

Communication

# BA.1 Omicron Variant of SARS-CoV-2: First Case Reported in Calabria Region, Italy

Cinzia Peronace <sup>1,\*</sup>, Rossana Tallerico <sup>1</sup>, Manuela Colosimo <sup>1</sup>, Marco De Fazio <sup>1</sup>, Federica Pasceri <sup>1</sup>, Ilenia Talotta <sup>1</sup>, Giuseppina Panduri <sup>1</sup>, Letizia Pintomalli <sup>2</sup>, Rosaria Oteri <sup>2</sup>, Valeria Calantoni <sup>2</sup>, Maria Teresa Fiorillo <sup>2</sup>, Luca Gallelli <sup>3</sup>, Erika Cione <sup>4,\*</sup> and Pasquale Minchella <sup>1</sup>

<sup>1</sup> Microbiology and Virology Unit, Pugliese-Ciaccio Hospital, 88100 Catanzaro, Italy; rossana.tallerico@gmail.com (R.T.); manuelacolosimo@hotmail.it (M.C.); marco\_2592@yahoo.it (M.D.F.); federicapasceri@gmail.com (F.P.); ilenia.talotta@gmail.com (I.T.); pandurigiuseppina@gmail.com (G.P.); pminchella@aocz.it (P.M.)

<sup>2</sup> Unit of Microbiology and Virology, North Health Center ASP 5, 89123 Reggio di Calabria, Italy; letizia.pintomalli@libero.it (L.P.); oterirosaria@gmail.com (R.O.); valeriacal@libero.it (V.C.); mariatfiorillo@hotmail.com (M.T.F.)

<sup>3</sup> Department of Health Science, University of Catanzaro, Mater Domini Hospital, 88100 Catanzaro, Italy; gallelli@unicz.it

<sup>4</sup> Department of Pharmacy, Health, and Nutritional Sciences, University of Calabria, 87036 Rende, Italy

\* Correspondence: cinziaperonace@hotmail.it (C.P.); erika.cione@unical.it (E.C.); Tel.: +39-0961-883-202 (C.P.); +39-0844-93193 (E.C.)

**Abstract:** In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), emerged in Wuhan, China. Several variants of concern (VOCs) have been identified so far. Recently, the B.1.1.529 (Omicron) variant of SARS-CoV-2 spread rapidly worldwide. We describe the first case of the Omicron genetic lineage BA.1 in our region. The patient is a physician who traveled to Johannesburg (South Africa) and returned to Reggio Calabria (Italy). He underwent a SARS-CoV-2 screening before leaving—a required procedure where travelers present a negative PCR test one-day prior to departing. Three days after arriving in Italy, he started experiencing cold symptoms. Clinically, he was without fever or severe respiratory symptoms and reported suffering from a cold and sore throat. The nasopharyngeal swab specimen was tested by TaqPath COVID-19 RT-PCR and sequenced by Sanger sequencing, and next-generation sequencing (NGS) data were processed with their relative software. A peculiar drop-off of the S gene was obtained with TaqPath COVID-19 RT-PCR. S gene mutations indicative of the Omicron variant were obtained with both sequencing methods, pointing out 17 mutations in the 29 recognized by Sanger and the 28 recognized by NGS.

**Keywords:** Omicron; NGS; mutations



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## 1. Introduction

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), emerged in Wuhan, China. Several variants of concern (VOCs) have been identified so far. Recently, the discovery of the highly mutated Omicron variant of concern (VOC) G.R./484A (B.1.1.529) of SARS-CoV-2 was firstly detected in specimens collected on 11th November 2021, in Botswana and three days later (November 14th) in South Africa [1,2], which raised many questions. With the confirmed cases of Omicron reported in the European Union, there was an urgent need to trace it to monitor its spread [3]. This is of fundamental importance for setting up an effective countermeasure. More than half a million genome sequences were submitted to the Global Initiative on Sharing Avian Influenza Data (GISAID) from all six continents to confirm variants before December 2021, including this case from our laboratories the Department of Microbiology and Virology, Pugliese Ciaccio's Hospital, Catanzaro, Italy,

and the Unit of Microbiology and Virology, North Health Center ASP 5, Reggio Calabria, Italy, respectively. At the time of writing this report, twenty-seven cases were reported to be the Omicron variant in Italy, of which one comes from the Calabria Region [4,5]. Besides that, a recent report indicates that two patients tested positive with Omicron on November 13 and 18 in Hong Kong, China [6]. It is believed that the first passenger transmitted the virus to the other passenger despite being in a hotel quarantine in different rooms [6,7]. Therefore, more studies are needed to better understand its transmissibility, immunity escape potential, clinical presentation and disease severity and the role of other available diagnostic and therapeutic countermeasures. Herein, we describe the first case of the Omicron genetic lineage BA.1 associated with mild clinical symptoms.

