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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 [2,3]. 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...
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 obtaining 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: CAACCCCGTTTTGAAAGTCTTG, and probe C_SA_3_MBP: TGA- CATCTTCAATGGGAATGT. 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 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 Comoros in January 2021 [16] (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. Bootstrav 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.)

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-
Epidemiological and genomic surveillance is needed to decipher the multiple travel routes and that closing national borders is complicated. Albeit through travel, thus causing distinct epidemics according to 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. Loutfi 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.

References

[1] European Centre for Disease Prevention and Control. COVID-19 situation update worldwide. European Centre for Disease Prevention and Control; 2021. as of week 36, updated 16 September, https://www.ecdc.europa.eu/en/geographical-dis tribution-2019-ncov-cases.

[2] Colson P, Fournier PE, Chaudet H, Delerce J, Giraud-Gatineau A, Houhamdi L, et al. Analysis of SARS-CoV-2 variants from 24,181 patients exemplifies the role of globalisation and zoonosis in pandemics. Front Microbiol 2022;12:786233. https://doi.org/10.3389/fmicb.2021.786233.

[3] Colson P, Levasseur A, Delerce J, Chaudet H, Bossi V, Ben Khedher M, et al. Dramatic increase in the SARS-CoV-2 mutation rate and low mortality rate during the second epidemic in summer in Marseille. BIU Preprints 2020. doi:10.35068/68c3-ew82.

[4] Dao TL, Hoang VT, Nguyen NN, Delerce J, Chaudet H, Levasseur A, et al. Clinical outcomes in COVID-19 patients infected with different SARS-CoV-2 variants in Marseille, France. Clin Microbiol Infect 2021;27:1516.e1-1516.e6.

[5] Davies NG, Abbott S, Barnard GC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372. eaab3055.

[6] Oude Munnink BB, Worp N, Nieuwenhuijse DF, Sikkema RS, Haagmans B, Fouche R, et al. The next phase of SARS-CoV-2 surveillance: real-time molecular epidemiology. Nat Med 2021;27:1518–24.

[7] Fink S. South Africa announces a new coronavirus variant. The New York Times; 2020. Available at: https://www.nytimes.com/2020/12/19/world/south-africa-announces-a-new-coronavirus-variant.html.

[8] Tegally H, Wilkinson E, Giovanetti M, Iranazadeh A, Fomecha V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 2021;592:438-43.

[9] Cov-lines.org. Report B.1.351 variant. Available at: https://cov-lines.org/global_report_B.1.351.html; 2021.

[10] Colson P, Levasseur A, Gautret P, Fournier E, Chaudet H, Delerce J, et al. Introduction into the Marseille geographical area of a mild SARS-CoV-2 variant originating from sub-Saharan Africa: an investigational study. Trav Med Inf Dis 2021;40:101980.

[11] La Scola B, Lavrard P, Fournier P-E, Colson P, Lacoste A, Raoult D. SARS-CoV-2 variant from India to Marseille: the still active role of ports in the introduction of epidemics. Trav Med Inf Dis 2021;42:102085.

[12] Colson P, Levasseur A, Delerce J, Pinault L, Dudouet D, Devaux E, et al. Spreading of a new SARS-CoV-2 NS051Y spike variant in a new lineage. Clin Microbiol Infect 2021;27:1352.e1–5.

[13] Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 2018;34:3094–100. Sep. 15.

[14] Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 2018;34:4121–3.

[15] Rambaut A, Holmes EC, O’Toole A, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol 2020;5:1403–7.

[16] Shu Y, McCauley J. GISAID: global initiative on sharing all influenza data - from surveillance to reality. Euro Surveill 2017;22:30494.

[17] Kumar S, Stecher G, Tamura K. MEGA6: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2016;33:1547–50.

[18] Agotì CN, Githinji G, Mohammed KS, Lambisia AW, de Laurent ZR, Mburu MW, et al. Detection of SARS-CoV-2 variant 501Y.V2 in Comoros islands in January 2021. Wellcome Open Res 2021;6:192.

[19] Wurtz N, Penant G, Jarot P, Durlot N, La Scola B. Culture of SARS-CoV-2 in a panel of laboratory cell lines, permissivity, and differences in growth profile. Eur J Clin Microbiol Infect Dis 2021;40:477–84.

[20] Marseille Population. Demographics, Maps, Graphs. 2021. n.d. https://worldpopul ationreview.com/world-cities/marseille-population.
[21] Katibou A. Les migrations Comoriennes en France. Available at: https://www.recherches-internationales.fr/R90/R90-Katibou.pdf; 2021.

[22] Griffiths RM, Savini H, Brouqui P, Simon F, Parola P, Gautret P. Surveillance of travel-associated diseases at two referral centres in Marseille, France: a 12-year survey. J Trav Med 2018;25:tay007.

[23] Parola P, Gutzin P, Pradines B, Parzy D, Delmont J, Brouqui P. Marseille: a surveillance site for malaria from the Comoros Islands. J Trav Med 2004;11:184–6.

[24] Gautret P, Simon F, Askling HH, Bouchaud O, Leparc-Goffart I, Ninove L, et al. Dengue type 3 virus infections in European travellers returning from the Comoros and Zanzibar. Euro Surveill 2010;15:19541. February-April 2010.

[25] Dao TL, Hoang VT, Colson P, Lagier JC, Million M, Rasolt D, Levasseur A, Gautret P. SARS-CoV-2 infectivity and severity of COVID-19 according to SARS-CoV-2 variant: current evidence. J Clin Med 2021;10:2635.

[26] Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med 2021;27:622–5.

[27] Lemey P, Ruktanonchai N, Hong SL, Colizza V, Poletto C, Van den Broeck F, et al. Untangling introductions and persistence in COVID-19 resurgence in Europe. Nature 2021;595:713–7.