ISSN: 2755-0125

Journal of Virology Research & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

A Genomic Surveillance During the Evolution of SARS-CoV-2 Omicron Variants in Sri Lanka

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ABSTRACT

Objective: To illustrate the genomic evolution and mutation pattern of the SARS-CoV-2 Omicron variant and its sub-variants in Sri Lanka from December 2021 to March 2024, examine the association with the sample characteristics, and summarize the principle of the Oxford Nanopore Technology sequencing platform.

Methods: The study retrospectively analyzed 189 SARS-CoV-2 RNA-positive swab samples received at the sequencing laboratory from December 2021 to March 2024. SARS-CoV-2 positive samples sequenced as the Omicron variant was considered the major inclusion criteria. The viral RNA was extracted, and the DNA library was prepared according to the ONT protocol and subjected to the Nanopore sequencing. Consensus sequences were generated and analyzed.

Results: Of the total 189 Omicron cases, 8 major Omicron sub-lineages including BA.1(30.2%), BA.2(14.3%), BA.5(16.4%), BA.2.12.1(0.5%), BA.2.75(1.6%), CH.1.1(4.2%), XBB(16.9%), JN.1(15.9%) were identified, and the spike protein showed the highest number of mutations in all sub-lineages. The Omicron variant was circulated among all age groups, predominated in the 20 ≤ age <40 group and Western province reported the highest cases.

Conclusion: The present study examined the genomic evolution and mutation pattern of the SARS-CoV-2 Omicron variant locally and the principle and application of Oxford Nanopore Technology in the sequencing platform. Further, the study strongly convinced the importance of continuous surveillance systems and strengthened sequencing facilities in the country.

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Received: October 12, 2024; **Accepted:** October 18, 2024; **Published:** November 05, 2024

Keywords: Genome Sequencing, Nanopore Technology, Omicron, SARS-CoV-2 Variants

Introduction

The coronavirus disease 2019 (COVID-19) is a global outbreak caused by SARS-CoV-2 (nCoV) virus, first detected in China in December 2019. With the virus spreading rapidly across the world the World Health Organization (WHO) declared a public health emergency of international concern (PHEIC) on 11th March 2020, officially characterizing the outbreak as a pandemic [1,2]. Since then, more than 750 million confirmed cases of SARS-CoV-2 have been reported to WHO and it has consistently mutated throughout its global transmission giving rise to many COVID-19 waves [3]. WHO has established a classification system for SARS-CoV-2 variant surveillance to monitor and assess the properties of the virus such as transmissibility, associated disease severity, the efficacy of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures. The WHO defined a SARS-CoV-2 variant with genetic changes potentially affecting virus characteristics and growth advantage requires enhanced monitoring and reassessment, especially if it has many mutations in known antigenic sites but few sequences, such a variant can be designated a variant under monitoring (VUM). SARS-CoV-2 variants with genetic changes affecting virus traits and showing a global growth advantage, increasing prevalence and cases, pose an emerging risk to public health classified as variant of interest (VOI). VOIs that cause detrimental changes in clinical disease severity or change in COVID-19 epidemiology resulting in substantial impact on public health or not controlled through vaccination or medical therapy interventions are designated as variants of concern (VOC) [4].

As of 10th January 2022, the WHO has designated five VOCs namely the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) variants first detected in the United Kingdom, South Africa, Brazil, India and Botswana respectively [5]. Sri Lanka also encountered several Covid-19 waves parallel to the global transmission of the disease and the

first Omicron case in Sri Lanka was reported in early December 2021 [6]. The Omicron variant which possesses the highest number of mutations among VOCs, rapidly became the dominant variant worldwide supplanting the highly transmissible Delta variant [7]. SARS-CoV-2 is one of the largest spherical, enveloped, single-stranded, non-segmented, positive sense RNA viruses with a complete genome size of ~30,000 bases in length. Ten open reading frames (ORFs) including four major structural protein coding ORFs for the spike, envelop, membrane, and nucleocapsid have been characterized in the SARS-CoV-2 genome [8]. Overall more than 60 substitutions, deletions, and insertions, have been identified in the Omicron variant influencing its enhanced transmissibility, disease severity, and immune escape as evidenced by the exponential increase in cases over shorter periods compared to prior VOCs [9,10].

