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**Flash survey on SARS-CoV-2 variants in urban wastewater in Italy  
20<sup>th</sup> Report  
(Study period: April 3<sup>rd</sup> to April 7<sup>th</sup>, 2023)**

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## Main findings:

- During the week of April 3rd to April 7th, 2023, a total of 133 wastewater samples were collected from 16 Regions and 2 Autonomous Provinces (AP).
- Mutations characteristic of the Omicron variant were identified in 11 of these Regions/AP, while no sequencing data was obtained from the remaining areas.
- Analysis of the sequences obtained by Sanger sequencing showed that 78.9% of the positive samples had amino acid substitutions belonging to the Omicron XBB.1.5\*/XBB.1.9\* lineages while 18.4% of the positive samples had mutations representative of Omicron CH.1.1\* 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. Responding to this recommendation, the Istituto Superiore di Sanità (ISS) initiated a series of "flash surveys". These surveys involve monthly sampling campaigns carried out over a short period of time at different locations throughout Italy. The primary objective of these flash surveys is to gather supplementary information on SARS-CoV-2 variants in the population to complement the data obtained through clinical surveillance. The aim of this report is to summarize the results from the 20th national flash survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from April 3rd to April 7th, 2023.

## Methodology

During the period from April 3rd to April 7th, 2023, the 20th national flash survey on SARS-CoV-2 variants in wastewater samples was conducted in Italy. The survey involved the collection of 133 wastewater samples from 129 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<sup>1</sup>. The samples collected during the survey were processed and the virus 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"<sup>2</sup>. The purified RNAs extracted from the samples were then sent to ISS for variant detection.

A real-time RT-PCR assay was used to screen for the presence of the Omicron variant<sup>3</sup>. In addition, a long-nested PCR assay of approximately 1600 base pairs was used, covering amino acid residues 58 to 573 of the spike protein. The assay was designed to detect multiple nucleotide changes characteristic of Variants of Concern (VoCs) and Variants of Interest (VoIs) in the spike protein<sup>4</sup>. Following amplification of the target DNA sequences, Sanger sequencing was performed on individual samples. For variant classification we adopted a lineage classification

<sup>1</sup> 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 \(iss.it\)](https://doi.org/10.5281/zenodo.5758724)

<sup>2</sup> DOI [10.5281/zenodo.5758724](https://doi.org/10.5281/zenodo.5758724).

<sup>3</sup> La Rosa G, Iaconelli M, Veneri C, Mancini P, Bonanno Ferraro G, Brandtner D, Lucentini L, Bonadonna L, Rossi M, Grigioni M; SARI network; Suffredini E. The rapid spread of SARS-COV-2 Omicron variant in Italy reflected early through wastewater surveillance. *Sci Total Environ*. 2022 Sep 1;837:155767. doi: 10.1016/j.scitotenv.2022.155767. Epub 2022 May 6. PMID: 35533857; PMCID: PMC9074219.

<sup>4</sup> G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferrero, 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>.

based on 'outbreak.info'<sup>5</sup> 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 assignment to sublineages, based solely on the mutations observed in the spike region, not feasible.

## Results

### *Real Time qPCR*

Out of the 133 samples collected, a total of 127 (95.5%) 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 1.84E+02 to 2.24E+05 genome copies (g.c.) per liter of wastewater. Additionally, 125 out of the 133 samples (94.0%) were found to be positive for the omicron variant using the RT-qPCR assay, with cycle threshold (CT) values ranging from 32.0 to 39.5.

### *Sanger Sequencing*

Table 1 provides a summary of the results obtained from the real-time PCR assays, long nested PCR, and sequencing methods. A total of 38 samples (28.6%) from 11 Regions/AP were successfully amplified using the long-nested PCR assay. Sanger sequencing confirmed that all sequences corresponded to the omicron variant.

Analysis of the wastewater samples revealed the presence of two SARS-CoV-2 lineages, as shown in Tables 1 and 2. Among these lineages, the XBB.1.5\*/XBB.1.9\* lineages (which were not distinguishable in the sequenced region), were dominant, detected in 78.9% of the positive samples (30 samples). In addition, the CH.1.1\* lineage of the omicron variant was identified in 18.4% of the samples (7 samples). One sample could not be assigned due to partial sequence.

