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Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

The dynamic change of SARS-CoV-2 variants in Sierra Leone

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ARTICLE INFO

Keywords:
SARS-CoV-2
Lineage
Delta variant
Sierra Leone

ABSTRACT

Since the beginning of the SARS-CoV-2 pandemic, the emergence of multiple new variants posed an increased risk to global public health. The aim of this study is to investigate SARS-CoV-2 variants and possible transmission of variants of concern (VOCs) in Sierra Leone. A total of 65 nasal swab samples were collected from COVID-19 cases in Sierra Leone, among which 24 samples were collected during the second wave and 41 samples were collected during the third wave. Nanopore sequencing generated 54 SARS-CoV-2 whole genomes. The second COVID-19 wave was mainly caused by R.1 lineage while the third COVID-19 wave was dominated by B.1.617.2 lineage (Delta variant). The phylogenetic analysis suggested multiple introductions of SARS-CoV-2 Delta variant into Sierra Leone and subsequent local transmission in this country. Our findings highlight the importance of genomic surveillance of SARS-CoV-2 variants and the urgent need for implementation of strengthened public health and social measures (PHSM) to control the spread of virus variants.

1. Introduction

Owing to the error prone nature of the viral replication process, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), like other RNA viruses, accumulates mutations over time resulting in the emergence of multiple genetic variants. The vast majority of these variants appear to be of little or no biological significance (<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>). However, a small number of variants, termed variants of concern (VOCs), are associated with increase in transmissibility or virulence (Buchan et al., 2021; Campbell et al., 2021; Tegally et al., 2021; Bager et al., 2021), decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics (Planas et al., 2021; Sabino et al., 2021; Davis et al., 2021). In addition, variants of interest (VOIs) are defined as variants with specific genetic changes that are predicted or known to affect virus characteristics and identified to cause apparent epidemiological impacts to suggest an emerging risk to global public health. According to the data from WHO, there are four currently designated

VOCs (Alpha, Beta, Gamma and Delta) and four currently designated VOIs (Eta, Iota, Kappa and Lambda). The number of countries reporting VOCs continues to increase. By 3 August 2021, Alpha had been reported from 182 countries, Beta from 132 countries, Gamma from 81 countries and Delta from 135 countries (COVID-19 weekly epidemiological update, 2021). Due to the high transmissibility of VOCs (Buchan et al., 2021; Campbell et al., 2021; Tegally et al., 2021), the replacement of locally circulating variants by VOCs poses a serious public health threat.

According to WHO Weekly Epidemiological Update (Edition 51), as of 16 August 2021, there have been 4,955,648 cumulative cases of coronavirus disease (COVID-19), including cumulative 117,282 deaths, in African region (COVID-19 weekly epidemiological update, 2021). The Region reported relatively similar numbers of weekly cases and deaths as the previous week, with just over 182,000 new cases and over 4800 new deaths reported this week. In Sierra Leone, as of 6 August 2021, there have been 6303 confirmed cases of COVID-19 with 120 deaths, reported to WHO. On 17 June 2021, National COVID-19 Emergency Response Centre (NaCOVERC) announced that Sierra Leone was

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<https://doi.org/10.1016/j.meegid.2022.105208>

Received 19 September 2021; Received in revised form 24 December 2021; Accepted 3 January 2022