The WHO designated Pango lineage B.1.1.529 as Omicron which is classified into several lineages BA.1, BA.2, BA.3, BA.4, BA.5, and a series of sub lineages mainly including BA.1.1, BA.2.12.1, BA.2.75, BA.2.86, and XBB. Although BA.3 became highly restricted, other lineages showed increased transmissibility, and immune escape and became the globally dominant variants[11-14]. Currently circulating VOIs are designated as Omicron JN.1 and Omicron BA.2.86 by the WHO [15]. Nucleic acid amplification tests (NAAT) are the reference standard for diagnosis of acute SARS-CoV-2 infection [16]. The identification and classification of SARS-CoV-2 variants mainly relied on partial or whole genome sequencing [5]. Next-generation sequencing (NGS) technologies particularly Oxford nanopore sequencing technology (ONT) have been validated as a potent instrument for tracing origins, dissemination, and transmission chains of the Covid-19 outbreak, as well as for monitoring the evolution of SARS-CoV-2 variants [17]. ONT has emerged as one of the most powerful sequencing platforms since its inception. It cost-effectively provides rapid and real-time data. It has a reduced error rate, increased throughput, short turnaround time, and furnished portable device for whole genome sequencing [18,19].

ONT sequencing platforms were established in the state sector laboratories in Sri Lanka in the latter part of 2021. Sequencing was carried out during both the pandemic and post-pandemic period to enhance awareness of circulating SARS-CoV-2 variants. This study aims to describe the evolution of SARS-CoV-2 Omicron sub-variants during a pandemic and post-pandemic period in Sri Lanka examining their association with sample characteristics and clinical profiles. Furthermore, explore the principles of sequencing with ONT MinION Mk1C device.

Material and Method

Sample Collection and Extraction

The study retrospectively analyzed 189 swab samples (nasopharyngeal/oropharyngeal) received at the National Virus Reference Laboratory (NVRL) using ONT-based MinION Mk1C genomic sequencer, from December 2021 to March 2024 during pandemic and post-pandemic periods in Sri Lanka. The NVRL received swab samples from SARS-CoV-2 positive patients in viral transport media covering all the provinces in the country through healthcare institutions for SARS-CoV-2 gene sequencing. The selected samples were extracted using the QIAamp® Viral RNA Mini Kit following the manufacturer's instructions.

Library Preparation and Sequencing

ONT library was prepared according to PCR tiling of SARS-CoV-2 virus with rapid barcoding and Midnight RT-PCR Expansion (SQK-RBK110.96 and EXP-MRT001) protocol. RNA samples were first reverse transcribed using the LunaScript RT SuperMix (LS RT) kit and the resulting cDNA was used as the template for genome amplification with Q5 Hot Start (Q5 HS) master mix in a two-pool PCR strategy based on primal scheme approach(20). Two mid-night primer pools were used to generate 1.2 kb amplicons that overlap by approximately 20 bp(21). The protocol produces amplicons in a tiled manner across the whole SARS-CoV-2 genome. Then the primer pools were combined, and rapid barcodes were attached to the DNA ends.A two-step clean-up was performed using SPRI beads or AMPure XP beads and freshly prepared 80% ethanol. After DNA was extracted in the Elution buffer, sequencing adapters were ligated onto the DNA ends. DNA quantification was done at this point using Qubit™ 4 Fluorometer. The DNA library was then mixed with sequencing buffer II (SBII) and loading beads II (LBII) before loading into the primed R9 Spot-On flowcell (FLO-MIN106D). ONT MinION Mk1C device was used as the sequencer.

ONT Principle and SARS-CoV-2 Sequence Analysis

ONT employs a single-molecule sequencing technology based on nanopores. The reaction system for nanopore sequencing is carried out in a flowcell, where two compartments filled with ionic solution are separated by electrically resistant synthetic membranes containing nanopores. Flowcell consists of 2048 individually addressable protein nanopores that can be controlled in an array of 512 sensors, each connected to four nanopores. Nanopore technology operates by applying a cathode and anode to the solution on the forward and reverse sides of the membrane, respectively. Negatively charged biomolecules, such as DNA are introduced on the forward side, allowing them to traverse the protein pores of the membrane, under the influence of an electrophoretic force generated by an applied voltage. Different nanopores are equipped with distinct readers which is the part of the nanopore that significantly contributes to the modulation of ionic current as DNA passes through. The nanopores of the R9 version of flowcell feature a single reader located centrally in the barrel whereas the nanopores of the R10 version of flowcell are designed with two readers positioned along its length, enhancing their ability to capture and interpret the signal more effectively [18].

