



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy

41st Report

(Study period: January 13th to January 17th, 2025)

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Main findings:

- During the week of 13 January to 17 January 2025, a total of 109 wastewater samples were collected from 16 Regions and two Autonomous Provinces (A.P.s).
- Mutations characteristic of the Omicron variant were identified in five regions, while sequencing data were not available from the remaining regions and A.P.s
- Sanger sequencing analysis showed that all positive samples carried amino acid substitutions typical of the Omicron JN.1* lineage, including mutations associated with the XEC*, KP.3*/JN.1.16* and XDY.1 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. 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 41st national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 13 January to 17 January 2025.

Methodology

The 41st national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 13 January to 17 January 2025. The survey involved the collection of 109 wastewater samples from 104 wastewater treatment plants (WTPs) located in 16 Regions and two 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 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, a long-nested PCR assay was employed, covering approximately 1330 base pairs and spanning from amino acid residues 34 to 475 of the spike protein (PCR ID 1033/1034). After the target sequences were amplified, individual samples were subjected to Sanger sequencing.

For variant classification, a lineage classification based on 'outbreak.info'³ was adopted instead of specifying sublineages. This choice was made because numerous sublineages 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 unfeasible.

Results

Real Time qPCR

Real-time PCR was performed on only 86 of the 109 samples. Out of the 86 samples analysed, a total of 71 (82.5%) 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 5.00E + 01 to 4.58E + 04 genome copies (g.c.) per liter of sewage.

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>

Table 1 summarises the results of the long-nested PCR assay and sequencing methods. A total of 6/109 samples (5.5%) from five Regions were successfully amplified using the long-nested PCR assay described above.

Analysis of the wastewater samples confirmed the exclusive presence of the Omicron JN.1* lineage, as shown in Tables 1 and 2. This lineage was detected in five regions: Emilia-Romagna, Liguria, Molise, Puglia and Veneto.

Within this lineage, 66.6% of the samples (4/6 samples) showed mutation associated with the XEC* sublineage, 16.7% (1/6 samples) with the KP.3*/JN.1.16* sublineage and 16.7% (1/6 samples) with the XDY.1 sublineage.

The observed mutations are grouped into a single panel, referred to as a "mutation package", listed below:

Package A (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

Table 1. PCR and sequencing results

ID ISS	ID SARI	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)
76	28489	Abruzzo	Pescara	Via Raiale	<LOD		
77	28490		Pescara	Villa Carmine	<LOD		
78	28491		Pescara	Villa Carmine	<LOD		
79	28492		L'Aquila	Pile	<LOD		
1	28424	Basilicata	Potenza	Tiera di Vaglio	6,31E+03		
2	28425		Matera	Pantano	2,05E+04		
104	28548	Campania	Napoli	Napoli EST	2,85E+03		
105	28549		Napoli	Napoli OVEST - Ingresso Principale	7,93E+03		
106	28550		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,12E+04		
3	28385	Emilia-Romagna	Piacenza	Borgoforte	<LOD		
4	28386		Parma	Parma ovest	1,85E+03		
5	28387		Reggio Emilia	Mancasale	1,80E+03		
6	28431		Ferrara	Ferrara - Linea 1	1,73E+04		
7	28432		Ferrara	Ferrara - Linea 2	<LOD		
8	28433		Modena	Carpi	<LOD		
83	28454		Modena	Naviglio	1,05E+03	Package A + F59S + F456L ^a + E298K	Omicron JN.1*
84	28455		Bologna	IDAR	1,22E+03		
85	28479		Forlì-Cesena	Cesena	1,88E+03		
86	28480		Bologna	Imola	3,69E+02		
87	28481		Ravenna - Forlì-Cesena	Ravenna	2,50E+02		
88	28482		Rimini - Forlì-Cesena	S. Giustina	4,72E+02		
89	28483		Forlì-Cesena	Forlì	3,77E+02		
9	28381	Friuli Venezia Giulia	Pordenone	Cordenons	1,04E+04		
10	28382		Trieste	Servola	1,80E+03		
11	28383		Udine	Udine	2,90E+02		
12	28389	Lazio	Roma	Civitavecchia Fiumaretta	<LOD		

57	29059		Roma	Anzio - Colle Cocchino	NA		
58	29060		Latina	Aprilia (Via del Campo)	NA		
59	29061		Latina	Latina Loc Latina Est	NA		
60	29062		Roma	Velletri (LA CHIUSA-SORBO)	NA		
61	29063		Roma	Pomezia - Via Cincinnato	NA		
62	29064		Roma	Guidonia - Ponte Lucano	NA		
63	29058		Viterbo	Viterbo - Strada Bagni	NA		
13	28388		Genova	Recco	1,38E+03		
14	28398		Genova	Pegli	2,36E+04		
15	28399		Genova	Voltri	1,99E+04		
16	28400		Genova	Quinto	4,32E+03		
17	28401		Genova	Sestri P	1,49E+04		
18	28402		Genova	Valpolcevera	1,12E+04		
19	28403	Liguria	Genova	Rapallo	1,74E+04	Package A+ Q183H + R190T +A435S + F456L ^b	Omicron JN.1*
20	28404		Imperia	Sanremo - località Capo Verde	4,58E+04		
21	28405		Savona	Savona	<LOD		
22	28406		Genova	Punta Vagno Genova	1,09E+04		
23	28407		Genova	Darsena	1,17E+04		
24	28408		Genova	Sturla	2,33E+04	Package A + F59S + F456L ^a	Omicron JN.1*
25	28409		Imperia	Imperia	7,75E+03		
26	28410		Savona	Borghetto Santo Spirito	2,89E+04		
64	NA		Pavia	Pavia	NA		
65	NA		Como	Como	NA		
66	NA		Milano	Milano S. Rocco	NA		
67	NA		Milano	Milano Nosedo	NA		
68	NA	Lombardia	Sondrio	Sondrio	NA		
69	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA		
70	NA		Monza	Monza	NA		
71	NA		Lecco	Lecco	NA		

