

Respiratory Pathogen Genomic Surveillance in Bangladesh

October 2022- March 2023 Report



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National Respiratory Pathogen Genomic Surveillance in Bangladesh

Who We Are

The National Respiratory Pathogen Genomic Surveillance (NRPGS) in Bangladesh is conducted by a consortium of three institutes: (1) Institute of Epidemiology, Disease Control and Research (IEDCR), a Bangladesh government research institute, under the Ministry of Health and Family Welfare (MoHFW), and also the Govt mandated institute for outbreak investigation and response and communicable disease surveillance; (2) International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b); and (3) Institute for Developing Science and Health Initiatives (ideSHi). These institutes worked closely to monitor genomic variants of SARS-CoV-2 across the country. Leveraging existing influenza virus surveillance platforms, the consortium is working to scale-up whole genome sequencing (WGS) capacity not only for SARS-CoV-2 but also for other respiratory pathogens, most importantly, RSV and influenza virus to support the country's epidemic and pandemic preparedness.

What We Do

Our overall goal is to conduct the national respiratory virus genomic surveillance of influenza virus, SARS-CoV-2 and RSV in Bangladesh through institutional collaborative efforts and systematic approach. The specific objectives are:

1. to continue and expand genomic surveillance of respiratory viral pathogens, leveraging existing surveillance platforms for severe acute respiratory illness (SARI) and Influenza-like illness (ILI) at sentinel sites across Bangladesh.
2. to continue the existing consortium approach for specimen and data sharing, and ensure timely reporting and uploading of sequence data.

Why We Do

To support the country's epidemic preparations, it is important to scale-up whole genome sequencing (WGS) capacity following a systematic approach to monitor influenza, RSV and SARS-CoV-2 variants continuously circulating in Bangladesh. Utilization and strategic strengthening of this capacity of the consortium institutes will maximize the information we can obtain about the epidemic and outbreaks, and will help in country wide interpretation.

How We Do

A total of 18000 samples through NISB and HBIS are collected each year. Among them, 40% are children less than 5 years of age (n=7200). All samples will be tested for influenza and SARS-CoV-2; however, only children less than 5 years of age will be tested for RSV. The consortium will sequence 720 samples for Influenza virus, 720 RSV and 360 SARS-CoV-2.

The genomic surveillance data and the outcomes of the study will be disseminated by sharing the research findings with the national and international stakeholders; Institute of Epidemiology, Disease Control and Research (IEDCR), World Health Organization (WHO), Directorate General of Health Services (DGHS) of GoB. The report of the circulatory influenza virus, SARS-COV-2 and RSV variants will be submitted bi-weekly to GISAID.ORG database, which is an open platform from where all the stakeholders can avail genetic data of the contemporary variants as and when required.

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A. BACKGROUND

Respiratory viruses are among the leading causes of morbidity and mortality worldwide. Seasonal viruses such as influenza and RSV, as well as highly virulent types of influenza viruses (H5N1, H1N1, and H7N9), coronaviruses (SARS, SARS-CoV-2, MERS), Chikungunya virus, dengue virus, Zika virus, and Nipah virus, have been identified as a major concern for global public health. In most cases, health professionals were unable to quickly identify and report the source of the disease due to a lack of technology and insufficient resources. The SARS-CoV-2 pandemic has increased awareness of the value of genomic surveillance and genomic epidemiology to help guide the public health response to emerging infectious diseases.

Leveraging existing surveillance platforms for severe acute respiratory illness (SARI) and Influenza-like illness (ILI) at sentinel sites across Bangladesh, the consortium implemented a strategic sequencing approach to maximize information regarding the virus evolution within the country and shared data with stakeholders who could use these data for a broad range of pathogen threats in the future, building a sustainable platform for genomic surveillance in Bangladesh.

