



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

42nd Report

(Study period: February 3rd to February 7th, 2025)

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Main findings:

- During the week of 3 February to 7 February 2025, a total of 109 wastewater samples were collected from 16 Regions and two Autonomous Provinces (A.P.).
- Mutations characteristic of the Omicron variant were identified in 3 regions, while sequencing data were not available from the remaining regions/AP.
- 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* and KP.3*/JN.1.16* 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 42nd national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 3 February to 7 February 2025.

Methodology

The 42nd national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 3 February to 7 February 2025. The survey involved the collection of 109 wastewater samples from 105 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. 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 86 of the 109 samples. Out of the 86 samples analysed, a total of 62 (72.1%) 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 1.12E + 01 to 1.22E + 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 5/109 samples (4.6%) from 3 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 three regions: Abruzzo, Emilia-Romagna and Sicilia.

Within this lineage, 40.0% of the samples (2/5 samples) showed mutation associated with the XEC* sublineage and 60.0% (3/5 samples) with the KP.3*/JN.1.16* 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)
77	28783	Abruzzo	Pescara	Via Raiale	<LOD	Package A + F59S + F456L ^a	Omicron JN.1*
78	28784		Pescara	Villa Carmine	<LOD		
79	28782		Chieti	S. Martino	<LOD		
80	28785		L'Aquila	Pile	<LOD		
81	28786		Teramo	Villa Pavone	<LOD		
72	28681	Basilicata	Matera	Pantano	7,86E+03		
73	28680		Potenza	Tiera di Vaglio	9,54E+03		
98	28705	Campania	Napoli	Napoli EST	<LOD	1,85E+03	
96	28706		Napoli	Napoli OVEST - Ingresso Principale	1,85E+03		
97	28707		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,23E+03		
1	28618	Emilia-Romagna	Piacenza	Borgoforte	2,06E+02	Package A + F59S + F456L ^a	Omicron JN.1*
2	28619		Parma	Parma ovest	3,50E+03		
3	28620		Reggio Emilia	Mancasale	2,50E+03		
65	28708		Bologna	IDAR	1,01E+04		
69	28712		Forlì-Cesena	Cesena	7,23E+03		
71	28714		Rimini - Forlì-Cesena	S. Giustina	3,54E+03		
66	28709		Modena	Naviglio	5,59E+03		
67	28710		Ravenna - Forlì-Cesena	Ravenna	3,29E+03		
68	28711		Forlì-Cesena	Forlì	1,61E+03		
70	28713		Bologna	Imola	3,36E+03		
54	28873		Ferrara	Ferrara - Linea 1	<LOD		
55	28874		Ferrara	Ferrara - Linea 2	<LOD		
56	28875		Modena	Carpi	<LOD		
4	28673	Friuli Venezia Giulia	Pordenone	Cordenons	<LOD		
5	28674		Udine	Udine	<LOD		
6	28675		Trieste	Servola	<LOD		

7	28583	Lazio	Roma	Civitavecchia Fiumaretta	<LOD
99	29065		Viterbo	Viterbo - Strada Bagni	NA
100	29066		Latina	Latina Loc Latina Est	NA
101	29067		Latina	Aprilia (Via del Campo)	NA
102	29068		Roma	Anzio - Colle Cocchino	NA
103	29069		Roma	Velletri (LA CHIUSA-SORBO)	NA
104	29070		Roma	Pomezia - Via Cincinnato	NA
105	29071		Roma	Guidonia - Ponte Lucano	NA
8	28621	Liguria	Genova	Recco	4,63E+03
9	28626		Genova	Pegli	9,10E+03
11	28628		Genova	Quinto	4,04E+03
12	28629		Genova	Sestri P	5,61E+03
13	28630		Genova	Valpolcevera	4,51E+03
14	28631		Genova	Rapallo	5,24E+03
15	28632		Imperia	Sanremo - località Capo Verde	1,00E+04
16	28633		Savona	Savona	3,38E+03
17	28634		Genova	Punta Vagno Genova	5,15E+03
18	28635		Genova	Darsena	4,68E+03
10	28627		Genova	Voltri	<LOD
19	28636		Genova	Sturla	4,53E+03
20	28637		Imperia	Imperia	7,81E+03
21	28638		La Spezia	La Spezia	4,91E+03
22	28639		Savona	Borghetto Santo Spirito	2,73E+03
50	NA	Lombardia	Bergamo	Bergamo	NA
51	NA		Cremona	Cremona	NA
52	NA		Mantova	Mantova	NA
53	NA		Verziano	Brescia	NA
57	NA		Pavia	Pavia	NA
58	NA		Como	Como	NA
59	NA		Milano	Milano S. Rocco	NA

