Brief Report No. 31: Active surveillance of SARS-CoV-2 variants from Buenos Aires City (CABA), Argentina. Genomic analysis of cases of emerging lineages of Omicron variant in CABA and province of Chaco in Argentina. Update to 05/10/2022.

The extended version of this report with additional data will be published shortly.

ABSTRACT

From epidemiological weeks 6-7, the Omicron BA.2 variant has been detected in cases of local transmission, and it became predominant for weeks 14-15 in CABA, Argentina. In the last analyzed weeks (EW 16-17) Omicron BA.2 was detected in 87% of the cases.

The genomic analysis of three cases of the Omicron variant in Argentina are reported: one belonging to the BA.4 lineage and two to BA.2.12.1, emerging lineages of Omicron that have not been reported in Argentina to date. The case of BA.4, confirmed by phylogenetic analysis, corresponds to an individual from CABA with no known links to international travel, representing the first detection of this lineage in South America. The two cases of BA.2.12.1 correspond to a individual from CABA with no available information of links with international trips, and another from the province of Chaco with an indirect link with a traveler to Mexico.

The phylogenetic analysis of the BA.2.12.1 lineage cases showed no evidence that they belong to a common chain of transmission within Argentina. Both presented phylogenetic associations with different North American sequences, which is compatible with independent entries to the country and consistent with the available epidemiological information.

Epidemiological context

The emergence, spread, and establishment of the Omicron variant of concern has driven waves of COVID-19 globally, generating an unprecedented number of infections. Its extensive circulation has resulted in the existence of several derived lineages (dubbed BA.1-BA.5), some with intrinsic diversification. The BA.1 lineage has spread worldwide at the end of 2021 and has driven the latest wave in Argentina and in South America, while the more recently expanded BA.2 lineage has already displaced BA.1 in various regions of the world.

Some Omicron lineages could differ in their potential public health risk, depending on the epidemiological impact of their higher transmission or association to a different severity profile. Among the main characteristics of Omicron, its greater transmission capacity over other variants stands out.

In particular, the BA.4, BA.5 and BA.2.12.1 lineages have received special interest worldwide in recent weeks because they have been associated with an increase in the number of cases (BA.4 and BA. 5 in South Africa (1) and BA.2.12.1 in the United States (2) and a displacement of the BA.2 lineage, which in turn was reported to be more transmissible than BA.1 (3). These lineages have acquired some additional mutations that could affect their biological characteristics (BA.4 and BA.5 include mutations of interest in the Spike protein: del69/70, L452R and F486V, and BA.2.12.1, mutations L452Q and S704L). From GISAID and WHO reports, the BA.4 lineage has not yet been

reported in South America, while the BA.2.12.1 lineage has only recently been detected in Peru and Colombia.

To date there is no evidence of an increase in hospitalizations or association with differential severity related to these lineages. However, it is possible that its transmission is favored by an immune escape against the immune response developed by previous infection with other variants or by vaccination (4).

In **order** to study the evolution of the Omicron variant and the distribution of its derived lineages in CABA, Argentina, a total of 237 samples were analyzed between 01/23/2022 and 04/30/2022 , of which 210 had no known history of travel or close contact with travelers. Among these, two cases of the emerging lineages BA.4 and BA.2.12.1 were detected, which were studied by complete genome sequencing for their characterization by phylogenetic analysis. Likewise, as part of the genomic surveillance of the province of Chaco, a case of the BA.2.12.1 lineage was detected in an individual who had a history of indirect contact with a traveler abroad, which was also included in the evolutionary analyses.

RESULTS:

The sequencing of 237 cases from the CABA, Argentina between 01/23/2022 and 04/30/2022 was carried out, of which 210 had no known history of travel or close contact with travelers. Figure 1 shows the number of samples sequenced by epidemiological week between EW26-27 of 2021 and EW16-17 of 2022. Table 1 and Figure 2 show the frequency of the variants found by epidemiological week (EW) 2021-2022 in CABA.

In report No. 30 of the PAIS Project, we reported that for EW 3 of 2022, the Omicron lineage BA.1 variant constituted 100% of the cases analyzed in the CABA. This report shows that during EW 6-7 the BA.2 lineage begins to be detected in this jurisdiction, becoming the dominant one in EW 14-15 of 2022 with 69.4%, a value that rose to 87% in EW 16-17.

It should be noted that in EW 16 of 2022, the first case of the emerging BA.4 lineage was detected, through the analysis of marker mutations in the RBD region of the Spike protein. Their complete genome was then sequenced, which in principle could not be designated as such by the Pangolin rapid lineage assignment tool, but the correct classification was achieved through phylogenetic analysis (Figure 3). Then, in EW 17, the first case of the emerging lineage BA.2.12.1 in CABA was detected in the same way.

On the other hand, as part of the genomic surveillance carried out by the IMR sequencing node of the province of Chaco, between the months of March and April 2022, 16 samples were sequenced from the localities of Sáenz Peña (4), Resistencia (8), Fontana (1), General San Martín (1) and Colonia Elisa (2) between the dates 02/03/2022 and 04/14/2022. Five corresponded to the BA.1 lineage, nine to the BA.1.1 lineage, one to the BA.2 lineage (03/28/2022) and one to the emerging BA.2.12.1 lineage of 03/07/2022.

Phylogenetic analysis

Phylogenetic analysis showed that the BA.4 lineage sequence clustered with lineage reference sequences, confirming their assignment. This sequence presents an additional non-synonymous change to those reported for this lineage in the Spike protein (S640F).

The cases of variant BA.2.12.1 presented phylogenetic association with different sequences from North America (one case with sequences from the United States, and the other with sequences from Canada), which is compatible with independent entries to the country, and it is consistent with the epidemiological information available for these cases.

CONCLUSIONS

Omicron's BA.2 lineage is predominant in the CABA, Argentina since epidemiological weeks 14-15 (first fortnight of April), where it displaced the BA.1 lineage, associated with the last wave of COVID-19 in our country. In the last weeks analyzed (end of April), the BA.2 lineage was detected in 87% of the cases.

Three cases were detected from two emerging Omicron lineages of recent worldwide attention due to their association with an increase in cases and immune evasion, one belonging to the BA.4 lineage and two to the BA.2.12.1 lineage.

The case of BA.4 constitutes the first detection in South America and corresponds to an individual from CABA, with no known link to international travel.

The two cases of BA.2.12.1 constitute the first reports in Argentina and correspond to an individual from CABA and one from the province of Chaco, the latter with an epidemiological link with a traveler to Mexico.

The three cases described constitute sporadic cases so far, however, their evolution over time must be followed to analyze whether these lineages will be relevant in our country.

In the current global epidemiological context, with a rise in cases and in the context of the emergence of these new Omicron lineages, it is recommended to reinforce care measures (ventilation of environments, use of masks, physical distancing) to prevent further spread of these lineages in Argentina, as well as completing vaccination schedules and accessing booster doses.

The Argentine Interinstitutional SARS-CoV-2 Genomics Consortium will continue to carry out molecular surveillance and genomic analysis on cases of community circulation, in order to rapidly monitor the presence, emergence and evolution of variants of national and international epidemiological interest.

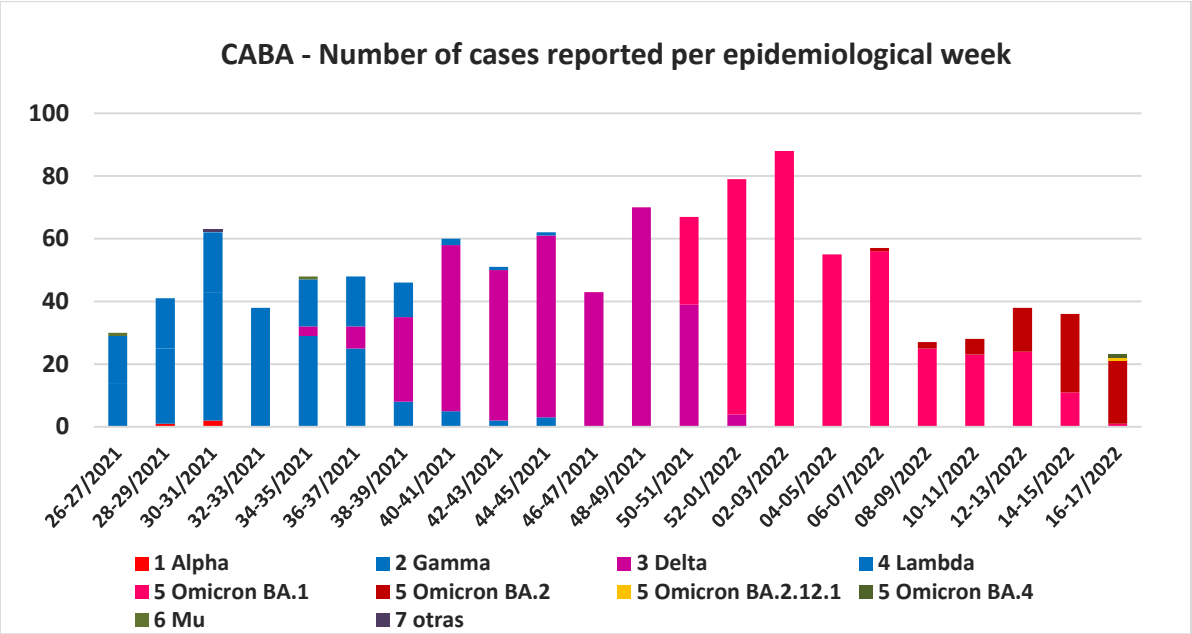

Figure 1: Number of cases reported by epidemiological week 2021-2022. Only cases from the **CABA** that did not present a history of travel or close contact with travelers are included.

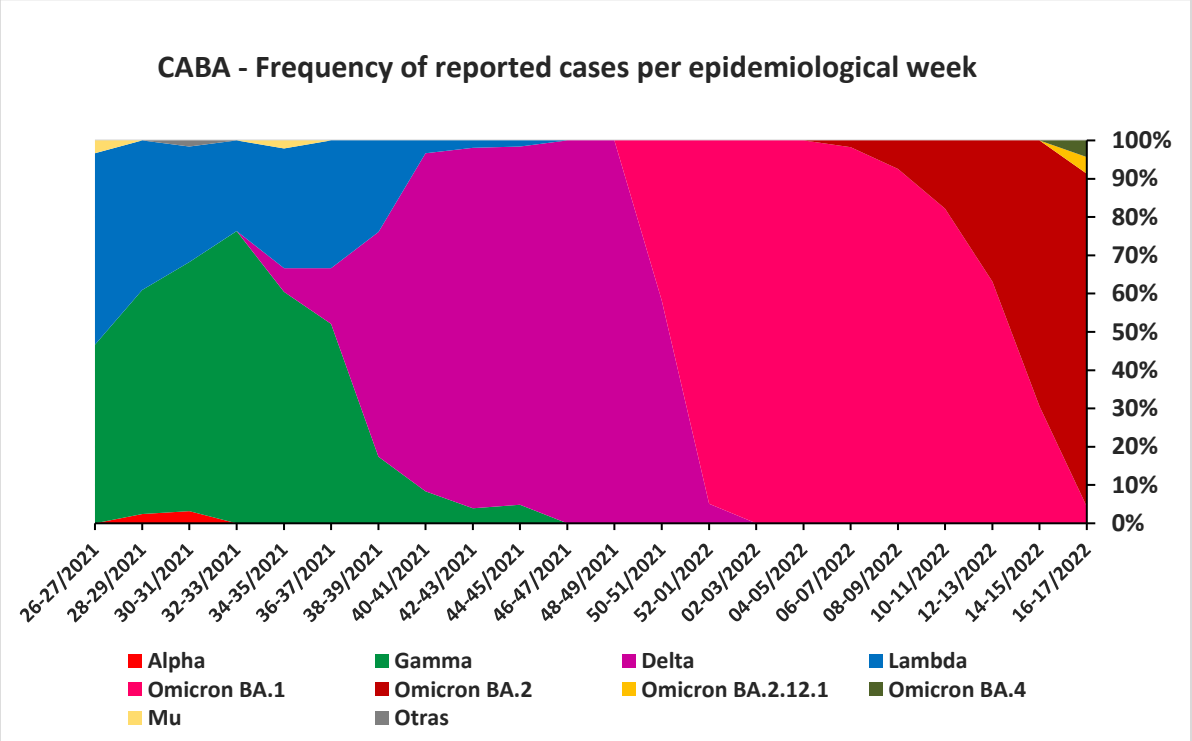

Figure 2: Frequency of SARS-CoV-2 variants by epidemiological week. Only cases from the **CABA** that did not present a history of travel or close contact with travelers are included.