For ease of understanding, the mutations have been grouped into two panels or 'mutation packages' as follows:

- **Package A (assigned to the lineages Omicron XBB.1.5\*/XBB.1.9\*)** = V83A, G142D, DEL144, H146Q/H146K, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H
- **Package B (assigned to the lineage Omicron CH.1.1\*)** = G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, G446S, L452R, N460K, S477N, T478K, E484A, F486S, Q498R, N501Y, Y505H

'Package A' combined with the 'F456L' mutation, suggests the presence of the Omicron EG.5 sublineage. This sublineage is characterized by having the 'F456L' mutation in approximately 80.5% of its sequences (<https://outbreak.info/situation-reports>, date: 05/10/2023).

Package A was detected in nine Regions (Abruzzo, Emilia-Romagna, Friuli-Venezia Giulia, Liguria, Lombardia, Molise, Puglia, Sicilia, Veneto) and two A.P. (Bolzano and Trento). Characteristic amino acid substitutions of package B (lineage CH.1.1\*) were observed in Puglia and Sicilia.

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<sup>5</sup> <https://outbreak.info/situation-reports>, date: 05/10/2023

**Table 1. PCR and sequencing results**

| Sample ID | Region/A.P. | City           | WTP                    | RT-qPCR (c.g./L)                     | RT-PCR Omicron-ID 999 (CT value) | mutations found by Sanger sequencing (long PCR ID_980) | SARS-CoV-2 lineages            |  |
|-----------|-------------|----------------|------------------------|--------------------------------------|----------------------------------|--|--------------------------------|--|
| 108       | 19966       | Abruzzo        | Pescara                | Villa Carmine                        | 1,95E+02                         | 36.30  |                                |  |
| 109       | 19968       |                | Pescara                | Via Raiale                           | 1,84E+02                         | 38.37  |                                |  |
| 110       | 19969       |                | Chieti                 | S. Martino                           | <LOD                             | 38.06  |                                |  |
| 111       | 19965       |                | L'Aquila               | Pile                                 | 6,34E+02                         | 34.47  | Package A                      | Omicron XBB.1.5*/XBB.1.9*              |
| 112       | 19964       |                | Teramo                 | Villa Pavone                         | 5,68E+02                         | 36.13  |                                |  |
| 1         | 19869       | Basilicata     | Potenza                | Tiera di Vaglio                      | 1,16E+04                         | 38.90  |                                |  |
| 2         | 19871       |                | Matera                 | Pantano                              | 7,39E+03                         | 38.10  |                                |  |
| 3         | 19807       | Campania       | Napoli                 | Napoli EST                           | 3,09E+03                         | 36.05  |                                |  |
| 4         | 19808       |                | Napoli                 | Napoli OVEST - Ingresso Principale   | 5,08E+04                         | 37.19  |                                |  |
| 5         | 19809       |                | Napoli                 | Napoli OVEST - ex ingresso Camaldoli | 1,72E+04                         | 39.14  |                                |  |
| 6         | 19742       | Emilia-Romagna | Ferrara                | Ferrara - Linea 1                    | 2,93E+04                         | ND   |                                |  |
| 7         | 19743       |                | Ferrara                | Ferrara - Linea 2                    | 9,78E+03                         | ND   |                                |  |
| 8         | 19744       |                | Modena                 | Carpi                                | 2,11E+04                         | 37.11  |                                |  |
| 9         | 19854       |                | Piacenza               | Borgoforte                           | 3,73E+04                         | ND   |                                |  |
| 10        | 19855       |                | Parma                  | Parma ovest                          | 8,13E+03                         | 37.02  |                                |  |
| 11        | 19857       |                | Reggio Emilia          | Mancasale                            | 3,85E+04                         | 33.46  | Package A + F456L <sup>a</sup> | Omicron XBB.1.5*/XBB.1.9* <sup>a</sup> |
| 117       | 19925       |                | Bologna                | IDAR                                 | 1,21E+05                         | 35.06  | Package A                      | Omicron XBB.1.5*/XBB.1.9*              |
| 118       | 19963       |                | Ravenna - Forlì-Cesena | Ravenna                              | 1,10E+05                         | 34.36  |                                |  |
| 119       | 19945       |                | Modena                 | Naviglio                             | 2,93E+04                         | 36.43  |                                |  |
| 120       | 19946       |                | Ravenna                | Faenza                               | 3,06E+04                         | 36.09  |                                |  |
| 121       | 19949       | Bologna        | Imola                  | 3,76E+04                             | 35.37                            | Package A  | Omicron XBB.1.5*/XBB.1.9*      |  |
| 124       | 19950       | Forlì-Cesena   | Forlì                  | 5,44E+04                             | 34.18                            | Package A  | Omicron XBB.1.5*/XBB.1.9*      |  |

