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Rapid Report

First identification of SARS-CoV-2 Omicron BA.4 lineage in Italy: genomic comparison with omicron lineages

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Abstract: The rapid emergence and worldwide detection of the SARS-CoV-2 omicron variant underscore the importance of robust genomic surveillance systems and prompt information sharing among global public health partners. The Omicron variant has rapidly replaced the delta variant as a dominating SARS-CoV-2 variant because of natural selection, favoring the variant with higher infectivity and more strong vaccine breakthrough ability. Omicron has three lineages or sub-variants, BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), and BA.3 (B.1.1.529.3). Among them, BA.1 is the currently prevailing sub-variant. BA.2 is found to be able to alarmingly re-infect patients originally infected by omicron BA.1. BA.3 lineage is a combination of mutations in BA.1 and BA.2 spike proteins. Today any data are reported on BA.4 lineage. Here we describe the new emerging BA.4 lineage, first detected in Italy, and its genomic comparison with the other three lineages of the omicron variant, which harbor a deletion of the ORF1 ab gene never reported till now. We can speculate that omicron BA.4 will become a new dominating "variant of concern". Besides that, we show also the capability of five different types of rapid antigenic tests to recognize it.

Keywords: SARS-CoV-2, Variant of Concern, BA.4, pandemic.

1. Introduction

A new variant of SARS-CoV-2, B.1.1.529 (Omicron) (1-2), was first reported to the World Health Organization (WHO) by South Africa on November 24, 2021, and designated as a variant of concern (3). We reported the first case in our region (Calabria, Italy) on December 5, 2021 (4). The variant carries an unusually high number of mutations, 32 mutations located within S protein, which is the key viral component that determines the infectivity and antigenicity of the virus. Furthermore, 15 of 32 mutations are located right at the receptor-binding region (RBD) of Spike protein that interacts with human cells before cell entry, possibly enhancing the transmissibility (5). Omicron was recently divided into four lineages: BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), BA.3 (B.1.1.529.3), and B.1.1.529 lineages, (6-7). The differences between the BA.1 and BA.2 lineages are explored (8). The data reported in the literature confirm Omicron's high infectivity (9-10), high vaccine breakthrough rate (11-12), and severe antibody escape rate (13-15). Though all the

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three lineages have spread worldwide, the rate of spread of these three lineages is different. Of the Omicron sequences submitted to GISAID, the BA.1 lineage is approximate>98%, the BA.2 to approximately 1% sequence, and the BA.3 around 0.1% sequence (www.gisaid.org). Of these three lineages, only BA.1 dominates much more than the other lineages that have ousted Delta. This is likely due to differences in mutations in the spike protein required for virus transmission and host cell entry (16-17). Design et al., report that of these four lineages, B.1.1.529 appears to be the parental lineage of the Omicron variant, and then the BA.1 lineage seems to be the closest to this B.1.1.529 lineage. BA.2 has significant diversity from the B.1.1.529 and BA.1 lineage, while BA.3 has the intermediate lineage to BA.1 and BA.2 (8). Today, any published data are reported in the literature about BA.4 lineage. We first identified by sequencing this lineage on April, 25 by depositing it in ICOGEN Platform by Istituto Superiore di Sanità (ISS). The novel omicron lineage BA.4 is here studied in comparison with the other three lineages and together with the capability of five different type of Rapid Antigenic Tests (RATs) to recognize them.

2. Materials and Methods

Sample collection and viral RNA extraction

Positive nasopharyngeal and oropharyngeal swab collected in UTM was carried out with the TaqPath COVID-19 CE-IVD RT-PCR kit, which targets the following genes of SARS-CoV-2: i) open reading frame (ORF)1ab; ii) nucleocapsid (N) and iii) spike (S), coupled with QuantStudio 5 DX Thermo-Fisher Real-Time PCR (RT-PCR) as describe in pour previous study (18).
A total of 180 µl of the sample was used for RNA extraction by automated instrument (MGISP-100, MGI) using the MGIEasy Nucleic Acid Extraction Kit with superparamagnetic beads technology (MGI). Before RNA extraction, 10 µL of Proteinase K was added to each well in the King-Fisher™ Deep well 96 Plate. In addition, 10 µL of the MS2 Phage Control was added to all specimens together with 10 µL of magnetic beads. RNA extracted was used for whole-genome sequencing of SARS-CoV-2.

