E+03	38.02				
4	15647		L'Aquila	Pile	9,61E+03	35.68				
5	15648		Teramo	Villa Pavone	4,42E+03	38.39	-			
6	15699	Basilicata	Potenza	Tiera di Vaglio	< LOD	ND				
7	15700		Matera	Pantano	2,53E+03	39.55				
8	15757		Cosenza	Cosenza - Sant'Angelo	1,77E+05	34.61			• PACKAGE A • PACKAGE B • PACKAGE C	• BA.4/5 • BA.4/5 + R346T • BQ.1
9	15758		Cosenza	Cosenza - Code di volpe	1,63E+05	ND				
10	15759		Crotone	Crotone - località Papaniciaro	1,84E+05	39.35				
11	15760	Calabria	Reggio Calabria	Ravagnese - località Aeroporto	2,07E+05	ND	PACKAGE B	Omicron BA.4/5 + R346T		
12	15761		Catanzaro	Catanzaro Lido - Loc. Verghello	2,32E+05	ND	PACKAGE A	Omicron BA.4/5[a]		
13	15762		Catanzaro	Catanzaro - Zona industriale	1,64E+05	38.59				
14	16221		Salerno	Salerno	< LOD	37.66				
15	16222		Salerno	Nocera Sup	< LOD	38.08				
16	16223	Campania	Napoli	Napoli OVEST - ex ingresso Camaldoli	< LOD	ND				
17	16224		Napoli	Napoli OVEST - Ingresso Principale	< LOD	37.42				

#	ID	Region	Province	Location	Conc	Ct	Package	Variant		Region Packages		Region Variants
18	16225		Napoli	Area Nolana	< LOD	ND						
19	16226		Napoli	Napoli EST	< LOD	36.37						
20	16227		Caserta	Area Casertana	< LOD	ND						
21	16228		Caserta	Villa Literno	< LOD	37.26						
22	15616		Ferrara	Ferrara - Linea 1	2,27E+03	ND				• PACKAGE A		• BA.4/5
23	15617		Ferrara	Ferrara - Linea 2	< LOD	ND				• PACKAGE B		• BA.4/5 + R346T
24	15618		Modena	Carpi	5,75E+03	36.88				• PACKAGE C		• BQ.1
25	15667		Parma	Parma ovest	5,05E+04	38.76						
26	15670		Piacenza	Borgoforte	1,02E+04	39.11						
27	15671		Reggio Emilia	Mancasale	2,30E+04	34.36						
28	15713	Emilia Romagna	Bologna	IDAR	1,35E+04	37.03	PACKAGE C	Omicron BQ.1				
29	15714		Bologna	Imola	6,50E+03	36.74						
30	15715		Ravenna - Forlì-Cesena	Ravenna	7,10E+03	ND						
31	15716		Modena	Naviglio	3,75E+03	ND						
32	15717		Forlì-Cesena	Forlì	8,58E+03	34.50						
33	15718		Forlì-Cesena	Cesena	6,70E+03	ND						
34	15719		Ravenna	Faenza	1,00E+03	ND						
35	15720		Rimini - Forlì-Cesena	S. Giustina	5,83E+03	ND						
36	15735	Friuli-Venezia Giulia	Pordenone	Cordenons	3,53E+03	ND	PACKAGE C	Omicron BQ.1		• PACKAGE A		• BA.4/5
37	15736		Udine	Udine	2,15E+04	39.21				• PACKAGE B		• BA.4/5 + R346T
38	15737		Trieste	Servola	3,10E+04	36.01	PACKAGE A	Omicron BA.4/5[a]		• PACKAGE C		• BQ.1
									• PACKAGE D		• BQ.1.1
39	15451		Roma	Roma Est (linea 1 + linea 2)	1,79E+05	38.47				• PACKAGE A		• BA.4/5
40	15452		Roma	Roma Nord	1,34E+05	ND						
41	15453	Lazio	Roma	Roma Sud	3,67E+04	33.52						
42	15454		Roma	Ostia	1,19E+05	34.23						
43	15455		Roma	Fregene	2,85E+04	36.61						

