Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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First detection of SARS-CoV-2 A.23.1 sub-lineage in migrants arriving to Italy via the Mediterranean Sea and public health implications

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Dear Editor,

During the ongoing COVID-19 pandemic, the spread of new SARS-CoV-2 variants is grabbing the attention of the scientific community worldwide. Despite mutations of the SARS-CoV-2 are expected to occur, most of them are not worrisome; however, some variants are of Public Health relevance or even concern, since changes in the virus characteristics, such as increased transmissibility or partial immune escape to available vaccines have been documented.

Although more studies are needed to confirm these findings, the possibility that mutations could help the virus to evade the neutralizing effects of vaccine-induced immune responses is worrying. For the abovementioned reasons, the surveillance on emerging SARS-CoV-2 variants is of relevant interest and, in this framework, the actualization of genomic-based surveillance in low-income countries, where the use of NGS technology is still sparse can certainly represent an opportunity to improve existing Public Health systems [1].

In order to establish a mapping of the spread of the SARS-CoV-2 emerging variants in Italy, with particular regard to the B.1.1.7, P.1, P.2, B.1.351 and B.1.525 lineages, starting from the last February, periodic surveys have been carried out within the network of the Italian regional reference laboratories, coordinated by the Italian National Institute of Health [2]. In this context, the early identification of new variants could allow the introduction of special containment measures to reduce community transmission.

One aspect that still remains relatively unexplored regards the introduction of rare variants in high immigration areas, such as the southern part of Italy is. Therefore, under the supervision of the Italian Ministry of Health, a dedicated SARS-CoV-2 surveillance system has been implemented in Sicily and its smaller islands, representing the main entrance to Europe, through the Mediterranean Sea, from Northern African borders for migrant refugees or asylum-seekers [3], who are considered vulnerable populations to be targeted by specific prevention and control programs [4].

On February 24, 2021, SARS-CoV-2 infection was primarily detected by real-time reverse transcription-polymerase chain reaction (rt-RT-PCR) in nasopharyngeal swabs collected from two asymptomatic young men (aged 19 and 27) of Egyptian origin hosted in the Migrant Reception Center of Lampedusa island.

On March 6, 2021 whole next-generation genome sequencing was performed (Ion Torrent S5, Thermofisher), and the results were compared with full-length viral genomes available on GISAID. Phylogenetic analysis showed that the viral strains (EPI_ISL_1585281 and EPI_ISL_1585282) belonged to the A.23.1 Pangolin lineage nomenclature system. This sub-lineage was firstly detected in Uganda in autumn 2020. Afterward, it has been reported in almost all continents, with most cases identified in UK (n = 164), Central Africa (n = 136), and North America. In Italy, there has been only one notification in the northern part of the country in early February 2021.

The sub-lineage A.23.1 encodes multiple spike, nsp 6, ORF8 and ORF9 protein changes and some of the replacements are predicted to be functionally similar to those observed in lineage B VOCs.

In Uganda, it became predominant over the B.1 lineage in less than three months, suggesting a higher fitness and transmissibility and was reported to be capable of producing severe infections [5].

Of interest, the bioinformatic analysis carried out on the viral genomes from the two Egyptian migrants showed that the viruses EPI_ISL_1585281 had accumulated 15 amino acid substitutions, whereas the strain EPI_ISL_1585282 showed 18 amino acid substitutions and five amino acid deletions in the ORF1a and ORF7a genes (ORF1a:V86-, ORF1a:E87-, ORF7a:S98-, ORF7a:P99-, ORF7a:I100-). Altogether, the set of spike mutations included F157L, V367F, Q613H, and P681R, which have not yet been suggested to mediate escape from vaccine response. V367F change is reported to modestly increase infectivity, while the Q613H change is predicted to be functionally equivalent to the D614G mutation observed in the B.1 lineage, increasing infectivity, enhancing the stability of trimeric SARS-CoV-2 spike protein and furin

