Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
22nd Report
(Study period: June 5th to June 9th, 2023)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Claudia Del Giudice, Marcello Iaconelli, Luca Lucentini (Department of Enviroment and Health, Istituto Superiore di Sanità)
- David Brandtner (independent researcher)
- Mauro Grigioni, Mirko Rossi (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità

Main findings:

- During the week of June 5th to June 9th, 2023, a total of 129 wastewater samples were collected from 16 Regions and 2 Autonomous Provinces (AP).
- Mutations characteristic of the Omicron variant were identified in 14 of these Regions/AP, while no sequencing data was obtained from the remaining areas.
- Analysis of the sequences obtained by Sanger sequencing showed that 39.5% of the positive samples had amino acid substitutions belonging to the Omicron CM.7* lineage, 34.2% to the Omicron XBB.1.5*/1.9* lineage, 13.2% to the Omicron XBB.1.16* lineage, 7.9% to the Omicron XBB.2.3* lineage, and 2.6% to the Omicron GL.1 lineage.

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 22nd national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from June 5th to June 9th, 2023.

Methodology

During the period from June 5th to June 9th, 2023, the 22nd national Flash Survey on SARS-CoV-2 variants in wastewater samples was conducted in Italy. The survey involved the collection of 129 sewage samples from 125 wastewater treatment plants (WTPs) located across 16 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.

A real-time RT-PCR assay was used to screen for the presence of the Omicron variant[3]. For sequencing purposes, in addition to the long-nested PCR assay in use since October 2021, which covers approximately 1600 base pairs and spans amino acid residues 58 to 573 of the spike protein (PCR ID 979/980), we have designed a novel long-nested PCR assay covering approximately 1330 base pairs and spanning from amino acid residues 34 to 475 of the spike

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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.

protein (PCR ID 1033/1034). The assay was designed to better identify the latest Variants of Interest (VOI) and Variants under monitoring (VUM), which have characteristic mutations outside the region covered by PCR 980 (i.e. EG.5.1, which carries the additional spike mutation known as Q52H in addition to F456L). Both long nested PCRs were used for this flash survey.

Following the amplification of target sequences Sanger sequencing was employed for individual samples.

For variant classification we adopted a lineage classification based on 'outbreak.info'[4] rather than specifying sublineages. This choice was made because there are many sublineages that evolve rapidly, often converging on specific amino acid substitutions. In some cases, the differences between sublineages can be as small as a single nucleotide mutation in our target region, making a reliable assignation to sublineages, based solely on the mutations observed in the spike region, not feasible.

Results

Real Time qPCR

Out of the 125 samples analysed, a total of 107 (85.6%) 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 3.14E+02 to 7.04E+05 genome copies (g.c.) per liter of sewage. Additionally, 92 out of the 129 samples (71.3%) were found to be positive for the Omicron variant using the RT-qPCR assay, with cycle threshold (CT) values ranging from 34.65 to 39.95.

Sanger Sequencing

Table 1 provides a summary of the results obtained from the real-time PCR assays, long-nested PCR assays and sequencing methods. A total of 38 samples (29.5%) from 14 Regions/AP were successfully amplified using the long-nested PCR assays described above. Sanger sequencing confirmed that all the obtained sequences corresponded to the Omicron variant.

Analysis of wastewater samples revealed the presence of five SARS-CoV-2 lineages, as shown in Tables 1 and 2. Among these, the Omicron CM.7* lineage was the most common, detected in 39.5% (15 samples) of the positive samples. In addition, the Omicron XBB.1.5*/XBB 1.9* lineages (which were not distinguishable in the sequenced region) were detected in the 34.2% of the positive samples (13 samples), Omicron XBB.1.16* in 13.2% (5 samples) and Omicron XBB.2.3* in 7.9% (3 samples). Furthermore, the lineage GL.1 was found in only one sample (2.6%). One sample could not be assigned due to partial sequence.

For ease of understanding, the mutations have been grouped into panels or "mutation packages" that are different for each PCR assay. Packages A1 and B1 refer to the nested PCR (ID 980) spanning from amino acid residues 58 to 573 of the spike protein (1600 bp). Packages A2, B2, C, D and E refer to the second nested PCR (ID 1034), covering amino acid residues from 34 to 475 (1330 bp).