During the library preparation process, adapters are ligated to the ends of the DNA, and motor protein is added to the DNA. These adapters facilitate the capture of strands and loading of a processive enzyme at the 5' end of one strand. The adapters also concentrate DNA substrates at the membrane surface proximal to the nanopore, enhancing the DNA capture rate. When the sequencing starts, the motor protein unwinds the double strand and feeds a single strand through the nanopore, one base at a time. When the DNA molecules pass through the pore, it causes a deflection in the current across the pore, and this diversion of the current is related to the exact bases present in the pore at the moment. The translocation speed at which the bases pass through a nanopore is usually 350 to 450 bases per second and is controlled by the motor protein. The current in the nanopore is measured and recorded by a sensor several thousand times per second and the flow cell captures data from one nanopore of every channel at any time. At any given time, the status of each of the 512 channels can vary including molecules being sequenced, captured into the pores, or pores being empty or blocked [18,22,23].

The MinION Mk1C device operates using specialized software known as MinKNOW, which performs several essential functions. These include data acquisition, real-time analysis, basecalling, data streaming, providing sequencing feedback, device control,

configuring run parameters, and facilitating sample identification and tracking. When sequencing, the changes in current across the nanopore as the DNA strand passes, are recorded by MinKNOW software. The progressive passage of bases through the pore leads to continuous fluctuation in the electrical current referred to as the "squiggle". MinKNOW converts the squiggle into reads in real-time, each of which represents a single strand of DNA. These reads are formulated in raw data files known as FAST5. The signal stored in FAST5 files is processed by basecalling algorithms to decrypt the sequence of bases into FASTQ files. Dorado is integrated within MinKNOW to carry out basecalling, live during the run, after the run has finished, or a combination of both.A quality score is assigned by MinKNOW to quantify the accuracy of each read. FASTQ files are placed into a "pass" directory if the read has a mean Q-score more than 9 and if the Q-score is below 9, those FASTQ files are stored in a "failed" directory. MinKNOW also creates an "unclassified" folder containing reads that could not be confidently classified as either "pass" or "fail" based on their quality scores or other criteria, such as incomplete data, unresolved basecalls, and potential artifacts [19,22,23].

Successfully basedcalled reads were further analyzed following the ARTIC nCoV-2019 pipeline version 1.4.1, which included demultiplexing, read filtering, primers and barcode trimming, quality control, amplicon coverage normalization, and finally building a genome consensus sequence (FASTA) for each sample. The artic pipeline performs typing of SARS-CoV-2 sequencing data using both Pangolin and Nextstrain classification systems by aligning consensus data against reference SARS-CoV-2 genome (GenBank accession no.MN908947.3) [24]. Resulted consensus sequences and clinical history were also uploaded into the GISAID platform where the consensus data were published globally.

Results

189 SARS-CoV-2 positive nasopharyngeal/oropharyngeal swabs, received from December 2021 through March 2024 were analyzed and identified as Omicron variants by genomic sequencing in the study. The age of the analyzed samples ranged from 2 months to 82 years, while the majority (37.0%, n=70) belonged to the age group from >20 years to ≤40 years (Figure 1). In the sample population, 51.3% (n=97) were male, and 48.7% (n=92) were females. Among the sequenced samples, the highest number originated from the Western province (81%, n=153). The remaining samples were distributed across the Southern (6.3%, n=12), Sabaragamuwa (4.8%, n=9), North-western (3.7%, n=7), North (2.1%, n=4), Eastern (1.6%, n=3) and North-central (0.5%, n=1) provinces.