No.	Code	Region	Province	Location	Conc.	Ct	Package	Variant
44	15558		Viterbo	Viterbo - Strada Bagni	1,75E+04	33.12	PACKAGE A	Omicron BA.4/5[a]
45	15559		Latina	Latina Loc Latina Est	7,55E+03	34.12		
46	15560		Latina	Aprilia (Via del Campo)	1,45E+04	33.27		
47	15561		Roma	Anzio - Colle Cocchino	4,38E+03	33.66		
48	15562		Roma	Velletri (LA CHIUSA-SORBO)	1,02E+03	33.38		
49	15563		Roma	Pomezia - Via Cincinnato	4,30E+03	34.65		
50	15564		Roma	Guidonia - Ponte Lucano	9,93E+03	38.42		
168	15546		Roma	Civitavecchia	<LOD	34.70		
51	15833		La Spezia	Camisano	1,09E+05	34.97		
52	15834		La Spezia	Silea	1,80E+05	33.27	PACKAGE C	Omicron BQ.1
53	15835		La Spezia	La Spezia	8,83E+04	30.25		
54	15836		Genova	Pegli	2,83E+05	32.71	PACKAGE D	Omicron BQ.1.1
55	15837		Genova	Voltri	3,68E+05	34.18	PACKAGE A	Omicron BA.4/5[a]
56	15838		Genova	Quinto	3,70E+05	32.74		
57	15839		Genova	Rapallo	1,86E+05	33.13	PACKAGE A	Omicron BA.4/5[a]
58	15846		Genova	Sestri P	1,86E+05	32.42	PACKAGE A	Omicron BA.4/5[a]
59	15847	Liguria	Genova	Sturla	4,27E+04	32.92		
60	15848		Savona	Savona	2,23E+05	33.86		
61	15849		Savona	Borghetto Santo Spirito	4,06E+05	33.93	Mixed electopherograms[b]	
62	15850		Imperia	Imperia	5,17E+04	32.32		
63	15851		Imperia	Sanremo - località Capo Verde	2,00E+05	34.20	PACKAGE C	Omicron BQ.1
64	15852		Genova	Darsena	1,09E+05	32.87		
65	15853		Genova	Punta Vagno Genova	5,83E+05	ND		

- PACKAGE A
- PACKAGE B
- PACKAGE C
- PACKAGE D

- BA.4/5
- BA.4/5 + R346T
- BQ.1
- BQ.1.1

66	15854		Genova	Valpolcevera	1,71E+05	34.56	PACKAGE C	Omicron BQ.1		
67	15663		Genova	Punta Vagno Genova	6,05E+04	31.71	PACKAGE A	Omicron BA.4/5[a]		
68	15565		Milano	Bresso	8,10E+04	32.64			• PACKAGE B • PACKAGE C	• BA.4/5 + R346T • BQ.1
69	15566		Milano - Monza e della Brianza	Peschiera Borromeo	3,65E+04	32.69				
70	15567		Milano - Varese	Canegrate	1,07E+05	33.80				
71	15568		Varese	Varese	4,48E+04	32.82				
72	15569		Milano - Varese	Lonate Pozzolo	9,58E+04	34.04				
73	15637		Milano	Milano Nosedo	4,57E+04	33.08				
74	15638		Milano	Milano San Rocco	3,40E+04	36.72	PACKAGE C	Omicron BQ.1		
75	15639	Lombardia	Como	Como	3,82E+04	36.52				
76	15640		Pavia	Pavia	< LOD	36.52				
77	15641		Como - Lecco - Milano - Monza e della Brianza	Monza	6,00E+03	37.38				
78	15642		Pavia	Vigevano	< LOD	37.40				
79	15656		Bergamo	Bergamo	2,89E+04	37.71				
80	15657		Brescia	Verziano	1,76E+04	33.37				
81	15659		Cremona	Citta di Cremona	1,21E+04	34.67				
82	15781		Sondrio	Sondrio	1,51E+05	34.34				
83	15705		Pesaro-Urbino	Borgheria	2,34E+04	37.13	PACKAGE C	Omicron BQ.1	• PACKAGE A • PACKAGE B • PACKAGE C	• BA.4/5 • BA.4/5 + R346T • BQ.1
84	15706		Pesaro-Urbino	Ponte Metauro	2,57E+03	37.02				
85	15708	Marche	Ancona	Zipa	7,01E+04	38.11				
86	15709		Ancona	Falconara	2,11E+04	33.43				
87	15710		Ancona	Camerano	1,64E+04	34.99	PACKAGE B	Omicron.BA.4/5 + R346T		

