# Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
## 10th Report
## (Study period: 6 – 10 June 2022)

**Edited by:**

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Marcello Iaconelli, Luca Lucentini, Lucia Bonadonna (Department of Environment and Health, Istituto Superiore di Sanità)
- David Brandtner (independent researcher)
- Mauro Grigioni (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Mirko Rossi (independent researcher)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità)

**Main findings:**
- Overall, 167 wastewater samples were collected in the week 6-10 June from 18 Regions and 2 Autonomous Provinces (A.P.).
- Mutations characteristic of the Omicron variant were detected in 13 Regions and two A.P. while no sequences were obtained from the remaining Regions.
- Sublineages BA.4/BA.5 were detected in 54% of the samples by Sanger sequencing with a slight prevalence of BA.5.
- Aminoacid substitutions of sublineages BA.2 were detected in 43% of the sequences.
- NGS results confirmed the co-occurrence of sublineages BA.4/BA.5 and BA.2 in the vast majority of the Regions/A.P.

## Introduction

On 17 March 2021, the "EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU", strongly encouraged Member States to put in place, no later than 1 October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants[1].

Following the above EU Recommendation, the Istituto Superiore di Sanità (ISS) instituted "flash surveys", i.e. periodic (monthly) sampling campaigns to be held in different locations in Italy over the course of a brief period, aimed at assessing the diversity of SARS-CoV-2 in wastewater in the country.

The aim of this report is to summarize the results of the tenth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 6-10 June 2022.

## Methodology

The survey included 167 sewage samples collected between 6 and 10 June 2022 at 167 wastewater treatment plants (WTPs) located in 17 regions and 2 autonomous provinces (A.P.). Details on WTPs enrolled in the Surveillance of SARS-CoV-2 in urban wastewater in Italy can be found at the ISS website[2]. Samples were processed by the laboratories of the SARI network (see Acknowledgements). Viral concentration measurements and nucleic acid extraction were performed according to the protocol "Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3". Purified RNAs were shipped in dry ice to ISS, where samples were sequenced as previously described[3] with some modifications.

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[1] Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472&qid=1628798981209)

[2] 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)

[3] G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferro, D. Brandtner, A. Fasanella, L. Pace, A. Parisi, D. Galante, E. Suffredini. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein, Water Research, 2021, Volume 197, 1 June 2021, 117104. https://doi.org/10.1016/j.watres.2021.117104.

A long nested RT-PCR assay (ID_980, ~1600 bps, spanning amino acid residues 58 to 573 of the spike protein) was used to detect multiple key nucleotide changes (deletion and/or amino acid substitutions) distinctive of the Variants of Concern (VoCs) and Variants of Interest (VoIs).

The amplicons from the long nested assay were sequenced by both Sanger and Next Generation Sequencing (NGS), using the Oxford Nanopore Technology MinION platform. For NGS, the amplicons obtained from different samples collected in the same Region were mixed in a single pool. Bioinformatics analysis of NGS data was carried out as described in La Rosa et al., 2021[4]. Variant calling was performed for the currently recognized VoCs[5].

Since the Omicron sublineages BA.4 and BA.5 have identical spike proteins in the sequenced region, a new nested PCR was designed able to detect a mutation differentiating the Omicron sublineages BA.4 and BA.5, specifically D3N located in the M gene, in order to discriminate between the two subvariants.

## Results

Overall, 146 of the 167 samples (87%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 environmental surveillance (**Table 1**), viral concentrations ranging from 2.6 E+01 to 3.0 E+05 genome copies (g.c.)/L of sewage.

