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Introduction of the SARS-CoV-2 Beta variant from Comoros into the Marseille geographical area

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ABSTRACT

Background: We describe the epidemiology of the first cases diagnosed in our institute of infections with the SARS-CoV-2 Beta variant and how this variant was imported to Marseille.
Methods: The Beta variant was identified based on analyses of sequences of viral genomes or of a spike gene fragment obtained by next-generation sequencing using Illumina technology, or by a real-time reverse-transcription-PCR (qPCR) specific of the Beta variant.
Results: The first patient diagnosed as infected with the SARS-CoV-2 Beta variant was sampled on January 15, 2021. Twenty-nine patients were diagnosed in January 2021 (two weeks). Fifteen (52%) patients were of Comorian nationality. Eight (28%) had travelled abroad, including six who had returned from Comoros. Phylogeny based on SARS-CoV-2 genomes from 11 of these patients and their best BLAST hits from the GISAID database showed that seven patients, including the four returning from Comoros, were clustered with 27 other genomes from GISAID that included the six first Beta variant genomes described in Comoros in January 2021.
Conclusions: Our analyses highlight that, as for the case of other SARS-CoV-2 variants that have been diagnosed in Marseille, the Beta variant was imported to Marseille through travel from abroad. It had limited spread in our geographical area.

1. Introduction

Since its emergence, SARS-CoV-2 has spread worldwide and has led to a COVID-19 pandemic [1]. Due to the considerable variability and spread of this virus, multiple variants have emerged that have been and continue to be responsible for distinct epidemics that occurred concurrently or successively [2]. At the University Hospital Institute (IHU) - Méditerranée Infection, in Marseille, southern France, we described 10 variants as early as during the summer of 2020 by performing genomic surveillance using next-generation sequencing and variant specific qPCR [2,3]. In addition, we observed differences in clinical severity between some of these variants [4]. However, it took until December 2020 for such variants to be considered as being worthy of detection and monitoring, when the emergence of another variant, currently named Alpha (or B.1.1.7 or 20I), was described in the United Kingdom and then spread worldwide [5]. Subsequent SARS-CoV-2 variants have been increasingly scrutinised through genomic surveillance, and three other major variants were first described in South Africa (Beta, or B.1.351 or 20H), Brazil (Gamma, or P.1 or 20J), and India (Delta, or B.1.617 or 21A) [6]. The Beta variant, first described in late 2020 [7,8], was

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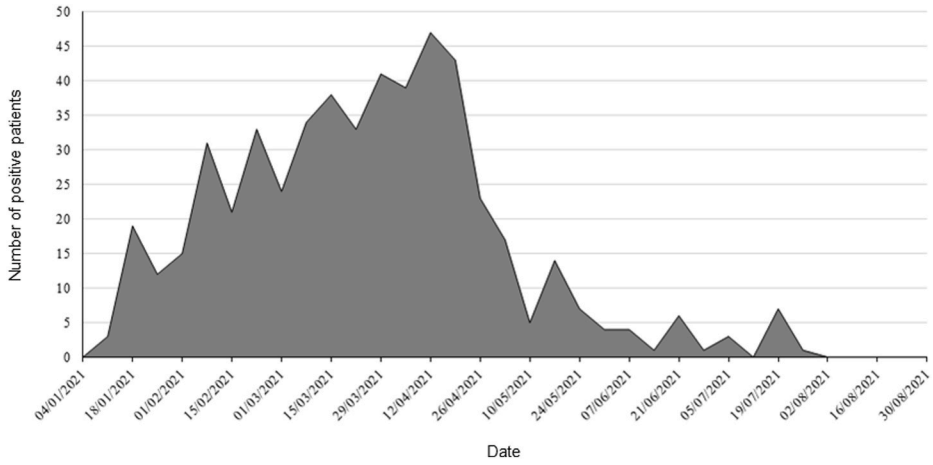

Fig. 1. Weekly distribution of the numbers of patients diagnosed with the SARS-CoV-2 Beta variant.

classified by the World Health Organization as a variant of concern, and spread to 105 countries worldwide with >25,000 sequences published [9].

At the IHU Méditerranée Infection, we previously reported how several variants had been imported from abroad, including the Marseille-1 variant, of sub-Saharan origin, from North Africa by boat [10]; the Delta variant from India through a sailor who was to embark on a boat in Marseille [11]; or the Marseille-501 variant from Mayotte, a French overseas department in the Comoros archipelago [12]. Here we describe the epidemiology of the first cases which were diagnosed by our institute as being infected with the Beta variant in January 2021, and how this variant was imported to Marseille.

