



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
30th Report
(Study period: February 5th to February 9th, 2024)

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Main findings:

- During the week of 5 February to 9 February 2024, a total of 102 wastewater samples were collected from 16 Regions and 2 Autonomous Province (A.P.).
- Mutations characteristic of the Omicron variant were identified in 7 of these Regions/AP, while no sequencing data were collected from the remaining areas.
- Analysis of the sequences obtained by Sanger sequencing showed that 93% of the positive samples displayed amino acid substitutions that are typical of the Omicron JN.1*lineage and the 7% had substitutions of the XBB.1.5/1.9* lineages.

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) started a series of "flash surveys". These surveys consist of monthly sampling campaigns carried out over short periods in different locations throughout Italy. 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 summarise the results of the 30th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 5 to 9 February 2024.

Methodology

The 30th national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 5 to 9 February 2024. The survey involved the collection of 102 wastewater samples from 98 wastewater treatment plants (WTPs) located in 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 website¹. 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. Purified RNA extracts from the samples were delivered to ISS for variant detection.

For sequencing purposes, we employed a long-nested PCR assay covering approximately 1330 base pairs and spanning from amino acid residues 34 to 475 of the spike protein (PCR ID 1033/1034). After amplification of the target sequences, we used Sanger sequencing on individual samples.

For variant classification, we adopted a lineage classification based on 'outbreak.info'³ 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

Of the 102 samples analysed, a total of 92 (90.2%) 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 8.20E + 01 to 6.20E + 05 genome copies (g.c.) per liter of wastewater.

Sanger Sequencing

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 <https://outbreak.info/situation-reports>, date: 05/12/2023

Table 1 summarises the results of the long-nested PCR assay and sequencing methods. A total of 15 samples (14.7%) from 7 Regions/AP were successfully amplified using the long-nested PCR assay described above. High-quality sequences were obtained from 15 of these samples by Sanger sequencing. All of these sequences were classified as belonging to the Omicron variant. Analysis of the wastewater samples revealed the presence of two SARS-CoV-2 lineages, as shown in Tables 1 and 2. Among these, the Omicron JN.1* lineage was the most common, detected in 93% of the positive samples (14 samples), followed by the Omicron XBB.1.5/1.9* lineages (which are not distinguishable in the sequenced region), detected in 7% (1 sample). For ease of understanding, the mutations have been grouped into panels or "mutation packages". These are listed below:

- **Package A (assigned to the lineage Omicron XBB.1.5*/XBB.1.9*)** = V83A, G142D, DEL144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K
- **Package B (assigned to the Omicron JN.1*)** = DEL69/70, V127F, G142D, DEL144, F157S, R158G, DEL211/212, V213G, L216F, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445H, G446S, N450D, L452W, L455S, N460K

The Omicron XBB.1.5*/XBB.1.9* lineage was detected in Liguria. Instead, the Omicron JN.1* lineage was found in 7 regions: Liguria, Lombardia, Marche, Molise, Piemonte, Sicilia and Veneto.

Table 1. PCR and sequencing results

ID ISS	Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_1034)	Sars-CoV-2 lineages (Sanger sequencing)
1	24466	Abruzzo	Chieti	S. Martino	<LOD		
2	24467		Pescara	Via Raiale	<LOD		
3	24468		Pescara	Villa Carmine	2,47E+02		
4	24469		L'Aquila	Pile	1,97E+02		
5	24470		Teramo	Villa Pavone	3,01E+02		
81	24543	Basilicata	Potenza	Tiera di Vaglio	1,14E+04		
82	24544		Matera	Pantano	6,36E+03		
6	24540	Campania	Napoli	Napoli EST	5,91E+03		
7	24541		Napoli	Napoli OVEST - Ingresso Principale	2,84E+04		
8	24542		Napoli	Napoli OVEST - ex ingresso Camaldoli	2,46E+04		
9	24463	Emilia-Romagna	Ferrara	Ferrara - Linea 1	<LOD		
10	24464		Ferrara	Ferrara - Linea 2	<LOD		
11	24465		Modena	Carpi	<LOD		
12	24502		Piacenza	Borgoforte	4,70E+03		
13	24503		Parma	Parma ovest	7,55E+04		
14	24504		Reggio Emilia	Mancasale	2,60E+04		
83	24563		Bologna	Imola	1,12E+05		
84	24564		Ravenna	Faenza	6,20E+05		
85	24565		Ravenna - Forlì-Cesena	Ravenna	1,10E+05		
86	24566		Bologna	IDAR	6,18E+04		
87	24567		Forlì-Cesena	Forlì	1,10E+05		
88	24568		Forlì-Cesena	Cesena	2,45E+05		
89	24569		Modena	Naviglio	1,60E+04		