Table 1. CABA: Frequency of variants by epidemiological week (EW) 2021-2022 [1].

Epi Week	Delta			Omicron BA.1			Omicron BA.2			Omicron BA.2.12.1			Omicron BA.4			Other [3]		
	Freq (%)	CI95% [2]		Freq (%)	CI95% [2]		Freq (%)	CI95% [2]		Freq (%)	CI95% [2]		Freq (%)	CI95% [2]		Freq (%)	CI95% [2]	
26-27/2021																**100.0**	88.6	100.0
28-29/2021																**100.0**	91.4	100.0
30-31/2021																**100.0**	94.3	100.0
32-33/2021																**100.0**	90.8	100.0
34-35/2021	**6.3**	2.1	16.8													**93.8**	83.2	97.9
36-37/2021	**14.6**	7.2	27.2													**85.4**	72.8	92.8
38-39/2021	**58.7**	44.3	71.7													**41.3**	28.3	55.7
40-41/2021	**88.3**	77.8	94.2													**11.7**	5.8	22.2
42-43/2021	**94.1**	84.1	98.4													**5.9**	1.6	15.9
44-45/2021	**93.5**	84.6	97.5													**6.5**	2.5	15.4
46-47/2021	**100.0**	91.8	100.0															
48-49/2021	**100.0**	94.8	100.0															
50-51/2021	**58.2**	46.3	69.3	**41.8**	30.7	53.7												
52-01/2022	**5.1**	2.0	12.3	**94.9**	87.7	98.0												
02-03/2022				**100.0**	95.8	100.0												
04-05/2022				**100.0**	93.5	100.0												
06-07/2022				**98.2**	90.7	99.9	**1.8**	0.1	9.3									
08-09/2022				**92.6**	76.6	98.7	**7.4**	1.3	23.4									
10-11/2022				**82.1**	64.4	92.1	**17.9**	7.9	35.6									
12-13/2022				**63.2**	47.3	76.6	**36.8**	23.4	52.7									
14-15/2022				**30.6**	18.0	46.9	**69.4**	53.1	82.0									
16-17/2022				**4.3**	0.2	21.0	**87.0**	67.9	95.5	**4.3**	0.2	21.0	**4.3**	0.2	21.0			

[1] Only cases from those with no known history of travel or close contact with travelers are included.
[2] The frequency confidence interval was estimated using the Wilson/Brown method, implemented in the Graph Pad Prism v.8.3 program (California, United States, www.graphpad.com).
[3] Other variant detections (Alpha, Gamma, Lambda, Mu) are included.

