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BRIEF RESEARCH REPORT
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SARS-CoV-2 Genomic Surveillance Enables the Identification of Delta/Omicron Co-Infections in Argentina

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Molecular surveillance of SARS-CoV-2 is crucial for the early detection of new variants and lineages. In addition, detection of co-infections with more than one SARS-CoV-2 lineage has been sporadically reported. In this work, surveillance of SARS-CoV-2 variants was performed on 2,067 RNA samples (Ct > 30) obtained during December 2021 and January 2022 from Córdoba province, Argentina, by real-time RT-PCR specific for variants of concern (VOCs) and variants of interest (VOIs) relevant mutations (TaqMan™ SARS-CoV-2 Mutation Panel, Applied Biosystems). The following distribution of variants was obtained: Omicron (54.9%), Delta (44.2%), and Lambda (0.8%). Three samples (0.1%), from the last week of December, were compatible with a Delta/Omicron co-infection. One of them was sequenced by NGS-Illumina, obtaining reads for both VOCs. One of the co-infected patients presented with severe symptoms, was not vaccinated, and had risk factors (older than 60 years and arterial hypertension). We describe for the first time in Argentina the identification of cases of co-infection with two SARS-CoV-2 lineages, VOCs Delta and Omicron, during the third COVID-19 wave in the country (a high viral circulation period), when Delta and Omicron co-circulated. Our findings highlight the importance of continuing molecular surveillance, in order to elucidate possible recombination events and the emergence of new variants.

Keywords: SARS-CoV-2, co-infection, Delta, Omicron, Argentina, molecular surveillance

INTRODUCTION

During the 2 years of the COVID-19 pandemic, the original SARS-CoV-2 that was identified at the end of 2019 has evolved into various lineages (1), presenting characteristic mutations. Among them, variants that posed an increased risk to global public health have been identified as variants of interest (VOIs) and variants of concern (VOCs), which present a defined pattern of mutations (2).

Five VOCs—Alpha (lineage B.1.1.7), Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2), and Omicron (lineage B.1.1.529)—and two VOIs—Lambda (C.37) and Mu (B.1.621)—have been reported to date (2).

Whole-genome sequencing (WGS) has been widely used since the beginning of the pandemic to monitor virus variants, to obtain a better understanding of the virus biology and epidemiology (3). However, it is a time-consuming and expensive technique that requires trained staff and specific equipment, restricting its access in resource-limited settings (4). As an alternative, reverse transcription real-time polymerase chain reaction (real-time RT-PCR) assays for the detection of relevant mutations associated with SARS-CoV-2 variants have been developed, to typify circulating variants, as a more accessible tool for the monitoring of VOCs (4, 5).

Molecular SARS-CoV-2 surveillance has allowed the identification of the simultaneous infection (co-infection) of a single individual by two distinct SARS-CoV-2 lineages, an event that has been sporadically reported (3, 6–8). These cases constitute an opportunity for viral genetic recombination and the emergence of new lineages with different phenotypes (6), which may cause more severe clinical symptoms (7). The frequency of co-infected patients and their role in promoting recombination-driven SARS-CoV-2 evolution is still unknown and poorly understood (6).

In Argentina, the profile of circulating lineages and variants has been changing throughout the pandemic, as has happened in the rest of the world (9). Molecular surveillance in the country started with WGS carried out by the Ministries of Science and Technology, and Health, at the national level (10, 11), but then, given the increase in the number of COVID-19 cases, the appearance of VOCs/VOIs, and the need for rapid results that enable public health decision-making, some provinces implemented different strategies based on real-time RT-PCRs for the detection of VOC/VOI relevant mutations, as additional techniques to WGS. This was the case of the province of Córdoba, in the central region of the country, where a strategy that combined detection of point mutations was developed (12). This strategy was used for the molecular surveillance of SARS-CoV-2 variants during 2021, enabling the typing of a greater number of samples with less processing time (12, 13). Moreover, this strategy allowed the detection of the Omicron variant for the first time in Cordoba on December 2021, when the third wave of COVID-19 started in the country, displacing the VOC Delta, the major variant circulating at that time (13).

In this report, we describe for the first time in Argentina cases of co-infection with the Delta and Omicron variants of SARS-CoV-2 that were detected by molecular surveillance in December 2021 during the third COVID-19 wave in the country.

METHODS

Samples Obtained During SARS-CoV-2 Genomic Surveillance

A total of 2,067 SARS-CoV-2 RNA-positive samples obtained from oropharyngeal swabs collected during December 2021 and January 2022 in the province of Córdoba (central area of Argentina) were analyzed for VOC/VOI detection as part of the molecular surveillance program of the local government of the province. The samples had originally been extracted with the MegaBio plus Virus RNA Purification Kit II (BioFlux) on the GenePure Pro Nucleic Acid Purification System NPA-32P and amplified by real-time RT-PCR using the DisCoVery SARS-CoV-2 Nucleic Acid Detection Kit.