72	NA		Brescia	Verziano	NA		
73	NA		Mantova	Mantova	NA		
74	NA		Cremona	Cremona	NA		
75	NA		Bergamo	Bergamo	NA		
27	28436	Marche	Pesaro-Urbino	Borgheria	8,84E+03		
28	28437		Pesaro-Urbino	Ponte Metauro	2,67E+02		
29	28438		Ancona	Zipa	3,86E+03		
30	28439		Ancona	Falconara	1,24E+03		
31	28440		Ascoli Piceno	Marino del Tronto	1,51E+04		
32	28441		Fermo	Salvano	1,58E+04		
80	28495	Molise	Campobasso	Termoli - località Pantano Basso	<LOD		
81	28494		Campobasso	Termoli - località Porto	3,03E+02	Package A + F456L ^c + A435S	Omicron JN.1*
82	28493		Campobasso	Campobasso - San Pietro	3,00E+02		
107	28657	A.P. Bolzano	Bolzano	IDA Termeno	3,68E+03		
108	28656		Bolzano	IDA Merano	2,06E+03		
109	28655		Bolzano	IDA Bolzano	2,68E+03		
33	28374	A.P. Trento	Trento	Trento nord	9,00E+03		
34	28375		Trento	Trento sud	3,96E+03		
35	28376		Trento	Rovereto	1,60E+04		
36	28360	Piemonte	Torino	Castiglione Torinese	9,72E+02		
37	28361		Biella	Biella Nord	3,00E+02		
38	28362		Biella	Biella Sud	4,78E+03		
39	28363		Novara	Novara	2,50E+02		
40	28378		Alessandria	Alessandria	3,10E+03		
41	28379		Asti	Asti	6,80E+03		
42	28380		Cuneo	Cuneo	6,95E+03		
43	28364	Puglia	Bari	Bari Est	<LOD		
44	28365		Bari	Bari Ovest	7,64E+02	Package A + F59S + F456L ^a	Omicron JN.1*
45	28366		Taranto	Taranto Bellavista	5,11E+02		
46	28367		Taranto	Taranto Gennarini	9,86E+02		

90	28393	Sicilia	Catania	Pantano d'Arci	5,30E+03		
91	28394		Catania	Giarre	<LOD		
92	28395		Siracusa	Siracusa	3,24E+03		
93	28368		Trapani	Trapani	2,88E+03		
94	28369		Trapani	Mazara del Vallo	9,08E+02		
95	28370		Trapani	Marsala	4,32E+03		
98	28435		Caltanissetta	Gela Macchitella	5,00E+01		
99	28465		Palermo	Acqua dei Corsari	1,59E+03		
100	28466		Palermo	Fondo Verde	2,37E+03		
101	28467		Caltanissetta	Caltanissetta e San Cataldo	7,20E+03		
102	28468	Toscana	Enna	Enna	1,25E+04		
103	28469		Agrigento	Agrigento	5,44E+03		
96	28443		Lucca	Pontetetto	<LOD		
97	28442		Pisa	Pisa Nord - S. Jacopo	4,37E+03		
47	28317	Umbria	Perugia	Perugia - Pian della Genna	<LOD		
48	28292	Veneto	Padova	Padova Ca' Nordio - centro storico	NA	Package A + F59S + F456L ^a + G181V	Omicron JN.1*
49	28293		Padova	Padova Ca' Nordio - zip	NA		
50	28294		Padova	Padova Guizza	NA		
51	28295		Padova	Abano Terme	NA		
52	28371		Treviso	Treviso	1,90E+03		
53	28372		Venezia	Venezia Fusina	6,08E+03		
54	28373		Vicenza	Vicenza Casale	<LOD		
55	28391		Verona	Verona_collettore 1M	7,49E+03		
56	28392		Verona	Verona_collettore 8M	3,42E+04		

NA= Not available

^a The key mutations of Omicron JN.1* in association with F59S and F456L may indicate the presence of the Omicron XEC* sublineage.

^b The key mutations of Omicron JN.1* in association with Q183H, R190T, A435S and F456L may indicate the presence of the Omicron XDY.1 sublineage.

^c The key mutations of Omicron JN.1* in association with F456L may indicate the presence of the Omicron KP.3* or JN.1.16* sublineages.

Table 2. Sanger sequencing results

ID SAMPLES	F59S	DEL69/70	V127F	G142D	DEL 144	F157S	R158G	Q183H	R190T	DEL211/212	V213G	L216F	H245N	A264D	I332V	G339H	K356T	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	A435S	N440K	V445H	G446S	N450D	L452W	L455S	F456L	N460K	VARIANTS	
81																																				Package A (Omicron JN.1* + F456L)
24, 44, 83																																				Package A (Omicron JN.1* + F59S + F456L)
19																																				Package A (Omicron JN.1* + Q183H + R190T + A435S + F456L)

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 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 findings from this survey confirm that the Omicron JN.1 lineage is the only SARS-CoV-2 variant detected in wastewater in Italy, with mutations associated with various sublineages. 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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