Packages related to the long-nested PCR (ID 980):

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[4] https://outbreak.info/situation-reports, date: 05/10/2023

- **Package A1 (assigned to the lineage Omicron XBB.1.5*/XBB.1.9*)** = V83A; G142D; DEL144; H146Q/H146K; 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 B1 (assigned to the lineage Omicron XBB.1.16*)** = V83A; G142D; DEL 144; H146Q; E180V; Q183E; V213E; G252V; G339H; R346T; L368I; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; V445P; G446S; N460K; S477N; T478R; E484A; F486P; F490S; Q498R; N501Y; Y505H

Packages related to the newly designed long-nested PCR (ID 1034):

- **Package A2 (assigned to the lineage Omicron XBB.1.5*/XBB.1.9*)** = V83A; G142D; DEL144; H146Q/H146K; Q183E; V213E; G252V; G339H; R346T; L368I; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; V445P; G446S; N460K
- **Package B2 (assigned to the lineage Omicron XBB.1.16*)** = V83A; G142D; DEL 144; H146Q; E180V; Q183E; V213E; G252V; G339H; R346T; L368I; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K
- **Package C (assigned to the lineage Omicron XBB.2.3*)** = V83A, G142D, DEL144, H146Q, Q183E, V213E, D253G, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K
- **Package D (assigned to the lineage Omicron CM.7*)** = G142D, K147E, M153T, N164K, V213G, H245N, G257D, G339D, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, K444R, N450D, L452M, N460K
- **Package E (assigned to the lineage Omicron GL.1)** = T95I; G142D; DEL144; W152L; E156G; DEL157; F186L; V213G; D253G; G339D; R346T; S371F; S373P; S375F; T376A; D405N; R408S; K417N; D420N; N440K; G446D; L452R

The lineages Omicron XBB.1.5*/XBB.1.9* were detected in 9 regions/A. P. (Campania, Emilia-Romagna, Liguria, Lombardia, Molise, Puglia, Valle d'Aosta, Veneto and P.A. Bolzano). Key mutations of lineage Omicron XBB.1.16* were observed in 5 regions/A.P. (Emilia Romagna, Lazio, Liguria, Sicilia and A.P. Bolzano). Amino acid substitutions characteristic of lineage Omicron GL.1 were detected only in Veneto. Key mutations of lineage Omicron XBB.2.3* were found in 3 regions (Abruzzo, Lombardia and Veneto). Finally, the lineage Omicron CM.7* was detected in 6 regions (Abruzzo, Basilicata, Emilia Romagna, Lazio, Liguria and Veneto).

Table 1. PCR and sequencing results

	Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	RT-PCR Omicron ID-999 (CT value)	Mutations found by Sanger sequencing (long PCR ID_980)	Mutations found by Sanger sequencing (long PCR ID_1034)	SARS-CoV-2 lineages
1	21027	Abruzzo	Chieti	S. Martino	3,14E+02	39.41		Package D	Omicron CM.7*
2	21028		Pescara	Via Raiale	1,73E+03	37.10		Package D	Omicron CM.7*
3	21029		Pescara	Villa Carmine	2,18E+03	35.49			
4	21030		L'Aquila	Pile	1,30E+03	38.08			
5	21031		Teramo	Villa Pavone	6,45E+02	ND		Package C	Omicron XBB.2.3*
6	21050	Basilicata	Potenza	Tiera di Vaglio	3,83E+03	37.17		Package D	Omicron CM.7*
7	21051		Matera	Pantano	4,15E+03	37.35		Package D	Omicron CM.7*
125	21225	Campania	Napoli	Napoli OVEST - Ingresso Principale	1,04E+03	39.08		Package A2	Omicron XBB.1.5*/XBB.1.9*
126	21226		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,04E+03	39.46			
127	21227		Napoli	Napoli EST	<LOD	ND			
8	20915	Emilia-Romagna	Ferrara	Ferrara - Linea 1	<LOD	ND			
9	20916		Ferrara	Ferrara - Linea 2	<LOD	ND			
10	20918		Modena	Carpi	<LOD	ND		Package D	Omicron CM.7*
11	20972		Bologna	IDAR	5,50E+03	36.03	Package B1		Omicron XBB.1.16 *
12	20974		Modena	Naviglio	2,75E+03	ND			
13	21001		Forlì-Cesena	Forlì	1,05E+03	ND			
14	21002		Bologna	Imola	2,80E+03	37.67			
15	21003		Forlì-Cesena	Cesena	2,70E+03	38.25			
16	21004		Rimini - Forlì-Cesena	S. Giustina	7,43E+03	38.71	Package A1		Omicron XBB.1.5*/XBB.1.9*
17	21038		Piacenza	Borgoforte	1,40E+04	ND		Package D	Omicron CM.7*
18	21040		Parma	Parma ovest	3,95E+04	38.25			
19	21041		Reggio Emilia	Mancasale	3,35E+04	ND			
20	20993	Friuli-Venezia Giulia	Pordenone	Cordenons	1,78E+03	ND			
21	20994		Udine	Udine	1,13E+03	39.54			
22	20995		Trieste	Servola	5,58E+03	39.12	Partial sequence, from H146Q to G339H [a]		Not assigned
23	20891	Lazio	Viterbo	Viterbo - Strada Bagni	<LOD	39.59			
24	20892		Latina	Latina Loc Latina Est	3,85E+03	37.46		Package D	Omicron CM.7*
25	20893		Latina	Aprilia (Via del Campo)	1,59E+03	38.18			

