Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
40th Report
(Study period: December 2nd to December 6th, 2024)

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Main findings:

- During the week of 2 December to 6 December 2024, a total of 111 wastewater samples were collected from 16 Regions and 2 Autonomous Province (A.P.).
- Mutations characteristic of the Omicron variant were identified in 9 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 XEC*, KP.3*, LF.7* and LP.8.1 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 40th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, conducted from 2 December to 6 December 2024.

Methodology

The 38th national Flash Survey on SARS-CoV-2 variants in wastewater samples was carried out in Italy from 2 December to 6 December 2024. The survey involved the collection of 111 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 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 85 of the 111 samples. Out of the 85 samples analysed, a total of 71 (83.5%) 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 1.05E + 02 to 3.62E + 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 17/111 samples (15.3%) from 9 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 nine regions/A.P.: Campania, Emilia-Romagna, Lazio, Liguria, Piemonte, Sicilia, Veneto and the A.Ps of Trento and Bolzano.

Within this lineage, 47.1% of the samples (8/17 samples) showed mutation associated with the XEC* sublineage, 41.1% (7/17 samples) with the KP.3*/JN.1.16* sublineage and 5.9% (1/17 samples) with the LF.7* sublineage and 5.9% (1/17 samples) with the LP.8.1* 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)
83	28131	Abruzzo	Teramo	Villa Pavone	<LOD		
84	28130		L'Aquila	Pile	2,98E+02		
85	28128		Pescara	Villa Carmine	1,30E+02		
86	28127		Pescara	Via Raiale	<LOD		
87	28129		Chieti	S. Martino	<LOD		
1	28058	Basilicata	Potenza	Tiera di Vaglio	8,92E+03		
2	28059		Matera	Pantano	9,21E+03		
112	NA	Campania	Napoli	Napoli OVEST - Ingresso Principale	NA		
113	NA		Napoli	Napoli OVEST - ex ingresso Camaldoli	NA	Package A + F59S[a]	Omicron JN.1*
114	NA		Napoli	Napoli EST	NA		
3	28035	Emilia-Romagna	Piacenza	Borgoforte	<LOD		
4	28036		Parma	Parma ovest	<LOD	Package A + F59S + K150N[a]	Omicron JN.1*
5	28037		Reggio Emilia	Mancasale	3,10E+02		
73	28230		Ferrara	Ferrara - Linea 1	1,45E+04		
74	28231		Ferrara	Ferrara - Linea 2	<LOD		
75	28232		Modena	Carpi	6,45E+03		
76	28208		Modena	Naviglio	3,18E+03		
77	28209		Bologna	IDAR	3,95E+03		
78	28210		Ravenna - Forlì-Cesena	Ravenna	7,33E+03	Package A + F456L[b]	Omicron JN.1*
79	28211		Forlì-Cesena	Forlì	1,20E+03	Package A + F182R + R190S + R346T + K444R + F456L[c]	Omicron JN.1*
80	28212		Forlì-Cesena	Cesena	1,09E+04		
81	28213		Bologna	Imola	7,60E+03		
82	28214		Rimini - Forlì-Cesena	S. Giustina	1,19E+04		
109	28161	Friuli-Venezia Giulia	Udine	Udine	6,08E+03		
110	28162		Trieste	Servola	8,95E+03		
111	28163		Pordenone	Cordenons	1,42E+03		

6	27967	Lazio	Roma	Civitavecchia Fiumaretta	<LOD		
54	NA		Viterbo	Strada Bagni	NA		
55	NA		Anzio	Anzio-Colle Cocchino	NA		
56	NA		Aprilia	Aprilia-Via del Campo	NA		
57	NA		Latina	Latina Est	NA	Package A + F59S[a]	Omicron JN.1*
58	NA		Velletri	La Chiusa	NA		
59	NA		Tivoli	Ponte Lucano di Guidonia	NA		
60	NA		Pomezia	Pomezia Capoluogo	NA		
7	28004	Liguria	Genova	Pegli	8,60E+03		
8	28005		Genova	Voltri	1,55E+04	Package A + F59S[a]	Omicron JN.1*
9	28006		Genova	Quinto	2,36E+04		
10	28007		Genova	Rapallo	2,58E+04		
11	28008		Genova	Sestri P	9,09E+03		
12	28009		Genova	Valpolcevera	1,63E+03		
13	28010		Imperia	Sanremo - località Capo Verde	<LOD		
14	28011		Genova	Punta Vagno Genova	2,51E+04		
15	28012		Genova	Darsena	2,48E+04		
16	28013		Genova	Sturla	3,48E+04	Package A + A435S + F456L[b]	Omicron JN.1*
17	28014		Savona	Savona	<LOD		
18	28015		La Spezia	La Spezia	3,69E+03		
19	28016		Imperia	Imperia	1,19E+04	Package A + F456L[b]	Omicron JN.1*
20	28028		Savona	Borghetto Santo Spirito	1,70E+03		
21	28038		Genova	Recco	4,03E+03		
61	NA	Lombardia	Como	Como	NA		
62	NA			Pavia	NA		
63	NA		Milano	Milano Nosedo	NA		
64	NA		Milano	Milano S. Rocco	NA		
65	NA		Milano - Monza e della Brianza	Peschiera Borromeo	NA		
66	NA		Sondrio	Sondrio	NA		
67	NA		Lecco	Lecco	NA		

