Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
38th Report
(Study period: September 30th to October 4th, 2024)

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Main findings:

- During the week of 30 September to 4 October 2024, a total of 106 wastewater samples were collected from 16 Regions and 2 Autonomous Province (A.P.).
- Mutations characteristic of the Omicron variant were identified in 7 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 38th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 30 September to 4 October 2024.

Methodology

The 38th national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 30 September to 4 October 2024. The survey involved the collection of 106 wastewater samples from 102 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 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]. 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 amplifyed, 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 91 of the 106 samples. Out of the 91 samples analysed, a total of 84 (92%) 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 8.49E + 01 to 4.87E + 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 15/106 samples (14.2%) from 7 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 seven regions/A.P.: Emilia-Romagna, Liguria, Molise, Piemonte, Sicilia, Veneto and the A.Ps of Trento.

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% (7/15 samples) with 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)
85	27502	Abruzzo	Teramo	Villa Pavone	3,27E+02		
86	27503		L'Aquila	Pile	1,87E+02		
87	27506		Pescara	Villa Carmine	<LOD		
88	27504		Pescara	Via Raiale	3,27E+02		
89	27505		Chieti	S. Martino	<LOD		
49	27400	Basilicata	Potenza	Tiera di Vaglio	1,42E+04		
50	27402		Matera	Pantano	3,36E+04		
101	27696	Campania	Napoli	Napoli EST	1,61E+04		
102	27697		Napoli	Napoli OVEST - Ingresso Principale	4,70E+04		
103	27698		Napoli	Napoli OVEST - ex ingresso Camaldoli	5,82E+04		
1	27342	Emilia-Romagna	Ferrara	Ferrara - Linea 1	4,07E+03		
2	27343		Ferrara	Ferrara - Linea 2	6,34E+03		
3	27344		Modena	Carpi	2,33E+03		
5	27380		Piacenza	Borgoforte	<LOD		
47	27381		Parma	Parma ovest	9,93E+02		
48	27382		Reggio Emilia	Mancasale	1,95E+03		
70	27487		Modena	Naviglio	5,78E+03		
71	27488		Ravenna - Forlì-Cesena	Ravenna	2,03E+03		
72	27489		Bologna	Imola	1,28E+04	Package A + F456L[a]	Omicron JN.1*
73	27493		Forlì-Cesena	Cesena	2,13E+03		
74	27492		Bologna	IDAR	1,06E+04		
75	27491		Forlì-Cesena	Forlì	7,75E+03		
76	27490		Rimini - Forlì-Cesena	S. Giustina	3,28E+03		
39	27430	Friuli-Venezia Giulia	Trieste	Servola	1,49E+04		
40	27431		Udine	Udine	4,30E+04		
41	27432		Pordenone	Cordenons	5,85E+03		

51	27447	Lazio	Viterbo	Viterbo - Strada Bagni	NA		
52	27448		Roma	Anzio - Colle Cocchino	NA		
53	27449		Latina	Aprilia (Via del Campo)	NA		
54	27450		Latina	Latina Loc Latina Est	NA		
55	27451		Roma	Pomezia - Via Cincinnato	NA		
56	27452		Roma	Velletri (LA CHIUSA-SORBO)	NA		
57	27453		Roma	Guidonia - Ponte Lucano	NA		
93	27395		Roma	Civitavecchia Fiumaretta	<LOD		
6	27352	Liguria	Genova	Pegli	1,81E+05	Package A + F59S + F456L[b]	Omicron JN.1*
7	27353		Genova	Voltri	7,66E+04		
8	27354		Genova	Quinto	1,37E+05	Package A + F59S + F456L[b]	Omicron JN.1*
9	27355		Genova	Rapallo	5,37E+04		
10	27356		Genova	Sestri P	4,81E+04	Package A + F59S + F456L[b]	Omicron JN.1*
11	27358		Genova	Punta Vagno Genova	8,62E+04	Package A + F456L[a]	Omicron JN.1*
12	27359		Genova	Darsena	2,79E+04		
13	27360		Genova	Sturla	5,45E+04	Package A + F456L[a]	Omicron JN.1*
14	27362		Savona	Savona	4,40E+04		
15	27363		Savona	Borghetto Santo Spirito	2,51E+04		
16	27364		Imperia	Sanremo - località Capo Verde	1,09E+05		
17	27383		Genova	Recco	4,10E+03		
31	27357		Genova	Valpolcevera	4,73E+04		
32	27361		La Spezia	La Spezia	2,85E+04		
33	NA	Lombardia	Bergamo	Bergamo	NA		
34	NA		Cremona	Città di Cremona	NA		
35	NA		Mantova	Mantova	NA		
36	NA		Brescia	Verziano	NA		
104	NA		Sondrio	Sondrio	NA		
105	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA		
106	NA		Como - Lecco - Milano - Monza e della Brianza	Monza	NA		
18	27391	Marche	Pesaro-Urbino	Borgheria	5,36E+04		
19	27392		Pesaro-Urbino	Ponte Metauro	1,10E+04		