26	20894		Roma	Anzio - Colle Cocchino	1,86E+03	39.01			
27	20895		Roma	Velletri (LA CHIUSA-SORBO)	<LOD	39.68			
28	20896		Roma	Pomezia - Via Cincinnato	1,11E+03	39.03		Package D	Omicron CM.7*
29	20897		Roma	Civitavecchia Fiumaretta	<LOD	ND			
30	20898		Roma	Guidonia - Ponte Lucano	<LOD	39.28			
128	21351		Roma	Roma Nord	8,07E+03	ND			
129	21352		Roma	Roma Sud	<LOD	ND		Package D	Omicron CM.7*
130	21353		Roma	Fregene	9,38E+04	37.60			
131			Roma	Ostia	NA	ND		Package B2	Omicron XBB.1.16 *
31	20946	Liguria	Genova	Pegli	1,69E+04	37.94		Package D	Omicron CM.7*
32	20947		Genova	Voltri	2,27E+04	36.62	Package A1		Omicron XBB.1.5*/XBB.1.9*
33	20948		Genova	Quinto	6,32E+04	35.94		Package D	Omicron CM.7*
34	20949		Genova	Rapallo	5,57E+04	35.42		Package D	Omicron CM.7*
35	20950		Genova	Sestri P	9,46E+04	35.40	Package A1		Omicron XBB.1.5*/XBB.1.9*
36	20951		Genova	Sturla	3,27E+04	38.24			
37	20952		Savona	Savona	8,95E+03	ND			
38	20953		Savona	Borghetto Santo Spirito	3,74E+04	36.57	Package B1		Omicron XBB.1.16 *
39	20954		Imperia	Imperia	3,10E+04	ND			
40	20955		Imperia	Sanremo - località Capo Verde	3,34E+04	36.65		Package D	Omicron CM.7*
41	20956		La Spezia	Camisano	1,52E+04	39.91		Package D	Omicron CM.7*
42	20957		La Spezia	Silea	3,25E+04	37.34			
43	20958		La Spezia	La Spezia	8,67E+03	ND			
44	20959		Genova	Darsena	3,22E+04	36.61			
45	20960		Genova	Punta Vagno Genova	1,27E+05	35.37		Package A2	Omicron XBB.1.5*/XBB.1.9*
46	20961		Genova	Valpolcevera	1,01E+05	36.48	Package A1		Omicron XBB.1.5*/XBB.1.9*
47	21035		Genova	Punta Vagno Genova	1,25E+05	38.80			
48	20935	Lombardia	Sondrio	Sondrio	2,57E+03	39.05			
49	20969		Bergamo	Bergamo	2,04E+03	ND			
50	20970		Brescia	Verziano	9,87E+02	ND			
51	20975		Cremona	Citta di Cremona	4,28E+03	ND		Package C	Omicron XBB.2.3*
119	20936		Milano	Milano Nosedo	3,79E+05	37.32			
120	20937		Milano	Milano San Rocco	7,04E+05	ND			
121	20938		Como	Como	4,50E+05	39.51			
122	20939		Pavia	Pavia	<LOD	39.40			

