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SHORT COMMUNICATION

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First cases of infection with the 21L/BA.2 Omicron variant in Marseille, France

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Abstract

The SARS-CoV-2 21K/BA.1, 21L/BA.2, and BA.3 Omicron variants have recently emerged worldwide. To date, the 21L/BA.2 Omicron variant has remained very minority globally but became predominant in Denmark instead of the 21K/BA.1 variant. Here, we describe the first cases diagnosed with this variant in south-eastern France. We identified 13 cases using variant-specific qPCR and next-generation sequencing between 28/11/2021 and 31/01/2022, the first two cases being diagnosed in travelers returning from Tanzania. Overall, viral genomes displayed a mean (±standard deviation) number of 65.9 ± 2.5 (range, 61–69) nucleotide substitutions and 31.0 ± 8.3 (27–50) nucleotide deletions, resulting in 49.6 ± 2.2 (45–52) amino acid substitutions (including 28 in the spike protein) and 12.4 ± 1.1 (12–15) amino acid deletions. Phylogeny showed the distribution in three different clusters of these genomes, which were most closely related to genomes from England and South Africa, from Singapore and Nepal, or from France and Denmark. Structural predictions highlighted a significant enlargement and flattening of the surface of the 21L/BA.2 N-terminal domain of the spike protein compared to that of the 21K/BA.1 Omicron variant, which may facilitate initial viral interactions with lipid rafts. Close surveillance is needed at global, country, and center scales to monitor the incidence and clinical outcome of the 21L/BA.2 Omicron variant.

KEYWORDS

emergence, Omicron, SARS-CoV-2, southern France, travel, variant

1 | INTRODUCTION

SARS-CoV-2 variants have been detected since summer 2020[1,2] and have been of critical interest with regard to viral transmissibility, viral load, and escape to natural or vaccine immunity.[3,4] The Omicron variant is currently the predominant variant of concern in many countries worldwide (https://covariants.org/per-country).[5,6] It has been reported to show considerable escape to antibodies elicited by vaccination[7,8] and to be associated with lower clinical severity including in our center.[8,9] It was first detected in early November in Botswana and thereafter, in many countries, its incidence has rapidly exceeded that of the Delta variant that had predominated since the summer of 2021 (https://covariants.org/per-country).[5,6] In fact, Omicron, or clade 21M, is composed of three branches corresponding to three variants named Nextstrain clade[10,11] 21K (or Pangolin lineage[12] BA.1), 21L (or BA.2), and lineage BA.3. Primarily and until recently, unlike the 21K/BA.1 Omicron variant, the 21L/BA.2 Omicron variant has remained minoritary in most countries worldwide, including in South Africa from where it seems to originate, although its incidence grew substantially in few countries and it even became predominant in Denmark.[8,13] Here, we describe the emergence of this variant in south-eastern France.

2 | MATERIALS AND METHODS

Nasopharyngeal samples were collected from patients in our university hospital institute (Méditerranée Infection; https://www.mediterranee-infection.com/) and tested for SARS-CoV-2 infection by real-time reverse transcription PCR (qPCR) as previously described.[2,14] Then qPCR assays specific of variants were performed according to French recommendations, as previously reported.[2,14,15] This included detection of spike mutations L452R, K417N, E484K, and/or P681H (Thermo Fisher Scientific), combined with testing with the TaqPath COVID-19 Kit (Thermo Fisher Scientific) that target viral genes ORF1, N (nucleocapsid), and S (spike).

Genomic identification of the 21L/BA.2 Omicron variant was performed by next-generation sequencing with the Oxford Nanopore Technology (ONT) on a GridION instrument (Oxford Nanopore Technologies Ltd.) or with the Illumina COVID-seq protocol on the NovaSeq 6000 instrument (Illumina Inc.), as previously described.[2,14,15] Sequence read processing and genome analysis were performed as previously described.[2,14,15] Fastq files were processed differently according to the sequencing technology. Briefly, for ONT reads, Fastq files were processed with the ARTIC field bioinformatics pipeline (v1.1.0; https://github.com/artic-network/fieldbioinformatics). Sequencing reads were basecalled with Guppy (v.4.0.14) and aligned to the Wuhan-Hu-1 genome GenBank accession no. NC_045512.2 using minimap2 (v2.17-r941) (https://github.com/lh3/minimap2). Reads were cleaned with Guppyplex. Mapping was cleaned with ARTIC align_trim. Variant calling was performed using Medaka and Longshot. Consensus genome sequences were built with Bcftools (https://samtools.github.io/bcftools/bcftools.html). Illumina NovaSeq reads were basecalled with the Dragen Bcl Convert pipeline (v3.9.3; https://emea.support.illumina.com/sequencing/sequencing_software/bcl-convert.html; Illumina Inc.), mapping was performed with the bwa-mem2 tool (https://github.com/bwa-mem2/bwa-mem2) on the Wuhan-Hu-1 genome. Mapping was cleaned with Samtools (https://www.htslib.org/). Variant calling was performed with freebayes (https://github.com/freebayes/freebayes) and consensus genomes were built with Bcftools.

