



# Coding-Complete Sequence of a SARS-CoV-2 B.1.1.25 Lineage Obtained from an 8-Day-Old Deceased Neonate

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**ABSTRACT** We report the complete genome sequence of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strain, hCoV-19/Bangladesh/icddrb-CHAMPS-BDAA02205/2021, obtained from a nasopharyngeal swab from a deceased neonate from Faridpur, Bangladesh. The strain belongs to lineage B.1.1.25 but contains some notable mutations similar to the B.1.1.7 lineage.

The Child Health and Mortality Prevention Surveillance (CHAMPS) project aims to determine the cause of deaths among stillbirths, neonates, infants, and children <5 years of age in sub-Saharan Africa and South Asia (1). Postmortem tissue and swab specimens were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the *Betacoronavirus* genus of the broad family *Coronaviridae*. On 6 February 2021, an 8-day-old male neonate died at Faridpur Medical College Hospital. The postmortem blood specimen, cerebrospinal fluid (CSF), nasopharyngeal and oropharyngeal swab, lung tissue, and rectal swab were tested for SARS-CoV-2 RNA by real-time reverse transcription-PCR (RT-PCR). The blood (RNA-dependent RNA polymerase [RdRp] cycle threshold [ $C_T$ ], 34.3), CSF (RdRp  $C_T$ , 34.4), nasopharyngeal swab (RdRp  $C_T$ , 13.8), and lung tissue (RdRp  $C_T$ , 32.6) specimens all had evidence of SARS-CoV-2 RNA. The nasopharyngeal swab specimen was selected for whole-genome sequencing because of its low  $C_T$  value.

Total nucleic acid was extracted from the nasopharyngeal swab using the DNeasy blood and tissue kit (Qiagen, Germany) following the manufacturer's guidelines. The presence of two targets, the N gene (nucleocapsid) and RdRp, was detected by real-time RT-PCR using the AgPath-ID One-Step RT-PCR kit (Thermo Scientific, USA) (2). Upon primary confirmation, a total of 98 primer sets were used to amplify the relevant gene fragments covering the whole genome (3, 4). Sequencing was done using BigDye Terminator v3.1 cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, CA, USA) through an Applied Biosystems 3500XL genetic analyzer. The raw sequence data were analyzed using Chromas v2.23, and consensus sequences were assembled using SeqMan II (DNASTAR, WI, USA). MEGA 7.0 software was used to perform multiple sequence alignment using default parameters (5).

The assembled SARS-CoV-2 hCoV-19/Bangladesh/icddrb-CHAMPS-BDAA02205/2021 genome was identified to be from lineage B.1.1.25 using pangolin COVID19 lineage assigner (6). It consisted of 29,752 nucleotides with 99.89% similarity with the reference strain Wuhan-Hu-1 (GenBank accession number [NC\\_045512](https://doi.org/10.1016/j.nm.2020.05.012)). Using the Nextclade tool, the sequence was compared to the Wuhan-Hu-1 strain; a total of 23 mutations with 10 amino acid substitutions, three deletions, and one stop codon were observed in our analyzed sequence (Table 1) (<https://clades.nextstrain.org>). The mutations were compared with the contemporary variants circulating in Bangladesh (7), such as VOC-202012/01, belonging to lineage B.1.1.7 (alpha variant; GISAID accession number EPI\_ISL\_601443), and hCoV-19/Bangladesh/CHRF-0149/2021, belonging to Bangladeshi lineage B.1.1.25 (EPI\_ISL\_1360425).

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**TABLE 1** Comparison of mutations of the sequence with the B.1.1.7 lineage and another B.1.1.25 lineage from Bangladesh<sup>a</sup>

| Gene segment name   | Position                 | Nucleotide <sup>b</sup> | Amino acid <sup>b</sup> | B.1.1.7 <sup>c</sup> | B.1.1.25 <sup>c</sup> |
|---------------------|--------------------------|-------------------------|-------------------------|----------------------|-----------------------|
| 5' UTR <sup>d</sup> |                          | C241T                   | -                       | Y                    | N                     |
| ORF1a               | 266–13483                | T982C                   | -                       | N                    | N                     |
|                     |                          | A1163T                  | I300F                   | N                    | Y                     |
|                     |                          | T1921G                  | -                       | N                    | N                     |
|                     |                          | C2094T                  | S610L                   | N                    | N                     |
|                     |                          | C3037T                  | -                       | Y                    | Y                     |
|                     |                          | C4901T                  | -                       | N                    | N                     |
|                     |                          | C5388A                  | A1708D                  | Y                    | N                     |
|                     |                          | C5986T                  | -                       | Y                    | N                     |
|                     |                          | T7171G                  | -                       | N                    | N                     |
|                     |                          | T7984C                  | -                       | N                    | N                     |
|                     |                          | G10193A                 | E3110K                  | N                    | N                     |
|                     |                          | 11288-97 <sup>Δ</sup>   | SGF3675 <sup>Δ</sup>    | Y                    | N                     |
|                     |                          | C12473T                 | -                       | N                    | N                     |
| RdRp                | 13442–13468, 13468–16236 | C14408T                 | P314L                   | Y                    | Y                     |
|                     |                          | T15244A                 | -                       | N                    | N                     |
|                     |                          | C15279T                 | L593I                   | Y                    | N                     |
| Helicase            | 16237–18039              | C17012T                 | S1182L                  | N                    | N                     |
| Spike               | 21563–25384              | A23403G                 | D614G                   | Y                    | Y                     |
| ORF8                | 27894–28259              | C27972T                 | Q27 <sup>*</sup>        | Y                    | N                     |
| N gene              | 28274–29533              | GGG28881AAC             | RG203KR                 | Y                    | Y                     |

<sup>a</sup> The positions and changes are indicated by comparison with strain Wuhan-Hu-1.

<sup>b</sup> <sup>Δ</sup>, deletion; <sup>\*</sup>, stop codon; -, synonymous mutation.

<sup>c</sup> Y, present; N, absent.

<sup>d</sup> UTR, untranslated region.

A total of 10 changes were shared with lineage B.1.1.7, including 9 nucleotide (3 amino acid) deletions (11288 to 11297). In addition, a Q27<sup>\*</sup> stop codon was found in ORF8 which is responsible for a truncation of 78.5% of the protein that supports the viral adaptation to the host (8). This stop codon was reported across different lineages from the early days of the pandemic, including in Bangladesh. Among other amino acid substitutions, one was found in the spike protein (D614G), five in ORF1a, three in RdRp, one in the helicase, and two in the N gene. This is one of the few SARS-CoV-2 whole-genome sequences identified from a neonatal postmortem specimen in Bangladesh. Further investigations are required to understand the role of SARS-CoV-2 in this neonate's death.

**Data availability.** The whole-genome sequence is available under GISAID accession number EPI\_ISL\_2003459 and GenBank accession number [MZ401437](https://www.ncbi.nlm.nih.gov/nuccore/MZ401437).

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