*Sanger Sequencing*

Real-time PCR, long nested PCR, and sequencing results are summarized in **Tables 1-2**. Overall, 38 samples from 15 Regions/A.P. were amplified by the long PCR assay. Long amplicons could not be obtained from samples collected in the Regions of Abruzzo, Campania, Marche, Molise and Valle d'Aosta. Sanger sequences were obtained for 35 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained for three samples which, therefore, could not be assigned. All amplicons were characterized as Omicron variant by Sanger sequencing. Specifically, amino acid substitutions of the sublineage Omicron **BA.4/5** (characterized by the amino-acid substitutions L452R and F486V in addition to those typical of BA.2) were detected in 19 samples (54%) from 8 Regions/A.P. (Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Puglia, Veneto and A.P. of Bolzano). Using the newly designed assay targeting the M gene, 8 of these 19 samples were assigned to Omicron BA.5 (presence of mutation D3N in the M gene), 7 to BA.4, and 4 showed the presence of both subvariants (presence of a double A/G peak in correspondence to the mutation site). Mutation of the Omicron sublineage **BA.2** were detected in 15 samples (43%) from 9 Regions/A.P. (Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Sicilia, Umbria and A.P. of Bolzano and Trento). Moreover, one sample, collected in the Region of Calabria, showed the presence of the characteristic mutation of BA.2, plus L452R (but not F486V) and could not be assigned since this panel of mutations is shared by BA.2.11, BA.2.35, and BA.2.28.

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[4] G La Rosa, D. Brandtner, P. Mancini, C. Veneri, G. Bonanno Ferraro, L. Bonadonna, L. Lucentini, E. Suffredini. Key SARS-CoV-2 mutations of Alpha, Gamma and Eta variants detected in urban wastewaters in Italy by long-read amplicon sequencing based on nanopore technology. *Water* 2021, 13(18), 2503; https://doi.org/10.3390/w13182503

[5] SARS-CoV-2 variants of concern as of 9 June 2022. SARS-CoV-2 variants of concern as of 9 June 2022 (europa.eu)

*Next Generation Sequencing*

NGS results could be successfully obtained for all the 15 tested pools, all of which showing characteristic mutations of the Omicron variant. Overall, 57.9% of the reads were assigned to Omicron BA.4/5, and 42.1% to BA.2.

Key mutations of the Omicron sublineages BA.4/5 and BA.2 were detected simultaneously in 13 Regions (Calabria, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Piemonte, Puglia, Sicilia, Toscana, Umbria, Veneto and A.P. of Trento and Bolzano). In two Regions, only one Omicron sublineage was identified (BA.4/5 in Lombardia and BA.2 in Basilicata).

Sequencing results are summarized in **Table 1**. To improve readability of the table, mutations were combined into panels ('mutation packages') as follow:

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, **F486V**, Q498R, N501Y, Y505H)
- **Package B** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)
- **Package C** (Unassigned, shared among Omicron BA.2.11/BA.2.35/ BA.2.28, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, **L452R**, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)

**Table 1. PCR and sequencing results (long PCR ID 980)**

| | Sample ID | Region/A.P. | City | WTP | RT-qPCR (c.g./L) | Mutations found by Sanger sequencing (long PCR ID_980) | SARS-CoV-2 variant (Sanger sequencing) | Sequencing results (NGS) | SARS-CoV-2 variant (NGS) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 10988 | Abruzzo | Chieti | S. Martino | <LOD | - | | | |
| 2 | 10992 | | Pescara | Via Raiale | <LOD | - | | | |
| 3 | 10994 | | Pescara | Villa Carmine | 1,25E+02 | - | | | |
| 4 | 10996 | | L'Aquila | Pile | <LOD | - | | | |
| 5 | 10997 | | Teramo | Villa Pavone | <LOD | - | | | |
| 6 | 11132 | Basilicata | Potenza | Tiera di Vaglio | 1,31E+03 | M.E. [a] | | • Package B | • Omicron BA.2 |
| 7 | 11133 | | Matera | Pantano | <LOD | - | | | |
| 9 | 11178 | Calabria | Cosenza | Cosenza - Sant'Angelo | 5,82E+01 | - | | • Package A | • Omicron BA.4/5 |
| 10 | 11179 | | Crotone | Crotone - località Papaniciaro | 4,61E+01 | - | | • Package B | • Omicron BA.2 |
| 11 | 11180 | | Catanzaro | Catanzaro Lido - Loc. Verghello | 2,93E+01 | - | | | |
| 159 | 11181 | | Catanzaro | Catanzaro - Zona industriale | 2,98E+01 | - | | | |
| 160 | 11182 | | Reggio Calabria | Ravagnese - località Aeroporto | 2,61E+01 | Package C | unassigned [b] | | |
| 13 | 11149 | Campania | Salerno | Salerno | 2,88E+03 | - | | | |
| 14 | 11150 | | Salerno | Nocera Sup | 4,39E+04 | - | | | |
| 15 | 11151 | | Avellino | Manocalzati | <LOD | - | | | |
| 16 | 11152 | | Napoli | Napoli OVEST - Ingresso Principale | 3,22E+04 | - | | | |
| 17 | 11153 | | Napoli | Napoli OVEST - ex ingresso Camaldoli | 1,51E+04 | - | | | |
| 18 | 11155 | | Napoli | Area Nolana | 3,37E+04 | - | | | |
| 19 | 11156 | | Napoli | Napoli EST | 4,88E+03 | - | | | |
| 20 | 11161 | | Caserta | Area Casertana | 1,14E+04 | - | | | |