90	24570		Rimini - Forlì-Cesena	S. Giustina	1,00E+05		
100	24728	Friuli-Venezia Giulia	Pordenone	Cordenons	1,05E+04		
101	24729		Udine	Udine	1,71E+04		
102	24730		Trieste	Servola	8,31E+03		
77	24462	Lazio	Roma	Civitavecchia Fiumaretta	2,50E+02		
15	24501	Liguria	Genova	Recco	6,28E+04		
16	24505		Genova	Pegli	3,80E+04		
17	24506		Genova	Voltri	3,78E+04	Package B	Omicron JN.1*
18	24507		Genova	Rapallo	3,75E+04		
19	24508		Genova	Sestri P	3,58E+04		
20	24509		Genova	Sturla	1,07E+04		
21	24510		Genova	Darsena	2,32E+04	Package B	Omicron JN.1*
22	24511		Genova	Punta Vagno Genova	4,50E+04		
23	24512		Genova	Valpolcevera	1,95E+04		
24	24513		Savona	Savona	2,67E+03		
25	24514		Savona	Borghetto Santo Spirito	2,04E+04	Package A + F456L + L455F	Omicron XBB.1.5*/XBB.1.19*
26	24515		Imperia	Imperia	2,37E+04		
27	24516		Imperia	Sanremo - località Capo Verde	4,41E+04	Package B	Omicron JN.1*
28	24492	Lombardia	Bergamo	Bergamo	1,26E+04		
29	24493		Cremona	Città di Cremona	5,69E+03	Package B	Omicron JN.1*
30	24496		Brescia	Verziano	1,06E+04		
70	24612		Sondrio	Sondrio	6,85E+03		
71	24482		Milano	Milano Nosedo	<LOD	Package B	Omicron JN.1*
72	24483		Milano	Milano San Rocco	<LOD		
73	24484		Como	Como	<LOD	Package B	Omicron JN.1*
74	24485		Pavia	Pavia	<LOD		
75	24486		Como - Lecco - Milano - Monza e della Brianza	Monza	<LOD		
76	24487		Pavia	Vigevano	6,69E+04	Package B	Omicron JN.1*
31	24536	Marche	Pesaro-Urbino	Borgheria	2,59E+03	Package B	Omicron JN.1*