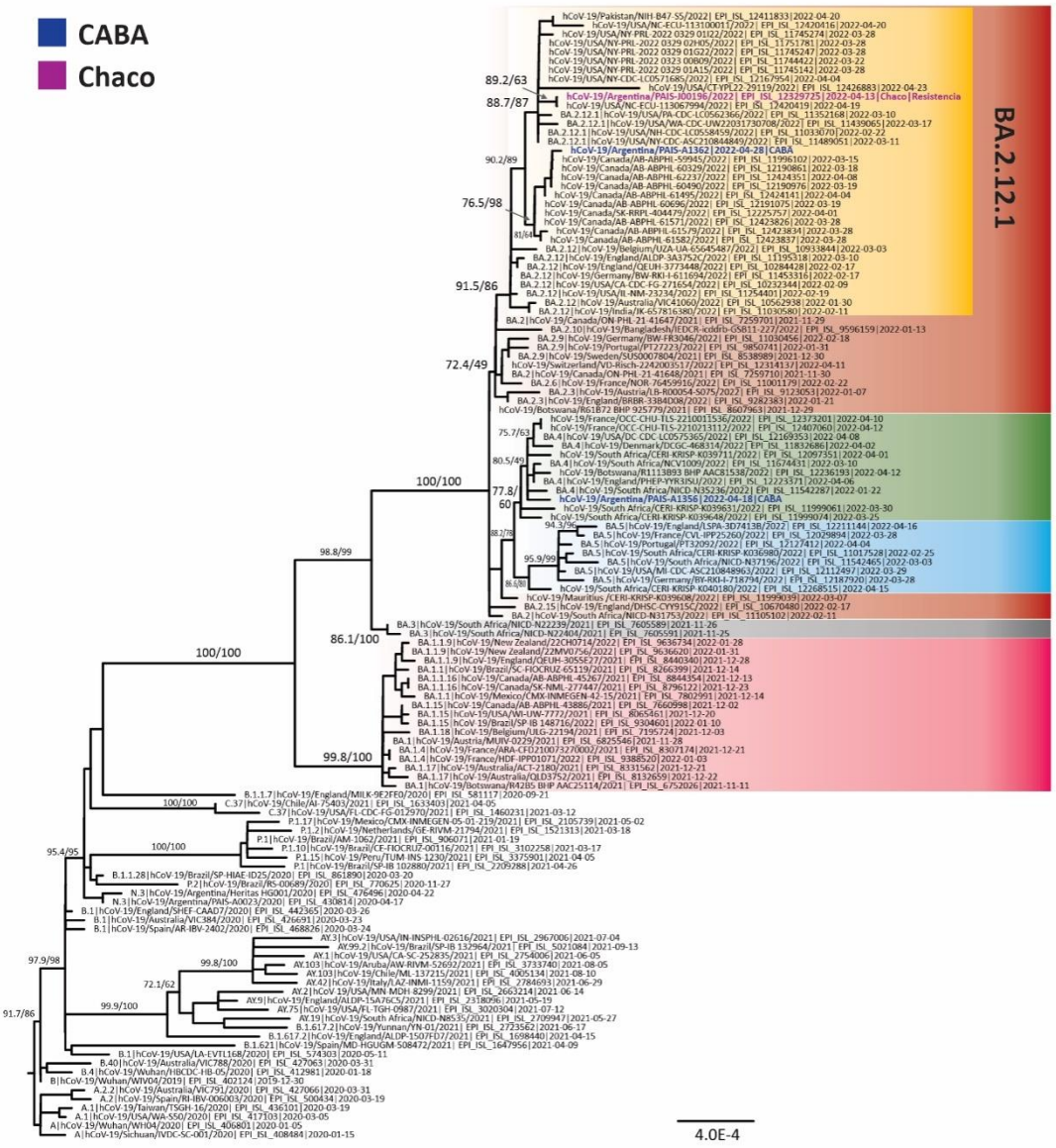

Figure 3: Phylogenetic tree of SARS-CoV-2 sequences. The names of the studied sequences of CABA and the province of Chaco are indicated in colors. Omicron-derived lineages are indicated with sidebars. The support value (SH- *to* LRT/UFB) is shown only for some selected groups.

MATERIALS AND METHODS

Sequencing strategy used:

For partial **sequencing of the gene that codes for the *Spike protein,*** the traditional Sanger method was used, with the sequencing protocol recommended by the CDC (https://wwwnc.cdc.gov/eid/article/26/10_/20-1800_article_), by amplifying segment 29 of the mentioned protocol (fragment comprised between amino acids S_428 and S_750).

In the case of the **genomes**, the **complete sequencing** of SARS-CoV-2 was performed with a Midnight RT-PCR protocol, for use in Oxford Nanopore MinION (PCR tiling of SARS-CoV-2 virus with rapid barcoding and Midnight RT PCR Expansion -SQK-RBK110.96 and EXP-MRT001): https://community.nanoporetech.com/protocols/pcr-tiling-of-sars-cov-2-virus-with-rapid-barcoding-and-midnight.

Phylogenetic analysis:

A phylogenetic analysis was performed with SARS-CoV-2 sequences that included reference sequences from several lineages, Omicron reference sequences (lineages BA.1-BA.5) and some of its derivatives (from the Pango-designation v1.8, available at https://github.com/cov-lineages/pango-designation), the three reported Omicron sequences from Argentina, and the ten sequences with the least number of changes of each, obtained by Audacity*Instant* (against the GISAID database as of May 6, 2022). The alignment was built with MAFFT v7.486, the maximum likelihood tree was built with IQ-TREE v.2.1. The reliability of the branches and clusters was evaluated using the *SH-approximate likelihood ratio test (SH-aLRT)* (1000 replications) and *Ultrafast bootstrap Approximation* (UFB) (10000 replications) methods. We thank the laboratories that generated and shared sequence data through the GISAID Initiative, whose data was included in this analysis.

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[3] https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---4-may-2022 _

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