123	20940		Como - Lecco - Milano - Monza e della Brianza	Monza	<LOD	ND		Package A2	Omicron XBB.1.5*/XBB.1.9*
52	21015	Marche	Pesaro-Urbino	Borgheria	<LOD	ND			
53	21016		Pesaro-Urbino	Ponte Metauro	<LOD	ND			
54	21017		Ancona	Zipa	2,29E+03	37.51			
55	21018		Ancona	Falconara	4,54E+03	38.69			
56	21032	Molise	Campobasso	Campobasso - San Pietro	5,61E+02	39.86			
57	21033		Campobasso	Termoli - località Porto	7,05E+02	39.76			
58	21034		Campobasso	Termoli - località Pantano Basso	1,85E+03	38.58		Package A2 + F456L[b]	Omicron XBB.1.5*/XBB.1.9*
59	21067	P.A. Bolzano	Bolzano	IDA Bolzano	1,91E+04	38.17		Package B2	Omicron XBB.1.16 *
60	21068		Bolzano	IDA Merano	1,06E+04	36.91			
61	21069		Bolzano	IDA Termeno	4,75E+04	36.11	Package A1		Omicron XBB.1.5*/XBB.1.9*
62	20869	P.A. Trento	Trento	Trento nord	2,98E+04	37.00			
63	20870		Trento	Trento sud	3,63E+04	36.47			
64	20871		Trento	Rovereto	3,43E+04	38.44			
65	20841	Piemonte	Torino	Castiglione Torinese	8,45E+02	39.05			
66	20842		Biella	Biella Nord	<LOD	ND			
67	20843		Biella	Biella Sud	2,02E+03	ND			
68	20844		Novara	Novara	1,92E+03	ND			
69	20912		Alessandria	Alessandria	1,61E+03	39.05			
70	20913		Asti	Asti	2,29E+03	37.53			
71	20914		Cuneo	Cuneo	1,07E+03	37.26			
72	20839	Puglia	Bari	Bari Est	5,53E+02	ND			
73	20840		Bari	Bari Ovest	3,89E+02	39.95			
74	20845		Bari	Altamura	4,79E+03	35.61			
75	20846		Lecce	Lecce	4,00E+03	37.15			
76	20847		Brindisi	Brindisi Fiume Grande	1,91E+03	39.54			
77	20848		Taranto	Taranto Bellavista	6,80E+02	ND			
78	20849		Taranto	Taranto Gennarini	7,69E+02	ND			
79	20872		Bari	Bitonto	7,87E+02	38.37			
80	20873		Foggia	Foggia	8,21E+02	37.25			
81	20874		Foggia	Cerignola	1,68E+03	ND			
82	20875		Foggia	Manfredonia	9,40E+02	38.71			
83	20876		Barletta-Andria-Trani	Barletta	1,78E+03	ND		Package A2 + F456L[b]	Omicron XBB.1.5*/XBB.1.9*
84	20877		Barletta-Andria-Trani	Andria	9,85E+02	ND		Package A2	Omicron XBB.1.5*/XBB.1.9*

85	20908		Barletta-Andria-Trani	Bisceglie	4,97E+02	37.26			
86	20909		Bari	Molfetta	1,04E+03	38.38			
87	20910		Barletta-Andria-Trani	Trani	7,96E+02	38.77			
88	20880	Sicilia	Trapani	Trapani	1,85E+03	38.52			
89	20881		Trapani	Mazara del Vallo	2,17E+03	37.15			
90	20882		Trapani	Marsala	1,40E+03	37.01			
91	20924		Ragusa	Modica	<LOD	37.37			
92	20925		Ragusa	Vittoria	1,40E+03	ND			
93	20926		Ragusa	Ragusa	6,50E+02	36.11			
94	20927		Caltanissetta	Gela Macchitella	6,00E+03	ND			
95	20928		Messina	Mili Marina	3,18E+03	34.65			
96	20977		Agrigento	Agrigento	8,62E+04	36.46			
97	20978		Enna	Enna	3,14E+04	38.29			
98	20979		Palermo	Bagheria	8,34E+03	36.76			
99	20980		Palermo	Acqua dei Corsari	1,28E+04	36.32			
100	20981		Palermo	Fondo Verde	1,41E+04	34.97			
101	20982		Caltanissetta	Caltanissetta e San Cataldo	2,08E+04	39.18			
115	20987		Catania	Pantano d'Arci	1,17E+04	37.37			
116	20988		Catania	Giarre	9,73E+03	36.90			
117	20989		Siracusa	Siracusa	1,13E+04	38.31		Package B2	Omicron XBB.1.16 *
118	20929		Palermo	Mili Marina	2,98E+03	ND			
124	20962	Umbria	Perugia	Perugia - Pian della Genna	<LOD	37.72			
102	20967	Valle d'Aosta	Aosta	La Salle	<LOD	36.21			
103	20968		Aosta	Brissogne	3,90E+02	38.02		Package A2	Omicron XBB.1.5*/XBB.1.9*
104	20865	Veneto	Padova	Padova Ca' Nordio - centro storico	NA	ND		Package C	Omicron XBB.2.3*
105	20866		Padova	Padova Ca' Nordio - zip	NA	38.12			
106	20867		Padova	Padova Guizza	NA	ND			
107	20868		Padova	Abano Terme	NA	37.06			
108	20899		Vicenza	Vicenza Casale	1,77E+03	34.74		Package E	Omicron GL.1
109	20900		Treviso	Treviso	1,72E+04	35.33		Package D	Omicron CM.7*
110	20901		Venezia	Venezia Fusina	7,39E+03	35.49			
111	20943		Verona	Verona_collettore 1M	6,99E+03	36.20	Package A1 + F456L[b]		Omicron XBB.1.5*/XBB.1.9*
112	20944		Verona	Verona_collettore 3M	3,81E+03	38.85			
113	20945		Verona	Verona_collettore 8M	<LOD	37.39			