## 2. Case Presentation Section

On 5 December 2021, a 30-year-old man without comorbidities presented voluntarily to the hospital for a SARS-CoV-2 screening. He informed the hospital he had traveled to Johannesburg (South Africa) and back home to Reggio Calabria (Italy). In Johannesburg, he worked at a Trauma Unit as a physician for about one month. He had a SARS-CoV-2 screening before leaving—a procedure that requires travelers to present a negative PCR test one-day prior to departing. Three days after arriving in the Italy, he was started experiencing cold symptoms. Clinically, he was without fever or any severe respiratory symptoms. However, he was suffering from a cold and a sore throat. The patient had received two doses of the COVID-19 vaccine (BNT162b2) and received the booster dose on 6 November 2021. Therefore, blood specimens and nasopharyngeal swabs were collected. Markers of inflammation, including pro-calcitonin, C-reactive protein (CRP), natural killer cells and B and T lymphocytes, were immediately evaluated. The serological tests showed that he was positive for SARS-CoV-2-specific IgG. Unfortunately, the molecular nasopharyngeal swab resulted positive for SARS-CoV-2 RNA. Consequently, the patient was immediately notified.

## 3. Methods

Testing was carried out with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (hereinafter referred to as TaqPath™ COVID-19) (Thermo Fisher Scientific, Waltham, MA, USA), which targets the following genes: (i) open reading frame (ORF)1ab; (ii) nucleocapsid (N); and (iii) spike (S), coupled with QuantStudio 5 DX ThermoFisher Real-Time PCR (RT-PCR) (Thermo Fisher Scientific, Waltham, MA, USA). At the time of the study, the nasopharyngeal sample was collected in Universal Transport Media (UTM), opened in biosafety cabinet class-II and then 200 µL of the UTM was further processed for viral nucleic acid extraction. Briefly, 200 µL of UTM was extracted for the TaqPath™ COVID-19 (Thermo Fisher Scientific, Waltham, MA, USA). in the semi-automatic KingFisher Duo Prime by ThermoFisher using the MagMAX™ Viral/Pathogen Nucleic Acid isolation kit on the KingFisher Flex Purification system (Thermo Fisher Scientific, Waltham, MA, USA). Before RNA extraction, 10 µL of Proteinase K was added to each well in the KingFisher™ Deep 96-well Plate (Thermo Fisher Scientific, Waltham, MA, USA). In addition, 10 µL of the MS2 Phage Control, together with 10 µL of magnetic beads, was added to all specimens. In addition, the specimen underwent genomic characterization following two methodologies: the Sanger-based sequencing by the SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and whole-genome based on next-generation sequencing (NGS) by MiSeq System (Illumina, San Diego, CA, USA). The latter was performed by the Regional Center at the Microbiology and Virology Laboratory of Catanzaro.

## 4. Results and Discussion

From the RT-PCR amplification curves, the shape highlights Ct values of 26.42 and 26.94 for ORF1ab and N genes, respectively, failing to identify the S gene (data not shown). This peculiar drop-off of the S gene was indicative of a possible variant of concern (VOC). Sequencing Sanger based on the S gene was performed using a validated, standard pro-