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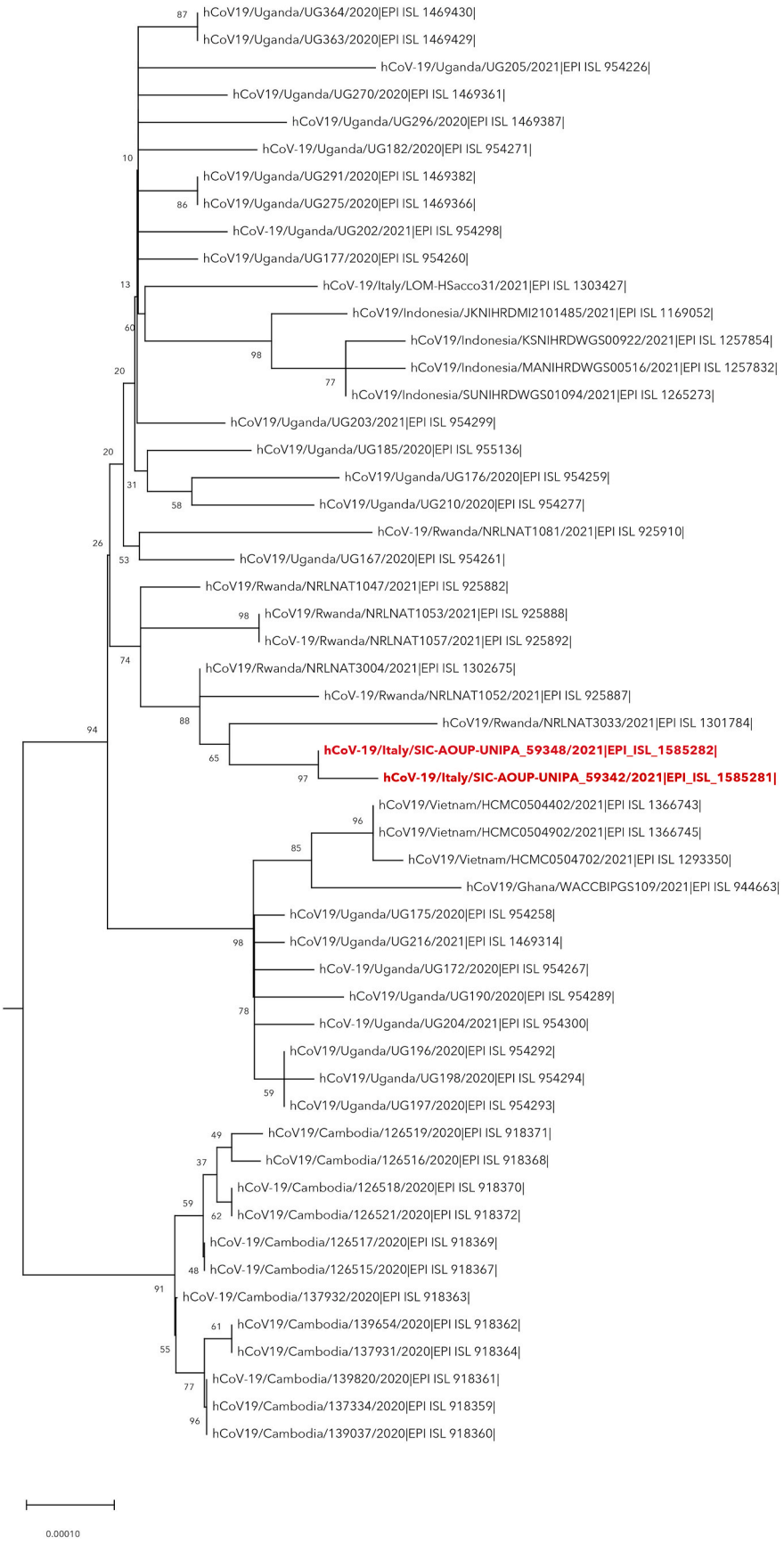

Fig. 1. Neighbor-Joining phylogenetic analysis of A.23.1 lineage SARS-CoV-2 genome sequences. The two complete genomes characterized in this study are indicated in red. The reference sequence Wuhan-Hu-1 (MN908947.3) was used to root the tree. The branch length is drawn to the scale of number of nucleotide

substitutions per site, indicated in lower left. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cleavage. Additionally, the P681R spike change has been shown *in vitro* to enhance the fusion activity of the SARS-CoV-2 spike protein, likely due to increased cleavage by the cellular furin protease; notably, a similar change (P681H) is becoming pervasive among viral sequences and it is encoded by the VOC B.1.1.7, that is now hugely spreading worldwide.

Conversely, the change in spike R102I observed in most of the Ugandan strains and also reported in the unique strain previously detected in Italy was not represented in either strains from Egyptian migrants.

Additionally, in EPI_ISL_1585282 we identified two different deletions in ORF1a and ORF7a genes. The first one is an already known non-codon-aligned deletion in nsp 1 (nucleotides 519–524), already documented in Europe and USA, although not in the A.23.1 sub-lineage. The second one is a 13-nt frameshift deletion (nucleotides 27,680–27692) in the ORF7a, a type I transmembrane protein playing an important role in virus-host interactions, which has never been described before.

The origin of deletion variants in ORF7a is still unknown and the occurrence of such SARS-CoV-2 viruses suggests that it is structurally tolerant to deletions. It is possible that most reported deletions are neutral or quasi-neutral, but the uneven deletion distribution and the fact that they are mostly in frame to keep the amino acid sequence of the remaining peptide suggest that this gene is important for viral fitness. Nevertheless, it has been proposed that this may still provide selective advantages influencing virulence and adaption to humans.