Nucleotide and amino acid changes in viral genomes relative to the Wuhan-Hu-1 isolate genome were obtained using the Nextclade tool (https://clades.nextstrain.org/).[10,11] Nextstrain clades and Pangolin lineages were determined using the Nextclade web application (https://clades.nextstrain.org/)[10,11] and Pangolin web application (https://cov-lineages.org/pangolin.html),[12] respectively. Genome sequences described here were deposited in the GISAID sequence database (https://www.gisaid.org/; Table 1A).[16] Finally, phylogeny was reconstructed with the nextstrain/ncov tool (https://github.com/nextstrain/ncov) then visualized with Auspice (https://docs.nextstrain.org/projects/auspice/en/stable/). The genomes the closest genetically to those obtained here were selected using Usher (https://genome.ucsc.edu/cgi-bin/hgPhyloPlace) and the GISAID BLAST tool (https://www.epicov.org/epi3/) then incorporated in phylogeny with all 21L/BA.2 Omicron variant genomes from France available in GISAID.

This study was approved by the ethics committee of University Hospital Institute Méditerranée Infection (No. 2022-008). Access to patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille and recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73.

3 | RESULTS

Thirteen infections with the 21L/BA.2 Omicron variant were diagnosed in our university hospital institute from patients sampled between 27/12/2021 and 31/01/2022 (Table 1A). First cases were in two spouses in their 60s diagnosed late December 2021 5 days after returning from a travel in Zanzibar, Tanzania. They received a third dose of Pfizer-BioNTech COVID-19 vaccine 3 weeks before diagnosis. The third case was a physician who has contacts with migrant patients and a family SARS-CoV-2-positive case (not tested in our institute) who met students from different countries. This third patient received a third dose of Pfizer-BioNTech COVID-19 vaccine 7 weeks before diagnosis. The fourth patient was another member from the same family as the third case. Two other patients were from the Netherlands and the United Kingdom. No information was available for the other seven patients.

All 21L/BA.2 Omicron variant-positive respiratory samples exhibited the same combination of spike mutations as screened by real-time qPCR: negativity for L452R, and, when performed,

TABLE 1 Main epidemiological and virological features of cases identified with infection with the SARS-CoV-2 21L/BA.2 Omicron variant (A), and nucleotide and amino acid changes in Omicron variants (lineages 21K/BA.1, 21L/BA.2, and 21M/BA.3) (B)

(A)

Case no.	Age	Epidemiological data	Clinical data	Date of sampling	Diagnostic qPCR Ct	Results of qPCR used to screen for the presence of SARS-CoV-2 spike substitutions	Results of the TaqPath COVID-19 qPCR assay (Targets: ORF1, S, and N genes)	Genome GISAID Id.
1	60s	Back to travel from Zanzibar (Tanzania)	Three doses of vaccine; mild symptoms	27/12/2021	21	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9161702
2	60s	Back to travel from Zanzibar (Tanzania)	Three doses of vaccine; mild symptoms	27/12/2021	12	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9184187
3	50s	No travel abroad	Three doses of vaccine; mild symptoms	27/12/2021	18	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9161106
4	20s	No data	No data	29/12/2021	16	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9184306
5	30s	Dutch nationality	No data	06/01/2022	20	L452R-Neg.; P681H: N.t.; E484K: N.t.	Pos. for all three genes	EPI_ISL_8709900
6	50s	No travel abroad	Not vaccinated; 4-day hospital-ization	29/12/2022	27	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9184305
7	20s	No data	No data	29/12/2021	31	L452R-Neg.; P681H-Neg.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9186024
8	30s	No data	No data	11/01/2022	18	L452R-Neg.; P681H: N.t.; E484K-Neg.	Pos. for all three genes	EPI_ISL_9486836
9	50s	No data	No data	31/01/2022	22	L452R-Neg.; K417N-Pos.; E484K: N.t.	Pos. for all three genes	EPI_ISL_9479322
10	30s	No data	No data	31/01/2022	23	L452R-Neg.; K417N-Pos.; E484K: N.t.	Pos. for all three genes	EPI_ISL_9479323
11	30s	UK nationality	No data	31/01/2022	32	L452R-Neg.; K417N-Pos.; E484K: N.t.	N gene-pos.; ORF1 and S genes-neg. *	EPI_ISL_9517119
12	20s	No data	No data	31/01/2022	30	L452R-Neg.; K417N-Pos.; E484K: N.t.	Pos. for all three genes	EPI_ISL_9468068
13	30s	No data	No data	31/01/2022	16	L452R-Neg.; K417N-Pos.; E484K: N.t.	Pos. for all three genes	EPI_ISL_9479324