68	NA		Monza	Monza	NA		
69	NA		Bergamo	Bergamo	NA		
70	NA		Brescia	Verziano	NA		
71	NA		Cremona	Cremona	NA		
72	NA		Mantova	Mantova	NA		
22	28052	Marche	Pesaro-Urbino	Borgheria	8,20E+03		
23	28053		Pesaro-Urbino	Ponte Metauro	1,44E+04		
24	28054		Ancona	Zipa	1,09E+04		
25	28055		Ancona	Falconara	3,60E+03		
26	28056		Ascoli Piceno	Marino del Tronto	1,71E+04		
27	28057		Fermo	Salvano	1,69E+04		
88	28134	Molise	Campobasso	Termoli - località Pantano Basso	<LOD		
89	28133		Campobasso	Termoli - località Pantano Basso	4,08E+02		
90	28132		Campobasso	Campobasso - San Pietro	1,05E+02		
94	28121	P.A. Bolzano	Bolzano	IDA Bolzano	2,90E+03	Package A + F59S[a]	Omicron JN.1*
95	28122		Bolzano	IDA Merano	6,47E+03		
96	28123		Bolzano	IDA Termeno	2,71E+03		
28	28001	P.A. Trento	Trento	Trento nord	2,13E+04		
29	28002		Trento	Trento sud	1,45E+04		
30	28003		Trento	Rovereto	1,80E+04	Package A + F456L[b]	Omicron JN.1*
31	27956	Piemonte	Torino	Castiglione Torinese	3,23E+03	Package A + F186L + R190S + R346T + A 411S + V445R + F456L[d]	Omicron JN.1*
32	27957		Biella	Biella Nord	1,05E+03		
33	27958		Biella	Biella Sud	3,40E+03		
34	27959		Novara	Novara	4,85E+03		
91	27994		Alessandria	Alessandria	7,35E+03		
92	27995		Asti	Asti	1,77E+03		
93	27996		Cuneo	Cuneo	1,43E+04		
35	27979	Puglia	Bari	Bari Est	3,93E+02		
36	27980		Bari	Bari Ovest	3,13E+02		
37	27981		Taranto	Taranto Bellavista	3,44E+02		

38	27982		Taranto	Taranto Gennarini	<LOD		
97	28039	Sicilia	Catania	Pantano d'Arci	NA		
98	28040		Catania	Giarre	NA		
99	28041		Siracusa	Siracusa	NA		
101	27984		Trapani	Mazara del Vallo	1,74E+03		
102	27983		Trapani	Trapani	1,43E+03		
103	27985		Trapani	Marsala	3,37E+03		
104	28105		Palermo	Acqua dei Corsari	1,20E+03		
105	28106		Palermo	Fondo Verde	5,85E+02		
106	28107		Caltanissetta	Caltanissetta e San Cataldo	2,07E+03		
107	28108		Enna	Enna	3,73E+03	Package A + F456L[b]	Omicron JN.1*
108	28109		Agrigento	Agrigento	4,84E+03		
42	28017	Toscana	Pisa	Pisa Nord - S. Jacopo	NA		
100	28084		Lucca	Pontetetto	<LOD		
43	28000	Umbria	Perugia	Perugia - Pian della Genna	1,34E+04		
44	27975	Veneto	Padova	Padova Ca' Nordio - centro storico	2,78E+04	Package A + Y248H + F456L[b]	Omicron JN.1*
45	27976		Padova	Padova Ca' Nordio - zip	3,18E+04		
46	27977		Padova	Padova Guizza	1,73E+04		
47	27978		Padova	Abano Terme	2,03E+04		
48	27986		Treviso	Treviso	<LOD		
49	27987		Venezia	Venezia Fusina	1,03E+04	Package A + F456L[b]	Omicron JN.1*
50	27988		Vicenza	Vicenza Casale	<LOD		
51	28019		Verona	Verona_collettore 1M	1,60E+04	Package A + F59S + V62F + K182R[a]	Omicron JN.1*
52	28020		Verona	Verona_collettore 3M	3,43E+04	Package A + F59S[a]	Omicron JN.1*
53	28021		Verona	Verona_collettore 8M	3,62E+04	Package A + F59S[a]	Omicron JN.1*

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.

[c] The key mutations of Omicron JN.1* in association with F182R, R190S, R346T, K444R, V455R and F456L may indicate the presence of the Omicron LF.7* sublineage.

[d] The key mutations of Omicron JN.1* in association with F186L, R190S, R346T, V455R and F456L may indicate the presence of the Omicron LP.8.1 sublineage

Table 2. Sanger sequencing results

ID SAMPLES	F59S	DEL69/70	V127F	G142D	DEL 144	F157S	R158G	F182R	F186L	R190S	DEL211/212	V213G	L216F	H245N	A264D	I332V	G339H	R346T	K356T	S371F	S373P	S375F	T376A	R403K	D405N	R408S	K417N	N440K	K444R	V445R	V445H	G446S	N450D	L452W	L455S	F456L	N460K	VARIANTS
16, 19, 30, 44, 49, 78, 107		■	■	■	■	■	■				■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	**Package A (Omicron JN.1* + F456L)**
4, 8, 51, 52, 53, 57, 94, 113	■	■	■	■	■	■	■				■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	**Package A (Omicron JN.1* + F59S + F456L)**
79		■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	**Package A (Omicron JN.1* + F182R + R190S + R346T + K444R + V455R + F456L)**
31		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	**Package A (Omicron JN.1* + F186L + R190S + R346T + V455R + 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:** Michele La Bianca (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 Elabori);
- **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); Manila Bianchi, Lucia Decastelli; Angelo Romano; Clara Tramuta (IZSTO - Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta SC Sicurezza e Qualità degli Alimenti); 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:** Mario Palermo (Regione Sicilia); Carmelo Massimo Maida, Walter Mazzucco (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), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento).