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

39th Report

(Study period: November 4th to November 8th, 2024)

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Main findings:

- During the week of 4 November to 8 November 2024, a total of 105 wastewater samples were collected from 16 Regions and 2 Autonomous Province (A.P.).
- Mutations characteristic of the Omicron variant were identified in 10 regions/A.P., while sequencing data were not available from the remaining regions.
- Sanger sequencing analysis showed that 100% of the positive samples carried amino acid substitutions typical of the Omicron JN.1* lineage, including mutations associated with the KP.3* and XEC* 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 39th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 4 November to 8 November 2024.

Methodology

The 38th national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 4 November to 8 November 2024. The survey involved the collection of 105 wastewater samples from 101 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 assignment to sublineages, based solely on the mutations observed in the spike region unfeasible.

Results

Real Time qPCR

Real-time PCR was conducted on 94 out of the 105 samples. Out of the 94 samples analysed, a total of 76 (80.9%) 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 $1.15\text{E} + 02$ to $2.68\text{E} + 05$ genome copies (g.c.) per liter of sewage.

Sanger Sequencing

Table 1 summarises the results of the long-nested PCR assay and sequencing methods. A total of 15/105 samples (14.3%) from 10 Regions/AP 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 ten regions/A.P.: Campania, Emilia-Romagna, Lazio, Liguria, Lombardia, Marche, Sicilia, Veneto and the A.Ps of Trento and Bolzano.

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>

Within this lineage, 53.3% of the samples (8/15 samples) showed mutation associated with the KP.3*/JN.1.16* sublineage and 46.7% (7 samples) to the XEC* 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	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_1034)	SARS-CoV-2 lineages (Sanger sequencing)
98	27828	Abruzzo	Teramo	Villa Pavone	<LOD		
99	27829		L'Aquila	Pile	2,12E+02		
100	27826		Pescara	Villa Carmine	<LOD		
101	27825		Pescara	Via Raiale	<LOD		
102	27827		Chieti	S. Martino	<LOD		
1	27760	Basilicata	Potenza	Tiera di Vaglio	1,00E+04		
2	27761		Matera	Pantano	2,22E+04		
67	27886	Campania	Napoli	Napoli OVEST - ex ingresso Camaldoli	<LOD		
68	27885		Napoli	Napoli OVEST - Ingresso Principale	<LOD		
69	27884		Napoli	Napoli EST	8,55E+03	Package A + F456L ^a	Omicron JN.1*
3	27771	Emilia-Romagna	Ferrara	Ferrara - Linea 1	1,20E+04		
4	27772		Ferrara	Ferrara - Linea 2	<LOD		
5	27773		Modena	Carpi	<LOD		
6	27778		Piacenza	Borgoforte	5,83E+03		
7	27779		Parma	Parma ovest	8,60E+03		
8	27780		Reggio Emilia	Mancasale	1,77E+04		
73	27960		Bologna	IDAR	2,93E+03	Package A + F456L ^a	Omicron JN.1*
74	27961		Modena	Naviglio	7,90E+03		
75	27962		Ravenna - Forlì-Cesena	Ravenna	6,25E+03		
76	27963		Bologna	Imola	2,68E+03	Package A + F59S + F456L ^b	Omicron JN.1*
77	27968		Forlì-Cesena	Forlì	4,48E+03		
78	27966		Forlì-Cesena	Cesena	2,38E+03		
79	27965		Rimini - Forlì-Cesena	S. Giustina	3,50E+03		
95	27881	Friuli-Venezia Giulia	Pordenone	Cordenons	<LOD		
96	27882		Udine	Udine	5,60E+02		
97	27883		Trieste	Servola	6,34E+02		

9	27730	Lazio	Roma	Civitavecchia Fiumaretta	<LOD	Package A + F456L ^a	Omicron JN.1*
10	27735	Liguria	Genova	Punta Vagno Genova	3,80E+04		
11	27736		Genova	Darsena	3,47E+04		
12	27737		Genova	Sturla	3,82E+04		
13	27738		La Spezia	La Spezia	6,99E+04		
14	27739		Imperia	Imperia	3,94E+04		
15	27740		Savona	Borghetto Santo Spirito	2,49E+04	Package A + F59S + F456L ^b	Omicron JN.1*
16	27741		Genova	Pegli	2,68E+05	Package A + F456L ^a	Omicron JN.1*
17	27742		Genova	Voltri	4,91E+04		
18	27743		Genova	Quinto	1,18E+04		
19	27744		Genova	Rapallo	6,59E+04		
20	27745		Genova	Sestri P	2,65E+04		
21	27746		Genova	Valpolcevera	4,48E+04	Package A + F59S + F456L ^b	Omicron JN.1*
22	27747		Savona	Savona	1,28E+04	Package A + F59S + F456L ^b	Omicron JN.1*
23	27781		Genova	Recco	7,38E+03		
55	NA	Lombardia	Sondrio	Sondrio	NA		
56	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA		
57	NA		Monza	Monza	NA	Package A + F456L ^a	Omicron JN.1*
58	NA		Lecco	Lecco	NA		
59	NA		Milano	Milano Nosedo	NA		
60	NA		Milano	Milano San Rocco	NA		
61	NA		Como	Como	NA		
62	NA		Cremona	Cremona	NA		
63	NA		Mantova	Mantova	NA		
64	NA		Brescia	Verziano	NA		
65	NA		Bergamo	Bergamo	NA		
24	27782	Marche	Pesaro-Urbino	Borgheria	2,73E+04		
25	27783		Pesaro-Urbino	Ponte Metauro	2,05E+04		
26	27784		Ancona	Zipa	3,44E+04	Package A + R237M + F456L ^a	Omicron JN.1*
27	27785		Ancona	Falconara	8,60E+03		
28	27786		Ascoli Piceno	Marino del Tronto	3,18E+04		
29	27787		Fermo	Salvano	3,64E+04		