(B)

Genes/regions	Nucleotide changes	Amino acid changes	Omicron variants/lineages		
			21 K/BA.1	21 L/BA.2	21 M/BA.3
5′UTR	C241U		Yes	Yes	Yes
ORF1a	U670G	S135R		Yes	Yes
ORF1a	C832U				Yes
ORF1a	C2790U	T842I		Yes	
ORF1a	A2832G	K856R	Yes		
ORF1a	C3037U		Yes	Yes	Yes
ORF1a	G4184A	G1307S		Yes	Yes
ORF1a	C4321U			Yes	Yes
ORF1a	U5386G		Yes		
ORF1a	Deletion 6513–6515	SL2083I	Yes		

(Continues)

TABLE 1 (Continued)

(B)					
Genes/regions	Nucleotide changes	Amino acid changes	Omicron variants/lineages		
			21 K/ BA.1	21 L/ BA.2	21 M/ BA.3
ORF1a	G8393A	A2710T	Yes		
ORF1a	C9344U	L3027F		Yes	
ORF1a	A9424G			Yes	
ORF1a	C9534U	T3090I		Yes	Yes
ORF1a	C9866U	L3201F		Yes	
ORF1a	C10029U	T3255I	Yes	Yes	Yes
ORF1a	C10198U			Yes	
ORF1a	G10447A			Yes	Yes
ORF1a	C10449A	P3395H	Yes	Yes	Yes
ORF1a	C11235U	-			Yes
ORF1a	G11287U	L3674F	Yes		
ORF1a	Deletion 11 288–11 296	SGF3675-	Yes	Yes	Yes
ORF1a	A11537G	I3758V	Yes		
ORF1a	C12880U			Yes	Yes
ORF1b	C14408U	P314L	Yes	Yes	Yes
ORF1b	C15240U		Yes		
ORF1b	C15714U			Yes	Yes
ORF1b	C17410U	R1315C		Yes	
ORF1b	A18163G	I1566V	Yes	Yes	Yes
ORF1b	C19955U	T2163I		Yes	
ORF1b	A20055G			Yes	
S	C21618U	T19I		Yes	
S	Deletion 21 633–61 641	LPP24-26-/A27S		Yes	
S	21642-21643	A27S		Yes	
S	C21762U	A67V	Yes		Yes
S	Deletion 21 765–21 770	HV69-	Yes		Yes
S	C21846U	T95I	Yes		Yes
S	G21987A	G142D	Yes	Yes	Yes
S	Deletion 21 988–21 996	VYY143-	Yes		Yes
S	Deletion 22 194–22 196	NL211I	Yes		Yes
S	U22200G	V213G		Yes	
S	Insertion22205GAGCCAGAA	215EPE	Yes		
S	G22578A	G339D	Yes	Yes	Yes
S	U22673C		Yes		
S	C22674U		Yes	Yes	Yes
S	U22679C	S373P	Yes	Yes	Yes
S	C22686U	S375F	Yes	Yes	Yes
S	A22688G	T376A		Yes	
S	G22775A	D405N		Yes	Yes
S	A22786U	R408S		Yes	
S	G22813U	K417N	Yes	Yes	Yes

TABLE 1 (Continued)

(B)					
Genes/regions	Nucleotide changes	Amino acid changes	Omicron variants/lineages		
			21 K/ BA.1	21 L/ BA.2	21 M/ BA.3
S	U22882G	N440K	Yes	Yes	Yes
S	G22898A	G446S	Yes		Yes
S	G22992A	S477N	Yes	Yes	Yes
S	C22995A	T478K	Yes	Yes	Yes
S	A23013C	E484A	Yes	Yes	Yes
S	A23040G	Q493R	Yes	Yes	Yes
S	G23048A	G496S	Yes		
S	A23055G	Q498R	Yes	Yes	Yes
S	A23063U	N501Y	Yes	Yes	Yes
S	U23075C	Y505H	Yes	Yes	Yes
S	C23202A	T547K	Yes		
S	A23403G	D614G	Yes	Yes	Yes
S	C23525U	H655Y	Yes	Yes	Yes
S	U23599G	N679K	Yes	Yes	Yes
S	C23604A	P681H	Yes	Yes	Yes
S	C23854A	N764K	Yes	Yes	Yes
S	G23948U	D796Y	Yes	Yes	Yes
S	C24130A	N856K	Yes		
S	A24424U	Q954H	Yes	Yes	Yes
S	U24469A	N969K	Yes	Yes	Yes
S	C24503U	L981F	Yes		
S	C25000U		Yes	Yes	
S	C25584U		Yes	Yes	
ORF3a	AC26059GU	T223V			Yes
ORF3a	C26060U	T223I		Yes	
E	C26270U	T9I	Yes	Yes	Yes
M	A26530G	D3G	Yes		
M	C26577G	Q19E	Yes	Yes	Yes
M	G26709A	A63T	Yes	Yes	Yes
M	C26858U			Yes	Yes
ORF6	A27259C		Yes	Yes	Yes
ORF6	GAU27382CUC	D61L		Yes	
ORF6	C27807U		Yes	Yes	Yes
-	A28271U		Yes	Yes	Yes
N	C28311U	P13L	Yes	Yes	Yes
N	Deletion 28 362–28 370	ERS31-	Yes	Yes	Yes
N	GGG28881AAC	RG203-204KR	Yes	Yes	Yes
N	A29510C	S413R		Yes	Yes