|     |       |                       |                       |                              |          |          |           |                           |
|-----|-------|-----------------------|-----------------------|------------------------------|----------|----------|-----------|---------------------------|
| 125 | 19952 |                       | Forlì-Cesena          | Cesena                       | 2,24E+05 | 37.01    | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 127 | 20039 |                       | Rimini - Forlì-Cesena | S. Giustina                  | 2,81E+04 | 35.54    | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 122 | 19909 | Friuli-Venezia Giulia | Pordenone             | Cordenons                    | 1,74E+05 | 35.89    | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 123 | 19910 |                       | Udine                 | Udine                        | 1,26E+05 | 33.02    |           |                           |
| 126 | 20046 |                       | Trieste               | Servola                      | 3,60E+04 | 33.67    | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 12  | 19620 | Lazio                 | Viterbo               | Viterbo - Strada Bagni       | 9,40E+03 | 35.72    |           |                           |
| 13  | 19621 |                       | Roma                  | Anzio - Colle Cocchino       | 1,66E+03 | 33.79    |           |                           |
| 14  | 19622 |                       | Latina                | Aprilia (Via del Campo)      | 7,38E+03 | 37.26    |           |                           |
| 15  | 19623 |                       | Latina                | Latina Loc Latina Est        | 2,14E+03 | 36.72    |           |                           |
| 16  | 19625 |                       | Roma                  | Pomezia - Via Cincinnato     | <LOD     | 35.90    |           |                           |
| 17  | 19626 |                       | Roma                  | Velletri (LA CHIUSA-SORBO)   | 2,31E+03 | 38.01    |           |                           |
| 18  | 19627 |                       | Roma                  | Guidonia - Ponte Lucano      | 1,10E+04 | 33.92    |           |                           |
| 19  | 19689 |                       | Roma                  | Civitavecchia Fiumaretta     | <LOD     | 36.61    |           |                           |
| 103 | 19882 |                       | Roma                  | Roma Est (linea 1 + linea 2) | 2,10E+04 | 36.96    |           |                           |
| 104 | 19883 |                       | Roma                  | Roma Nord                    | 1,61E+04 | 35.58    |           |                           |
| 105 | 19884 |                       | Roma                  | Roma Sud                     | 1,05E+04 | 36.71    |           |                           |
| 106 | 19885 |                       | Roma                  | Ostia                        | 2,47E+04 | 37.16    |           |                           |
| 107 | 19886 |                       | Roma                  | Fregene                      | 4,42E+04 | 38.76    |           |                           |
| 20  | 19766 |                       | Liguria               | La Spezia                    | Camisano | 2,21E+04 | ND        |                           |
| 21  | 19767 | La Spezia             |                       | Silea                        | 2,69E+04 | 34.29    |           |                           |
| 22  | 19768 | La Spezia             |                       | La Spezia                    | 5,68E+04 | 33.08    |           |                           |
| 23  | 19770 | Genova                |                       | Pegli                        | 3,81E+04 | 34.02    |           |                           |
| 24  | 19771 | Genova                |                       | Voltri                       | 2,10E+04 | 33.06    |           |                           |
| 25  | 19772 | Genova                |                       | Quinto                       | 2,19E+04 | 35.01    |           |                           |
| 26  | 19773 | Genova                |                       | Rapallo                      | 3,94E+04 | 34.78    |           |                           |
| 27  | 19774 | Genova                |                       | Sestri P                     | 1,24E+04 | 32.85    |           |                           |
| 28  | 19775 | Genova                |                       | Sturla                       | 1,71E+04 | 35.16    |           |                           |