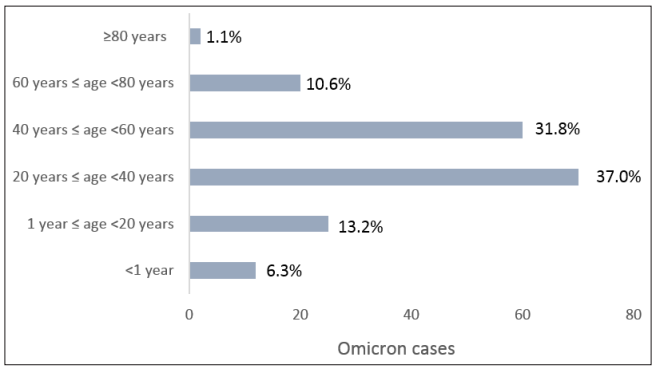

Figure 1: Age Distribution of the SARS-CoV-2 Omicron Cases

Following the initial detection of the Omicron variant in December 2021 in Sri Lanka, it was observed that Omicron and its sub-lineages rapidly supplanted other SARS-CoV-2 variants. During the onset of the Omicron wave, sub-lineages such as BA.1 (30.2%, n=57) and BA.5 (16.4%, n=31) were predominant in January 2022, and July 2022 respectively. However, from July 2022 onwards BA.2 (14.3%, n=27) and its sub-lineages including BA.2.12.1(0.5%, n=1), BA.2.75(1.6%, n=3), CH.1.1(4.2%, n=8), XBB (16.9%, n=32), and JN.1 (15.9%, n=30) became the dominant strains throughout the subsequent period. By March 2024, JN.1 had rapidly surpassed these variants, accounting for nearly all SARS-CoV-2 cases (Figure 2).

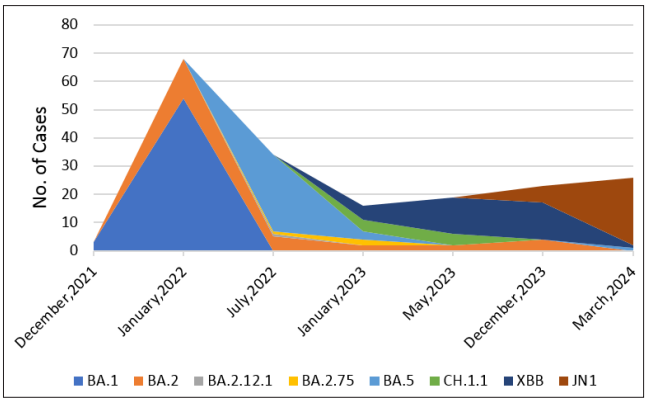

Figure 2: Prevalence of the Omicron Variants

For the study period, it was detected 8 major sub-lineages of the Omicron variant (BA.1.1.529 + BA. *) circulated in Sri Lanka. These sub-lineages and their NextStrain clades are listed in Table 1. Data in Table 2 illustrated how different Omicron variants and their sub-lineages circulated throughout the study period.

Table 1: Sub-Lineages of Omicron and their Designated Naming by WHO and Nextstrain

Lineage or sub-lineage of Omicron	WHO label	NextStrain clade
Omicron (B.1.1.529) BA.1 or B.1.1.529.1	BA.1	21K
Omicron (B.1.1.529) BA.2 or B.1.1.529.2	BA.2	21L
Omicron (B.1.1.529) BA.5 or B.1.1.529.5	BA.5	22B
Omicron (B.1.1.529) BA.2.12.1 or B.1.1.529. 2.12.1	BA.2.12.1	22C
Omicron (B.1.1.529) BA.2.75 or B.1.1.529. 2.75	BA.2.75	22D
Omicron (B.1.1.529) BA.2.75.5.1 or B.1.1.529. 2.75.5.1	CH.1.1	23C
recombinant of BA.2.10.1 and BA.2.75	XBB	22F
Omicron (B.1.1.529) BA.2.86.1.1 or B.1.1.529.2.86.1.1	JN.1	24A

Table 2: Distribution of Omicron Variants and their Sub-Lineages During the Period