Available online 6 January 2022

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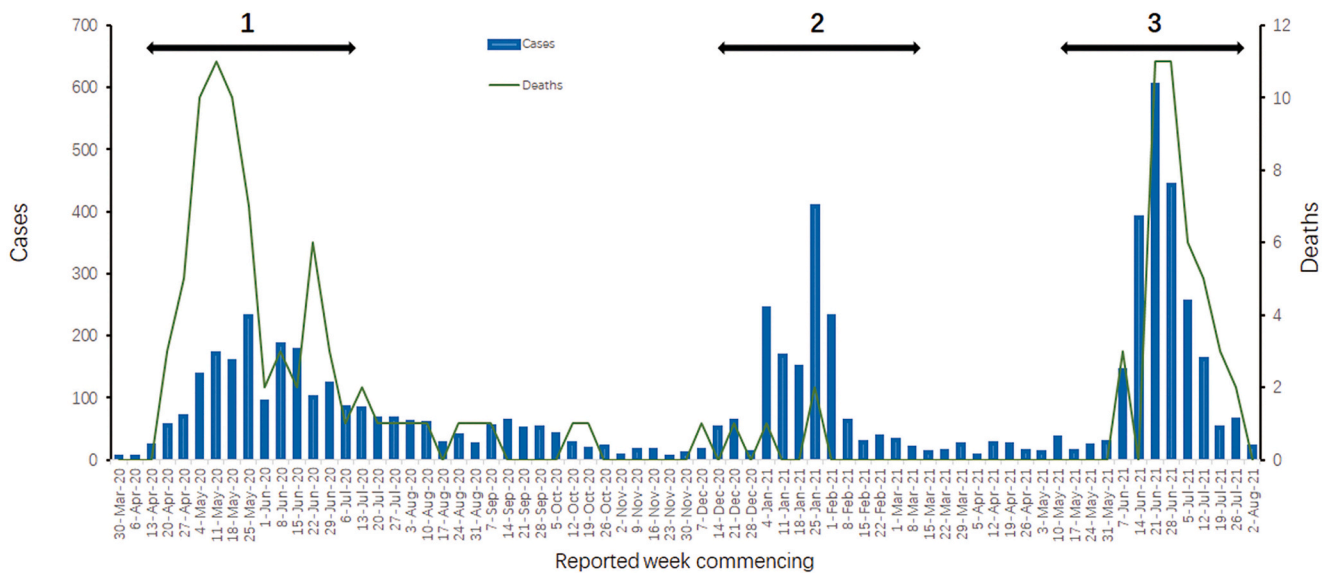


Fig. 1. COVID-19 cases and deaths reported weekly in Sierra Leone, as of 6 August 2021. The numbers 1, 2 and 3 at the top of the figure indicate three COVID-19 waves in Sierra Leone.

witnessing a third wave of COVID-19 infections. Genomic sequencing of SARS-CoV-2 can provide valuable information on the dynamics of viral epidemics and the efficacy of control measures (Long et al., 2020; Taboada et al., 2020). Since the start of the pandemic, millions of SARS-CoV-2 sequences have been submitted to and shared on public sequence databases such as GISAID, GenBank and COG-UK. However, prior to our study, there was only one piece of literature that reported 11 SARS-CoV-2 genome sequences from Sierra Leone, which were isolated from COVID-19 confirmed cases during the early period of the pandemic in Sierra Leone (unpublished data, <https://virological.org/t/sars-cov-2-genomic-epidemiology-in-sierra-leone/544>). The aim of this study is to investigate SARS-CoV-2 variants and possible transmission of VOCs in Sierra Leone.

2. Methods

A total of 65 nasal swab samples were collected from COVID-19 cases in Western Area Urban (WAU) district, which was the most severely affected district by the COVID-19 in Sierra Leone, accounting for 58.1% of cumulative confirmed cases and 82.5% of cumulative deaths in the country. A COVID-19 case was defined as a positive SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) test result. Among the 65 samples, 24 samples were collected during the second wave and

41 samples were collected during the third wave (Fig. 1). All study participants provided informed consent for the collection of samples and subsequent analyses. The study was approved by the Sierra Leone Ethics and Scientific Review Committee.

Viral RNA was extracted from 140 µl of nasal swab with QIAamp Viral RNA kit (Qiagen, GER) and reverse transcribed into cDNA using SuperScript™ III First-Strand Synthesis System (Thermo Fisher, USA) with random hexamers according to the manufacturer’s instructions. Ampliseq PCR was performed using Ion AmpliSeq HiFi Mix and Ion AmpliSeq™ 2019-nCoV Panel (Thermo Fisher, USA). The Ion AmpliSeq™ 2019-nCoV Panel consists of two pools with amplicons ranging from 125 bp to 275 bp in length and covers >99% of the SARS-CoV-2 genome. Two separate PCR reactions were done for each sample, and the pool 1 and pool 2 amplification products of each sample were mixed. The DNA concentration was determined with a Qubit 3.0 instrument using a dsDNA HS Assay Kit (Thermo Fisher, USA).