|     |       |           |  |                               |          |       |           |                           |
|-----|-------|-----------|--|-------------------------------|----------|-------|-----------|---------------------------|
| 29  | 19776 |           | Imperia  | Imperia                       | 1,54E+04 | 34.67 |           |                           |
| 30  | 19777 |           | Imperia  | Sanremo - località Capo Verde | 1,57E+04 | 34.34 |           |                           |
| 31  | 19778 |           | Genova   | Darsena                       | 2,16E+04 | 34.53 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 32  | 19779 |           | Genova   | Punta Vagno Genova            | 8,37E+03 | 33.50 |           |                           |
| 33  | 19780 |           | Genova   | Valpolcevera                  | 1,96E+04 | 34.68 |           |                           |
| 34  | 19781 |           | Savona   | Borghetto Santo Spirito       | 7,74E+03 | 36.99 |           |                           |
| 35  | 19851 |           | Genova   | Punta Vagno Genova            | 3,70E+04 | 37.30 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 116 | 19769 |           | Savona   | Savona                        | 2,34E+04 | 36.18 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 36  | 19745 |           | Sondrio  | Sondrio                       | 1,35E+04 | 38.32 |           |                           |
| 37  | 19749 |           | Milano   | Milano Nosedo                 | 1,12E+05 | 36.15 |           |                           |
| 38  | 19750 |           | Milano   | Milano San Rocco              | 1,20E+05 | 35.53 |           |                           |
| 39  | 19751 |           | Como   | Como                          | 8,99E+04 | ND    | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 40  | 19752 |           | Pavia  | Pavia                         | 7,75E+04 | 37.40 |           |                           |
| 41  | 19753 | Lombardia | Como - Lecco<br>- Milano -<br>Monza e della<br>Brianza | Monza                         | 5,90E+04 | 38.34 |           |                           |
| 42  | 19754 |           | Pavia  | Vigevano                      | 5,26E+04 | 36.20 |           |                           |
| 43  | 19783 |           | Bergamo  | Bergamo                       | 9,16E+03 | 35.44 |           |                           |
| 44  | 19785 |           | Cremona  | Citta di Cremona              | 1,88E+04 | 36.12 |           |                           |
| 45  | 19786 |           | Brescia  | Verziano                      | 8,40E+03 | 36.93 |           |                           |
| 46  | 19823 |           | Pesaro-<br>Urbino                                      | Borgheria                     | 3,23E+03 | 37.40 |           |                           |
| 47  | 19824 |           | Pesaro-<br>Urbino                                      | Ponte Metauro                 | 1,45E+03 | 37.51 |           |                           |
| 48  | 19825 | Marche    | Pesaro-<br>Urbino                                      | Ponte Sasso                   | 7,02E+03 | 33.48 |           |                           |
| 49  | 19826 |           | Ancona   | Zipa                          | 3,00E+04 | 35.02 |           |                           |
| 50  | 19827 |           | Ancona   | Falconara                     | 1,11E+04 | 37.21 |           |                           |
| 51  | 19828 |           | Ancona   | Camerano                      | 5,51E+03 | 33.69 |           |                           |
| 113 | 19974 | Molise    | Campobasso   | Campobasso - San Pietro       | 4,47E+02 | 37.11 |           |                           |

|     |       |              |                       |                                  |          |           |  |                           |
|-----|-------|--------------|-----------------------|----------------------------------|----------|-----------|--|---------------------------|
| 114 | 19975 |              | Campobasso            | Termoli - località Porto         | <LOD     | 36.62     |  |                           |
| 115 | 19976 |              | Campobasso            | Termoli - località Pantano Basso | 1,97E+02 | 35.57     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 131 | 19876 | P.A. Bolzano | Bolzano               | IDA Bolzano                      | 3,66E+04 | 35.50     | Partial sequence, from G339H to Y505H <sup>b</sup> | Not assigned <sup>b</sup> |
| 132 | 19877 |              | Bolzano               | IDA Merano                       | 6,71E+04 | 34.01     |  |                           |
| 133 | 19878 |              | Bolzano               | IDA Termeno                      | 9,20E+04 | 33.15     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 52  | 19690 | P.A. Trento  | Trento                | Trento nord                      | 1,05E+05 | 35.42     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 53  | 19691 |              | Trento                | Trento sud                       | 6,76E+04 | 33.65     |  |                           |
| 54  | 19692 |              | Trento                | Rovereto                         | 1,26E+05 | 38.14     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 55  | 19650 | Piemonte     | Torino                | Castiglione Torinese             | 9,15E+02 | ND        |  |                           |
| 56  | 19651 |              | Biella                | Biella Nord                      | 4,00E+02 | 38.75     |  |                           |
| 57  | 19652 |              | Biella                | Biella Sud                       | 2,23E+03 | 37.68     |  |                           |
| 58  | 19653 |              | Novara                | Novara                           | <LOD     | 36.24     |  |                           |
| 59  | 19733 |              | Alessandria           | Alessandria                      | 1,11E+04 | 34.70     |  |                           |
| 60  | 19734 |              | Asti                  | Asti                             | 1,23E+03 | 38.14     |  |                           |
| 61  | 19735 |              | Cuneo                 | Cuneo                            | 1,00E+03 | 36.80     |  |                           |
| 62  | 19618 | Puglia       | Bari                  | Bari Ovest                       | 1,17E+03 | 35.13     | Package B  | Omicron CH.1.1*           |
| 63  | 19619 |              | Bari                  | Bari Est                         | 7,80E+02 | 34.14     | Package B  | Omicron CH.1.1*           |
| 64  | 19624 |              | Bari                  | Molfetta                         | 6,06E+02 | 36.14     |  |                           |
| 65  | 19632 |              | Bari                  | Altamura                         | 7,29E+02 | 36.47     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 66  | 19634 |              | Brindisi              | Brindisi Fiume Grande            | 3,27E+03 | 35.36     | Package B  | Omicron CH.1.1*           |
| 67  | 19635 |              | Lecce                 | Lecce                            | 1,81E+04 | 33.00     | Package A  | Omicron XBB.1.5*/XBB.1.9* |
| 68  | 19637 |              | Taranto               | Taranto Bellavista               | 2,76E+03 | 35.72     | Package B  | Omicron CH.1.1*           |
| 69  | 19638 |              | Taranto               | Taranto Gennarini                | 9,51E+02 | 37.55     |  |                           |
| 70  | 19703 |              | Barletta-Andria-Trani | Andria                           | 7,92E+03 | 35.63     | Package B  | Omicron CH.1.1*           |
| 71  | 19704 |              | Barletta-Andria-Trani | Barletta                         | 1,30E+04 | 35.32     |  |                           |
| 72  | 19705 | Bari         | Bitonto               | 1,55E+04                         | 34.82    | Package A | Omicron XBB.1.5*/XBB.1.9*                          |                           |