Note: Some samples not tested for variant-specific qPCR assays were tested directly by next-generation sequencing.

Abbreviations: -, amino acid deletion; Ct, cycle threshold value; E, glutamic acid; H, histidine; Id., identifier; K, lysin; L, leucin; N, nucleocapsid; N.d., no data; Neg., negative; ORF, open reading frame; P, proline; Pos., positive; R, arginine; S, spike; UTR, untranslated region.

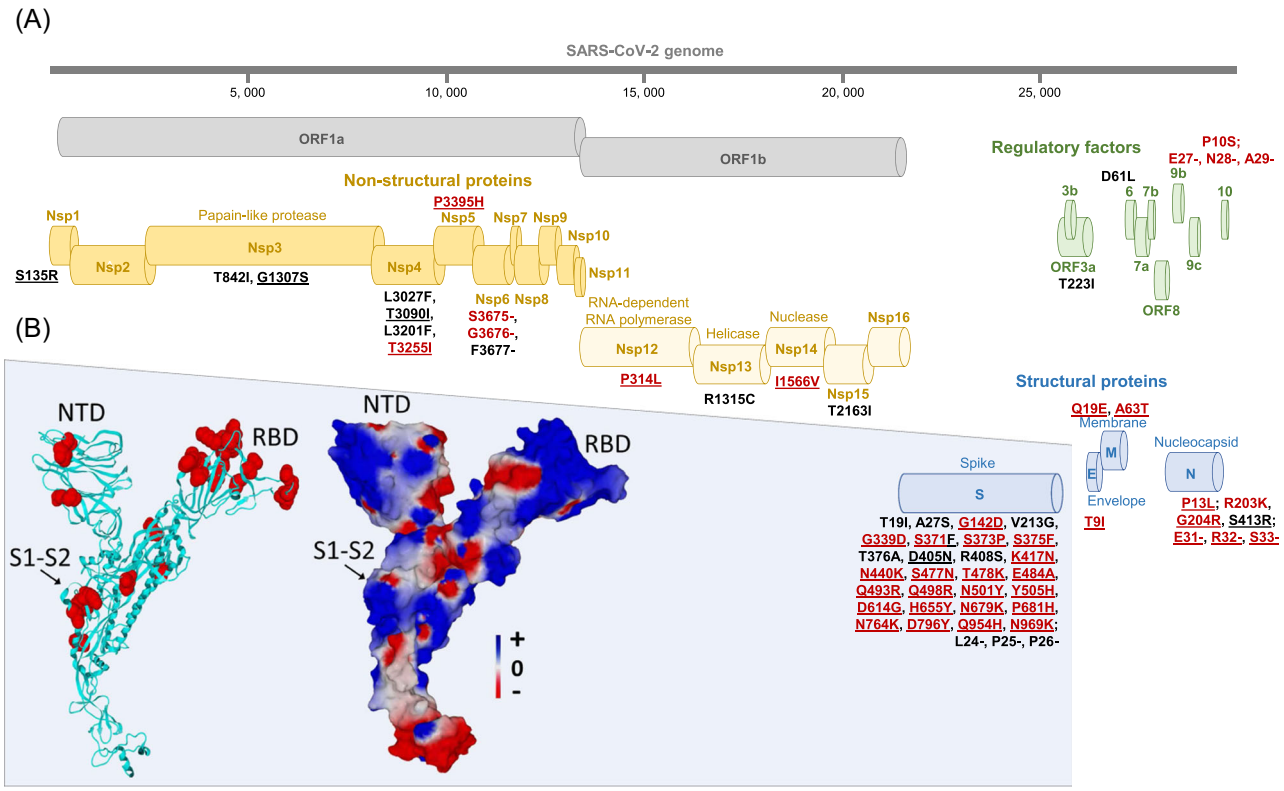

FIGURE 1 Map of the Omicron 21L/BA.2 spike protein with signature amino acid substitutions and deletions (A) and structural features of 21L/BA.2 Omicron variant spike protein (B). (A) Amino acid substitutions and deletions shared with the 21K/BA.1 Omicron variant are indicated by a red font. Amino acid substitutions and deletions shared with the 21M/BA.3 Omicron variant are underlined. See also Table 1B. (B) Structural model of the Omicron 21L/BA.2 spike protein with mutations highlighted in red atomic spheres (left panel) or in electrostatic surface rendering (right panel). Note the flat surface of the N-terminal domain that faces lipid rafts of the host cell membrane. The S1–S2 cleavage site is indicated by an arrow. The color scale for the electrostatic surface potential (negative in red, positive in blue, neutral in white) is indicated. NTD, N-terminal domain; RBD, receptor-binding domain