Month	Variant	Sub Lineages
December,2021	Omicron BA.1 (1.6%, n=3)	BA.1.1.14 (33.3%, n=1)
		BA.1.1 (33.3%, n=1)
		BA.1.1 (33.3%, n=1)
January,2022	Omicron BA.1 (28.6%, n=54)	BA.1 (24.1%, n=13)
		BA.1.1 (53.7%, n=29)
		BA.1.15 (3.7%, n=2)
		BA.1.16 (3.7%, n=2)
		BA.1.1.1 (1.9%, n=1)
		BA.1.1.10 (5.6%, n=3)
		BA.1.17.2 (7.4%, n=4)
	Omicron BA.2 (7.4%, n=14)	BA.2 (85.7%, n=12)
		BA.2.2 (7.1%, n=1)
		BA.2.3 (7.1%, n=1)
July,2022	Omicron BA.2 (3.7%, n=7)	BA.2 (57.1%, n=4)
		BA.2.12.1 (14.3%, n=1)
		BA.2.38 (14.3%, n=1)
		BA.2.76 (14.3%, n=1)
	Omicron BA.5 (14.3%, n=27)	BA.5 (7.4%, n=2)
		BA.5.1 (11.1%, n=3)
		BA.5.1.30 (3.7%, n=1)
		BA.5.2 (18.5%, n=5)
		BA.5.2.1 (59.3%, n=16)
January,2023	Omicron BA.2 (6.9%, n=13)	BA.2 (15.4%, n=2)
		BA.2.75.2 (7.7%, n=1)
		CH.1.1 (15.4%, n=2)
		CH.1.1.1 (15.4%, n=2)
		BL.1 (7.7%, n=1)
		XBB.1 (15.4%, n=2)
		XBB.1.5 (7.7%, n=1)
		XBB.3 (7.7%, n=1)
		XBB.4.1 (7.7%, n=1)
	Omicron BA.5 (1.6%, n=3)	BA.5.2 (33.3%, n=1)
		BA.5.2.1 (66.7%, n=2)
May,2023	Omicron BA.2 (10.1%, n=19)	BA.2 (10.5%, n=2)
		CH.1.1 (10.5%, n=2)
		CH.1.1.1 (10.5%, n=2)
		XBB.1.16 (47.4%, n=9)
		XBB.1.16.1 (10.5%, n=2)
		XBB.1.9.1 (10.5%, n=2)
December,2023	Omicron BA.2 (12.2%, n=23)	BA.2 (17.4%, n=4)
		XBB.1.16.6 (4.3%, n=1)
		XBB.2.3.5 (4.3%, n=1)
		XBB.1.5 (4.3%, n=1)
		HK.3 (8.7%, n=2)
		HV.1 (34.8%, n=8)
		JN.1 (26.1%, n=6)
March,2024	Omicron BA.2 (13.2%, n=25)	HV.1 (4.0%, n=1)
		JN.1 (76.0%, n=19)
		JN.1.18 (4.0%, n=1)
		JN.1.4 (4.0%, n=1)
		JN.1.39 (4.0%, n=1)
		JN.1.1 (4.0%, n=1)
		JN.1.28 (4.0%, n=1)
	Omicron BA.5 (0.5%, n=1)	BQ.1 (100%, n=1)

We analyzed the genome-wide mutations of BA.1, BA.2, BA.5, BA.2.75, XBB, and JN.1. All sub-lineages displayed the highest number of mutations in the spike protein. Here, we mapped the mutations and represented their distribution across different parts of the genomic regions (Figure 3). The mutations S371L, G446S, and G496S were identified as specific to the BA.1 lineage. A major mutation in BA.1, the deletion of Δ69-70 in the spike protein, was absent in the BA.2 which instead featured deletions at Δ24-26 in the spike protein. All these deletions and additional spike mutations L452R and F486V were found in BA.5. BA.2.75 is a descendant of the BA.2 lineage, but it harbors additional spike mutations including R493Q, G446S, W152R, and K147E. CH.1.1 was distinguished by the spike mutations of R346T, K444T, L452R, and F486S. JN.1 exhibited a unique spike protein mutation L455S and contained the highest number of mutations among the detected variants.