Library Preparation and Next-Generation Sequencing

RNA extracted from the sample was used for the next library preparation step for next-generation sequencing (NGS) on the Illumina sequencing platform (MiSeq System, Illumina USA). CleanPlex SARS-CoV-2 FLEX Paragon Genomics Panel performed a Reverse transcription of the whole-genome and library preparation. The thermal-cycling or incubation reaction is followed by a library purification using magnetic beads (CleanMag Magnetic Beads). The workflow involves just three steps: i) the first step is cDNA synthesis and purification from purified RNA sample; ii) the second step is a multiplex PCR reaction that uses target-specif primers to amply targets of interest, thus covering the entire SARS-CoV-2 genome with the 2-pool design, at this stage, internal human (host) housekeeping RNA control primer pair was also added; iii) the third step is a digestion reaction to amply and add-sample level indexes to the generated libraries. These libraries were quantified using Qubit dsDNA HS Assay Kit (Invitrogen by Thermo Fisher Scientific). The quality of the library was checked using the DNA high sensitivity assay kit on Bio-analyser 2100 (Agilent Technologies, United States) and sequenced by the MiSeq platform providing 2x250 bp reads length data. The SOPHIA DDM Platform analyzed FASTQ reads. Clade analyses were obtained by ICOGEN Platform (ISS) and the GISAID database.

Rapid SARS-CoV-2 antigen detection assays

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Rapid and accurate tests for SARS-CoV-2 screening are essential to expedite disease prevention and control. Thus, five Rapid Antigenic Tests (RATs) based on lateral flow immunoassay was carried out according to the manufacturer's instructions (read at 15 min), including: 1-GeneFinder COVID-19 Ag Plus Rapid Test manufactured by OSANG Healthcare Co., Ltd, South Korea; 2-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Vazyme) manufactured by Nevia Biotech; 3-SARS-CoV-2 Antigenic Rapid Test Flowflex manufactured by ACON Biotech (Hangzhou) Co., Ltd, China; 4-SARS-CoV-2 Antigen manufactured by Lifotronic Technology Co, Ltd, China; 5-InstaView COVID-19 Antigen manufactured by SG Medical, Inc., South Korea.

3. Results and Discussion

The uncertainty that what the next COVID variant will be, remains a significant cause of concern for the World Health Organization (WHO). Omicron is currently dominant worldwide and currently sub-lineages are tracked as well as and sister lineages of the variant - BA.2.12.1, BA.5 and BA.4. This latter was sequenced by Sanger as first level of screening based on S-gene that was performed using a standard protocol with 12 commercial primer pairings described by Paden et al. (19). Data analysis obtained by SeqScape software, revealed Omicron variant. The NGS approach provided 2x250 bp read length data. The SOPHIA DDM Platform analyzed FASTQ reads Then, lineage information was described using the Pangolin nomenclatures (20), and the omicron variant sequences were deposited in the ICOGEN Platform on the April, 25 2022 and GISAID on the April, 26 2022 (see supplementary materials). Clade analysis revealed 2 deletions and 13 mutations on the S gene corresponding to the following amino acids on the translated protein: T19I; LPPA24S_del; IHV68I_del; G142D; V213G; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; L452R; T47, of which some in common to other omicron, as shown in Table 1.