positivity for K417N and P681H and negativity for E484K and P681R (Table 1A). In addition, the TaqPath COVID-19 Kit (Thermo Fisher Scientific) provided positive signals for all three genes targeted (ORF1, S, and N), except for one sample that showed positivity for the N gene but negativity for both ORF1 and S genes, which was most likely due to a low viral load (qPCR cycle threshold, 32). Thus, 21L/BA.2 Omicron variant-infected patients could be distinguished by qPCR screening from the Delta (L452R-positive) and Omicron 21K/BA.1 (negative for S gene detection by the TaqPath COVID-19 assay) variants that co-circulated in southern France at the time of Omicron 21L/BA.2 emergence.

Thirteen 21L/BA.2 Omicron genomes were obtained. Analysis of those larger than 28 000 nucleotides showed the presence of a mean (±standard deviation) of 65.9 ± 2.5 (range, 61–69) nucleotide substitutions and 31.0 ± 8.3 (27–50) nucleotide deletions, which resulted in 49.6 ± 2.2 (45–52) amino acid substitutions and 12.4 ± 1.1 (12–15) amino acid deletions. All nine patients' viruses harbored the same set of 28 amino acid substitutions and three contiguous amino acid deletions in their spike protein (Figure 1A and Table 1B). These included (i) 7 substitutions located in other structural proteins (4, 2, and 1 in the nucleocapsid, membrane, and envelope proteins, respectively); (ii) 12 substitutions located in nonstructural proteins including 4 in Nsp4, 2 in Nsp3 (a papain-like protease with phosphatase activity,[17] and 1 each in Nsp1, Nsp5 (a 3C-like proteinase), Nsp12 (RNA-dependent RNA polymerase), Nsp13 (helicase), Nsp14 (3′-5′-exonuclease with proofreading activity), and Nsp15 (an endoribonuclease); and (iii) 1 substitution located in ORF9b, a regulatory protein (Table 1B). Finally, three contiguous amino acid deletions were located in the nucleocapsid protein and three others were located in ORF9b (Table 1B). Of the 28 amino acid substitutions present in the spike of the 21L/BA.2 Omicron variant, 21 are shared with the 21K/BA.1 as well as the 21M/BA.3 Omicron variants (https://covariants.org/variants/; Figure 1A and Table 1B).[5,6,18]

Phylogeny performed with the nextstrain/ncov tool (https://github.com/nextstrain/ncov) shows that the nine 21L/BA.2 Omicron variant genomes obtained in our institute were part of three clusters. Two genomes that were retrieved from the two patients who traveled in Tanzania were clustered with genomes obtained in England and South Africa (Figure 2). The genome retrieved from the Dutch patient was clustered with two genomes obtained in Nepal and Singapore. All other six genomes were most closely related to