Genetic variations have been very useful to classify the virus in different lineages and to perform phylodynamic analyses to understand viral evolution and epidemiology, and identify patterns of spread. Therefore, we used CoV-GLUE resource to generate phylogenetic placement of the A.23.1 strains, to annotate the sequences and check the prevalence of the deletions among worldwide sequences.

The phylogenetic analysis revealed that A.23.1 strains clustered in a separate subclade together with some recent strains collected in Rwanda at the beginning of 2021 (Fig. 1), whereas the other Italian single strain identified in the north of the country grouped with a set of sequences mostly from Indonesia. All sequences that dominated the Uganda epidemic in late December 2020 and which represent the ancestor of the A.23.1 sub-lineage, constituted independent homogeneous groups.

This is the first time the SARS-CoV-2 A.23.1 sub-lineage is detected in two migrants arriving to Italy via the Mediterranean Sea. The lineage isolated from the two strains differed from the single strain previously identified in the north of the country, indicating divergent evolutionary dynamics of the viruses from our geographic area.

Our findings support the use of full-genome sequencing and phylogenetic analysis to improve knowledge on global SARS-CoV-2 evolutionary trajectories and transmission dynamics.

The evolution of highly transmissible strains of SARS-CoV-2 and its global spread underlines the need to improve the surveillance and rapid detection of newly emerging variants, particularly focusing on those areas at higher migration rates from low-resources countries, where limited testing capacity and poor reporting systems are of Public Health concern [6]. This report may provide a reference to study the impact of the introduction of novel lineages in Italy and Europe and supports the need for continuous genomic surveillance in different geographic areas, with the aim of disclosing the significance of genetic mutations and their potential role in vaccine effectiveness.

Given the current uncontrollability of SARS-CoV-2 spread, the significant impact on transmissibility of some "variants of concern" and their potential effect on the effectiveness of current available vaccines, there is the necessity for wide-scale global vaccine rollout which includes low- and middle-income countries, in order to prevent these areas of the world for becoming the source of a long-lasting pandemic.

CRediT authorship contribution statement

Carmelo Massimo Maida: Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Fabio Tramuto: Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Daniela Di Naro: Microbiological characterization

Giulia Randazzo: Microbiological characterization

Paola Stefanelli: Supervision, Writing - review & editing.

Claudia Marotta: Supervision, Writing - review & editing.

Stefano Reale: Supervision, Writing - review & editing.

Achille Cernigliaro: Supervision, Writing - review & editing.

Teresa Barone: Microbiological characterization, Writing - review & editing.

Carlo Cesari: Supervision, Writing - review & editing.

Claudio Pulvirenti: Supervision, Writing - review & editing.

Ulrico Angeloni: Supervision, Writing - review & editing.

Angela Di Martino: Microbiological characterization.

Giovanni Rezza: Supervision, Writing - review & editing.

Francesco Vitale: Supervision, Writing - review & editing.

Walter Mazzucco: Conceptualization, Supervision, Writing - original draft, Writing - review &editing.

Declarations

Travel Medicine and Infectious Disease requires that all authors sign a declaration of conflicting interests. If you have nothing to declare in any of these categories then this should be stated.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the revised version of the paper.

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Declaration of competing interest

A conflicting interest exists when professional judgement concerning a primary interest (such as patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It may arise for the authors when they have financial interest that may influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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Carmelo Massimo Maida[1]
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Italy
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

Fabio Tramuto[*]
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Italy
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

Daniela Di Naro
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

Giulia Randazzo
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

Paola Stefanelli
Department of Infectious Diseases, National Institute of Health (ISS), Italy

Claudia Marotta
Ministry of Health, Rome, Italy

Stefano Reale
Zoo-prophilactic Experimental Institute of Sicily "A. Mirri", Palermo, Italy

Achille Cernigliaro
Regional Health Authority of Sicily, Palermo, Italy
Local Health Unit of Trapani, Trapani, Italy

Teresa Barone
Department of Laboratory Diagnostics, Local Health Unit of Palermo, Palermo, Italy

Carlo Cesari
Italian Red Cross, Rome, Italy

Claudio Pulvirenti
USMAF Sicily, Ministry of Health, Palermo, Italy

Ulrico Angeloni
Ministry of Health, Rome, Italy

Angela Di Martino
Department of Infectious Diseases, National Institute of Health (ISS), Italy

Giovanni Rezza
Ministry of Health, Rome, Italy

Francesco Vitale
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Italy
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

Walter Mazzucco
Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro", University of Palermo, Italy
Clinical Epidemiology Unit and Regional Reference Laboratory of Western Sicily for the Emergence of COVID-19, University Hospital "P. Giaccone", Palermo, Italy

For the SAMI-Surv Collaboration Annalisa Agnone
Local Health Unit of Palermo, Palermo, Italy

Davide Alba
University of Palermo, Palermo, Italy
E-mail address: davide.alba@unipa.it.