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

[b] Packages A1/A2 combined with the 'F456L' mutation, suggests the presence of the Omicron EG.5 sublineage. This sublineage is characterized by having the 'F456L' mutation in approximately 80.5% of its sequences. The Omicron XBB.1.5* lineage and the Omicron XBB.1.9*lineage may also carry the F456L mutation at rates of 3.0% and 26.6% respectively (https://outbreak.info/situation-reports, date: 05/10/2023);

ND: not detected

NA: not available

Table 2. Sanger sequencing results

ID SAMPLES	V83A	T95I	G142D	DEL144	H146Q/H146K	K147E	W152L	M153T	E156G	DEL157	N164K	E180V	Q183E	F186L	V213G	V213E	H245N	G252V	D253G	G257D	G339D	G339H	R346T	L368I	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	D420N	N440K	K444R	V445P	G446D	G446S	N450D	F456L	L452M	L452R	N460K	S477N	T478K	T478R	E484A	F486P	F490S	Q498R	N501Y	Y505H	PACKAGES	LINEAGES
16, 32, 35, 46, 61	■		■	■	■								■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■					■	■	■		■	■	■	■	■	■	Package A1	Omicron XBB.1.5*/XBB.1.9*
125, 45, 123, 84, 103	■		■	■	■								■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■					■										Package A2	
111	■		■	■	■								■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■		■			■	■	■		■	■	■	■	■	■	Package A1 + F456L[a]	
58, 83	■		■	■	■								■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■		■			■										Package A2 + F456L[a]	
11, 38	■		■	■	■							■	■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■					■	■		■	■	■	■	■	■	■	Package B1	Omicron XBB.1.16*
59, 117, 131	■		■	■	■							■	■			■		■				■	■	■	■	■	■	■		■	■	■		■		■		■					■										Package B2	
5, 51, 104	■		■	■	■								■			■			■			■	■	■	■	■	■	■		■	■	■		■		■		■					■										Package C	Omicron XBB.2.3*
1, 2, 6, 7, 10, 17, 24, 28, 31, 33, 34, 40, 41, 109, 129			■			■		■			■				■		■			■	■				■	■	■	■	■	■	■	■		■	■				■		■		■										Package D	Omicron CM.7*
108		■	■	■			■		■	■				■	■				■		■		■		■	■	■	■		■	■	■	■	■			■					■											Package E	Omicron GL.1

Limitations of the study

The geographical and population coverage of this flash survey was not comprehensive, as it encompassed 18 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. Our decision to adopt a broader lineage classification from 'outbreak.info' for variant classification, rather than specifying sublineage assignments, was influenced by the rapid evolution of numerous sublineages, often with minor differences, that hampered the reliable assignation to sublineages based solely on mutations observed in the spike region.

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 provide additional information on SARS-CoV-2 variants in the population, complementing data obtained through clinical surveillance. The results of this survey indicate that the Omicron variant is the sole of SARS-CoV-2 variant in Italy, with the CM.7* lineage being the most prevalent. However, mutations characteristic of other lineages, Omicron XBB.1.5*/XBB.1.9*, Omicron XBB.1.16*, Omicron GL.1 and Omicron XBB.2.3*, were also detected.

The sequencing of SARS-CoV-2 in wastewater samples provides valuable additional information alongside the sequencing of clinical cases. This approach provides a more complete and accurate understanding of the circulating variants in the country, contributing to a better characterization of the spread and evolution of this virus.

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