2. Patients and methods

Identification of the Beta variant was performed using different techniques, on viral RNA extracted from 200 μL of nasopharyngeal swab fluid collected from patients to diagnose SARS-CoV-2 infection by real-time reverse-transcription-PCR (qPCR) [2]. Extracted viral RNA was either reverse-transcribed using SuperScript IV (ThermoFisher Scientific) prior to cDNA second strand synthesis with Klenow Fragment DNA polymerase (New England Biolabs, Beverly, MA, USA), or processed for obtention of cDNA according to the COVIDSeq protocol (Illumina Inc.). In most cases, SARS-CoV-2 genotyping consisted of next-generation sequencing of viral genomes, or of a spike gene fragment (2129 nucleotides corresponding to positions 21,296–23,424 of the genome of the Wuhan-Hu-1 isolate GenBank Accession no. NC_045512.2) as previously described [12]. Next-generation sequencing was performed using the Illumina technology, with the Nextera XT paired-end procedure on a MiSeq instrument (Illumina Inc., San Diego, CA, USA) or with the Illumina COVIDSeq protocol on a NovaSeq 6000 instrument (Illumina Inc), as previously described [2,10]. Sequencing reads generated by next-generation sequencing were assembled by mapping on the SARS-CoV-2 genome GenBank Accession no. NC_045512.2 (Wuhan-Hu-1 isolate) with the CLC Genomics workbench v.7 software (https://digitalinsights.qiagen.com/) or with the Minimap2 software [13]. Assembled sequences were analysed for their classification using the Nextclade online tool (https://clades.nextstrain.org/) [14], the Pangolin online tool (https://cov-lineages.org/pangolin.html) [15], and an in-house script written in Python as previously described [2]. SARS-CoV-2 genome sequences obtained in the present study have been deposited on the GISAID sequence database (https://www.gisaid.org/) [16] (Supplementary Table S1). Alternative SARS-CoV-2 genotype identification used an in house-designed qPCR system specific of the Beta variant, as previously described [2]. This qPCR system targets the envelope gene and uses forward primer C_SA_3_MBF: TGAATTGCAGACACCTTTTGA, reverse primer C_SA_3_MBR: CAACCCTTGGTTGAATAGTCTTG, and probe C_SA_3_MBP: TGA-CATCTTCAATGGGGAATGT. Phylogenetic analyses were performed based either on SARS-CoV-2 genome sequences when available, or on spike gene sequences. Phylogeny reconstructions were performed using the MEGAX software v.10.2.6 (http://www.megasoftware.net/) [17] with the neighbour-joining method and the Kimura 2-parameter method. The five best BLAST hits from the GISAID sequence database (https://www.gisaid.org/) [16] for each SARS-CoV-2 genome analysed here, as well as the six first Beta variant genomes detected in Comoros in January 2021 [18] were incorporated into phylogenies. SARS-CoV-2 culture was performed by inoculating nasopharyngeal samples on Vero E6 cells, as previously described [19].

3. Results

The first SARS-CoV-2-infected patient to be diagnosed with the Beta variant was sampled on January 15, 2021. As of August 31, 2021, 611 patients had been diagnosed as being infected with this variant through routine SARS-CoV-2 genotyping (Fig. 1). Twenty-nine patients were diagnosed in January 2021 (over a two-week period), from whom the Beta variant was identified by genome or spike gene next-generation sequencing in 11 and 16 cases, respectively, and by qPCR specific of this variant, in two cases. Seventeen patients were male and 12 were female (sex ratio = 1.4). The median age was 37 years (interquartile = [27–50], range = 10–62 years). Additional epidemiological or clinical data were available for 26 of the 29 patients. They were tested because they had respiratory symptoms or were contact cases of patients diagnosed with SARS-CoV-2-diagnosed patients. Fifteen (52%) patients were of Comorian origin (Supplementary Table S1). Eight (28%) had travelled abroad, including six who had returned from Comoros (all were of Comorian nationality), and two who had returned from Italy and United Arab Emirates. Four of the six people returning from Comoros transited through Tunisia. Seven of the eight travellers (six returning from Comoros and one from United Arab Emirates) were diagnosed over a three-day period, between 16 and 18 January 2021, which corresponded to the first evidence of the introduction of the Beta variant in Marseille. Time between return to France and diagnosis ranged from 0 to 4 days for the eight patients who travelled abroad and from 0 to 1 day for the six patients who returned from Comoros (Supplementary Table S1). Among those patients who had not travelled, two had participated in mass events (a wedding and a religious festival), eleven were contact cases, and no specific exposure was reported in five symptomatic patients. SARS-CoV-2 culture was performed for a respiratory sample collected from twelve patients and was positive in all cases, a cytopathic effect being observed in a mean time of 5.7 ± 4.2 days (range, 2–16