Detection of VOC/VOIs by Real-Time RT-PCR

Detection of the relevant mutations L452R, P681R, P681H, K417N, and L452Q (within the spike protein) was carried out by real-time RT-PCR, using the TaqMan™ SARS-CoV-2 Mutation Panel (Applied Biosystems), following the strategy described by Castro et al. (12). Each reaction was performed as a multiplex, including probes simultaneously detecting the wild type (wt) and the mutant nucleotide sequences. Briefly, 7 µl of RNA was added to 8 µl of a mixture containing TaqPath™ 1-Step RT-qPCR Master Mix, CG (4×), TaqMan™ SARS-CoV-2 Mutation Panel Assay (40×), and nuclease-free water.

Whole-Genome Sequencing

Samples that were compatible with a co-infection profile in the real-time RT-PCRs for VOC/VOI screening were subjected to WGS by the Illumina platform, using the Illumina COVIDSeq RUO kit, version COVIDSeq Test Kit. Manual inspection of variant-specific mutation sites was accessed using the program Tablet (14). The sequenced sample was submitted to the GISAID database under the accession number EPI_ISL_8938300.

RESULTS

From the 2,067 samples analyzed using the mutation-specific real-time PCR strategy for detection of VOCs/VOIs, 913 (44.2%) belonged to VOC Delta, 1,135 (54.9%) belonged to VOC Omicron, 16 (0.8%) belonged to VOI Lambda, and 3 (0.1%) presented profiles compatible with co-infections. The distribution of variants abruptly changed during this 2-month study, with Delta comprising the majority of detections in early December, Omicron comprising the majority of detections in mid-December 2021 to mid-January 2022, and Omicron comprising all detections by late January 2022 (**Figure 1**).

The main features of the 3 co-infected patients are shown in **Table 1**. **Figure 2A** shows the mutation profile detected by real-time RT-PCRs on samples obtained from these patients. Wild-type and mutant RNA was simultaneously detected for L452R and K417N. Only mutant RNA was detected for P681R and P681H, without amplification of wild-type RNA, indicating the presence of a mutation in that position (**Figure 2A**, **Supplementary Table 1**). We ruled out cross-contamination by repeating nucleic acid extraction and VOC/VOI-specific qRT-PCR on samples from all 3 co-infected patients and arriving at the same results.

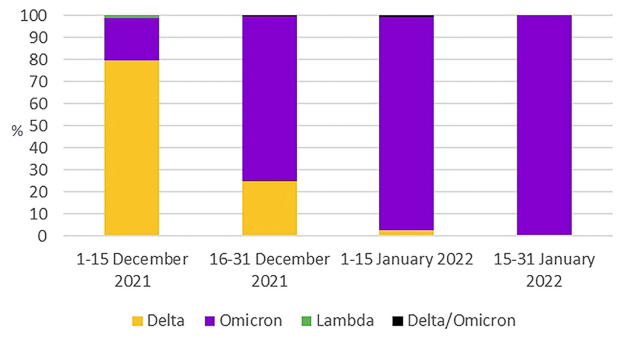

FIGURE 1 | Distribution of circulating VOCs/VOIs during December 2021 and January 2022 in the province of Cordoba, Argentina.

Sample no. 1 contained a sufficient viral load and RNA quantity to perform WGS for further investigation. The sequence obtained from this sample was 29,867 nucleotides in length. Compared to the WIV04 reference sequence (EPI_ISL_402124), it included 0.87% unidentified nucleotides (Ns) and 0.34% nucleotide mutations. The average percentage of reads matching the Omicron variant was higher than the average percentage of reads matching the Delta variant (**Figure 2B**). Positions in which the average percentage of reads matching the Delta variant was higher had lower coverage. Pangolin COVID-19 Lineage Assigner (Pangolin v3.1.19) could not assign a lineage to this sequence.

DISCUSSION

Co-infection with distinct SARS-CoV-2 lineages is considered a rare phenomenon. However, its likelihood increases as infection prevalence increases and is thought to be underestimated (6).

In this work, we report the co-infection with Delta and Omicron VOCs. This is the first description of co-infected individuals carrying two distinct lineages of SARS-CoV-2 in Argentina. VOC Delta was first described in our province in July 2021, when it was detected in a traveler and his close contacts. Due to the efforts carried out by the health authorities of the province, which included tracking and isolating Delta-positive cases and their close contacts, the spread of this VOC was delayed, so its increase was gradual, until reaching its highest proportion of circulation (85%) in November 2021 (12), but without a substantial increase in the number of cases (13).

VOC Omicron was detected in Argentina—and particularly in Córdoba province—during the first few days of December 2021, in a traveler from Dubai, and it quickly spread throughout the province (13). The sharp increase in Omicron frequency was accompanied by an increase in the number of cases, giving rise to the third wave of COVID-19 in the province and the entire country (11, 13).