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Table 1. Amino acidic mutations on omicron lineages obtained by full genome

Omicron lineages	Mutations										
	ORF1ab	**S-protein**	**ORF3a**	**E-protein**	**M-protein**	**ORF6**	**ORF7a**	**ORF7b**	**ORF8**	**N-protein**	**ORF10**
BA.1	E563D; K38R; F106; SL1265I_del; T492I; P132H; LSGF105F_del; I189V; V57; P323L; N600	A67V; IHV68I_del; T95I; GVYY142D_del; NL211I_del; D215EPED; S371L; S373P; S375F; K417N; N440K; G446S; T547	T64	T9I	D3G; Q19E; A63T	R20		L18		P13L; GERS30G_del; R203K;G204R	
BA.2	S135R; T24I; F106; G489S; A534; A1526V; L264F; V290; T327I; T492I; D48; R131; P132H; SGF106_del; F251L; R252T; Y253S; I65; S11; P323L; L758; I258; R392C; I42V; T112I; E145	T19I; LPPA24S_del; G142D; V213G; D405N; R408S; K417N; N440K; S477N; T478K; E484A; Q493R; Q498R; N501Y; Y505H; T547K; D614G; H655Y; N679K; P681H; N764K; D796Y; Q954H; N969K; D1146	T64; T223I	T9I	Q19E; A63T; F112	R20; D61L		L18		P13L; GERS30G_del; R203K; G204R	
BA.3	R27C; K38R; F106; L264F; V290; T327I; R131; P132H; SGF106_del; P323L; L758; R392C; I42V; E145	T19I; LPPA24S_del; G142D; S371F; S373P; S375F; T376A; N440K; F456; D614G; H655Y; N679K; P681H; D796Y; Q954H	T64	T9I	F112	D61L		L18		P13L	
BA.4	S135R; KSF141_del; T24I; F106; G489S; A534; L264F; V290; T327I; L417; T492I; D48; R131; P132H; SGF106_del; E23; I65	T19I; LPPA24S_del; IHV68I_del; G142D; V213G; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; L452R; T47	T64; T223I	T9I	Q19E; A63T; F112	R20; D61L		L11F; L18; T40I		P13L; GERS30G_del; P151S; R203K; G204R; S413R; S416L	

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As expected, the NGS data pointed out other mutations in additional gene region of the virus herein also reported for ORF1ab protein: S135R; KSF141_del; T24I; F106; G489S; A534; L264F; V290; T327I; L417; T492I; D48; R131; P132H; SGF106_del; E23; I65. The report pointed out in ORF1 ab gene a mutation in S135R, reported in Africa on 4th April 2022, see figure 1 (GISAID EPI ISL: EPI_ISL_12243764 for verification).