tol with 12 commercial primer pairings, as described by Paden et al. [7], for generating high-quality, full-length SARS-CoV-2 genomes from primary samples. The protocol uses multiplex reverse transcription PCR, followed by Sanger and MiSeq sequencing. Data analysis conducted on SeqScape software revealed the Omicron variant, lineage BA.1 (clade 21k), recognizing 29 mutations. The NGS approach provided  $2 \times 250$  bp read-length data. The SOPHIA DDM Platform analyzed the FASTQ reads. Clade analysis conducted on the ICOGEN Platform revealed 28 mutations in the S gene, of which 17 were common to both systems, as shown in Table 1. As expected, the NGS data pointed out other mutations in the genome region of the virus: E T9I, M A63T, M D3G, M Q19E, N E31del, N G204R, N P13L, N R32del, N R203K, N S33del, NSP2 E563D, NSP3 A1892T, NSP3 K38R, NSP3 L1266I, NSP3 S1265del, NSP4 T492I, NSP5 P132H, NSP6 G107del, NSP6 I189V, NSP6 L105del, NSP6 S106del, NSP12 P323L and NSP14 I42V. Then, the lineage information was described using the Pangolin nomenclatures [7], and the Omicron variant sequences were deposited in the GISAID database on 9 December 2021. Consensus accuracy, including single-nucleotide polymorphisms and indels, is critical for determining coronavirus lineage and transmission networks. VOC are still integral to controlling virus spread. Therefore, a small aliquot of the collected specimen was processed through a rapid antigen test (AgRT) device based on a lateral-flow immuno-chromatographic assay (GeneFinder COVID-19 Ag Plus Rapid Test) (Osang Healthcare Co., Ltd, Daegu, Republic of Korea) [8]. The device showed positive results, indicating how an AgRT with high sensitivity and specificity represents an excellent screening method, especially in high prevalence areas. Additionally, as in this case, an AgRT can detect the Omicron variant. Further, nasopharyngeal swabs were collected to monitor the viral load up to a negative result, which was achieved in about ten days. From a clinical point of view, symptomatology did not worsen, which is in agreement with other clinical characteristics of the Omicron variant infection, which consisted, for the most part, of mild symptoms [9]. However, as with all variants, a lag exists between infection and more severe outcomes, and symptoms are expected to be milder in vaccinated persons and those with previous SARS-CoV-2 infection than in unvaccinated persons [10]. Our patient received two doses of the COVID-19 vaccine (BNT162b2) and received the booster dose on 6 November 2021. He was without fever or any severe respiratory symptoms. He was only suffering from a cold and a sore throat. Although mRNA vaccines provide protection mediated by T-cells, the response may still provide partial protection against mutations that allow the virus to escape from antibodies, significantly decreasing the severity and hospitalization rate, as in our case. With this evidence, we speculate that the immune responses elicited by vaccines could have alleviated the disease progression course, as reported in our case. The trials of vaccines performed in 2020–2021 clearly show the efficacy against SARS-CoV-2 infection. However, the vaccine effectiveness with the arrival of the new variant, Omicron, remains unclear. It is worth noting that the clinical features and epidemiologic data of the BA.1 Omicron infection are still being defined. This study, which included one patient who experienced reinfection after having been fully vaccinated, suggests also a shorter incubation period and a clinical condition associated with the other variant in persons who have been vaccinated, and the study adds to existing evidence of potential reinfection. The average incubation period has been described as  $\geq 5$  days for SARS-CoV-2 and nearer to 4 for the B.1.617.2 (Delta) SARS-CoV-2 variant [10–12]. In this case, the average incubation period observed was about 3 days. Few clinical descriptions of Omicron infections are available, and mild illness amongst vaccinated subjects has been reported in California [13]. Although the vaccine produces a whole array of antibodies against the RBD-S spike protein, there are still many unknown mutations associated with the Omicron variant; therefore, partial immune escape may be expected. Lastly, more studies are needed to better understand the Omicron variant's transmissibility, clinical presentation, immunity escape potential and disease severity, as well as the role of other available diagnostic and therapeutic countermeasures.

**Table 1.** Spike amino acid Omicron variation (ND = Not Detected).

#	Sanger-Based Sequencing	Next-Generation Sequencing
1	A67V	A67V
2	T95I	T95I
3	D215EPED	ND
4	S371L	S371L
5	S373P	S373P
6	S375F	S375F
7	K417N	K417N
8	N440K	N440K
9	G446S	G446S
10	S477N	ND
11	T478K	ND
12	E484A	ND
13	Q493R	ND
14	G496S	ND
15	Q498R	ND
16	N501Y	ND
17	Y505H	ND
18	T547K	T547K
19	D614G	D614G
20	H655Y	H655Y
21	N679K	N679K
22	P681H	ND
23	N764K	N764K
24	D796Y	ND
25	N856K	N856K
26	Q954H	Q954H
27	N969K	N969K
28	L981F	L981F
29	D1146	ND
30	ND	G142D
31	ND	G339D
32	ND	L212I
33	ND	P681H
34	ND	V70del
35	ND	V143del
36	ND	Y144del
37	ND	H69del
38	ND	N211del
39	ND	Y145del
40	ND	ins214EPE

## 5. Conclusions

In light of the emergence of a new SARS-CoV-2 variant associated with increased transmissibility and partially immune-escape, the new Omicron variant of COVID-19 has been detected in more than 40 countries on five continents. Although the drop-off of the S gene was recognized by the TaqPath COVID-19 RT-PCR kit assay, genomic characterization is crucial for identifying emerging viral variants and remains essential for determining the neutralization capacity against all variants, including Omicron. Full-genome sequencing is a critical tool in understanding emerging viruses. In fact, the initial sequencing of SARS-CoV-2 showed limited genetic variation before, which increased rapidly.

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**Institutional Review Board Statement:** All the procedures were following the 1964 Helsinki Declaration and its later amendments. In any case the study did not involve humans or animals.

**Informed Consent Statement:** The study was conducted using residual specimens. According to the Italian health public law, this type of study did not require specific, informed consent and/or ethics committee approval.

**Data Availability Statement:** Data supporting the reported results can be found at the Department of Microbiology and Virology, Pugliese Ciaccio's Hospital, Catanzaro, Italy, and at the Unit of Microbiology and Virology, North Health Center ASP 5, Reggio Calabria, Italy.

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