#	ID	Region	Province	Location	Copies	Ct	Package	Variant	Packages	Variants
167	15707		Pesaro-Urbino	Ponte Sasso	1,11E+04	35.13				
88	15650	Molise	Campobasso	Campobasso - San Pietro	1,04E+03	39.50				
89	15651		Campobasso	Termoli - località Porto	6,11E+03	36.17				
90	15652		Campobasso	Termoli - località Pantano Basso	9,37E+03	35.21				
91	15638	P.A. Bolzano	Bolzano	IDA Bolzano	1,00E+05	35.02	PACKAGE C	Omicron BQ.1	• PACKAGE A • PACKAGE B • PACKAGE C	• BA.4/5 • BA.4/5 + R346T • BQ.1
92	15663		Bolzano	IDA Merano	8,11E+04	33.15	PACKAGE B	Omicron BA.4/5 + R346T		
93	15692		Bolzano	IDA Termeno	4,02E+04	33.27	PACKAGE B	Omicron BA.4/5 + R346T		
94	15552	P.A. Trento	Trento	Trento nord	2,17E+05	33.81	PACKAGE D	Omicron BQ.1.1	• PACKAGEA • PACKAGE D	• BA.4/5 • BQ.1.1
95	15553		Trento	Trento sud	1,28E+05	37.19				
96	15554		Trento	Rovereto	3,65E+05	34.71				
97	15511	Piemonte	Torino	Castiglione Torinese	1,72E+04	36.87				
98	15512		Biella	Biella Nord	2,78E+03	ND				
99	15513		Biella	Biella Sud	5,60E+03	38.24				
100	15514		Novara	Novara	4,58E+03	ND				
101	15587		Alessandria	Alessandria	8,45E+03	35.95				
102	15588		Asti	Asti	1,37E+03	38.03				
103	15589		Cuneo	Cuneo	7,08E+03	36.45				
104	15506	Puglia	Brindisi	Brindisi Fiume Grande	1,98E+03	36.06			• PACKAGE A • PACKAGE B • PACKAGE C • PACKAGE D	• BA.4/5 • BA.4/5 + R346T • BQ.1 • BQ.1.1
105	15507		Bari	Bari Est	6,18E+03	33.79				
106	15508		Lecce	Lecce	4,96E+03	35.04				
107	15509		Taranto	Taranto Bellavista	7,18E+03	36.16				
108	15510		Taranto	Taranto Gennarini	3,20E+03	34.33	PACKAGE A	Omicron BA.4/5[a]		
109	15547		Foggia	Cerignola	2,49E+03	37.46	PACKAGE C	Omicron BQ.1		
110	15548		Foggia	Foggia	2,69E+03	35.50				