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|---|---|---|---|---|---|---|---|---|---|
| 21 | 11162 | | Caserta | Villa Literno | 3,91E+04 | - | | | |
| 22 | 11030 | | Ferrara | Ferrara - Linea 1 | <LOD | - | | • Package A | • Omicron BA.4/5 |
| 23 | 11031 | | Ferrara | Ferrara - Linea 2 | <LOD | - | | • Package B | • Omicron BA.2 |
| 24 | 11032 | | Modena | Carpi | <LOD | - | | | |
| 25 | 11117 | | Parma | Parma ovest | 4,73E+04 | - | | | |
| 26 | 11119 | | Reggio Emilia | Mancasale | 1,71E+04 | - | | | |
| 27 | 11121 | | Piacenza | Borgoforte | 3,10E+03 | - | | | |
| 28 | 11093 | Emilia-Romagna | Bologna | IDAR | 2,38E+04 | - | | | |
| 29 | 11094 | | Ravenna | Faenza | 1,40E+04 | - | | | |
| 30 | 11095 | | Bologna | Imola | 2,98E+04 | - | | | |
| 31 | 11097 | | Ravenna - Forlì-Cesena | Ravenna | 1,33E+04 | - | | | |
| 32 | 11103 | | Forlì-Cesena | Cesena | 2,20E+04 | - | | | |
| 33 | 11104 | | Forlì-Cesena | Forlì | 1,34E+04 | Package B | Omicron BA.2 | | |
| 34 | 11106 | | Modena | Naviglio | 2,07E+04 | - | | | |
| 35 | 11107 | | Rimini - Forlì-Cesena | S. Giustina | 2,55E+04 | Package A | Omicron BA.4/5[c] | | |
| 36 | 11136 | | Pordenone | Cordenons | 1,15E+05 | Package A | Omicron BA.4/5[d] | • Package A | • Omicron BA.4/5 |
| 37 | 11137 | Friuli-Venezia Giulia | Udine | Udine | 1,17E+04 | - | | • Package B | • Omicron BA.2 |
| 38 | 11138 | | Trieste | Servola | 3,23E+04 | Package B | Omicron BA.2 | | |
| 39 | 10937 | | Viterbo | Viterbo - Strada Bagni | 4,88E+03 | Package A | Omicron BA.4/5[c] | • Package A | • Omicron BA.4/5 |
| 40 | 10938 | | Roma | Anzio - Colle Cocchino | 6,20E+02 | - | | • Package B | • Omicron BA.2 |
| 41 | 10939 | | Latina | Aprilia (Via del Campo) | 7,90E+03 | - | | | |
| 42 | 10940 | | Latina | Latina Loc Latina Est | 1,93E+03 | - | | | |
| 43 | 10941 | Lazio | Roma | Guidonia - Ponte Lucano | 7,70E+03 | - | | | |
| 44 | 10942 | | Roma | Velletri (LA CHIUSA-SORBO) | 4,98E+03 | Package A | Omicron BA.4/5[e] | | |
| 45 | 10943 | | Roma | Pomezia - Via Cincinnato | 2,70E+03 | - | | | |
| 46 | 11057 | | Roma | Civitavecchia Fiumaretta | 2,50E+02 | - | | | |

