



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

34th Report

(Study period: June 3th to June 7th, 2024)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Marcello Iaconelli, Luca Lucentini (National Center for Water Safety, Istituto Superiore di Sanità)
- David Brandtner (Department of Infectious Diseases, Istituto Superiore di Sanità)
- Giuseppe D'Avenio, Mirko Rossi (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità)

Main findings:

- During the week of 3 June to 7 June 2024, a total of 100 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 no sequencing data were collected from the remaining areas.
- Analysis of the sequences obtained by Sanger sequencing showed that 100% of the positive samples had amino acid substitutions typical of the Omicron JN.1* lineage.

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 34th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 3 to 7 June 2024.

Methodology

The 34th national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 3 to 7 June 2024. The survey involved the collection of 100 wastewater samples from 96 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, 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 amplifying 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

Out of the 70 samples analysed, a total of 56 (80%) 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 2.69×10^2 to 6.11×10^4 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>, date: 05/12/2023

Table 1 summarises the results of the long-nested PCR assay and sequencing methods. A total of 8 samples (8.0%) from 8 Regions/AP were successfully amplified using the long-nested PCR assay described above. High-quality sequences were obtained from all 8 samples by Sanger sequencing. Analysis of the wastewater samples revealed the presence of one SARS-CoV-2 lineage, as shown in Tables 1 and 2. The Omicron JN.1* lineage was detected in 100% of the positive samples. Within the JN.1* lineage, 37.5% of the samples (3 samples) showed mutation associated with the KP.2* sublineage, 25% (2 samples) to the KP.3*/JN.1.16* sublineage and 12.5% (1 sample) to the JN.1.18* sublineage. Two samples could not be assigned to specific sublineage due to the partial sequence.

For ease of understanding, the mutations have been grouped into panels or "mutation packages". These are 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

The Omicron JN.1* lineage was detected in 8 regions/A.P.: Emilia-Romagna, Liguria, Lombardia, Piemonte, Puglia, Sicilia, Veneto and the A.P of Bolzano.

Table 1. PCR and sequencing results

ID ISS	Sample ID	Region	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_1034)	SARS-CoV-2 lineages (Sanger sequencing)
61	26023	Abruzzo	Pescara	Via Raiale	<LOD		
62	26022		Pescara	Villa Carmine	<LOD		
63	26021		Chieti	S. Martino	<LOD		
64	26020		L'Aquila	Pile	<LOD		
65	26024		Teramo	Villa Pavone	<LOD		
72	26018	Basilicata	Potenza	Tiera di Vaglio	2,75E+03		
73	26019		Matera	Pantano	2,72E+03		
84	26066	Campania	Napoli	Napoli OVEST - Ingresso Principale	1,56E+04		
85	26065		Napoli	Napoli OVEST - ex ingresso Camaldoli	6,83E+03		
86	26064		Napoli	Napoli EST	2,35E+03		
1	25936	Emilia-Romagna	Parma	Parma ovest	<LOD		
2	25937		Reggio Emilia	Mancasale	<LOD		
3	25961		Ferrara	Ferrara - Linea 1	<LOD		
4	25962		Ferrara	Ferrara - Linea 2	<LOD		
5	25963		Modena	Carpi	1,30E+04		
87							
88							
89							
90						Package A + R346T (Partial sequence, from H69del to K417N) ^a	Omicron JN.1*
91							
92							
93							
94							
95							
98	26091	Friuli-Venezia	Udine	Udine	6,44E+03		
99	26092	Giulia	Trieste	Servola	2,74E+03		

100	26093		Pordenone	Cordenons	<LOD		
6	25904	Lazio	Roma	Civitavecchia Fiumaretta			
7	25938	Liguria	Genova	Recco	2,06E+03	Package A + F456L ^b	Omicron JN.1*
8	25939		Savona	Savona	8,22E+03		
9	25940		Imperia	Sanremo - località Capo Verde	4,90E+04		
10	25941		La Spezia	La Spezia	2,26E+04		
11	25942		Genova	Pegli	6,10E+03		
12	25943		Genova	Voltri	1,62E+04		
13	25944		Genova	Quinto	5,50E+04		
14	25945		Genova	Rapallo	5,14E+04		
15	25946		Genova	Sestri P	5,45E+04		
16	25947		Genova	Sturla	6,11E+04		
17	25948		Genova	Darsena	1,35E+04		
18	25949		Genova	Punta Vagno Genova	1,83E+04		
19	25950		Genova	Valpolcevera	1,12E+04		
20	25951		Savona	Borghetto Santo Spirito	6,47E+03		
52	25952	Lombardia	Milano	Milano Nosedo	NA		
53	25953		Milano	Milano San Rocco	NA		
54	25954		Como	Como	NA	Package A + R190S + R346T + F456V ^c	Omicron JN.1*
55	25955		Pavia	Pavia	NA		
56	25956		Como - Lecco - Milano - Monza e della Brianza	Monza	NA		
57	26012		Sondrio	Sondrio	<LOD		
58	25966		Bergamo	Bergamo	5,30E+03		
59	25967		Brescia	Verziano	<LOD		
60	25970		Cremona	Citta di Cremona	5,03E+03		
21	25989	Marche	Pesaro-Urbino	Borgheria	3,38E+03		
22	25990		Pesaro-Urbino	Ponte Metauro	<LOD		
23	25991		Ancona	Zipa	9,78E+03		
24	25992		Ancona	Falconara	1,09E+03		
66	26027	Molise	Campobasso	Termoli - località Pantano Basso	2,69E+02		
67	26025		Campobasso	Termoli - località Porto	6,14E+02		