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#	Code	Region	Province	Location	Value	Ct	Package	Variant		
111	15549		Foggia	Manfredonia	1,60E+03	35.88				
112	15590		Bari	Altamura	1,42E+04	31.19	PACKAGE A	Omicron BA.4/5ᵃ		
113	15591		Barletta-Andria-Trani	Andria	7,79E+03	32.01	PACKAGE A	Omicron BA.4/5ᵃ		
114	15592		Barletta-Andria-Trani	Barletta	1,03E+04	33.82	PACKAGE C	Omicron BQ.1		
115	15593		Barletta-Andria-Trani	Bisceglie	3,41E+03	34.66	PACKAGE D	Omicron BQ.1.1		
116	15594		Bari	Molfetta	1,84E+04	35.21				
117	15595		Barletta-Andria-Trani	Trani	1,16E+04	32.80	PACKAGE D	Omicron BQ.1.1		
118	15596		Bari	Bitonto	7,06E+03	34.40				
119	15598		Bari	Bari Ovest	2,32E+03	33.41				
120	15550		Trapani	Mazara del Vallo	4,08E+03	39.41			• PACKAGE D	• BQ.1.1
121	15551		Trapani	Trapani	1,14E+03	ND				
122	15608		Agrigento	Agrigento	3,83E+04	35.22				
123	15609		Enna	Enna	1,66E+05	32.87				
124	15610		Palermo	Acqua dei Corsari	5,58E+04	34.68				
125	15611		Palermo	Fondo Verde	8,92E+04	34.73				
126	15612		Palermo	Bagheria	6,39E+04	35.62				
127	15613		Caltanissetta	Caltanissetta e San Cataldo	8,48E+04	34.36				
128	15665	Sicilia	Catania	Pantano d'Arci	1,03E+04	36.29				
129	15672		Catania	Giarre	1,24E+04	35.03				
130	15674		Siracusa	Siracusa	4,48E+03	35.27				
131	15675		Ragusa	Vittoria	3,43E+03	37.72				
132	15676		Ragusa	Modica	9,20E+03	36.67				
133	15677		Ragusa	Ragusa	3,48E+03	38.29				
134	15678		Caltanissetta	Gela Macchitella	1,93E+03	ND				
135	15680		Messina	Mili Marina	1,16E+04	36.98	PACKAGE D	Omicron BQ.1.1		

#	ID	Region	City	Site	Conc.	Ct	Package	Variant	Packages	Variants
136	15691		Firenze	San Colombano	5,05E+03	38.73			• PACKAGE A • PACKAGE B • PACKAGE C • PACKAGE D • PACKAGE E	• BA.4/5 • BA.4/5 + R346T • BQ.1 • BQ.1.1 • XBB.1
137	15692		Firenze	San Colombano	1,85E+03	37.59				
138	15693		Prato	Baciacavallo	2,13E+03	37.98				
139	15694		Prato	Baciacavallo	4,48E+03	37.89				
140	15695		Arezzo	Casolino - San Leo	6,95E+03	36.07				
141	15696		Grosseto	San Giovanni - Pianetto	1,08E+04	37.44	PACKAGE B	Omicron BA.4/5 +R346T		
142	15697		Pistoia	Centrale Pistoia	9,63E+03	35.37				
143	15698	Toscana	Siena	Ponte a Tressa	1,37E+04	35.05				
144	15724		Pisa	Pisa Nord - S. Jacopo	1,09E+05	35.66	PACKAGE E	XBB.1		
145	15725		Firenze	Empoli Pagnana	1,34E+04	ND				
146	15726		Massa	Lavello 2	2,65E+05	33.72				
147	15727		Lucca	Viareggio	2,64E+05	34.04				
148	15728		Massa	Lavello 1	6,31E+04	35.33				
149	15729		Lucca	Pontetetto	< LOD	ND				
150	15730		Livorno	Rivellino	1,10E+05	34.04				
151	15731		Livorno	Rivellino	1,14E+05	34.58				
152	15487		Perugia	Perugia - Pian della Genna	9,34E+04	35.37	PACKAGE B	Omicron BA.4/5 + R346T	• PACKAGE B • PACKAGE D	• BA.4/5 + R346T • BQ.1.1
153	15603	Umbria	Perugia	Foligno Casone	4,61E+04	36.05				
154	15607		Terni	Terni	1,83E+05	33.31	PACKAGE D	Omicron BQ.1.1		
155	15661	Valle d'Aosta	Aosta	La Salle	5,33E+02	ND				
156	15662		Aosta	Brissogne	7,29E+04	ND				
157	15534		Padova	Padova Ca' Nordio - centro storico	2,25E+05	35.61	PACKAGE A	Omicron BA.4/5[a]	• PACKAGE A • PACKAGE B • PACKAGE C • PACKAGE D • PACKAGE F	• BA.4/5 • BA.4/5 + R346T • BQ.1 • BQ.1.1 • BA.2.75
158	15535	Veneto	Padova	Padova Ca' Nordio - zip	3,02E+05	35.13	PACKAGE B	Omicron BA.4/5 + R346T		
159	15536		Padova	Padova Guizza	2,36E+05	35.59	PACKAGE A	Omicron BA.4/5[a]		
160	15537		Padova	Abano Terme	2,77E+05	33.90	PACKAGE A	Omicron BA.4/5[a]		

