Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
19th Report
(Study period: March 6th to March 10th, 2023)

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Main findings:
- During the week of March 6th to March 10th, 2023, 150 wastewater samples were collected from 17 Regions and 2 Autonomous Provinces.
- Mutations characteristic of the Omicron variant were detected in 12 Regions/Autonomous Provinces, while no sequences were obtained from the remaining regions.
- Sanger sequencing revealed that 55.5% of the obtained sequences showed amino acid substitutions of sublineage XBB.1.5, 27.8% of BQ.1.1, 11.1% of CH.1.1 and 5.6% of XBB.1.17.1.
- Next-generation sequencing (NGS) results confirmed the widespread presence of key mutation of sublineages XBB.1.5 and BQ.1.1 across the majority of the Regions/Autonomous provinces and the circulation of other less prevalent sublineages

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. Responding to this recommendation, the Istituto Superiore di Sanità (ISS) initiated a series of "flash surveys". These surveys involve monthly sampling campaigns conducted at various locations across Italy over a short period of time. The primary objective of these flash surveys is to gather supplementary information on SARS-CoV-2 variants in the population, complementing data obtained through clinical surveillance. The aim of this report is to summarize the findings from the 19th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from March 6th to March 10th, 2023.

Methodology

During the period from March 6th to March 10th, 2023, the 19th national Flash Survey on SARS-CoV-2 variants in wastewater samples was conducted in Italy. The survey involved the collection of 150 sewage samples from 148 wastewater treatment plants (WTPs) located across 17 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 collected during the survey were processed and the viral concentration was determined by laboratories within the SARI network using the protocol "Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3"[2]. The purified RNAs extracted from the samples were then sent to ISS for variant detection. Both Sanger sequencing and NGS sequencing were employed for this purpose.

A real-time RT-PCR assay was used to screen for the presence of the Omicron variant[3]. A long nested RT-PCR assay covering approximately 1600 base pairs was employed, spanning amino acid residues 58 to 573 of the spike protein. The assay was used to detect multiple nucleotide changes

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[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.

distinctive of Variants of Concern (VoCs) and Variants of Interest (VoIs) in the spike protein[4]. Following the amplification of target sequences Sanger sequencing was employed for individual samples, while Next Generation Sequencing (NGS) was used for pooled samples based on Regions/Autonomous Provinces. NGS was conducted using the Oxford Nanopore platform. Bioinformatics analysis was carried out on the obtained sequencing data, and variant calling was performed for recognized VoCs following established protocol as previously described[5].

Results

Out of the 150 samples collected, a total of 140 (93.3%) tested positive for SARS-CoV-2 using the real-time RT-qPCR method employed for environmental surveillance (Table 1). The viral concentrations detected in these samples varied, ranging from 1,5E+02 to 3,1E+05 genome copies (g.c.) per liter of sewage. Additionally, 124 out of the 150 samples (82.7%) were found to be positive for the Omicron variant using the RT-qPCR assay, with cycle threshold (Ct) values ranging from 32.3 to 39.7.

Sanger Sequencing

Table 1 provides a summary of the results obtained from the real-time PCR assays, long nested PCR, and sequencing methods. A total of 19 samples from 12 Regions/Autonomous Provinces were successfully amplified using the long nested PCR assay. Among these samples, high-quality sequences were obtained from 18 of them through Sanger sequencing. However, one sequence was unsuccessful due to high background noise or a noisy sequencing signal. Sanger sequencing confirmed that all the obtained sequences 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 the XBB.1.5 sublineage, which was detected in 55.5% of the total samples (10 samples) The Omicron BQ.1.1 variant was the next most common variant, identified in 27.8% (5 samples) of the samples. Two other sublineages were found, albeit in smaller proportions: Omicron CH.1.1 in two samples (11.1%) and XBB.1.17.1 in one sample (5.6%).

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 facilitate comprehension, the mutations were grouped into panels or "mutation packages" as follows:

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[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 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 A** (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 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
- **Package C** (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 D** (Omicron XBB.1.17.1) = V83A, G142D, DEL144, H146Q, Q183E, **D215H**, 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 E** (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

The defining mutations of the Omicron variant (Table 1) were successfully detected in 11 tested Regional pools through NGS. For clarity, the mutations were grouped into five panels, or "mutation packages." The findings are as follows:

- Package A (sublineage XBB.1.5): This sublineage was detected in five Regions (Friuli Venezia Giulia, Liguria, Piemonte, Puglia, Veneto) and two autonomous provinces (Bolzano and Trento),

- Package B (sublineage BQ.1.1): Amino acid substitutions characteristic of this sublineage were found in six Regions (Calabria, Liguria, Puglia, Sicilia, Umbria, Valle d'Aosta) and the A.P. of Bolzano.