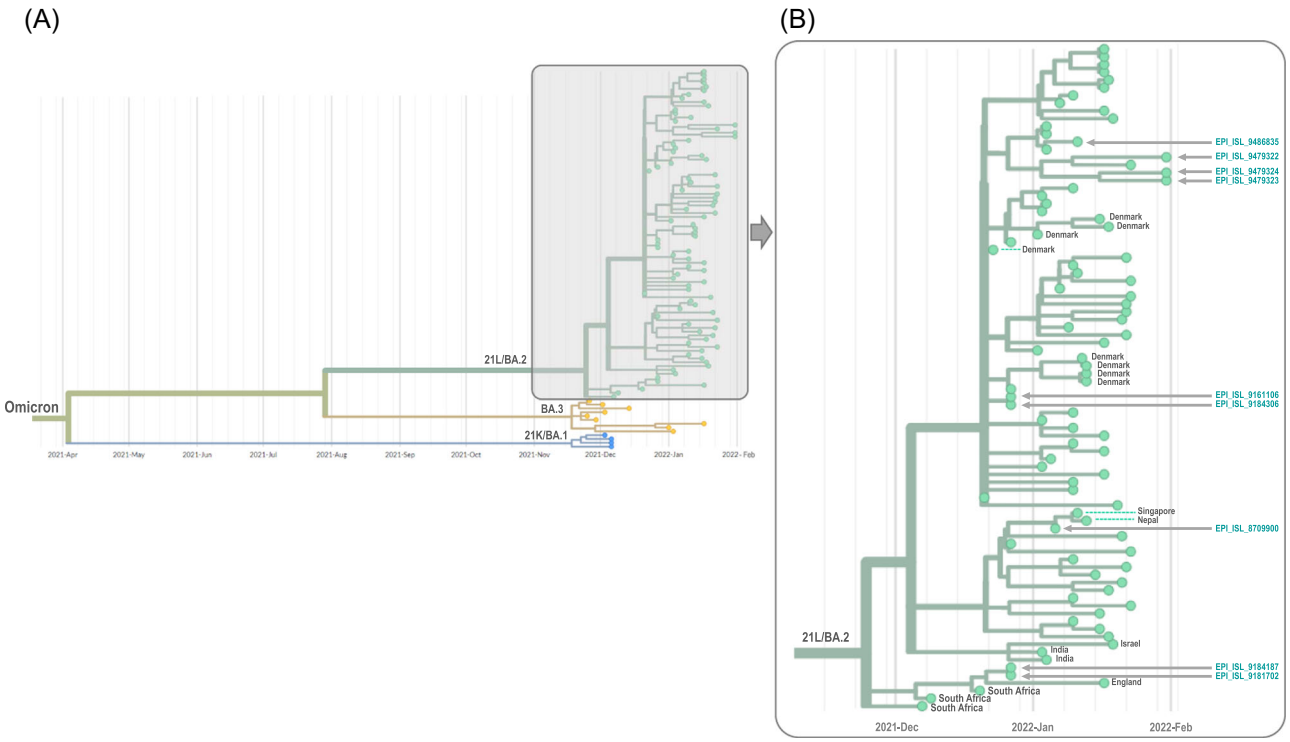

FIGURE 2 Phylogeny reconstruction based on genomes of the 21L/BA.2 Omicron variant were obtained in the present study. (A) incorporated genome sequences of 21K/BA.1, 21L/BA.2, and BA.3 Omicron variants. (B) is a zoom of the 21L/BA.2 Omicron cluster of (A). Phylogenetic tree was built using the nextstrain/ncov tool (https://github.com/nextstrain/ncov) then visualized with Auspice (https://docs.nextstrain.org/projects/auspice/en/stable/). *X*-axis shows time. The 21L/BA.2 Omicron genomes the closest genetically to those obtained in our institute were selected using the Usher tool (https://genome.ucsc.edu/cgi-bin/hgPhyloPlace) and the GISAID BLAST tool (https://www.epicov.org/epi3/) and they were incorporated in the phylogenetic analysis in addition to all 21L/BA.2 Omicron variant genomes from France are available in GISAID as of 02/02/2022. Sequences obtained in our laboratory (IHU Méditerranée Infection) are indicated by a dark blue arrow and their GISAID identifier is indicated. Countries are indicated when they are not France. Gisaid hcov-19 acknowledgment table is provided as supplementary file.

genomes from France and Denmark. As the first two cases we diagnosed were most likely infected with the 21L/BA.2 Omicron variant during their travel in Tanzania, we sought for this variant in GISAID among genomes from this country, but as of 02/02/2022 only three genomes (EPI_ISL_8917336, EPI_ISL_8917337, and EPI_ISL_9391124) were available from this country: they were obtained from samples collected in December 2021 and belong to the 21K/BA.1 Omicron variant.

The earliest 21L/BA.2 Omicron variant genome available from GISAID was obtained in South Africa from a sample collected on 17/11/2021 (EPI_ISL_6795834). As of 02/02/2022, most of the 37 521 21L/BA.2 Omicron variant genomes were obtained in Denmark ($n = 24$ 138; 64%; Figure 3A). Other countries with the greatest number of genomes were United Kingdom ($n = 4637$ cases; 12%), India ($n = 3073$ cases; 8%), Germany ($n = 1104$ cases; 2.9%), and Philippines ($n = 890$ cases; 2.4%). Overall, Europe, Asia, North America, Africa, and Oceania accounted for 34 498, 5071, 398, 377, and 184 genomes, respectively. South Africa, where the 21L/BA.2 Omicron was first described, and Botswana accounted for only 304 and 62 genomes, respectively, while 5550 and 1449 genomes were deposited in GISAID and obtained from samples collected since 01/12/2021, respectively. Finally, only 86 genomes (0.2%) were available for France out of 38 350 genomes deposited in GISAID and obtained from samples collected since 01/12/2021, while 18 219 21K/BA.1 Omicron variant genomes (48%) were available for the same period of time.