20	27393		Ancona	Zipa	6,15E+04		
21	27394		Ancona	Falconara	2,85E+04		
37	27497		Ascoli Piceno	Marino del Tronto	7,93E+04		
38	27498		Fermo	Salvano	5,44E+04		
90	27507	Molise	Campobasso	Termoli - località Porto	3,00E+02	Package A + F59S + F456L[b]	Omicron JN.1*
91	27508		Campobasso	Termoli - località Pantano Basso	5,73E+02		
92	27509		Campobasso	Campobasso - San Pietro	3,16E+02	Package A + F59S + F456L[b]	Omicron JN.1*
42	27484	P.A. Bolzano	Bolzano	IDA Bolzano	5,07E+04		
43	27481		Bolzano	IDA Merano	6,56E+04		
44	27483		Bolzano	IDA Termeno	5,00E+04		
22	27384	P.A. Trento	Trento	Trento nord	5,86E+04		
23	27385		Trento	Trento sud	4,38E+04	Package A + F456L[a]	Omicron JN.1*
24	27386		Trento	Rovereto	3,17E+04		
94	27377	Piemonte	Alessandria	Alessandria	1,23E+04		
95	27378		Asti	Asti	2,26E+04		
96	27379		Cuneo	Cuneo	1,30E+05	Package A + F59S + F456L[b]	Omicron JN.1*
97	27387		Torino	Castiglione Torinese	1,54E+04		
98	27388		Biella	Biella Nord	1,30E+03		
99	27389		Biella	Biella Sud	2,00E+04		
100	27390		Novara	Novara	1,31E+04		
25	27301	Puglia	Bari	Bari Est	5,25E+02		
26	27302		Bari	Bari Ovest	<LOD		
45	27303		Taranto	Taranto Bellavista	8,49E+01		
46	27304		Taranto	Taranto Gennarini	8,68E+01		
58	27369	Sicilia	Caltanissetta	Gela Macchitella	2,50E+02		
59	27420		Palermo	Acqua dei Corsari	1,10E+04		
60	27421		Palermo	Fondo Verde	5,07E+03		
61	27422		Caltanissetta	Caltanissetta e San Cataldo	1,49E+04		
62	27423		Palermo	Bagheria	2,87E+03		
63	27424		Enna	Enna	1,77E+04		
64	27313		Trapani	Marsala	9,70E+02		
65	27312		Trapani	Mazara del Vallo	1,27E+03		
66	27311		Trapani	Trapani	1,03E+03		

67	27405		Catania	Pantano d'Arci	2,59E+03		
68	27406		Catania	Giarre	2,47E+04	Package A + F456L[a]	Omicron JN.1*
69	27410		Siracusa	Siracusa	1,18E+04		
77	27428	Toscana	Pisa	Pisa Nord - S. Jacopo	<LOD		
78	27429		Lucca	Pontetetto	<LOD		
107	27341	Umbria	Perugia	Perugia - Pian della Genna	NA		
27	27294	Veneto	Padova	Padova Ca' Nordio - centro storico	1,39E+05		
28	27295		Padova	Padova Ca' Nordio - zip	2,25E+05	Package A + F456L[a]	Omicron JN.1*
29	27296		Padova	Padova Guizza	1,57E+05		
30	27297		Padova	Abano Terme	4,87E+05	Package A + F59S + F456L[b]	Omicron JN.1*
79	27401		Treviso	Treviso	5,57E+04		
80	27403		Venezia	Venezia Fusina	3,37E+03		
81	27404		Vicenza	Vicenza Casale	1,02E+04		
82	27461		Verona	Verona_collettore 1M	2,19E+05	Package A + F456L[a]	Omicron JN.1*
83	27462		Verona	Verona_collettore 3M	7,34E+04		
84	27463		Verona	Verona_collettore 8M	7,75E+04	Package A + F456L[a]	Omicron JN.1*

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
11, 13, 23, 28, 68, 72, 82, 84																																Package A (Omicron JN.1* + F456L)
6, 8, 10, 30, 90, 92, 96																																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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