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|---|---|---|---|---|---|---|---|---|---|
| 47 | 11109 | | Roma | Roma Est (linea 1 + linea 2) | 5,26E+04 | Package B | Omicron BA.2 | | |
| 48 | 11110 | | Roma | Roma Nord | 1,45E+04 | - | | | |
| 49 | 11111 | | Roma | Roma Sud | 4,10E+03 | - | | | |
| 50 | 11112 | | Roma | Ostia | 7,33E+03 | - | | | |
| 51 | 11113 | | Roma | Fregene | 3,23E+03 | - | | | |
| 52 | 11033 | | Savona | Savona | 3,46E+04 | - | | • Package A | • Omicron BA.4/5 |
| 53 | 11034 | | Genova | Pegli | 2,73E+04 | Package B | Omicron BA.2 | • Package B | • Omicron BA.2 |
| 54 | 11035 | | Genova | Voltri | 7,81E+03 | - | | | |
| 55 | 11036 | | Genova | Quinto | 1,37E+05 | Package B | Omicron BA.2 | | |
| 56 | 11037 | | Genova | Rapallo | 5,09E+04 | - | | | |
| 57 | 11038 | | Genova | Sestri P | 6,84E+04 | - | | | |
| 58 | 11039 | | Genova | Sturla | 5,73E+04 | Package B | Omicron BA.2 | | |
| 59 | 11040 | | La Spezia | Camisano | 1,13E+04 | - | | | |
| 60 | 11041 | | La Spezia | Silea | 1,19E+05 | Package B | Omicron BA.2 | | |
| 61 | 11042 | Liguria | La Spezia | La Spezia | 2,74E+04 | - | | | |
| 62 | 11043 | | Imperia | Sanremo - località Capo Verde | 2,08E+04 | - | | | |
| 63 | 11044 | | Savona | Borghetto Santo Spirito | 3,54E+03 | - | | | |
| 64 | 11045 | | Genova | Darsena | 1,03E+04 | - | | | |
| 65 | 11046 | | Genova | Punta Vagno Genova | 6,80E+04 | Package A | Omicron BA.4/5[e] | | |
| 66 | 11047 | | Genova | Valpolcevera | 2,75E+04 | Package A | Omicron BA.4/5[d] | | |
| 158 | 11114 | | Genova | Punta Vagno Genova | 3,08E+05 | Package A (Partial, from G339D to Y505H)[f] | Omicron BA.4/5[d] | | |
| 67 | 10962 | | Milano | Milano Nosedo | 1,17E+04 | Package A | Omicron BA.4/5[d] | • Package A | • Omicron BA.4/5 |
| 68 | 10963 | | Milano | Milano San Rocco | 7,35E+03 | - | | | |
| 69 | 10964 | Lombardia | Como | Como | 2,10E+04 | - | | | |
| 70 | 10965 | | Pavia | Pavia | 5,46E+03 | - | | | |

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|---|---|---|---|---|---|---|---|---|
| 71 | 10966 | Como - Lecco - Milano - Monza e della Brianza | Monza | 3,56E+04 | - | | | |
| 73 | 10976 | Milano | Bresso | 3,68E+04 | - | | | |
| 74 | 10977 | Milano - Monza e della Brianza | Peschiera Borromeo | 2,55E+04 | - | | | |
| 75 | 10978 | Milano - Varese | Canegrate | 8,68E+04 | - | | | |
| 76 | 10979 | Varese | Varese | 3,00E+04 | - | | | |
| 77 | 10980 | Milano - Varese | Lonate Pozzolo | 3,50E+04 | - | | | |
| 78 | 11091 | Sondrio | Sondrio | 7,13E+04 | - | | | |
| 79 | 11098 | Bergamo | Bergamo | 1,27E+04 | - | | | |
| 80 | 11099 | Cremona | Citta di Cremona | <LOD | - | | | |
| 81 | 11101 | Brescia | Verziano | 4,21E+03 | - | | | |
| 82 | 11048 | | Pesaro-Urbino | Borgheria | 1,81E+04 | - | | |
| 83 | 11049 | | Pesaro-Urbino | Ponte Metauro | 2,80E+03 | - | | |
| 84 | 11050 | Marche | Pesaro-Urbino | Ponte Sasso | 7,86E+03 | - | | |
| 85 | 11051 | | Ancona | Zipa | 1,88E+04 | - | | |
| 86 | 11052 | | Ancona | Falconara | 7,31E+03 | - | | |
| 87 | 11053 | | Ancona | Camerano | 3,54E+03 | - | | |
| 88 | 10982 | | Campobasso | Termoli - località Porto | <LOD | - | | |
| 89 | 10984 | Molise | Campobasso | Termoli - località Pantano Basso | 1,25E+02 | - | | |
| 90 | 10973 | | Bolzano | IDA Bolzano | 3,20E+04 | Package A | Omicron BA.4/5[e] | • Package A | • Omicron BA.4/5 |
| 91 | 10974 | A.P. Bolzano | Bolzano | IDA Merano | 1,61E+04 | Package B | Omicron BA.2 | • Package B | • Omicron BA.2 |
| 92 | 10975 | | Bolzano | IDA Termeno | 4,48E+04 | Package A | Omicron BA.4/5[d] | | |
| 93 | 10949 | | Trento | Trento nord | 4,16E+04 | - | | • Package A | • Omicron BA.4/5 |
| 94 | 10950 | A.P. Trento | Trento | Trento sud | 7,24E+04 | - | | • Package B | • Omicron BA.2 |
| 95 | 10951 | | Trento | Rovereto | 3,47E+04 | Package B | Omicron BA.2 | | |
| 96 | 10900 | | Torino | Castiglione Torinese | 7,50E+02 | M.E. [a] | | • Package A | • Omicron BA.4/5 |
| 97 | 10901 | Piemonte | Biella | Biella Nord | 3,08E+02 | - | | • Package B | • Omicron BA.2 |
| 98 | 10902 | | Biella | Biella Sud | 5,00E+01 | - | | | |