Molecular modeling of Omicron 21L/BA.2 variant spike protein was performed as previously described[19] by introducing the appropriate mutations and deletions in the framework of a complete 14–1200 amino acids structure of the original 20B SARS-CoV-2 (Wuhan-Hu-1 isolate with D614G substitution)[19] and by incorporating the missing amino acids with the Robetta protein structure prediction tool (https://robetta.bakerlab.org/) before energy minimization with the Polak-Ribière algorithm (Figure 1B).[20] The new 21L/BA.2 Omicron variant displays several common structural features with its close relative, the 21K/BA.1 Omicron variant: many mutations exist that are chiefly distributed in the N-terminal domain (NTD), the receptor binding domain (RBD), and the S1–S2 cleavage site. As for the 21K/BA.1 Omicron variant, the electrostatic surface potential of the RBD is mostly positive, whereas the NTD is constituted by a patchwork of electronegative, electropositive, and neutral regions. A key difference between both 21L/BA.2 and 21K/

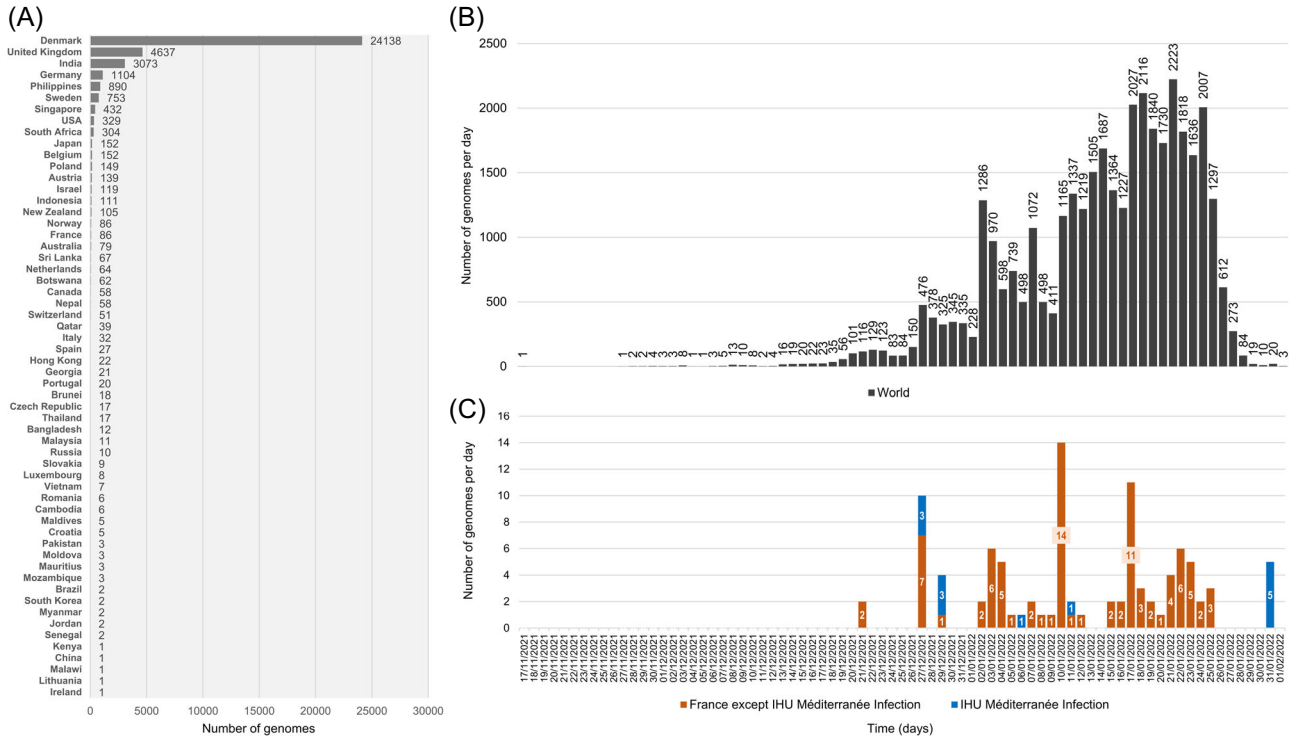

FIGURE 3 Number of genomes of the SARS-CoV-2 21L/BA.2 Omicron variant available in GISAID and chronology of collections of respiratory samples from where they were obtained. (A) Number of genomes of the SARS-CoV-2 21L/BA.2 Omicron variant are available in the GISAID sequence database (https://www.gisaid.org/)[16] as of 02/02/2022. (B) Chronology of SARS-CoV-2 diagnoses with the 21L/BA.2 Omicron variant for genomes were deposited in the GISAID sequence database and obtained worldwide. (C) Chronology of SARS-CoV-2 diagnoses with the 21L/BA.2 Omicron variant for genomes deposited in the GISAID sequence database and obtained in France or in our university hospital institute. The number of genomes was analyzed until 02/02/2022. Total number of genomes analyzed was 36 428. A total of 1093 genomes were excluded as the date of sample collection was uncomplete (days or months were lacking)

BA.1 Omicron spike proteins is the significant enlargement and flattening of the 21L/BA.2 Omicron NTD surface compared with that of the 21K/BA.1 Omicron variant.[19] This structural change is due to the lack of deletion 143–145 in the 21L/BA.2 Omicron variant. The flat surface of the 21L/BA.2 Omicron NTD may facilitate the initial interaction of the virus with lipid rafts,[20] especially since the surface gain corresponds to an electropositive area (located on the left of the NTD in Figure 1B). Overall, it could be hypothesized that the 21L/BA.2 Omicron variant NTD is better adapted to the electronegative surface of lipid rafts than that of the 21K/BA.1 Omicron variant.