60	NA		Milano	Milano Nosedo	NA
61	NA		Lecco	Lecco	NA
62	NA		Monza	Monza	NA
63	NA		Sondrio	Sondrio	NA
64	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA
23	28644	Marche	Pesaro-Urbino	Borgheria	1,34E+03
24	28650		Pesaro-Urbino	Ponte Metauro	2,04E+03
25	28651		Ancona	Zipa	1,67E+03
26	28652		Ancona	Falconara	3,86E+03
27	28653		Ascoli Piceno	Marino del Tronto	2,19E+03
28	28654		Fermo	Salvano	6,77E+03
82	28781	Molise	Campobasso	Termoli - località Pantano Basso	<LOD
83	28780		Campobasso	Termoli - località Porto	<LOD
84	28779		Campobasso	Campobasso - San Pietro	2,33E+02
85	28765	A.P. Bolzano	Bolzano	IDA Termeno	<LOD
86	28764		Bolzano	IDA Merano	3,28E+03
87	28763		Bolzano	IDA Bolzano	2,52E+03
29	28587	A.P. Trento	Trento	Trento nord	7,45E+03
30	28588		Trento	Trento sud	1,22E+04
31	28589		Trento	Rovereto	1,03E+04
32	28572	Piemonte	Torino	Castiglione Torinese	2,25E+03
33	28573		Biella	Biella Nord	1,57E+03
34	28574		Novara	Novara	5,10E+03
106	28580		Alessandria	Alessandria	8,33E+03
107	28581		Asti	Asti	6,13E+03
108	28582		Cuneo	Cuneo	1,11E+04
36	28608	Puglia	Bari	Bari Ovest	7,09E+02
35	28607		Bari	Bari Est	<LOD
37	28609		Taranto	Taranto Bellavista	1,12E+01
38	28610		Taranto	Taranto Gennarini	<LOD

88	28682	Sicilia	Palermo	Acqua dei Corsari	1,66E+03		
89	28683		Palermo	Fondo Verde	1,25E+03		
74	28640		Catania	Pantano d'Arci	<LOD		
75	28641		Catania	Giarre	5,43E+03		
76	28642		Siracusa	Siracusa	2,63E+03		
90	28684		Caltanissetta	Caltanissetta e San Cataldo	1,66E+03		
91	28685		Enna	Enna	1,79E+03		
92	28686		Agrigento	Agrigento	3,46E+03		
93	28604		Trapani	Trapani	1,39E+03		
95	28606		Trapani	Marsala	6,23E+02		
94	28605		Trapani	Mazara del Vallo	4,48E+02	Package A + F456L ^b + R273K	Omicron JN.1*
39	28643	Toscana	Pisa	Pisa Nord - S. Jacopo	<LOD		
109	28617	Umbria	Perugia	Perugia - Pian della Genna	<LOD		
44	28611	Veneto	Treviso	Treviso	2,09E+02		
48	28624		Verona	Verona_collettore 3M	4,34E+03		
49	28625		Verona	Verona_collettore 8M	4,70E+03		
40	28575		Padova	Padova Ca' Nordio - centro storico	NA		
41	28576		Padova	Padova Ca' Nordio - zip	NA		
42	28577		Padova	Padova Guizza	NA		
43	28578		Padova	Abano Terme	NA		
45	28612		Venezia	Venezia Fusina	2,10E+01		
46	28613		Vicenza	Vicenza Casale	<LOD		
47	28623		Verona	Verona_collettore 1M	<LOD		

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 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	DEL211/212	V213G	L216F	H245N	A264D	I332V	G339H	K356T	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	N440K	V445H	G446S	N450D	L452W	L455S	F456L	N460K	VARIANTS
65, 80																																Package A (Omicron JN.1* + F456L)
69, 71, 94																																Package A (Omicron JN.1* + F59S + 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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