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|---|---|---|---|---|---|---|---|---|---|
| 99 | 10903 | | Novara | Novara | 1,50E+02 | - | | | |
| 100 | 10987 | | Alessandria | Alessandria | 1,32E+03 | - | | | |
| 101 | 10989 | | Asti | Asti | 7,60E+02 | - | | | |
| 102 | 10990 | | Cuneo | Cuneo | 4,83E+03 | - | | | |
| 103 | 10871 | | Bari | Bari Est | 3,62E+03 | Package A | Omicron BA.4/5[c] | • Package A | • Omicron BA.4/5 |
| 104 | 10872 | | Bari | Bari Ovest | 1,91E+03 | - | | • Package B | • Omicron BA.2 |
| 105 | 10904 | | Brindisi | Brindisi Fiume Grande | 3,93E+03 | - | | | |
| 106 | 10905 | | Lecce | Lecce | 2,71E+03 | - | | | |
| 107 | 10906 | | Taranto | Taranto Bellavista | 9,10E+02 | - | | | |
| 108 | 10907 | | Taranto | Taranto Gennarini | 3,34E+03 | - | | | |
| 109 | 10952 | | Foggia | Foggia | 3,39E+03 | Package A (Partial, from G339D to Y505H)[f] | Omicron BA.4/5[e] | | |
| 110 | 10953 | | Foggia | Manfredonia | 8,34E+03 | - | | | |
| 111 | 10954 | Puglia | Foggia | Cerignola | 7,65E+03 | Package A | Omicron BA.4/5[c] | | |
| 112 | 10983 | | Barletta-Andria-Trani | Andria | 1,98E+03 | - | | | |
| 113 | 10985 | | Barletta-Andria-Trani | Barletta | 1,46E+04 | - | | | |
| 114 | 10991 | | Barletta-Andria-Trani | Trani | 1,19E+05 | - | | | |
| 115 | 10993 | | Bari | Molfetta | 1,86E+04 | - | | | |
| 116 | 10995 | | Barletta-Andria-Trani | Bisceglie | 2,89E+04 | Package A | Omicron BA.4/5[d] | | |
| 117 | 11007 | | Bari | Bitonto | 2,20E+04 | - | | | |
| 118 | 11092 | | Bari | Altamura | 5,60E+03 | Package A | Omicron BA.4/5[d] | | |
| 119 | 11022 | | Agrigento | Agrigento | 4,14E+04 | - | | • Package A | • Omicron BA.4/5 |
| 120 | 11023 | | Enna | Enna | 6,26E+04 | - | | • Package B | • Omicron BA.2 |
| 121 | 11025 | Sicilia | Palermo | Acqua dei Corsari | 3,11E+04 | - | | | |
| 122 | 11026 | | Palermo | Fondo Verde | 5,16E+04 | - | | | |
| 123 | 11027 | | Caltanissetta | Caltanissetta e San Cataldo | 6,21E+04 | Package B | Omicron BA.2 | | |