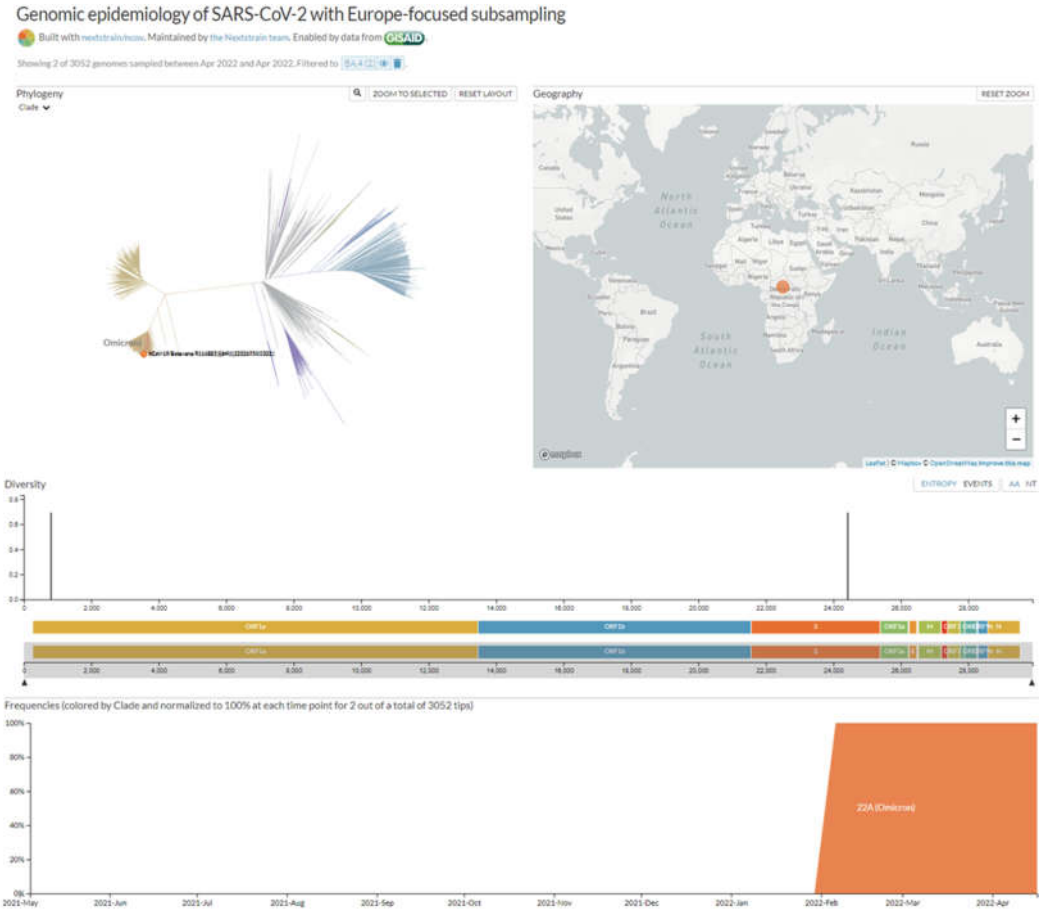

Figure 1. Nextstrain GSAID Built with nextstrain/ncov Accessed April 25, 2022 11:46 pm

The novelty form our NGS data is the evidence of a deletion on ORF1 ab gene which is the biggest gene in the virus genome (Figure 2 panel A) harbor a deletion identified as KSF141_del, peculiar of this new variant discovered in Italy. This was verified also by SNAPgene allineament with the other 3 omicron variant in which deletion in this genetic region is missing as shown in Figure 2 (panel b) (see also supplementary materials).

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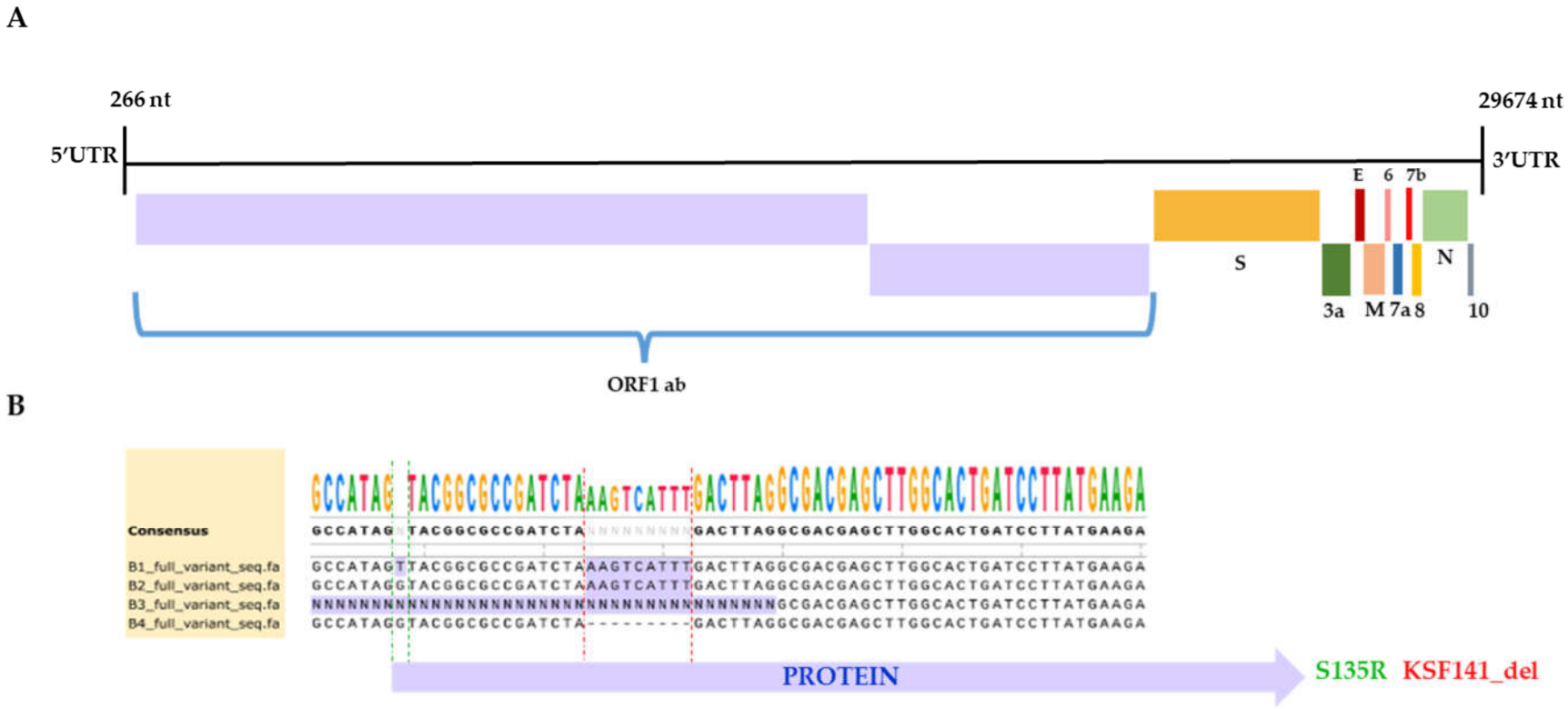