- Package C (sublineage CH.1.1): Key mutations were observed in three regions (Liguria, Lombardia and Puglia).

- Package D (sublineage XBB.1.17.1): Amino acid substitutions characteristic of this sublineage were found Lombardia

- Package E (sublineage BA.4/5): Key mutations were observed in Valle d'Aosta

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	19004	Abruzzo	Pescara	Via Raiale	3,53E+02	38.47				
2	19005	Abruzzo	Pescara	Villa Carmine	1,55E+02	37.99				
3	19006	Abruzzo	Chieti	S. Martino	<LOD	N/D				
4	19007	Abruzzo	L'Aquila	Pile	<LOD	36.66				
5	19008	Abruzzo	Teramo	Villa Pavone	3,41E+02	36.55				
6	19077	Basilicata	Potenza	Tiera di Vaglio	6,58E+03	N/D				
7	19080	Basilicata	Matera	Pantano	8,51E+03	N/D				
8	19019	Calabria	Cosenza	Cosenza - Code di volpe	9,59E+02	36.48			• Package B	• Omicron BQ.1.1
9	19020	Calabria	Cosenza	Cosenza - Sant'Angelo	1,87E+02	39.27				
10	19021	Calabria	Crotone	Crotone - località Papaniciaro	5,02E+02	36.72				
11	19023	Calabria	Catanzaro	Catanzaro Lido - Loc. Verghello	9,15E+02	35.71	Package B+K147I	Omicron BQ.1.1+K147I		
12	19029	Calabria	Reggio Calabria	Ravagnese - località Aeroporto	6,02E+02	37.45				
13	19032	Calabria	Catanzaro	Catanzaro - Zona industriale	1,00E+03	39.71				
16	18979	Emilia-Romagna	Ferrara	Ferrara - Linea 1	<LOD	39.28				
17	18980	Emilia-Romagna	Ferrara	Ferrara - Linea 2	6,30E+03	N/D				
18	18981	Emilia-Romagna	Modena	Carpi	1,51E+04	N/D				
19	19054	Emilia-Romagna	Piacenza	Borgoforte	1,43E+03	39.46				
20	19055	Emilia-Romagna	Parma	Parma ovest	5,43E+03	39.61				
21	19057	Emilia-Romagna	Reggio Emilia	Mancasale	2,32E+04	N/D				
22	19098	Emilia-Romagna	Bologna	IDAR	2,88E+04	34.78				
23	19099	Emilia-Romagna	Modena	Naviglio	9,65E+03	36.64				
24	19100	Emilia-Romagna	Ravenna - Forlì-Cesena	Ravenna	7,35E+03	N/D				
25	19101	Emilia-Romagna	Ravenna	Faenza	4,50E+03	38.28				

26	19102	Emilia-Romagna	Forlì-Cesena	Forlì	3,40E+04	34.92			
27	19103	Emilia-Romagna	Forlì-Cesena	Cesena	3,43E+04	35.03			
28	19104	Emilia-Romagna	Bologna	Imola	2,45E+04	34.89			
171	19108	Emilia-Romagna	Rimini - Forlì-Cesena	S. Giustina	8,80E+03	N/D			
29	19200	Friuli-Venezia Giulia	Pordenone	Cordenons	5,70E+03	38.74	•	Package A	• Omicron XBB.1.5
30	19201	Friuli-Venezia Giulia	Udine	Udine	2,73E+04	35.14			
31	19202	Friuli-Venezia Giulia	Trieste	Servola	1,23E+04	36.08		Package A	Omicron XBB.1.5
32	18966	Lazio	Viterbo	Viterbo - Strada Bagni	1,17E+04	36.23			
33	18967	Lazio	Roma	Anzio - Colle Cocchino	4,15E+03	35.36			
34	18968	Lazio	Latina	Aprilia (Via del Campo)	5,30E+03	35.93			
35	18969	Lazio	Latina	Latina Loc Latina Est	2,90E+03	36.27			
36	18970	Lazio	Roma	Velletri (LA CHIUSA-SORBO)	2,22E+03	39.11			
37	18971	Lazio	Roma	Pomezia - Via Cincinnato	3,50E+03	35.28			
38	18972	Lazio	Roma	Guidonia - Ponte Lucano	7,83E+03	N/D			
39	19088	Lazio	Roma	Civitavecchia Fiumaretta	<LOD	38.35			
40	19204	Lazio	Roma	Roma Est (linea 1 + linea 2)	3,11E+05	33.70			
41	19205	Lazio	Roma	Roma Nord	1,44E+05	34.59			
42	19206	Lazio	Roma	Roma Sud	9,09E+04	35.26			
43	19207	Lazio	Roma	Ostia	1,96E+05	34.81			
44	19208	Lazio	Roma	Fregene	2,11E+05	32.61			
45	19022	Liguria	Genova	Pegli	5,63E+04	35.20	• Package A • Package B • Package C		• Omicron XBB.1.5 • Omicron BQ.1.1 • Omicron CH.1.1
46	19024	Liguria	Genova	Voltri	4,68E+04	35.30			
47	19025	Liguria	Genova	Quinto	7,76E+04	34.16			
48	19026	Liguria	Genova	Rapallo	7,43E+04	34.50			
49	19027	Liguria	Genova	Sestri P	6,63E+04	34.22			
50	19028	Liguria	Genova	Sturla	1,14E+05	33.37			
51	19030	Liguria	La Spezia	Camisano	3,24E+04	35.55			