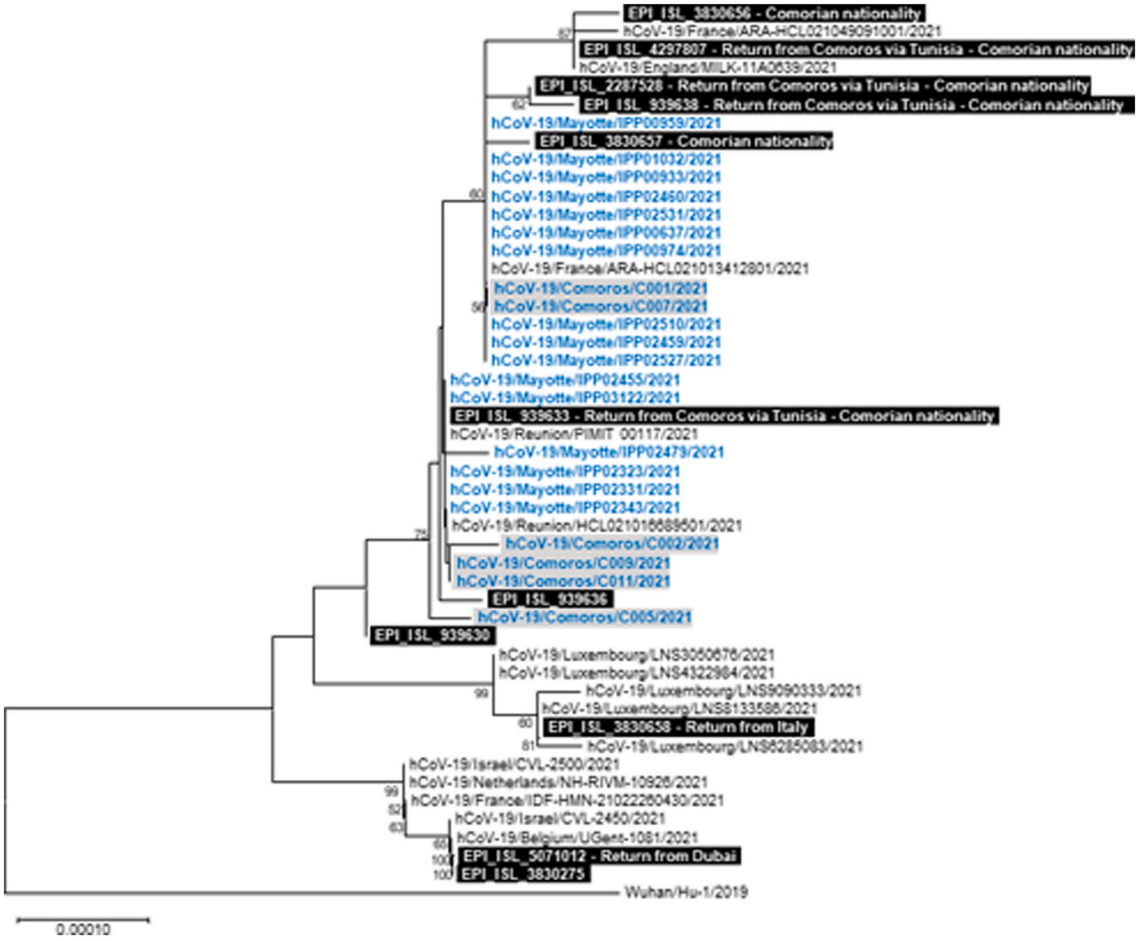

Fig. 2. Phylogenetic analysis based on SARS-CoV-2 genomes. The five best BLAST hits from the GISAID sequence database (https://www.gisaid.org/) [16] for SARS-CoV-2 genome analysed here (labelled with a white bold font and a black background), as well as the six first Beta variant genomes detected in Comoros in January 2021 [18] (labelled with a blue bold font and a grey background) and the genome of the Wuhan-Hu-1 isolate (GenBank Accession no. NC_045512.2) were incorporated into the phylogeny reconstruction. SARS-CoV-2 genomes obtained from patients sampled in the Comoros archipelago are labelled with a blue bold font. Evolutionary history was inferred using MEGAX software (http://www.megasoftware.net/) [17] using the neighbour-joining method and the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bars indicate the number of nucleotide substitutions per site. Bootstrap values > 50% are indicated on the tree. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

days).