Figure 2. A) Schematic rapresentation of SARS-CoV-2 full genome. B) SNAPgene allineament among omicron lineages.

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The variant of concern (VOC) is still an urgent need for controlling virus spread. Therefore, a small aliquot of the collected specimen was processed through a rapid antigen test (RATs) device based on lateral flow immuno-chromatographic assay, we tested five RATs reporting the capability of all of them to recognizing BA.4 new variants harboring on the nucleocapsid (N) protein the following mutations: P13L; GERS30G_del; P151S; R203K; G204R; S413R; S416L. The capability of lateral flow immunoassay are present in figure 3 and are showed as following: 1-GeneFinder COVID-19 Ag Plus Rapid Test manufactured by OSANG Healthcare Co., Ltd, South Korea; 2-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Vazyme) manufactured by Nevia Biotech; 3-SARS-CoV-2 Antigenic Rapid Test Flowflex manufactured by ACON Biotech (Hangzhou) Co., Ltd, China; 4-SARS-CoV-2 Antigen manufactured by Lifotronic Technology Co, Ltd, China; 5-InstaView COVID-19 Antigen manufactured by SG Medical, Inc., South Korea.

Figure 3. Capability of RATs to recognize omicron BA.4

The N protein from SARS-CoV-2 is recognized by capturing antigen-conjugate gold particle complexes. They migrate across a reaction area coated by antibodies to nucleocapsid proteins. Positive results display two-color related to control (C) and test (T) lines, while only one line in the C area is present for the negative one. The colored test (T) line's intensity depends on the amount of SARS-CoV-2 N antigen presented in the sample.

The devices showed positive results, indicating how RATs with high sensitivity and specificity represents an excellent screening method, especially in high prevalence areas, also for the omicron BA.4 variants, as it is in this case with mild symptoms (19). Although, as with all variants, a lag exists between infection and more severe outcomes, and symptoms would be expected to be milder in vaccinated persons and those with previous SARS-CoV-2 infection than in unvaccinated persons (20). Although the vaccine produces a whole array of antibodies against 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 understand better Omicron transmissibility, clinical presentation, immunity escape potential, and disease severity as well as the role of other available diagnostic and therapeutic countermeasures.

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Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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

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