



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
43rd Report
(Study period: March 3rd to March 7th, 2025)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Agata Franco, Daniele Congiu, 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 March to 7 March 2025, a total of 106 wastewater samples were collected from 16 Regions and two Autonomous Provinces (A.P.s).
- Mutations characteristic of the Omicron variant were identified in two 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 43rd national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 3 March to 7 March 2025.

Methodology

The 43rd national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 3 March to 7 March 2025. The survey involved the collection of 106 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 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 67 of the 103 samples. Out of the 67 samples analysed, a total of 38 (56.7%) 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 1.40E + 01 to 2.81E + 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 4/103 samples (3.9%) from 2 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 two regions: Liguria and Puglia. Within this lineage, 50.0% of the samples (2/4 samples) showed mutation associated with the XEC* sublineage and 50.0% (2/4 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)
1	28995	Abruzzo	Pescara	Villa Carmine	<LOD		
68	28996		Pescara	Via Raiale	<LOD		
69	28997		Chieti	S. Martino	<LOD		
70	28998		L'Aquila	Pile	<LOD		
71	28999		Teramo	Villa Pavone	<LOD		
2	28989	Basilicata	Potenza	Tiera di Vaglio	2,29E+04		
3	28990		Matera	Pantano	1,13E+04		
110	29107	Campania	Napoli	Napoli OVEST - Ingresso Principale	2,81E+04		
111	29109		Napoli	Napoli OVEST - ex ingresso Camaldoli	1,83E+04		
112	29104		Napoli	Napoli EST	<LOD		
4	28914	Emilia-Romagna	Piacenza	Borgoforte	<LOD		
5	28915		Parma	Parma ovest	1,78E+03		
6	28916		Reggio Emilia	Mancasale	<LOD		
81	29037		Forlì-Cesena	Forlì	1,30E+03		
82	29038		Forlì-Cesena	Cesena	<LOD		
83	29039		Ravenna - Forlì-Cesena	Ravenna	<LOD		
84	29040		Modena	Naviglio	<LOD		
85	29041		Bologna	IDAR	<LOD		
86	29042		Rimini - Forlì-Cesena	S. Giustina	<LOD		
87	29043		Bologna	Imola	5,12E+02		
57	29241		Ferrara	Ferrara - Linea 1	<LOD		
58	29242		Ferrara	Ferrara - Linea 2	<LOD		
59	29243		Modena	Carpi	<LOD		
89	28980	Friuli-Venezia Giulia	Pordenone	Cordenons	2,15E+04		
90	28981		Udine	Udine	1,44E+03		

91	28982	Trieste	Servola	<LOD		
88	28971	Roma	Civitavecchia Fiumaretta	<LOD		
103	29072	Viterbo	Viterbo - Strada Bagni	NA		
104	29073	Roma	Anzio - Colle Cocchino	NA		
105	29074	Latina	Aprilia (Via del Campo)	NA		
106	29075	Latina	Latina Loc Latina Est	NA		
107	29076	Roma	Guidonia - Ponte Lucano	NA		
108	29077	Roma	Velletri (LA CHIUSA-SORBO)	NA		
109	29078	Roma	Pomezia - Via Cincinnato	NA		
10	28917	Genova	Recco	<LOD		
41	28946	Genova	Pegli	NA		
42	28947	Genova	Voltri	NA		
43	28948	Genova	Quinto	NA		
44	28949	Genova	Sestri P	NA	Package A + F456L ^a	Omicron JN.1*
45	28950	Genova	Valpolcevera	NA		
46	28951	Genova	Rapallo	NA		
47	28952	Savona	Savona	NA		
48	28953	Genova	Punta Vagno Genova	NA		
49	28954	Genova	Darsena	NA		
50	28955	Genova	Sturla	NA	Package A + F456L ^a	Omicron JN.1*
51	28956	La Spezia	La Spezia	NA		
52	28957	Savona	Borghetto Santo Spirito	NA		
53	NA	Bergamo	Bergamo	NA		
54	NA	Brescia	Verziano	NA		
55	NA	Mantova	Mantova	NA		
56	NA	Cremona	Cremona	NA		
60	NA	Como	Como	NA		
61	NA	Pavia	Pavia	NA		
62	NA	Milano	Milano S. Rocco	NA		
63	NA	Milano	Milano Nosedo	NA		