Influenza virus

Influenza viruses contribute substantially to acute lower respiratory infections (ALRIs), a leading cause of morbidity and mortality among children, aged people and those with underlying health conditions (Lafond, Porter et al. 2021). In Bangladesh, the burden of influenza is moderately high, with an estimated annual incidence of 458 cases per 100,000 people, with 6 to 11 influenza-associated deaths per 100,000 people across all age groups (Troeger, Blacker et al. 2019). A retrospective, time-series study used data collected between January 2010 and December 2019 from 32 hospital-based influenza surveillance sites across the country and identified a distinct influenza season in Bangladesh, with an annual peak in June to July each year (Berry, Rahman et al. 2022).

icddr,b in collaboration with the IEDCR, the national influenza centre, has been conducting hospital-based influenza surveillance (HBIS) since 2007 across the country (IEDCR. 2021) and in collaboration with CDC, Atlanta, a subset of the sample has been sequenced and shared through EpiFlu database in GISAID.ORG. Until September 2022, there are 2290 influenza sequences submitted to GISAID.ORG; which is very low in number comparing the cases. Therefore, expanding the influenza genomic surveillance in Bangladesh is much needed to continuously monitor circulating strains with pandemic potential.

Respiratory syncytial virus (RSV)

Respiratory syncytial virus (RSV) is the leading viral agent causing lower respiratory tract infection among young children, especially during the first six months of life and in LMICs. Nearly all children are infected by RSV at least once by the age of 2 years (Zhu, Fu et al. 2017). Bangladesh has a high rate of RSV infection. A multicentre study found that the detection rate of RSV in Bangladeshi children with community-acquired pneumonia in 2014–2016 was 15.2%

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(Zhu, Fu et al. 2017). Another study reported that the serum positive rate of RSV reached 90% in children under three years old (Lu, Gonzalez et al. 2011).

Currently, no vaccine preventing RSV infection is available. The World Health Organization has listed RSV vaccine development as a recommended priority product (WHO, 2017). The development of RSV vaccines and drugs is based on understanding RSV epidemic genotypes and their sequence variation. Surveillance and large-scale whole-genome sequencing of RSV are currently limited but would help identify its evolutionary dynamics and sites of selective immune evasion. However, no sequences data from Bangladesh for RSV are available on GSIAD.org.

SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly worldwide, causing almost 6.5 million deaths as of 6 September 2022. For example, in Bangladesh, over 20 million individuals were infected, and 29,440 deaths occurred (Worldometer, January, 2023).

To date, 7243 SARS-CoV-2 genomes from Bangladesh have been uploaded to the public database, GISAID.ORG, which is less than 0.3% of the total number of COVID-19 positive cases identified in the country. As part of the SARS-CoV-2 genomic surveillance, our consortium generated 1,725 complete genome sequences of SARS-CoV-2 variants circulating across the country and reported to the GoB and submitted the data to the public database. This impacted taking the immediate decision by the policymakers to restrict movement and reduce the transmission of the virus. Still, very few organizations are conducting genomic sequencing, which is not enough to get a comprehensive scenario of the country.

B. SUMMARY REPORT

During October 2022-March 2023, RSV predominantly circulated in Bangladesh, whereas influenza virus and SARS-CoV-2 existence was minimum. This report is prepared to share respiratory pathogen genomic surveillance in Bangladesh during October 2022-March 2023.

Principal findings are:

- Overall, RSV was most prevalent in <6 months infants followed by 6-11 months.
- During October 2022-January 2023, RSV positivity was as high as 50-60% for SARI cases. The RSV positivity rate among ILI cases was lower compared to SARI.
- During February-March 2023, RSV positivity rate started to decline in both SARI and ILI cases.
- In October 2022, SARS-CoV-2 positivity rate was 7% for SARI and 3% for ILI cases. Only a few cases were found between November 2022 to March 2023.
- No influenza cases were identified in October 2022, and a few cases were found between November 2022 and March 2023.