Rapid barcoding workflow was used to prepare the sequencing library (Oxford Nanopore, UK). In each sequencing run, four samples were barcoded and mixed into one tube to generate one sequencing library. Sequencing was performed on the MinION platform, the final library was loaded onto the flow cell. Genome sequences were assembled using CLC software with the SARS-CoV-2 reference genome (accession number: NC_045512).

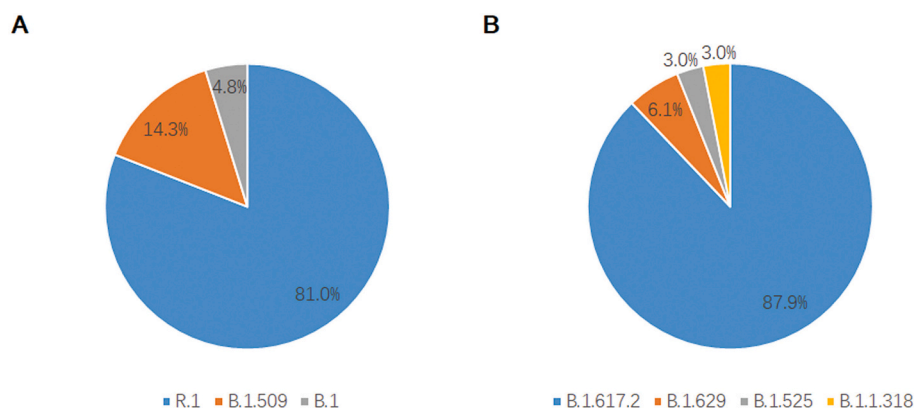


Fig. 2. The distribution of SARS-CoV-2 pango lineages circulating in Sierra Leone. (A) The proportion of SARS-CoV-2 lineages during the second wave; (B) The proportion of SARS-CoV-2 lineages during the third wave.