In this context of co-circulation of variants, 3 samples with Delta/Omicron SARS-CoV-2 co-infection were identified, all of them detected the last 2 weeks of December, when co-circulation of Delta and Omicron was registered (13). Co-infection events between dominant SARS-CoV-2 lineages have been previously reported, also in a very low proportion of the tested samples (3, 6–8). Although these events are rare, they are believed to be quite common during periods of high viral prevalence (15) and are believed to be underreported (6), as they are not easy to identify. Generally, one of the lineages is present in a greater proportion (3), which sometimes causes only one lineage to be detected, which has been registered for Delta/Omicron mixed infection (8). In addition, specialized personnel are required for the interpretation of variant-specific real-time PCR, which is not always capable of detecting subtleties in the reaction results. In our study, co-infections were detected during the third wave of COVID-19 that took place in our country, with very high levels of viral circulation, in accordance with previous reports (6, 15).

The report of co-infections of SARS-CoV-2, both locally and globally, becomes relevant in a context of changing circulation of variants and emergence of new ones. In this sense, recombination, already reported for other coronaviruses and also recently for SARS-CoV-2, is a possibility in individuals simultaneously infected with more than one lineage (3, 15). In turn, the emergence of newly recombined viruses might result in increased transmissibility or immune evasion (3), as recombination permits the combination of advantageous mutations from distinct variants (15). Since recombination is only possible with co-infection, decreasing the prevalence and circulation of SARS-CoV-2 will minimize the chance of forming recombinant lineages with genetic combinations that could potentially increase virus fitness (15).

Until now, no major clinical implications have been described in patients with co-infection with more than one SARS-CoV-2 lineage (3). In this study, only one of the patients presented severe symptoms (pneumonia and dyspnea), although they were probably due to the lack of vaccination and to the presence of risk factors (over 60 years of age and arterial hypertension) rather than the co-infection. However, more clinical research is needed and should be carried out on these patients.

TABLE 1 | Main characteristics of the patients with SARS-CoV-2 Delta/Omicron co-infections.

Sample ID	Date of swab collection/onset of symptoms	Sex	Age (years)	Vaccine	Co-morbidities	Clinical manifestations	Hospitalization
1	December 27, 2021	M	18	Yes[a]	No	Fever, throat pain	No
2	January 7, 2022	F	5	Yes[b]	No	Fever, dyspnea	Yes
3	December 29, 2021	M	64	No	Arterial hypertension	Asthenia, dyspnea, pneumonia	Yes

[a]*One dose of Recombinant Novel Coronavirus Vaccine (Adenovirus Type 5 Vector).*
[b]*One dose of SARS-CoV-2 inactivated (Vero cells) vaccine.*

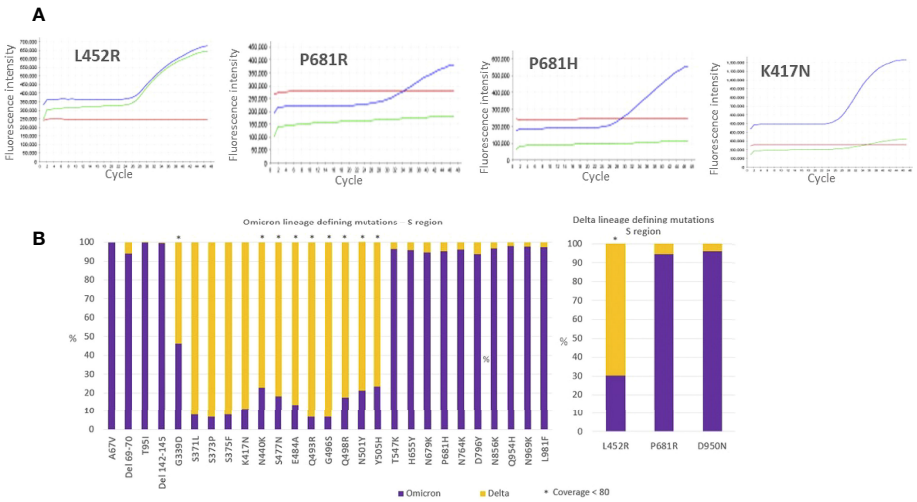

FIGURE 2 | **(A)** Real-time RT-PCR for VOC typing. Blue curve: presence of mutation; green curve: absence of mutation (presence of wild type); red curve: negative control. **(B)** Proportion of sequencing reads obtained for the S region by the whole-genome sequencing technique matching with VOCs Omicron or Delta for the analyzed mutations that define the lineage. *Positions in which the number of reads were less than 80.

In conclusion, we found, for the first time in Argentina, co-infections by two SARS-CoV-2 lineages (Delta/Omicron) during the third wave of COVID-19, the largest in our country (11). This highlights the importance of continuing molecular surveillance, especially in moments of high viral circulation, to detect both co-infections and recombinations. It is important to continue studying co-infection cases to determine if co-infection is associated with more severe disease and/or outcomes.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.gisaid.org/, EPI_ISL_8938300.

ETHICS STATEMENT

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MP performed the conceptualization, analyzed the data, and wrote the original draft. PS carried out molecular detections and analyzed the results. MZ, AL, FF, and GC carried out molecular detections. SG, MV, and LL contributed to the analysis of the data. MB supervised the work and revised and edited the manuscript. VR performed conceptualization and supervision, and revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fviro.2022.910839/full#supplementary-material

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