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|---|---|---|---|---|---|---|---|---|---|
| 124 | 11054 | | Trapani | Trapani | 7,31E+03 | - | | | |
| 125 | 11055 | | Trapani | Mazara del Vallo | 1,72E+03 | - | | | |
| 126 | 11056 | | Trapani | Marsala | 4,60E+03 | - | | | |
| 127 | 11058 | | Ragusa | Modica | 3,98E+04 | - | | | |
| 128 | 11059 | | Ragusa | Vittoria | 8,65E+03 | - | | | |
| 129 | 11060 | | Ragusa | Ragusa | 3,04E+04 | Package B | Omicron BA.2 | | |
| 130 | 11061 | | Caltanissetta | Gela Macchitella | 1,81E+04 | - | | | |
| 131 | 11062 | | Messina | Mili Marina | 1,29E+04 | - | | | |
| 132 | 11167 | | Catania | Pantano d'Arci | 1,68E+04 | - | | | |
| 133 | 11169 | | Catania | Giarre | 3,92E+04 | - | | | |
| 134 | 11170 | | Siracusa | Siracusa | 2,50E+04 | - | | | |
| 170 | 11024 | | Palermo | Bagheria | 3,11E+04 | - | | | |
| 171 | 11063 | | Messina | Mili Marina | 3,90E+03 | - | | | |
| 135 | 11064 | | Pisa | Pisa Nord - S. Jacopo | 2,92E+04 | - | | • Package A | • Omicron BA.4/5 |
| 136 | 11065 | | Firenze | Empoli Pagnana | <LOD | M.E.[a] | | • Package B | • Omicron BA.2 |
| 137 | 11066 | | Massa | Lavello 2 | <LOD | - | | | |
| 138 | 11067 | | Lucca | Viareggio | <LOD | - | | | |
| 139 | 11068 | | Massa | Lavello 1 | <LOD | - | | | |
| 140 | 11069 | | Lucca | Pontetetto | <LOD | - | | | |
| 141 | 11070 | | Livorno | Rivellino | <LOD | - | | | |
| 142 | 11071 | | Livorno | Rivellino | <LOD | - | | | |
| 161 | 11124 | Toscana | Firenze | San Colombano | <LOD | - | | | |
| 162 | 11125 | | Firenze | San Colombano | 3,30E+03 | - | | | |
| 163 | 11126 | | Prato | Baciacavallo | <LOD | - | | | |
| 164 | 11127 | | Prato | Baciacavallo | <LOD | - | | | |
| 165 | 11128 | | Arezzo | Casolino - San Leo | 6,35E+03 | - | | | |
| 166 | 11129 | | Grosseto | San Giovanni - Pianetto | 1,09E+04 | - | | | |
| 167 | 11130 | | Pistoia | Centrale Pistoia | 9,73E+03 | - | | | |
| 168 | 11131 | | Siena | Ponte a Tressa | 1,55E+04 | - | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 143 | 10899 | Umbria | Perugia | Perugia - Pian della Genna | 1,21E+05 | Package B | Omicron BA.2 | • Package A • Package B | | • Omicron BA.4/5 • Omicron BA.2 | |
| 144 | 11011 | | Perugia | Foligno Casone | 5,26E+04 | - | | | | | |
| 145 | 11012 | | Terni | Terni | 1,02E+05 | - | | | | | |
| 146 | 11087 | Valle d'Aosta | Aosta | La Salle | 6,87E+03 | - | | | | | |
| 147 | 11089 | | Aosta | Brissogne | 1,09E+04 | - | | | | | |
| 148 | 10945 | Veneto | Padova | Padova Ca' Nordio - centro storico | 2,06E+04 | - | | • Package A • Package B | | • Omicron BA.4/5 • Omicron BA.2 | |
| 149 | 10946 | | Padova | Padova Ca' Nordio - zip | 3,76E+04 | Package A | Omicron BA.4/5[c] | | | | |
| 150 | 10947 | | Padova | Padova Guizza | 3,35E+04 | - | | | | | |
| 151 | 10948 | | Padova | Abano Terme | 2,20E+04 | - | | | | | |
| 152 | 10998 | | Treviso | Treviso | 1,11E+04 | Package A (Partial, from G339D to Y505H)[f] | Omicron BA.4/5[c] | | | | |
| 153 | 10999 | | Vicenza | Vicenza Casale | 2,03E+03 | Package B | Omicron BA.2 | | | | |
| 154 | 11000 | | Venezia | Venezia Fusina | 6,35E+03 | Package B | Omicron BA.2 | | | | |
| 155 | 11073 | | Verona | Verona_collettore 1M | 9,08E+03 | Package A | Omicron BA.4/5[c] | | | | |
| 156 | 11074 | | Verona | Verona_collettore 3M | 1,44E+04 | Package A | Omicron BA.4/5[d] | | | | |
| 157 | 11075 | | Verona | Verona_collettore 8M | 2,27E+04 | Package B | Omicron BA.2 | | | | |