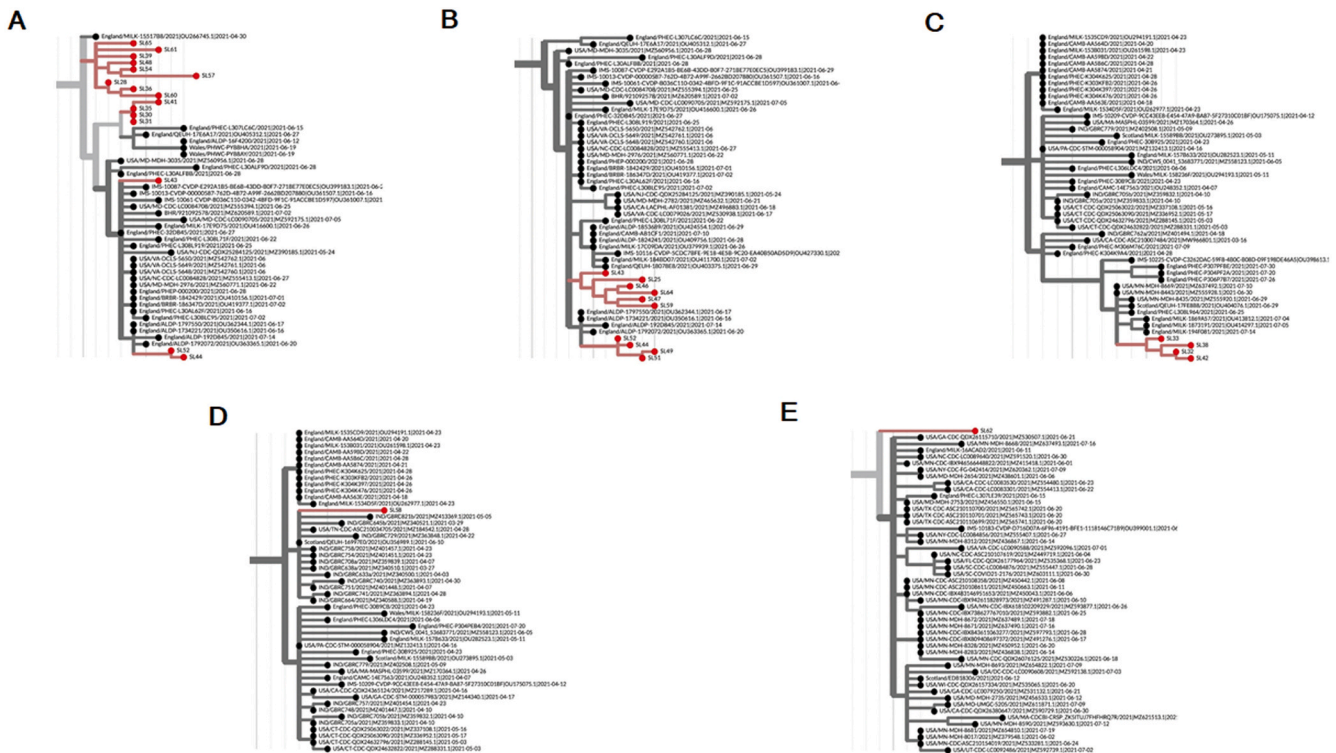


Fig. 3. Phylogenetic tree analysis of Delta variant. The subtrees were generated by Ultrafast Sample placement on Existing tRee (USHER) and explored using the Nextstrain platform. The uploaded Sierra Leone Delta variant sequences are denoted by red circle symbols and the public Delta variant sequences are indicated by black circle symbols. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The obtained SARS-CoV-2 sequences were assigned the most likely lineages by Phylogenetic Assignment of Named Global Outbreak Lineages (pangolin) web application (<https://pangolin.cog-uk.io/>) (Rambaut et al., 2020; O’Toole et al., 2021). Real-time phylogenetic was performed by Ultrafast Sample placement on Existing tRee (USHER) (Turakhia et al., 2021). USHER can generate local subtrees to show samples in the context of the most closely related sequences. The sequences reported in this article are available in GenBank under accession numbers of MZ411524-MZ411527, MZ436883-MZ436899, and MZ854386-MZ854418.

3. Results

Sierra Leone recorded its first confirmed COVID-19 case on 30 March 2020 in Freetown, the capital of Sierra Leone. Subsequently, COVID-19 cases were detected in all of 16 districts. Since the beginning of the pandemic, Sierra Leone has experienced three waves of COVID-19 infections (Fig. 1). At enrollment, the median age of participants was 39 years, ranging from 14 to 73. Of them, 32 were male and 33 were female. All of them were Sierra Leone citizens and reported no history of foreign travel in the 14 days prior to diagnosis.

A total of 21 genome sequences were obtained from 24 selected samples collected during the second wave. These sequences were also classified in pangolin as lineages: R.1 ($n = 17$), B.1.509 ($n = 3$) and B.1 ($n = 1$). As is shown in Fig. 2A, the percentage of R.1 lineage reached 81%, indicating R.1 lineage responsible for the second wave of COVID-19 infections in this country. There were no VOCs and VOIs detected in the 21 sequences. In addition, 33 genome sequences were determined from 41 selected samples representing the third wave of COVID-19. Among the 33 genome sequences, B.1.617.2 was the most common lineage (87.9%, 29/33), followed by B.1.629 (6.1%, 2/33), B.1.525 (3.0%, 1/33), B.1.1.318 (3.0%, 1/33) (Fig. 2B). Of great importance, a more highly transmissible variant of SARS-CoV-2, B.1.617.2 (Delta variant), has been detected and dominated the third wave of infections

in Sierra Leone. Among 29 B.1.617.2 sequences, three were further divided into AY.37, AY.43 and AY.44 sub-lineages respectively. The dynamic change of SARS-CoV-2 variants in Sierra Leone was observed.