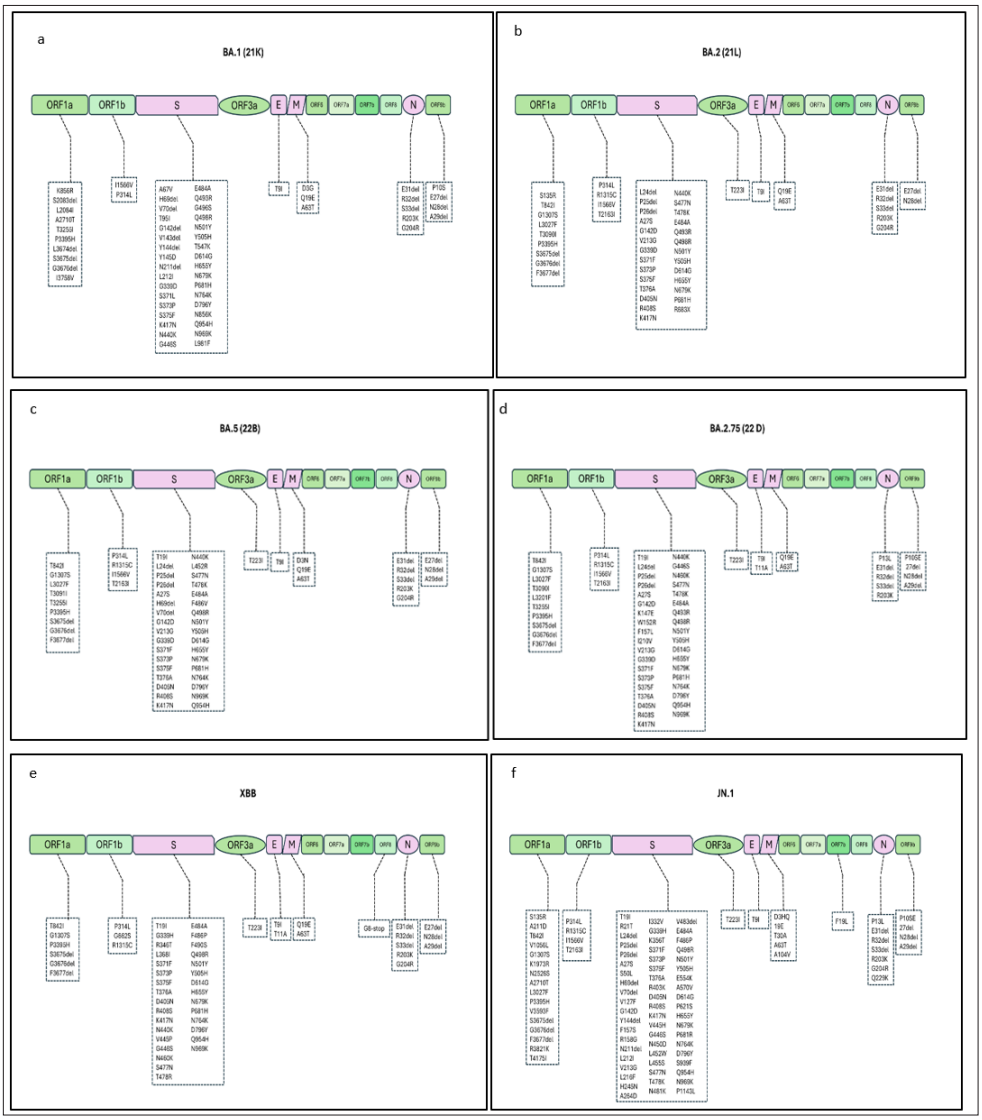

Figure 3: Schematic Diagram of Genome-Wide Mutations

The phylogenetic analysis of the identified sub-lineages is presented in Figure 4. The sub-lineages clustered into four major clades; 21k (BA.1), 21L (BA.2), 22F (XBB), and 24A (JN.1). Notably, the highest diversity was in 21L and 22F clades forming 22B(BA.5), 22D(BA.2.75), 23C(CH.1.1) and 23B(XBB.1.16), 23D(XBB.1.9), 23F(HV.1) respectively.