4 | DISCUSSION

It is currently unknown if this 21L/BA.2 Omicron variant would rise considerably in prevalence and compete with the currently predominant Omicron 21K/BA.1, which has spread massively and quickly in countries with a high level of vaccine coverage.[5] It was reported in February 2022 that it has spread to more than 150 countries/territories but their genome sequences represented about 1% of the Omicron genomes submitted to GISAID (https://www.gisaid.org/).[13,15] However, the very recent rise of the 21L/BA.2 Omicron variant in Denmark where it became predominant over the 21K/BA.1 Omicron variant that predominated until then suggests that such epidemiological change may occur in other countries worldwide (Figure 3B,C).[8,13,21] Interestingly Desingu and Nagarayab[13] reported that the Omicron 21L/BA.2 variant was comprised of five lineages that were each prevalent in a different geographical area worldwide, one of these latter being Denmark and Sweden. Two genomes obtained here (EPI_ISL_9161106 and EPI_ISL_9184306) that were clustered with Danish genomes harbored substitution H78K in ORF3a, which was reported by Desingu and Nagarayab[13] as a characteristic of the Sweden/Denmark lineage. In addition, another genome (EPI_ISL_8709900) that was clustered with a Singaporean genome harbored substitution S959P in Nsp13 (a helicase), which was reported by Desingu and Nagarayab[13] as mostly found in India and Singapore.[13] The significance of these amino acid changes is currently unknown.

In our institute, we diagnosed 16 285 SARS-CoV-2 infections between 28/11/2021 (first detection of the Omicron variant) and 02/02/2022, during which 66% of infections were identified as due to the 21K/BA.1 Omicron variant. A first study conducted in Denmark has reported a higher contagiousness with the Omicron 21L/BA.2 variant (n = 2122 primary household patients) than with the Omicron 21K/BA.1 variant (n = 5702 primary household patients).[21] Secondary attack rates were 39% and 29% among households, respectively, and susceptibility

to infection was reported to be significantly increased for unvaccinated (odds ratio [OR], 2.2) as well as full-vaccinated (2.5) and booster-vaccinated (3.0) people. No data to our knowledge is currently available regarding the frequency of asymptomatic and mild and severe clinical forms with this 21L/BA.2 Omicron variant.

As for the 21K/BA.1 and 21M/BA.3 Omicron variants, the origin of the 21L/BA.2 Omicron variant is currently unclear. The great number of amino acid substitutions in the spike protein and receptor binding domain of these viruses has fueled several hypotheses that include overlooked virus evolution in people with low access to viral diagnosis and genome sequencing, in an immunocompromized chronically infected patient, or in animals.[5] A closest known Omicron's ancestor has been estimated to date back to mid-2020.[5] Another finding is that despite the tremendous amount of genome sequences available in GISAID (7 790 928 as of 02/02/2022) we are still unable to predict the emergence, and outcome of new variants. This supports the real-time close surveillance of the emergence, spread, and vanishing of SARS-CoV-2 variants through molecular and genomic surveillance. It is also worthy of interest to assess phenotypically through inoculation on permissive cells the susceptibility of emerging variants to neutralization by anti-spike antibodies elicited by prior infection or by vaccination, which is ongoing in our laboratory for the 21L/BA.2 Omicron variant.

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CONFLICT OF INTERESTS

All authors have no conflicts of interest to declare. 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).

ETHICS STATEMENT

This study has been approved by the ethics committee of the University Hospital Institute (IHU) Méditerranée Infection (No. 2022-008). 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.

AUTHOR CONTRIBUTIONS

Study conception and design: Philippe Colson, Didier Raoult, Jacques Fantini, and Pierre-Edouard Fournier. *Materials, data and analysis tools*: Philippe Colson, Jeremy Delerce, Mamadou Beye, Anthony Levasseur, Céline Boschi, Linda Houhamdi, Hervé Tissot-Dupont, Nouara Yahi, Matthieu Milllion, and Jacques Fantini. *Data analyses*: Philippe Colson, Pierre-Edouard Fournier, Bernard La Scola, Didier Raoult, Jeremy Delerce, Mamadou Beye, Anthony Levasseur, Jacques Fantini, and Nouara Yahi. *Writing of the first draft of the manuscript*: Philippe Colson, Jacques Fantini, and Pierre-Edouard Fournier. All authors read, commented on, and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data set generated then analyzed during the current study is available in the GISAID database (https://www.gisaid.org/).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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