103	27831	Molise	Campobasso	Termoli - località Pantano Basso	<LOD		
104	27830		Campobasso	Termoli - località Porto	<LOD		
105	27832		Campobasso	Campobasso - San Pietro	1,15E+02		
70	27841	A.P. Bolzano	Bolzano	IDA Bolzano	9,70E+03	Package A + F456L ^a	Omicron JN.1*
71	27842		Bolzano	IDA Merano	1,53E+04		
72	27843		Bolzano	IDA Termeno	1,15E+04		
30	27702	A.P. Trento	Trento	Trento nord	6,42E+04	Package A + F456L ^a	Omicron JN.1*
31	27703		Trento	Trento sud	6,48E+04		
32	27704		Trento	Rovereto	6,36E+04	Package A + F59S + F456L ^b	Omicron JN.1*
33	27678	Piemonte	Torino	Castiglione Torinese	4,48E+03		
34	27679		Biella	Biella Nord	<LOD		
35	27680		Biella	Biella Sud	7,50E+02		
36	27681		Novara	Novara	2,50E+03		
37	27711		Alessandria	Alessandria	<LOD		
38	27712		Asti	Asti	7,15E+02		
39	27713		Cuneo	Cuneo	3,35E+04		
40	27686	Puglia	Bari	Bari Est	2,69E+02		
41	27687		Bari	Bari Ovest	3,18E+02		
42	27688		Taranto	Taranto Bellavista	8,60E+02		
43	27689		Taranto	Taranto Gennarini	<LOD		
80	27822	Sicilia	Catania	Pantano d'Arci	<LOD		
81	27823		Catania	Giarre	1,63E+03	Package A + F59S + F456L ^b	Omicron JN.1*
82	27824		Siracusa	Siracusa	3,09E+03		
84	27691		Trapani	Mazara del Vallo	8,20E+02		
85	27692		Trapani	Marsala	7,88E+02		
86	27690		Trapani	Trapani	9,55E+02		
87	27813		Palermo	Acqua dei Corsari	1,85E+03		
88	27814		Palermo	Fondo Verde	<LOD		
89	27815		Palermo	Bagheria	2,06E+03		
90	27816		Enna	Enna	1,38E+03		
91	27817		Agrigento	Agrigento	3,09E+03		
92	27818		Caltanissetta	Caltanissetta e San Cataldo	4,16E+03		
93	27750		Ragusa	Modica	5,75E+02		

94	27751		Ragusa	Vittoria	6,50E+02		
44	27715	Toscana	Pisa	Pisa Nord - S. Jacopo	1,61E+04		
83	27715		Pisa	Pisa Nord - S. Jacopo	1,61E+04		
66	27709	Umbria	Perugia	Perugia - Pian della Genna	4,71E+04		
45	27682	Veneto	Padova	Padova Ca' Nordio - centro storico	4,79E+04		
46	27683		Padova	Padova Ca' Nordio - zip	7,34E+04		
47	27684		Padova	Padova Guizza	5,96E+04		
48	27685		Padova	Abano Terme	2,42E+04		
49	27693		Treviso	Treviso	8,60E+03		
50	27694		Vicenza	Vicenza Casale	<LOD		
51	27695		Venezia	Venezia Fusina	2,62E+02		
52	27732		Verona	Verona_collettore 1M	3,57E+04		
53	27733		Verona	Verona_collettore 3M	4,49E+04	Package A + F59S + F456L ^b	Omicron JN.1*
54	27734		Verona	Verona_collettore 8M	4,12E+04		

NA= Not available

^a 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.

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

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
9, 16, 26, 30, 57, 69, 70, 73																																Package A (Omicron JN.1* + F456L)
15, 21, 22, 32, 53, 76, 81																																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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