64	NA		Sondrio	Sondrio	NA	
65	NA		Monza	Monza	NA	
66	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA	
67	NA		Lecco	Lecco	NA	
11	28974	Marche	Pesaro-Urbino	Borgheria	<LOD	
12	28975		Pesaro-Urbino	Ponte Metauro	1,37E+03	
13	28976		Ancona	Zipa	<LOD	
14	28977		Ancona	Falconara	1,49E+03	
15	28978		Ascoli Piceno	Marino del Tronto	1,24E+03	
16	28979		Fermo	Salvano	5,27E+03	
72	28993	Molise	Campobasso	Termoli - località Pantano Basso	<LOD	
73	28992		Campobasso	Termoli - località Porto	<LOD	
74	28994		Campobasso	Campobasso - San Pietro	<LOD	
75	29115	A.P. Bolzano	Bolzano	IDA Bolzano	2,92E+03	
76	29116		Bolzano	IDA Merano	4,50E-01	
77	29117		Bolzano	IDA Termeno	4,03E+02	
20	28921	A.P. Trento	Trento	Trento nord	3,18E+03	
21	28922		Trento	Trento sud	5,04E+03	
22	28923		Trento	Rovereto	1,31E+03	
23	28904	Piemonte	Alessandria	Alessandria	8,48E+03	
24	28905		Asti	Asti	3,90E+03	
25	28906		Cuneo	Cuneo	1,35E+03	
99	28888		Biella	Biella Nord	NA	
100	28889		Novara	Novara	NA	
101	28903		Torino	Castiglione Torinese	2,27E+03	
102	28887		Torino	Castiglione Torinese	NA	
26	28899	Puglia	Bari	Bari Est	1,90E+02	
27	28900		Bari	Bari Ovest	<LOD	
28	28901		Taranto	Taranto Bellavista	<LOD	Package A + F59S + F456L ^b Omicron JN.1*
29	28902		Taranto	Taranto Gennarini	<LOD	Package A + F59S + F456L ^b Omicron JN.1*

+ G72R

78	28972	Sicilia	Catania	Pantano d'Arci	2,19E+03
79	28973		Siracusa	Siracusa	1,92E+03
92	29020		Palermo	Acqua dei Corsari	1,18E+03
93	29022		Caltanissetta	Caltanissetta e San Cataldo	1,40E+02
94	29023		Enna	Enna	3,31E+03
95	29024		Agrigento	Agrigento	7,49E+03
96	28907		Trapani	Trapani	3,53E+02
97	28908		Trapani	Mazara del Vallo	3,30E+02
98	28909		Trapani	Marsala	5,85E+02
80	29000	Toscana	Pisa	Pisa Nord - S. Jacopo	<LOD
30	28933	Umbria	Perugia	Perugia - Pian della Genna	NA
31	28892	Veneto	Padova	Padova Ca' Nordio - centro storico	NA
32	28893		Padova	Padova Ca' Nordio - zip	NA
33	28894		Padova	Padova Guizza	NA
34	28895		Padova	Abano Terme	NA
35	28896		Treviso	Treviso	5,75E+03
36	28897		Venezia	Venezia Fusina	4,78E+03
37	28898		Vicenza	Vicenza Casale	1,54E+04
38	28926		Verona	Verona_collettore 1M	2,58E+03
39	28927		Verona	Verona_collettore 3M	9,38E+03
40	28928		Verona	Verona_collettore 8M	9,75E+02

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
44, 50																																Package A (Omicron JN.1* + F456L)
28, 29																																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.

Acknowledgements

We thank all of the members of the SARI network (“Sorveglianza Ambientale di SARS-CoV-2 attraverso i Reflui urbani in Italia”) for the cooperation in sample collection and processing, data gathering and management, organization and logistic support. The SARI network includes:

- **Abruzzo:** Giuseppe Bucciarelli, Paolo Torlontano (Regione Abruzzo); Giuseppe Aprea, Silvia Scattolini, Vicdalia Aniela Acciari (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale");
- **Basilicata:** Giovanni Canitano, Mariangela Mininni (Regione Basilicata); Rosa Anna Cifarelli, Achille Palma, Giovanna La Vecchia e Giuseppe Lauria (Agenzia Regionale per la Protezione dell'Ambiente Basilicata – ARPAB); Rosanna Brienza e Patrizia Montenegro (Acquedotto Lucano-AQL);
- **Campania:** Vincenzo Giordano (Regione Campania); Luigi Cossentino (Arpac - Agenzia Regionale per la Protezione Ambientale in Campania); Francesca Pennino, Annalisa Lombardi (Università degli Studi di Napoli “Federico II”);
- **Emilia-Romagna:** Paola Angelini, Lisa Gentili (Regione Emilia – Romagna); Laura De Lellis, Daniele Nasci (HERATech); Giovanni Alborali; Nicoletta Formenti, Flavia Guarneri (Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia-Romagna); Nadia Fontani, Giulia Nani, Gianluca Borlone, Marco Guercio (IREN);
- **Friuli Venezia Giulia:** Marika Mariuz e Gabriella Trani (Direzione Centrale Salute FVG); Fabrizio Agnoletti, Martina Ustulin, Chiara Targhetta (Istituto Zooprofilattico Sperimentale delle Venezie)
- **Lazio:** Carla Ancona (DEPLAZIO - Dipartimento di Epidemiologia del Servizio Sanitario Regionale - Regione Lazio); Alessandra Barca, Flavia Serio (Regione Lazio); Doriana Antonella Giorgi, Irene Ferrante, Monica Monfrinotti, Silvia Riosa, Valeria Capparuccini (ARPA Lazio - Agenzia Regionale per la Protezione Ambientale del Lazio); Maria Teresa Scicluna, Antonella Cersini (IZSLT - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana); Mariaconcetta Arizzi, Giancarlo Cecchini, Claudio Ottaviano (Acea Elabiori);
- **Liguria:** Elena Nicosia (Regione Liguria settore tutela della salute negli ambienti di vita e di lavoro); Nadia Fontani, Giulia Nani, Gianluca Borlone, Marco Guercio (Iren); Elena Grasselli; Giorgia Allaria, Lorenzo Dondero, Francesca Rispo (UNIGE - DISTAV); Alberto Izzotti (UNIGE – DIMES); Rosa Maria Bertolotto, Elena Nicosia, Stefano Rosatto, Marta Bellisomi, Irene Tomesani (ARPAL); Micaela Tiso (MICAMO srl);
- **Lombardia:** Emanuela Ammoni, Danilo Cereda (Regione Lombardia); Marina Nadia Losio, Barbara Bertasi (IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia); Desdemona Oliva, Maria Giovanna Guiso, Fabio Ferrari, Maria Mundo ed Antonino Martines (CAP Holding); Sara Castiglioni, Silvia Schiarea, Giulia Salmoiraghi (Istituto Mario Negri IRCCS); Manuela Antonelli, Arianna Azzellino, Francesca Malpei, Andrea Turolla (POLIMI); Sandro Binda, Pellegrinelli Laura, Valeria Primache (Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute), Clementina Cocuzza, Andrea Franzetti, Rosario Musumeci e Marianna Martinelli (Università di Milano-Bicocca); Giorgio Bertanza (Università di Brescia), Maria Luisa Callegari (Università Cattolica del Sacro Cuore);
- **Marche:** Luigi Bolognini, Fabio Filippetti (Regione Marche); Marta Paniccia', Francesca Ciuti, Sara Briscolini (IZSUM - Istituto Zooprofilattico Sperimentale Umbria Marche); Silvia Magi, Elena Ballarini, Annalisa Grucci (ARPAM);

- **Molise:** Michele Colitti (Regione Molise); Carmen Montanaro (ASReM); Giuseppe Aprea, Silvia Scattolini, Daniela D'Angelantonio, Giacomo Migliorati (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"); Maria Grazia Cerroni (Arpa Molise);
- **Piemonte:** Bartolomeo Griglio, Renza Berruti, Mauro Cravero, Angela Costa (Regione Piemonte); Daniela Manila Bianchi, Lucia Decastelli, Angelo Romano; Clara Tramuta (IZSPLV – Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta - SC Sicurezza Alimentare); Elisabetta Carraro, Cristina Pignata (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino), Silvia Bonetta (Dipartimento di Scienze della Vita e Biologia dei Sistemi), Lisa Richiardi (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino);
- **Puglia:** Nehludoff Albano, Giuseppe Di Vittorio, Onofrio Mongelli (Regione Puglia); Osvalda De Giglio, Francesca Apollonio, Francesco Triggiano (Università degli Studi di Bari Aldo Moro - Dipartimento Interdisciplinare di Medicina); Nicola Ungaro (ARPA Puglia);
- **Sicilia:** Carmelo Massimo Maida, Walter Mazzucco, Gina Andolina (Università degli Studi di Palermo-Dipartimento PROMISE - sezione di Igiene); Simona De Grazia, Giovanni Giammanco (Centro di Riferimento Regionale per la Sorveglianza delle Paralisi Flaccide Acute (PFA) e ambientale della circolazione di poliovirus in Sicilia - AOUP Palermo); Giuseppa Purpari (IZS - Istituto Zooprofilattico Sperimentale della Sicilia); Margherita Ferrante; Antonella Agodi, Martina Barchitta (Università degli Studi di Catania - Dipartimento "G. F. Ingrassia");
- **Toscana:** Piergiuseppe Cala' (Regione Toscana); Annalaura Carducci, Marco Verani, Ileana Federigi, Giulia Lauretani, Sara Muzio, Alessandra Pagani (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Università di Pisa);
- **Umbria:** Giovanni Santoro (Regione Umbria), Ermanno Federici, Maya Petricciuolo, Sofia Barigelli (Laboratorio Microbiologia Applicata e Ambientale, DCBB Università di Perugia);
- **Veneto:** Francesca Russo, Gisella Pitter, Vanessa Groppi (Regione Veneto); Franco Rigoli, Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto); Tatjana Baldovin, Irene Amoruso (Università di Padova);
- **P.A. Bolzano:** Lorella Zago (P.A. Bolzano); Alberta Stenico, Anna-Maria Prast, Morelli Marco, Dossena Matteo (A.P.P.A. Agenzia provinciale per l'ambiente e la tutela del clima, Laboratorio biologico)
- **P.A. Trento:** Francesco Pizzo; Andrea Anselmo, Elena Mengon (P. A. Trento); Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Paola Foladori, Serena Manara (UNITN – Università di Trento).