32	24537		Pesaro-Urbino	Ponte Metauro	1,87E+03		
33	24538		Ancona	Zipa	7,69E+03		
34	24539		Ancona	Falconara	5,22E+03		
35	24471	Molise	Campobasso	Campobasso - San Pietro	3,08E+02	Package B	Omicron JN.1*
36	24475		Campobasso	Termoli - località Porto	1,21E+03		
37	24481		Campobasso	Termoli - località Pantano Basso	4,70E+02		
78	24584	P.A. Bolzano	Bolzano	IDA Bolzano	6,14E+03		
79	24585		Bolzano	IDA Merano	2,02E+04		
80	24586		Bolzano	IDA Termeno	2,35E+04		
38	24423	P.A. Trento	Trento	Trento nord	1,38E+04		
39	24424		Trento	Trento sud	2,06E+04		
40	24425		Trento	Rovereto	7,51E+04		
41	24393	Piemonte	Torino	Castiglione Torinese	8,15E+03	Package B	Omicron JN.1*
42	24394		Biella	Biella Nord	5,15E+03		
43	24395		Biella	Biella Sud	8,55E+03		
44	24397		Novara	Novara	5,38E+03		
45	24438		Alessandria	Alessandria	1,11E+04		
46	24439		Asti	Asti	3,55E+03		
47	24440		Cuneo	Cuneo	2,63E+03		
48	24416	Puglia	Bari	Bari Est	3,21E+02		
49	24417		Bari	Bari Ovest	1,31E+03		
50	24418		Taranto	Taranto Bellavista	1,83E+03		
51	24419		Taranto	Taranto Gennarini	3,26E+03		
52	24428	Sicilia	Trapani	Trapani	2,84E+03	Package B	Omicron JN.1*
53	24429		Trapani	Mazara del Vallo	7,80E+02		
54	24430		Trapani	Marsala	1,28E+03		
55	24472		Ragusa	Modica	5,00E+02		
56	24473		Ragusa	Vittoria	4,50E+02		
57	24474		Ragusa	Ragusa	4,75E+02		
58	24478		Caltanissetta	Gela Macchitella	5,00E+02		
91	24559		Palermo	Acqua dei Corsari	1,65E+03		

92	24560		Palermo	Fondo Verde	5,08E+03		
93	24561		Caltanissetta	Caltanissetta e San Cataldo	7,42E+03		
94	24562		Palermo	Bagheria	2,74E+03		
95	24531		Catania	Pantano d'Archi	1,46E+04		
96	24532		Catania	Giarre	3,86E+03		
97	24533		Siracusa	Siracusa	5,59E+03		
98	24594	Toscana	Pisa	Pisa Nord - S. Jacopo	1,53E+02		
99	24595		Lucca	Pontetetto	8,20E+01		
59	24436	Umbria	Perugia	Perugia - Pian della Genna	9,72E+03		
60	24412	Veneto	Padova	Padova Ca' Nordio - centro storico	4,76E+04		
61	24413		Padova	Padova Ca' Nordio - zip	4,27E+04		
62	24414		Padova	Padova Guizza	3,19E+04		
63	24415		Padova	Abano Terme	4,86E+04	Package B	Omicron JN.1*
64	24433		Treviso	Treviso	1,09E+03	Package B + L176F	Omicron JN.1*
65	24434		Venezia	Venezia Fusina	5,30E+02		
66	24435		Vicenza	Vicenza Casale	2,83E+03		
67	24489		Verona	Verona_collettore 1M	4,28E+03		
68	24490		Verona	Verona_collettore 3M	6,53E+03	Package B	Omicron JN.1*
69	24491		Verona	Verona_collettore 8M	3,70E+03		

Table 2. Sanger sequencing results

ID SAMPLES	DEL69/70	V83A	V127F	G142D	DEL 144	H146Q/H146K	F157S	R158G	Q183E	DEL211/212	V213G	V213E	L216F	H245N	G252V	A264D	I332V	G339H	R346T	K356T	L368I	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	N440K	V445H	V445P	G446S	N450D	L452W	L455S	F456L	N460K	VARIANTS
25																																							Package A (Omicron XBB.1.5*/XBB.1.9*)
17, 21, 27, 29, 31, 36, 41, 55, 63, 64, 68, 71, 73, 76																																							Package B (Omicron JN.1*)

Limitations of the study

The geographical and population coverage of this flash survey is not representative of the entire territory of the country as it only covers 18 out of 21 of the Italian regions/Autonomous Provinces. It is important to highlight that the employment of molecular analytical methods in complex environmental matrices such as wastewater can be challenging due to a number of factors. These include low virus concentration, insufficient analytical recovery and/or PCR inhibitors. Consequently, both the detection/quantification and the PCR amplification required for the sequencing may produce false negatives, making molecular characterization and variant detection achievement difficult for all samples. In addition, obtainment of partial sequences from 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 assignment 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 SARS-CoV-2 variant in Italy, with the Omicron JN.1* lineage being the most prevalent. The Omicron XBB.1.5*/XBB.1.9* lineage has also been 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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