Emanuele Amodio
University of Palermo, Palermo, Italy
E-mail address: emanuele.amodio@unipa.it.

Rosario Asciutto
Local Health Unit of Trapani, Trapani, Italy
E-mail address: rosario.asciutto@asptrapani.it.

Ranieri Candura
Local Health Unit of Trapani, Italy
E-mail address: ranieri.candura@asptrapani.it.

Francesco Cascio
Local Health Unit of Palermo, Trapani, Italy
E-mail address: francesco.cascio@asppalermo.org.

Alessandra Casuccio
University of Palermo, Palermo, Italy
E-mail address: alessandra.casuccio@unipa.it.

Claudio Costantino
University of Palermo, Palermo, Italy
University Hospital "P. Giaccone", Palermo, Italy
E-mail address: claudio.costantino01@unipa.it.

Nadia D'Agostino
USMAF Lampedusa, Sicilia, Italy
E-mail address: n.dagostino-esterno@sanita.it.

Stefania D'Amato
Ministry of Health, Rome, Italy
E-mail address: stefania.damato@sanita.it.

Laura Di Quarto
Local Health Unit of Palermo, Palermo, Italy
E-mail address: lauradiquarto@libero.it.

Santo Fruscione
University of Palermo, Palermo, Italy
E-mail address: santi.fruscione@gmail.com.

Giorgio Graziano
University Hospital "P. Giaccone", Palermo, Italy
E-mail address: giorgio.graziano@policlinico.pa.it.

Daniele La Milia
USMAF UOT Palermo, Palermo, Italy
E-mail address: d.lamilia@sanita.it.

Mariano Lucchese
Local Health Unit Trapani, Italy
E-mail address: mariano.lucchese@asptrapani.it.

Giulia Mangano
Local Health Unit of Trapani, Trapani, Italy
E-mail address: giuliamangano96@gmail.com.

Maristella Messina
Local Health Unit of Trapani, Trapani, Italy
E-mail address: maristella00.mm@gmail.com.

Carmelo Migliorisi
Local Health Unit of Palermo, Palermo, Italy
E-mail address: carmelomigliorisi@gmail.com.

Giuseppa Mistretta
Local Health Unit of Trapani, Trapani, Italy
E-mail address: giusymistre@gmail.com.

Giulia Palmeri
Local Health Unit of Trapani, Trapani, Italy
E-mail address: giuliapalmeri25@gmail.com.

Laura Pecoraro
USMAF UOT Palermo, Palermo, Italy
E-mail address: l.pecoraro@sanita.it.

Vincenzo Restivo
University of Palermo, Palermo, Italy
University Hospital "P. Giaccone", Palermo, Italy
E-mail address: vincenzo.restivo@unipa.it.

Antonina Patrizia Rizzo
Local Health Unit of Trapani, Trapani, Italy
E-mail address: rizzoapatrizia@gmail.com.

Alessandra Savatteri
University of Palermo, Palermo, Italy
E-mail address: alesavatt@gmail.com.

Silvia Scibetta
Zoo-prophilactic Experimental Institute of Sicily "A. Mirri", Palermo, Italy
E-mail address: silvia.scibetta@hotmail.it.

Salvatore Scondotto
Regional Health Authority of Sicily, Palermo, Italy
E-mail address: salvatore.scondotto@regione.sicilia.it.

Antonino Sparaco
Local Health Unit of Trapani, Trapani, Italy
E-mail address: antonino.sparaco@asptrapani.it.

Vittorio Spoto
Local Health Unit of Agrigento, Agrigento, Italy
E-mail address: dp.direttore@aspag.it.

Domenico Stabile
USMAF UOT Palermo, Palermo, Italy
E-mail address: d.stabile@sanita.it.

Angela Mothia Tagliavia
Local Health Unit of Trapani, Trapani, Italy
E-mail address: angelamothiatagliavia@gmail.com.

Fabrizio Vitale
Zoo-prophilactic Experimental Institute of Sicily "A. Mirri", Palermo, Italy
E-mail address: fabrizio.vitale@izssicilia.it.

Mario Zappia
Local Health Unit of Agrigento, Agrigento, Italy
E-mail address: direzione.generale@aspag.it.

Salvatore Zichichi
USMAF UOT Palermo, Palermo, Italy
E-mail address: s.zichichi-esterno@sanita.it.

[*] Corresponding author. Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) "G. D'Alessandro" - University of Palermo, 133, via del Vespro I, 90127, Palermo, Italy.
E-mail address: fabio.tramuto@unipa.it (F. Tramuto).

[1] These authors have contributed equally.