C. Overall respiratory pathogen circulation in Bangladesh: from 1 October 2022 to 31 March 2023

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The consortium has tested 4893 SARI and 3620 ILI samples for influenza virus and SARS-CoV-2, and 2245 SARI and 1027 ILI (<5 years) for RSV using qPCR collected between 1 October 2022 to 31 March 2023. These samples were collected through HBIS and NISB platforms from all 8 divisions of Bangladesh. Table 1 and 2 show the total number of qPCR-positive respiratory viruses during Oct 2022-Mar 2023.

Table 1. Total number of confirmed influenza and COVID-19 using qPCR: October 2022-March 2023

Age groups	October 2022									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	198	0	0.0	18	9.1	125	0	0.0	3	2.4
1-<5 Years	132	0	0.0	3	2.3	148	0	0.0	3	2.0
5-14 Years	75	0	0.0	4	5.3	54	0	0.0	2	3.7
15-24 Years	59	0	0.0	4	6.8	46	0	0.0	4	8.7
25-39 Years	63	0	0.0	6	9.5	58	0	0.0	3	5.2
40-59 Years	78	0	0.0	5	6.4	31	0	0.0	2	6.5
≥60 Years	67	0	0.0	8	11.9	23	0	0.0	0	0.0
Total	672	0	0.0	48	7.1	485	0	0.0	17	3.5

Age groups	November 2022									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	196	0	0.0	1	0.5	94	0	0.0	0	0.0
1-<5 Years	114	0	0.0	0	0.0	153	2	1.3	0	0.0
5-14 Years	70	0	0.0	0	0.0	56	0	0.0	0	0.0
15-24 Years	66	0	0.0	0	0.0	54	0	0.0	0	0.0
25-39 Years	71	0	0.0	0	0.0	57	0	0.0	1	1.8
40-59 Years	77	1	1.3	0	0.0	45	0	0.0	0	0.0
≥60 Years	90	0	0.0	0	0.0	42	0	0.0	0	0.0
Total	687	1	0.1	1	0.1	501	2	0.4	1	0.2

Age groups	December 2022									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	200	0	0.0	0	0.0	160	0	0.0	0	0.0
1-<5 Years	105	1	1.0	0	0.0	183	0	0.0	0	0.0
5-14 Years	65	0	0.0	0	0.0	77	0	0.0	0	0.0
15-24 Years	43	0	0.0	1	2.3	62	1	1.6	0	0.0
25-39 Years	61	0	0.0	0	0.0	69	0	0.0	0	0.0
40-59 Years	90	0	0.0	0	0.0	68	0	0.0	0	0.0
≥60 Years	87	0	0.0	0	0.0	35	0	0.0	0	0.0
Total	651	1	0.2	1	0.2	654	1	0.2	0	0.0

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Age groups	January 2023									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	335	1	0.3	0	0.0	208	0	0.0	0	0.0
1-<5 Years	204	2	1.0	0	0.0	118	1	0.8	0	0.0
5-14 Years	121	0	0.0	0	0.0	70	1	1.4	0	0.0
15-24 Years	67	0	0.0	0	0.0	76	0	0.0	0	0.0
25-39 Years	67	1	1.5	0	0.0	74	0	0.0	0	0.0
40-59 Years	77	0	0.0	0	0.0	84	0	0.0	0	0.0
≥60 Years	75	1	1.3	0	0.0	82	0	0.0	0	0.0
Total	946	5	0.5	0	0.0	712	2	0.3	0	0.0

Age groups	February 2023									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	318	2	0.6	0	0.0	166	1	0.6	0	0.0
1-<5 Years	185	2	1.1	0	0.0	96	0	0.0	0	0.0
5-14 Years	159	1	0.6	0	0.0	80	1	1.3	0	0.0
15-24 Years	61	0	0.0	0	0.0	72	1	1.4	0	0.0
25-39 Years	68	1	1.5	0	0.0	71	2	2.8	0	0.0
40-59 Years	80	0	0.0	0	0.0	65	0	0.0	0	0.0
≥60 Years	92	0	0.0	0	0.0	72	0	0.0	0	0.0
Total	963	6	0.6	0	0.0	622	5	0.8	0	0.0