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161	15604	Verona	Verona_collettore 1M	4,14E+03	32.73		
162	15605	Verona	Verona_collettore 3M	3,49E+03	32.71	PACKAGE D	Omicron BQ.1.1
163	15606	Verona	Verona_collettore 8M	2,24E+03	33.20	PACKAGE F	Omicron BA.2.75
164	15557	Venezia	Vicenza Casale	7,40E+03	33.09		
165	15555	Treviso	Treviso	9,20E+03	33.15		
166	15556	Vicenza	Vicenza Casale	1,55E+04	32.55		

[a] the presence of mutation D3N in the M gene is suggestive of the presence of BA.5
[b] M.E. mixed electropherograms
ND: not detected

- **Package A** (<u>Omicron BA.4/5</u>, 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** (<u>Omicron BA.4/5 + R346T)</u>, long fragment from PCR ID_980) = DEL69/70, G142D, V213G, G339D, **R346T**, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, **F486V**, Q498R, N501Y, Y505H
- **Package C** (<u>Omicron BQ.1</u>, long fragment from PCR ID_980) = 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** (<u>Omicron BQ.1.1</u>, long fragment from PCR ID_980) = 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 XBB.1, long fragment from PCR ID_980) = **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 F** (Omicron BA.2.75, long fragment from PCR ID_980) = G142D, **K147E**, **W152R**, **F157L**, **I210V**, V213G, **G257S**, **G339H**, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **G446S**, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H

Table 2. Sanger sequencing results

CHARACTERISTIC MUTATIONS

ID SAMPLES	DEL69/70	V83A	G142D	DEL144	H146Q	K147E	W152R	F157L	Q183E	I210V	V213G	V213E	G252V	G257S	G339D	G339H	R346T	L368I	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	K444T	V445P	G446S	L452R	N460K	S477N	T478K	E484A	F486V	F486S	F490S	Q498R	N501Y	Y505H	VARIANTS
12-38-44-55-57-58-67-108-112-113-157-159-160	X	X	X								X				X				X	X	X	X	X	X	X	X				X		X	X	X	X			X	X	X	**Package A*** (Omicron BA.4/5)
11-87-92-93-141-152-158	X	X	X								X				X		X		X	X	X	X	X	X	X	X				X		X	X	X	X			X	X	X	**Package B** (Omicron BA.4/5+R346T)
28-36-52-63-66-74-83-91-109-114	X	X	X								X				X		X		X	X	X	X	X	X	X	X	X			X	X	X	X	X	X			X	X	X	**Package C** (Omicron BQ.1)
54-94-115-117-135-154-162	X	X	X								X				X		X		X	X	X	X	X	X	X	X				X	X	X	X	X	X			X	X	X	**Package D** (Omicron BQ.1.1)
144		X	X	X	X				X			X	X			X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X		X	X	X	X	X	**Package E** (Omicron XBB.1)
163			X			X	X	X		X				X		X			X	X	X	X	X	X	X	X			X		X	X	X	X				X	X	X	**Package F** (Omicron BA.2.75)

* Upon testing for the assay targeting the M gene, all the BA.4/BA.5 sequences, were assigned to Omicron BA.5.

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 is the fifteenth in a series of monthly reports on SARS-CoV-2 and its variants in wastewater, which will continue to be produced as part of the surveillance program established in Italy in accordance with the EU Commission Recommendation 2021/472.

The results of SARS-CoV-2 surveillance in wastewater indicate that the Omicron variant is the only one present in Italy in November 2022, with a prevalence of the sublineage BA.5. Mutations characteristic of the Omicron sublineages BA.4/5 + R346T, BQ.1, and BQ.1.1 are becoming more prevalent. For the first time, Omicron XBB.1 and Omicron BA.2.75 were also identified in two samples.

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.