68	26026		Campobasso	Campobasso - San Pietro	6,14E+02		
78	26051		Bolzano	IDA Bolzano	6,71E+03		
79	26053	P.A. Bolzano	Bolzano	IDA Termeno	5,90E+03	Package A + R346T + F456L ^d	Omicron JN.1* + R346T + F456L
80	26052		Bolzano	IDA Merano	8,18E+03		
25	25897		Trento	Trento nord	1,32E+04		
26	25898	P.A. Trento	Trento	Trento sud	9,16E+03		
27	25899		Trento	Rovereto	7,73E+03		
28	25889		Torino	Castiglione Torinese			
29	25890		Biella	Biella Nord			
30	25891		Biella	Biella Sud			
31	25892	Piemonte	Novara	Novara			
81	25925		Alessandria	Alessandria	<LOD		
82	25926		Asti	Asti	1,08E+04	Package A + R346T + F456L ^d	Omicron JN.1* + R346T + F456L
83	25927		Cuneo	Cuneo	1,75E+04		
32	25906		Bari	Bari Est			
33	25907		Bari	Bari Ovest			
34	25908	Puglia	Taranto	Taranto Bellavista		Package A + R346T (Partial sequence, from H69del to K417N) ^a	Omicron JN.1*
35	25909		Taranto	Taranto Gennarini			
36	25910		Trapani	Trapani	2,08E+03		
37	25911		Trapani	Mazara del Vallo	2,10E+03		
38	25993		Palermo	Acqua dei Corsari	3,29E+03		
39	25994		Palermo	Fondo Verde	1,51E+03		
40	25995		Caltanissetta	Caltanissetta e San Cataldo	7,57E+03		
69	25964	Sicilia	Catania	Pantano d'Archi	2,33E+03		
70	25965		Catania	Giarre	1,10E+03	Package A + R346T + F456L ^d	Omicron JN.1*+ R346T + F456L
71	25969		Siracusa	Siracusa	2,89E+03		
74	25985		Ragusa	Modica	4,25E+02		
75	25986		Ragusa	Vittoria	9,25E+02		
76	25987		Ragusa	Ragusa	7,75E+02		

77	25988		Caltanissetta	Gela Macchitella	2,38E+03		
96	26030	Toscana	Pisa	Pisa Nord - S. Jacopo	NA		
97	26031		Lucca	Pontetetto	NA		
41	25930	Umbria	Perugia	Perugia - Pian della Genna			
42	25900	Veneto	Padova	Padova Ca' Nordio - centro storico			
43	25901		Padova	Padova Ca' Nordio - zip			
44	25902		Padova	Padova Guizza			
45	25903		Padova	Abano Terme			
46	25912		Treviso	Treviso	2,83E+04		
47	25913		Vicenza	Vicenza Casale	4,20E+03		
48	25914		Venezia	Venezia Fusina	3,89E+04		
49	25958		Verona	Verona_collettore 1M	4,79E+04	Package A + F456L ^b	Omicron JN.1*
50	25959		Verona	Verona_collettore 3M	5,34E+04		
51	25960		Verona	Verona_collettore 8M	5,74E+04		

NA= Not available

^a Partial sequence due to mixed electropherograms and/or high signal noise; within brackets the region for which a sequence was provided

^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.

^c The key mutations of Omicron JN.1* in association with R190S, R346T and F456V may indicate the presence of the Omicron JN.1.18* sublineage.

^d The key mutations of Omicron JN.1* in association with R346T and F456L may indicate the presence of the Omicron KP.2* sublineage.

Table 2. Sanger sequencing results

ID SAMPLES	DEL69/70	V127F	G142D	DEL 144	F157S	R158G	R190S	DEL211/212	V213G	L216F	H245N	A264D	I332V	G339H	R346T	K356T	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	N440K	V445H	G446S	N450D	L452W	L455S	F456L	F456V	N460K	VARIANTS
34, 90																																		Package A (Omicron JN.1*, partial sequence)
7, 49																																		Package A (Omicron JN.1* + F456L)
54																																		Package A (Omicron JN.1* + R190S + R346T + F456V)
70, 79, 82																																		Package A (Omicron JN.1* + R346T + 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 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* lineages being the most prevalent. 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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