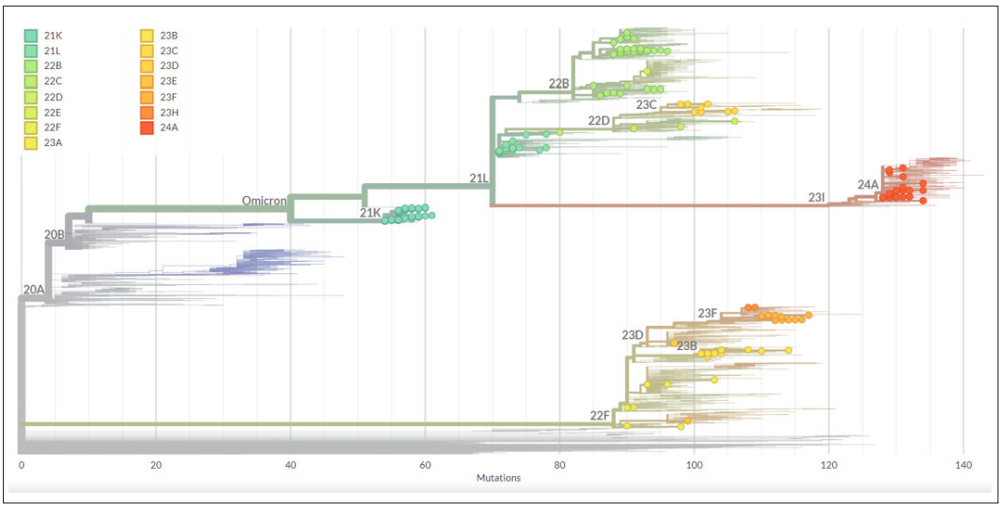

Figure 4: Phylogenetic Tree showing the Distribution of Clades

Discussion

The study provides a comprehensive analysis of SARS-CoV-2 variants circulating in Sri Lanka during December 2021 and March 2024, with focus on the Omicron variant and its sub-lineages using the next-generation sequencing platform; Oxford Nanopore Technology. The manuscript explains the working principle behind the nanopore sequencing including the library preparation method and the evolution of the Omicron variant through genome-wide mutation analysis of 189 nasopharyngeal swab samples received at the virus reference laboratory during the post-pandemic period of Sri Lanka.

The emergence of SARS-CoV-2 and its rapid evolution into highly mutated variants emphasize the importance of genomic surveillance in monitoring virus evolution and identifying novel mutations [17,18]. Genomic surveillance using Oxford Nanopore Technology has proven its effectiveness in the SARS-CoV-2 outbreak. ONT has unique advantages including generating short to ultra-long reads making it much easier to sequence an entire genome, providing real-time analysis for rapid insight, high throughput, portability, and low material requirements making it cost-effective especially for a low-resource setting country [17,18,22,23,25].

After its first detection in December 2021, The Omicron variant rapidly replaced the Delta variant and became the predominant variant in Sri Lanka in par with globe. In January 2022, BA.1 became the leading variant and by July 2022 BA.5 dominated the active SARS-CoV-2 cases. Then BA.2 and its sub-lineages including XBB started dominating the outbreak from January 2023 onwards. The rapid spread of the JN.1 variant supplanted all the other Omicron variants dominating almost all the SARS-CoV-2 active cases by March 2024.This rapid shift among the leading variants of the Omicron within two years showed its continuing adaptation and evolution.

The demographic data discloses that the majority of cases were in the 20–40-year age group (37%) indicating that this group is more vulnerable to SARS-CoV-2 infection or more possibly to be tested during the study period. The pattern is similar in other South Asian countries(26,27). The distribution of cases between males (51.3%) and females (48.7%) suggests no significant variation between genders in the study population confirming the global pattern of SARS-CoV-2 prevalence by gender [28]. Western province accounted for most cases (81%), reflecting its dense population and active surveillance program.

The mutation analysis of the SARS-CoV-2 genome reveals the Spike protein of Omicron showed the highest number of mutations compared to any other region of the genome. These mutations have led the Omicron variants to the most transmissible variant among all SARS-CoV-2 variants [29]. BA.2 (14.3%) and its descendants including BA.5 (16.4%), XBB (16.9%), and JN.1 (15.9%) were the most prevalent during the study. However, BA.2.75 (1.6%), BA.2.12.1 (0.5%), and CH.1.1 (4.2%) sub-variants were detected at very low frequencies during the study period. The JN.1 variant, carrying the unique L455S mutation in the spike protein highlighted the continuing evolution of the virus and the potential for the emergence of novel variants.

Conclusively, the document summarized the principle of nanopore technology and its application in the SARS-CoV-2 genome sequencing. The present study declared the emergence, prevalence, distribution, and evolution of the mutation pattern of the SARS-CoV-2 Omicron variant and its sub-lineages locally in Sri Lanka. Strengthening the continuous surveillance of the SARS-CoV-2 throughout the country and empowering genomic sequencing facilities will contribute to a better understanding of the virus and its evolution.

Conflict of Interest Statement

All authors would like to declare no potential conflicts of interest.

Acknowledgments

The authors would like to acknowledge the staff of the Department of Virology, Medical Research Institute, Sri Lanka.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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