|     |       |             |                       |                                    |          |       |           |                           |
|-----|-------|-------------|-----------------------|------------------------------------|----------|-------|-----------|---------------------------|
| 73  | 19706 |             | Foggia                | Cerignola                          | 1,44E+04 | 35.25 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 74  | 19707 |             | Foggia                | Foggia                             | 1,50E+04 | 35.02 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 75  | 19708 |             | Foggia                | Manfredonia                        | 6,17E+03 | ND    |           |                           |
| 98  | 19728 |             | Barletta-Andria-Trani | Bisceglie                          | 3,63E+03 | 37.14 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 99  | 19729 |             | Barletta-Andria-Trani | Trani                              | 1,58E+03 | 35.10 |           |                           |
| 76  | 19788 |             | Ragusa                | Modica                             | 5,03E+02 | 37.54 |           |                           |
| 79  | 19791 |             | Caltanissetta         | Gela Macchitella                   | 1,53E+03 | ND    |           |                           |
| 80  | 19792 |             | Messina               | Mili Marina                        | 2,25E+02 | 38.21 |           |                           |
| 81  | 19793 |             | Messina               | Mili Marina                        | 1,06E+03 | 34.88 |           |                           |
| 82  | 19839 |             | Agrigento             | Agrigento                          | 2,41E+04 | 37.43 | Package B | Omicron CH.1.1*           |
| 83  | 19841 |             | Enna                  | Enna                               | 4,88E+03 | 36.36 |           |                           |
| 84  | 19842 |             | Palermo               | Bagheria                           | 1,17E+04 | 37.94 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 85  | 19843 |             | Palermo               | Acqua dei Corsari                  | 7,20E+03 | 37.08 | Package B | Omicron CH.1.1*           |
| 86  | 19844 | Sicilia     | Palermo               | Fondo Verde                        | 3,20E+03 | 35.09 |           |                           |
| 87  | 19846 |             | Caltanissetta         | Caltanissetta e San Cataldo        | 1,35E+04 | 39.53 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 100 | 19700 |             | Trapani               | Mazara del Vallo                   | 6,34E+03 | 37.11 |           |                           |
| 101 | 19701 |             | Trapani               | Marsala                            | 3,89E+03 | 39.30 |           |                           |
| 102 | 19702 |             | Trapani               | Trapani                            | 1,05E+04 | 39.02 |           |                           |
| 128 | 19763 |             | Catania               | Pantano d'Arci                     | 2,10E+04 | 36.77 |           |                           |
| 129 | 19764 |             | Catania               | Giarre                             | 1,80E+04 | 37.43 |           |                           |
| 130 | 19765 |             | Siracusa              | Siracusa                           | 2,54E+04 | 39.03 |           |                           |
| 135 | 19697 | Umbria      | Perugia               | Perugia - Pian della Genna         | 6,95E+04 | 34.49 |           |                           |
| 134 | 20007 | Val d'Aosta | Aosta                 | Brissogne                          | 4,25E+02 | 37.02 |           |                           |
| 88  | 19673 |             | Padova                | Padova Ca' Nordio - centro storico | 8,33E+04 | 35.39 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 89  | 19674 |             | Padova                | Padova Ca' Nordio - zip            | 8,95E+02 | 37.48 |           |                           |
| 90  | 19675 | Veneto      | Padova                | Padova Guizza                      | 1,44E+05 | 33.58 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 91  | 19676 |             | Padova                | Abano Terme                        | 1,01E+05 | 37.22 |           |                           |