We investigated the introduction of B.1.617.2 lineage into Sierra Leone. A total of five local subtrees were generated, and each one showed the most closely related B.1.617.2 sequences from the public sequence databases with those from the study. Moreover, the local subtrees showed evidence of different geographic origins of B.1.617.2 lineage. As is shown in the subtrees (Fig. 3A, B), the majority of Sierra Leone B.1.617.2 sequences were most closely related with B.1.617.2 sequences from England, indicating their origin in England. Fig. 3C and E illustrated five Sierra Leone B.1.617.2 sequences (samples 32, 33, 38, 42, and 62) were most closely related with B.1.617.2 sequences from USA. The remained one Sierra Leone B.1.617.2 sequences (sample 58) was most closely related with one Scotland sequence (Fig. 3D). The different geographic origins suggested multiple introductions of SARS-CoV-2 lineage into Sierra Leone. Finally, we found that most of Sierra Leone B.1.617.2 sequences were each other closely related, and five monophyletic clusters were observed (Fig. 3). For example, nine Sierra Leone B.1.617.2 sequences (samples 28, 36, 39, 48, 54, 57, 60, 61 and 65) formed a monophyletic cluster (Fig. 3A). All B.1.617.2 sequences in this cluster were from Sierra Leone and each other closely related. Given all the participants reported no history of foreign travel in the 14 days prior to diagnosis, the observed five Sierra Leone B.1.617.2 clusters indicated local transmission of B.1.617.2 lineage in the country. Taken together, the phylogenetic analysis suggested multiple introductions of SARS-CoV-2 lineage into Sierra Leone and subsequent local transmission in the country.

4. Discussion

In this work, we analyzed the molecular genomics features of two waves of COVID-19 infections occurring in Sierra Leone. We discovered that the second COVID-19 wave was mainly caused by R.1 lineage while

the third COVID-19 wave was dominated by B.1.617.2 lineage. The dynamic change of SARS-CoV-2 lineages indicated the continuously introductions of different SARS-CoV-2 lineages into this country. We further showed that B.1.617.2 lineage was introduced into Sierra Leone from different geographical regions independently. These results strongly suggest the active transmission of virus variants.

Although the R.1 lineage is not currently classified as a VOC or VOI, it does have several mutations of importance. For example, the E484K mutation has also been identified in SARS-CoV-2 VOCs, such as Beta (B.1.351) and Gamma (P.1), which showed evidence of reduced neutralization by convalescent and postvaccination sera (Wang et al., 2021a; Wang et al., 2021b). Since R.1 lineage was identified on 14 January 2020, the lineage variant has been detected in at least 34 countries. A recent study reported that the relative instantaneous reproduction numbers (R_{RI}) of the R.1 with respect to other strains circulating in Japan were estimated at 1.25, which indicating the higher transmissibility of R.1 lineage (Ito et al., 2021). The first 11 sequences obtained from samples collected during the first COVID-19 wave in Sierra Leone belonged to A.12, B.1, B.1.1, B.6 and B.1.379 lineages. Indeed, we observed the replacement of initially circulating variants by mainly R.1 lineage during the second wave.

The SARS-CoV-2 B.1.617 lineage was initially identified in October 2020 in India (Ferreira et al., 2021). B.1.617 viruses are divided in three lineages: B.1.617.1, B.1.617.2 and B.1.617.3. B.1.617.2, also referred to as the Delta variant, rapidly spread around the world prompting the WHO to classify it as a VOC in May 2021. The world is still grappling with the Delta variant. Recently, Finlay Campbell et al. reported a statistically significant increase in the pooled mean effective reproduction number relative to non-VOC/VOI of Alpha at 29%, Beta at 25%, Gamma at 38% and Delta at 97%, which indicating the highest transmissibility of Delta variant (Campbell et al., 2021). Moreover, reduced sensitivity of SARS-CoV-2 Delta variant to antibody neutralization was illustrated (Planas et al., 2021). By 27 August 2021, only 2.0% of people in Sierra Leone have received at least one dose of a COVID-19 vaccine, and only 0.4% of people in Sierra Leone have been fully vaccinated (<https://ourworldindata.org/covid-vaccinations>). With the low percentage of the population that is completely immunized in Sierra Leone, there is a possibility of a new COVID-19 wave in the country. In our study, we found the replacement of previously circulating SARS-CoV-2 variants by Delta variant in Sierra Leone, which poses a serious public health threat. Our findings highlight the importance of genomic surveillance of SARS-CoV-2 variants and the urgent need for implementation of strengthened public health and social measures (PHSM) to control the spread of virus variants.

Funding

This work was supported by grant from the State Key Laboratory of

Pathogen and Biosecurity (SKLPBS2126).

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank all the participants for providing samples that enabled the conduction of this study.

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