The phylogenetic tree based on SARS-CoV-2 genomes showed that seven of the genomes that were obtained in our institute from 10 patients, including the four from patients returning from Comoros, were clustered (with a bootstrap value of 75%) with 27 other genomes from GISAID, which included the first six Beta variant genomes detected in January 2021 and described in Comoros [16], 16 genomes obtained from patients in Mayotte, a French overseas department in the Comoros archipelago, and two genomes obtained from patients in Reunion Island, another French overseas department in the Indian Ocean (Fig. 2). Three other genomes that were obtained in our institute, including one from a patient returning from Italy and one from a patient returning from Dubai, were clustered with genomes obtained from patients sampled in Luxembourg, France, Belgium, the Netherlands and Israel, while the last genome was not robustly clustered with another genome, although it was also most closely related to genomes from Comoros. The phylogenetic tree based on the SARS-CoV-2 spike gene fragment showed lower bootstrap values than that based on genomes. Nonetheless, sequences from all fifteen patients of Comorian nationality including those from all six patients returning from Comoros were clustered (bootstrap value, 54%) with all other sequences from patients sampled in Comoros islands (Supplementary Figure S1).

4. Discussion

Our analyses highlight that, as is the case of other SARS-CoV-2 variants that we have diagnosed in Marseille [2,10–12], the Beta variant was imported to Marseille through travel from abroad, primarily from the Comoros. This coincided with the initial detection of genomes of the Beta variant in Comoros in January 2021, which comprised two clusters with the most recent common ancestor dating to October 30, 2020, and which were close to genomes of viruses identified in Mayotte, part of the Comoros archipelago [18]. In the present study, sources other than Comoros, including return from Italy, United Arab Emirates and unknown sources, contributed to the early emergence or spread of the Beta variant in our geographical area.

Marseille includes a large community of more than 50,000 people originating from the Comoros [20,21]. Comorians often travel between Comoros and Marseille, which can spread infectious diseases. The transmission of several infectious agents between Comoros and Marseille has previously been described [22–24]. Furthermore, Marseille is France's second largest city and is located on the Mediterranean coast. Daily passenger traffic takes place by air and by boat, especially between Marseille and African countries [22].

The Beta variant, along with other spike N501Y substitution-

harboring variants including the Alpha, Gamma, and Marseille-501 variants, was part of the third period of high SARS-CoV-2 incidence in Marseille [4]. It has been described as being more infectious than previous SARS-CoV-2 variants [25], and as displaying decreased susceptibility to neutralising antibodies, including from the convalescent plasma of South African patients [26]. However, following its introduction, it had limited spread in our geographical area, with only 611 cases as of August 31, 2021, compared to 9436 cases (15 times more) with the Alpha variant, first detected in late December 2020; 6742 cases (11 times more) with the Marseille-4 variant (or B.1.160 lineage according to the Pangolin classification) that we first detected in July 2020; and 6079 cases (10 times more) with the Delta variant, that we first detected in April 2021. The extent of the Beta variant in the Marseille area was, in contrast, higher than that of the Gamma variant (87 cases as of August 31, 2021). The causes of such differences in the spread of SARS-CoV-2 variants on the local and national scales merit further study. These causes may include viral features but also various epidemiological parameters, including the frequency of events introducing these variants from abroad and the effect of the co-circulation of several variants during the same period of time.

Travels abroad during the SARS-CoV-2 pandemic, including after the reopening of international borders, create favourable conditions for the spread of SARS-CoV-2 variants on a global scale [2,25,27]. Overall, our data exemplify that such viral variants can rapidly spread internationally through travel, thus causing distinct epidemics according to geographic location and over time. They also show that there are multiple travel routes and that closing national borders is complicated. Epidemiological and genomic surveillance is needed to decipher the dynamic of these variants and their epidemiological and clinical specificities.

Ethics

This study has been approved by the ethics committee of our institution (N°2020-016-03). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019–73.

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CRediT authorship contribution statement

Van Thuan Hoang: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Loutfia Assoumani:** Methodology, Investigation, Formal analysis. **Jérémy Delerce:** Methodology, Investigation. **Linda Houhamdi:** Methodology, Investigation. **Marielle Bedotto:** Methodology, Investigation. **Jean-Christophe Lagier:** Investigation. **Matthieu Million:** Investigation. **Anthony Levasseur:** Methodology, Validation. **Pierre-Edouard Fournier:** Methodology, Validation. **Bernard La Scola:** Methodology, Investigation. **Didier Raoult:** Conceptualization, Formal analysis, Writing – review & editing, Supervision. **Philippe Gautret:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Supervision. **Philippe Colson:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest. Didier Raoult has been a consultant for Hitachi High-Technologies Corporation, Tokyo, Japan from 2018 to 2020. He is a scientific board member of Eurofins company and a founder of a microbial culture company (Culture Top). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2022.102277.

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