[a] M.E. mixed electropherograms

[b] Panel C, containing mutation L452R but not F486V is shared by Omicron BA.2.11/BA.2.35/BA.2.28

[c] the absence of mutation D3N in the M gene is suggestive of the presence of BA.4

[d] the presence of mutation D3N in the M gene is suggestive of the presence of BA.5

[e] the presence of a double A/G peak in correspondence to the mutation site (corresponding to presence/absence of mutation D3N in the M gene) is suggestive of the simultaneous presence of BA.4 and BA.5

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

- **Package A** (Omicron BA.4/5, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H)
- **Package B** (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)
- **Package C** (Unassigned, shared among Omicron BA.2.11/BA.2.35/ BA.2.28, long fragment from PCR ID_980) = G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H)

# Table 3. Sanger sequencing results (long PCR ID980)

| | G142D | V213G | G339D | S371F | S373P | S375F | T376A | D405N | R408S | K417N | N440K | L452R | S477N | T478K | E484A | F486V | Q493R | Q498R | N501Y | Y505H | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35, 36, 39, 44, 65, 66, 67, 90, 92, 103, 109*, 111, 116, 118, 149, 152*, 155, 156, 158* | | | | | | | | | | | | | | | | | | | | | **Package A\*\*** (Omicron BA.4/5) |
| 33, 38, 47, 53, 55, 58, 60, 91, 95, 123, 129, 143, 153, 154, 157 | | | | | | | | | | | | | | | | | | | | | **Package B** (Omicron BA.2) |
| 160 | | | | | | | | | | | | | | | | | | | | | **Package C** (unassigned) |

\* Partial sequence

\*\* Upon testing for the newly designed assay targeting the M gene, of the 19 BA.4/BA.5 sample 8 were assigned to Omicron BA.5, 7 to BA.4, and 4 showed the presence of both sublineages

## Limitations of the study

This flash survey's geographical and population coverage was incomplete, as it covered 20/21 of the Italian regions/Autonomous Provinces.

Molecular analytical methods applied to complex environmental matrices as wastewaters may be hampered by low viral concentration, poor recovery of the analyte, and/or inhibition of PCR amplification. Therefore, both detection/quantification and PCR amplification for sequencing purposes may produce false negatives. Consequently, molecular characterization and variant detection may not be achieved for all samples.

Partial sequencing of the Spike region does not allow conclusive assignation of sublineages. However, the detection within the Spike region of multiple, linked mutations associated to specific lineages/sublineages is strongly suggestive of their presence. Therefore, the detection, either by Sanger or NGS sequencing, of defined mutation panels characteristic of certain lineages/sublineages should be considered as a presumptive detection.

## Conclusions and final considerations

This is the tenth of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters that will continue to be issued as a part of the surveillance established in Italy under EU Commission Recommendation 2021/472, with the aim of providing information on SARS-CoV-2 variants in the population to supplement information acquired through the clinical surveillance.

The results of SARS-CoV-2 surveillance in wastewaters showed the presence of characteristic mutations of the Omicron variant, sublineages BA.2 and BA.4/BA.5 (with BA.5 slightly prevalent compared to BA.4).

Results suggest that we are now in a replacement phase of the Omicron subvariants, with BA.4/BA.5 expected to replace the BA.2 variant as the dominant strains in the country.