52	19031	Liguria	La Spezia	Silea	3,48E+04	35.36				
53	19033	Liguria	La Spezia	La Spezia	1,18E+04	38.43				
54	19034	Liguria	Savona	Savona	<LOD	39.54				
55	19035	Liguria	Imperia	Imperia	3,30E+04	36.62				
56	19036	Liguria	Genova	Darsena	3,32E+04	35.80				
57	19037	Liguria	Genova	Punta Vagno Genova	2,84E+04	36.78				
58	19038	Liguria	Genova	Valpolcevera	7,93E+04	34.38	Package C	Omicron CH.1.1		
59	19039	Liguria	Savona	Borghetto Santo Spirito	1,54E+04	38.03				
60	19051	Liguria	Genova	Punta Vagno Genova	1,68E+04	N/D				
62	18922	Lombardia	Milano	Milano Nosedo	1,63E+05	37.03			• Package C • Package D	• Omicron CH.1.1 • Omicron XBB.1.17.1
63	18923	Lombardia	Milano	Milano San Rocco	1,17E+05	36.37				
64	18924	Lombardia	Como	Como	7,96E+04	36.61				
65	18925	Lombardia	Como - Lecco - Milano - Monza e della Brianza	Monza	1,76E+04	39.63				
66	18926	Lombardia	Pavia	Pavia	1,37E+05	39.15				
67	18927	Lombardia	Pavia	Vigevano	9,03E+04	38.34	Package D	Omicron XBB.1.17.1		
68	19042	Lombardia	Bergamo	Bergamo	5,54E+04	35.00				
69	19043	Lombardia	Brescia	Verziano	3,67E+04	36.32				
70	19045	Lombardia	Cremona	Citta di Cremona	2,34E+04	37.39				
71	19113	Lombardia	Sondrio	Sondrio	<LOD	N/D				
72	19255	Lombardia	Milano - Varese	Lonate Pozzolo	7,75E+04	34.09				
73	18998	Marche	Pesaro-Urbino	Borgheria	8,20E+03	39.27				
74	18999	Marche	Pesaro-Urbino	Ponte Metauro	8,52E+03	N/D				
75	19000	Marche	Pesaro-Urbino	Ponte Sasso	1,82E+03	N/D				
76	19001	Marche	Ancona	Zipa	2,17E+04	35.52				
77	19002	Marche	Ancona	Falconara	9,45E+03	35.68				
78	19003	Marche	Ancona	Camerano	3,04E+03	36.61				
79	19009	Molise	Campobasso	Campobasso - San Pietro	<LOD	N/D				
80	19010	Molise	Campobasso	Termoli - località Porto	5,10E+02	33.64				

#	ID	Region	Province	Location	Value	Ct	Package	Variant	Package	Variant
81	19011	Molise	Campobasso	Termoli - località Pantano Basso	7,34E+02	33.12				
82	19089	P.A. Bolzano	Bolzano	IDA Bolzano	3,76E+04	32.84			• Package A	• Omicron XBB.1.5
83	19090	P.A. Bolzano	Bolzano	IDA Merano	2,46E+04	33.57	Package A	Omicron XBB.1.5	• Package B	• Omicron BQ.1.1
84	19091	P.A. Bolzano	Bolzano	IDA Termeno	1,03E+05	32.96	Package A	Omicron XBB.1.5		
85	18928	P.A. Trento	Trento	Trento nord	2,10E+05	33.10	Package A	Omicron XBB.1.5	• Package A	• Omicron XBB.1.5
86	18929	P.A. Trento	Trento	Trento sud	1,54E+05	34.15	Package A	Omicron XBB.1.5		
87	18930	P.A. Trento	Trento	Rovereto	2,05E+05	33.01				
88	18839	Piemonte	Torino	Castiglione Torinese	5,73E+03	36.13			• Package A	• Omicron XBB.1.5
89	18840	Piemonte	Biella	Biella Nord	7,68E+02	N/D				
90	18841	Piemonte	Biella	Biella Sud	1,35E+03	39.59				
91	18842	Piemonte	Novara	Novara	2,09E+03	38.13				
92	18976	Piemonte	Alessandria	Alessandria	6,53E+03	34.85	Package A	Omicron XBB.1.5		
93	18977	Piemonte	Asti	Asti	4,80E+03	33.79				
94	18978	Piemonte	Cuneo	Cuneo	3,80E+03	34.14				
95	18843	Puglia	Bari	Bari Est	2,69E+03	37.82	M.E.[a]		• Package A	• Omicron XBB.1.5
96	18844	Puglia	Bari	Bari Ovest	3,92E+02	37.14	Package C	Omicron CH.1.1	• Package B	• Omicron BQ.1.1
97	18845	Puglia	Bari	Altamura	1,65E+03	34.15			• Package C	• Omicron CH.1.1
98	18861	Puglia	Taranto	Taranto Gennarini	5,73E+02	37.62				
99	18864	Puglia	Lecce	Lecce	8,76E+02	37.71				
100	18865	Puglia	Brindisi	Brindisi Fiume Grande	2,97E+03	38.70				
101	18973	Puglia	Barletta-Andria-Trani	Bisceglie	8,35E+02	N/D				
102	18974	Puglia	Barletta-Andria-Trani	Trani	<LOD	38.82				
103	18982	Puglia	Bari	Bitonto	8,63E+02	36.59				
104	18983	Puglia	Bari	Molfetta	1,67E+02	35.34				
105	18947	Puglia	Barletta-Andria-Trani	Andria	2,92E+03	38.76	Package A	Omicron XBB.1.5		
106	18946	Puglia	Barletta-Andria-Trani	Barletta	4,41E+02	36.22				
107	18945	Puglia	Foggia	Foggia	9,15E+02	36.23				

108	18944	Puglia	Foggia	Manfredonia	<LOD	38.17				
109	18943	Puglia	Foggia	Cerignola	6,11E+02	N/D				
110	18858	Puglia	Taranto	Taranto Bellavista	3,16E+03	35.48	Package B	Omicron BQ.1.1		
127	19047	Sicilia	Catania	Pantano d'Arci	1,55E+04	N/D			• Package B	• Omicron BQ.1.1
128	19049	Sicilia	Catania	Giarre	1,62E+04	39.53				
129	19050	Sicilia	Siracusa	Siracusa	3,60E+04	36.72				
130	19013	Sicilia	Ragusa	Modica	5,43E+02	N/D				
131	18932	Sicilia	Trapani	Mazara del Vallo	3,03E+03	37.11				
132	18931	Sicilia	Trapani	Trapani	9,90E+03	33.90	Package B	Omicron BQ.1.1		
133	19122	Sicilia	Enna	Enna	1,27E+04	35.09				
134	19123	Sicilia	Palermo	Bagheria	1,22E+04	38.71				
135	19124	Sicilia	Palermo	Acqua dei Corsari	2,02E+04	37.96				
136	19125	Sicilia	Palermo	Fondo Verde	1,53E+04	38.46				
137	19126	Sicilia	Caltanissetta	Caltanissetta e San Cataldo	2,77E+04	39.38				
111	19059	Toscana	Pisa	Pisa Nord - S. Jacopo	1,56E+05	37.31				
112	19060	Toscana	Firenze	Empoli Pagnana	1,95E+05	37.67				
113	19061	Toscana	Massa	Lavello 2	2,76E+05	37.91				
114	19062	Toscana	Lucca	Viareggio	2,93E+05	37.18				
115	19063	Toscana	Massa	Lavello 1	8,36E+04	36.28				
116	19064	Toscana	Lucca	Pontetetto	6,77E+04	38.68				
117	19065	Toscana	Firenze	San Colombano	7,50E+03	38.61				
118	19066	Toscana	Livorno	Rivellino	1,75E+05	N/D				
119	19067	Toscana	Firenze	San Colombano	2,60E+04	37.35				
120	19068	Toscana	Livorno	Rivellino	2,46E+05	34.41				
121	19069	Toscana	Prato	Baciacavallo	8,25E+03	37.53				
122	19070	Toscana	Prato	Baciacavallo	3,48E+04	N/D				
123	19071	Toscana	Arezzo	Casolino - San Leo	1,68E+04	N/D				
124	19072	Toscana	Grosseto	San Giovanni - Pianetto	1,26E+05	38.05				
125	19073	Toscana	Pistoia	Centrale Pistoia	4,08E+04	N/D				

#	ID	Region	Province	Location	Conc.	Ct	Package	Variant		
126	19074	Toscana	Siena	Ponte a Tressa	<LOD	N/D				
156	18911	Umbria	Perugia	Perugia - Pian della Genna	4,28E+04	34.82			• Package B	• Omicron BQ.1.1
157	18760	Umbria	Perugia	Foligno Casone	1,09E+04	35.00				
158	18761	Umbria	Terni	Terni	5,89E+04	32.49	Package B	Omicron BQ.1.1		
159	19040	Valle d'Aosta	Aosta	La Salle	3,05E+02	38.03			• Package B	• Omicron BQ.1.1
160	19041	Valle d'Aosta	Aosta	Brissogne	3,40E+02	35.54	Package B	Omicron BQ.1.1	• Package E	• Omicron BA.4/5
161	18907	Veneto	Padova	Padova Ca' Nordio - centro storico	1,82E+05	N/D			• Package A	• Omicron XBB.1.5
162	18908	Veneto	Padova	Padova Ca' Nordio - zip	1,91E+05	38.58				
163	18909	Veneto	Padova	Padova Guizza	8,30E+04	N/D				
164	18910	Veneto	Padova	Abano Terme	4,78E+04	37.41				
165	18954	Veneto	Treviso	Treviso	8,19E+03	39.34				
166	18955	Veneto	Vicenza	Vicenza Casale	7,79E+03	32.30	Package A	Omicron XBB.1.5		
167	18956	Veneto	Venezia	Venezia Fusina	1,18E+04	32.42				
168	18992	Veneto	Verona	Verona_collettore 1M	2,28E+04	33.98	Package A	Omicron XBB.1.5		
169	18993	Veneto	Verona	Verona_collettore 3M	1,23E+04	34.01				
170	18994	Veneto	Verona	Verona_collettore 8M	6,91E+03	32.29	Package A	Omicron XBB.1.5		

[a] M.E. mixed electropherograms (noisy sequencing signal)
N/D: not detected

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Table 2. Sanger sequencing results

	DEL 69/70	V83A	G142D	DEL 144/144	H146Q	K147E	W152R	F157L	Q183E	I210V	V213G	D215H	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	F486P	F490S	Q498R	N501Y	Y505H	VARIANTS
31, 83, 84, 85, 86, 92, 105, 166, 168, 170		■	■	■				■				■	■			■	■	■	■	■	■	■	■	■	■	■			■	■		■	■	■	■		■		■	■	■	■	Package A (Omicron XBB.1.5)
11, 110, 132, 160, 158	■									■				■				■	■	■	■	■	■	■	■	■				■	■	■	■	■	■	■			■	■	■	■	Package B (Omicron BQ.1.1)
58, 96		■			■	■	■		■	■					■	■		■	■	■	■	■	■	■	■	■			■		■	■	■				■		■	■	■	■	Package C (Omicron CH.1.1)
67		■	■	■	■			■			■	■	■			■	■	■	■	■	■	■	■	■	■	■			■	■		■	■	■	■				■	■	■	■	PacKage D (Omicron XBB.1.17.1)

Limitations of the study

The geographical and population coverage of this flash survey was not comprehensive, as it encompassed 19 out of 21 of the Italian regions/Autonomous Provinces.

It is important to note that the molecular analytical methods used for complex environmental matrices, such as wastewater, can face challenges due to factors such as low viral concentrations, inadequate analyte recovery, and/or PCR amplification inhibition. Consequently, both the detection/quantification and PCR amplification for sequencing may yield false negatives, making it challenging 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 specific 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 focusing on SARS-CoV-2 and its variants in wastewater samples in Italy, in accordance with the EU Commission Recommendation 2021/472. The primary objective is to gather supplementary information on SARS-CoV-2 variants in the population, complementing data obtained through clinical surveillance. The findings from this survey indicate that the Omicron variant is the sole of SARS-CoV-2 variant in Italy, with the sublineages XBB.1.5 and BQ.1.1 being the most prevalent. However, mutations characteristic of other sublineages, namely Omicron CH.1.1, XBB.1.17.1 and BA.4/5 were also detected.

Sequencing SARS-CoV-2 in wastewater samples provides valuable additional insights alongside the sequencing of clinical cases. This approach enables a more comprehensive and accurate understanding of the circulating variants in the country, contributing to a better characterization of the virus's spread and evolution.