|    |       |         |                      |          |       |           |                           |
|----|-------|---------|----------------------|----------|-------|-----------|---------------------------|
| 92 | 19718 | Treviso | Treviso              | 1,22E+04 | 33.74 |           |                           |
| 94 | 19720 | Venezia | Venezia Fusina       | 7,53E+03 | 33.18 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 93 | 19719 | Vicenza | Vicenza Casale       | <LOD     | 34.89 |           |                           |
| 95 | 19756 | Verona  | Verona_collettore 1M | 1,40E+04 | 32.36 |           |                           |
| 96 | 19757 | Verona  | Verona_collettore 3M | 4,45E+04 | 32.01 | Package A | Omicron XBB.1.5*/XBB.1.9* |
| 97 | 19758 | Verona  | Verona_collettore 8M | 2,68E+04 | 35.56 | Package A | Omicron XBB.1.5*/XBB.1.9* |

<sup>a</sup> 'Package A' combined with the '**F456L**' mutation, suggests the presence of the Omicron EG.5 sublineage. This sublineage is characterized by having the 'F456L' mutation in approximately 80.5% of its sequences. The Omicron XBB.1.5\* and Omicron XBB.1.9\* lineages may also carry the F456L mutation, but at lower rates of 3.0% and 26.6%, respectively (<https://outbreak.info/situation-reports>, date: 05/10/2023);

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

ND: Not Detected

**Table 2. Sanger sequencing results**

| ID SAMPLES  | V83A | G142D | DEL144 | H146Q/H146K | K147E | W152R | F157L | Q183E | I210V | V213G | V213E | G252V | G257S | G339H | R346T | L368I | S371F | S373P | S375F | T376A | D405N | R408S | K417N | N440K | K444T | V445P | G446S | F456L | L452R | N460K | S477N | T478K | E484A | F486S | F486P | F490S | Q498R | N501Y | Y505H | PACKAGES | LINEAGES |                                   |  |           |                 |                          |
|---|------|-------|--------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|-----------------------------------|--|-----------|-----------------|--------------------------|
| 31, 35, 39, 52, 54, 65, 67, 72, 73, 74, 84, 87, 88, 90, 94, 96, 97, 98, 111, 115, 116, 117, 121, 122, 124, 125, 126, 127, 133 |      |       |        |             |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |          |          |                                   |  |           | Package A       | Omicron<br>XBB.1.5*/1.9* |
| 11  |      |       |        |             |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |          |          | Package A +<br>F456L <sup>a</sup> |  |           |                 |                          |
| 62, 63, 66, 68, 70, 82, 85  |      |       |        |             |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |          |          |                                   |  | Package B | Omicron CH.1.1* |                          |

## Limitations of the study

The geographic and population coverage of this flash survey was not comprehensive, as it included 18 out of 21 of the Italian Regions/Autonomous Provinces.

It is important to note that the molecular analytical methods used for complex environmental matrices, such as wastewater, can be challenged by factors such as low viral concentrations, insufficient analyte recovery, and/or inhibition of PCR amplification. As a result, both the detection/quantification and PCR amplification for sequencing may yield false negatives, making it difficult to achieve molecular characterization and variant detection for all samples.

Partial sequencing of 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 supplementary 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 only SARS-CoV-2 variant in Italy, with the XBB.1.5\*/XBB.1.9\* lineages being the most common. However, mutations characteristic of the Omicron CH.1.1\* lineage have also been detected.

The sequencing SARS-CoV-2 in wastewater samples provides valuable additional information alongside the sequencing of clinical cases. This approach provides a more comprehensive and complete understanding of the circulating variants in the country, contributing to a better characterization of the spread and evolution of the virus.

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