Age groups	March 2023									
	SARI					ILI				
	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)	Sample (n)	Flu (n)	Flu (%)	COVID (n)	COVID (%)
<1 Year	336	1	0.3	0	0.0	120	1	0.8	0	0.0
1-<5 Years	165	2	1.2	0	0.0	151	3	2.0	0	0.0
5-14 Years	106	1	0.9	0	0.0	115	3	2.6	0	0.0
15-24 Years	67	1	1.5	0	0.0	94	2	2.1	0	0.0
25-39 Years	70	1	1.4	0	0.0	72	0	0.0	0	0.0
40-59 Years	115	1	0.9	0	0.0	59	0	0.0	0	0.0
≥60 Years	118	0	0.0	0	0.0	35	0	0.0	0	0.0
Total	977	7	0.7	0	0.0	646	9	1.4	0	0.0

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Table 2: Total number of confirmed RSV cases using qPCR: October 2022-March 2023

Age groups	October 2022					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	160	112	70.0	12	4	33.3
6-11 months	135	89	65.9	28	9	32.1
12-23 months	98	54	55.1	24	10	41.7
2-<5 years	50	12	24.0	17	5	29.4
Total	443	267	60.3	81	28	34.6

Age groups	November 2022					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	171	116	67.8	18	7	38.9
6-11 months	143	104	72.7	23	11	47.8
12-23 months	80	48	60.0	24	10	41.7
2-<5 years	51	19	37.3	35	12	34.3
Total	445	287	64.5	78	40	51.3

Age groups	December 2022					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	134	94	70.1	20	7	35.0
6-11 months	90	48	53.3	48	13	27.1
12-23 months	68	23	33.8	41	12	29.3
2-<5 years	40	12	30.0	55	11	20.0
Total	332	187	56.3	164	43	26.2

Age groups	January 2023					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	158	89	56.3	19	4	21.1
6-11 months	92	50	54.3	29	5	17.2
12-23 months	64	28	43.8	25	4	16.0
2-<5 years	27	10	37.0	36	13	36.1
Total	341	177	51.9	109	26	23.9

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Age groups	February 2023					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	163	82	50.3	18	2	11.1
6-11 months	90	28	31.1	27	7	25.9
12-23 months	58	12	20.7	31	4	12.9
2-<5 years	37	10	27.0	40	4	10.0
Total	348	132	37.9	116	17	14.7

Age groups	March 2023					
	SARI			ILI		
	Sample (n)	RSV (n)	RSV (%)	Sample (n)	RSV (n)	RSV (%)
<6 months	145	45	31.0	22	1	4.5
6-11 months	88	14	15.9	25	6	24.0
12-23 months	63	13	20.6	21	3	14.3
2-<5 years	40	6	15.0	26	4	15.4
Total	336	78	23.2	94	14	14.9

Sequencing

59 (54 RSV and 7 influenza viruses) qPCR-positive samples were sequenced using Paragon Genomics library preparation kit in Illumina MiSeq V3 system. 16 SARS-CoV-2 positive samples were sequenced in Oxford Nanopore platform.

- The RSV samples were collected between October 2022 and March 2023. Sequence analysis revealed that **all were RSV B Victoria**.
- The influenza-positive samples were collected from November 2022 to February 2023. Out of 7, **one was influenza A, and 6 were influenza B Victoria**.
- 16 SARS-CoV-2 samples collected in October 2022 were sequenced. Among those, **one turned out to be Omicron XBB.3 and another one was BM.1.1.1. All others were either XBB.1 or XBB**.

Viruses	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23
SARS-CoV-2	16					
Influenza				4	3	
RSV	22					32

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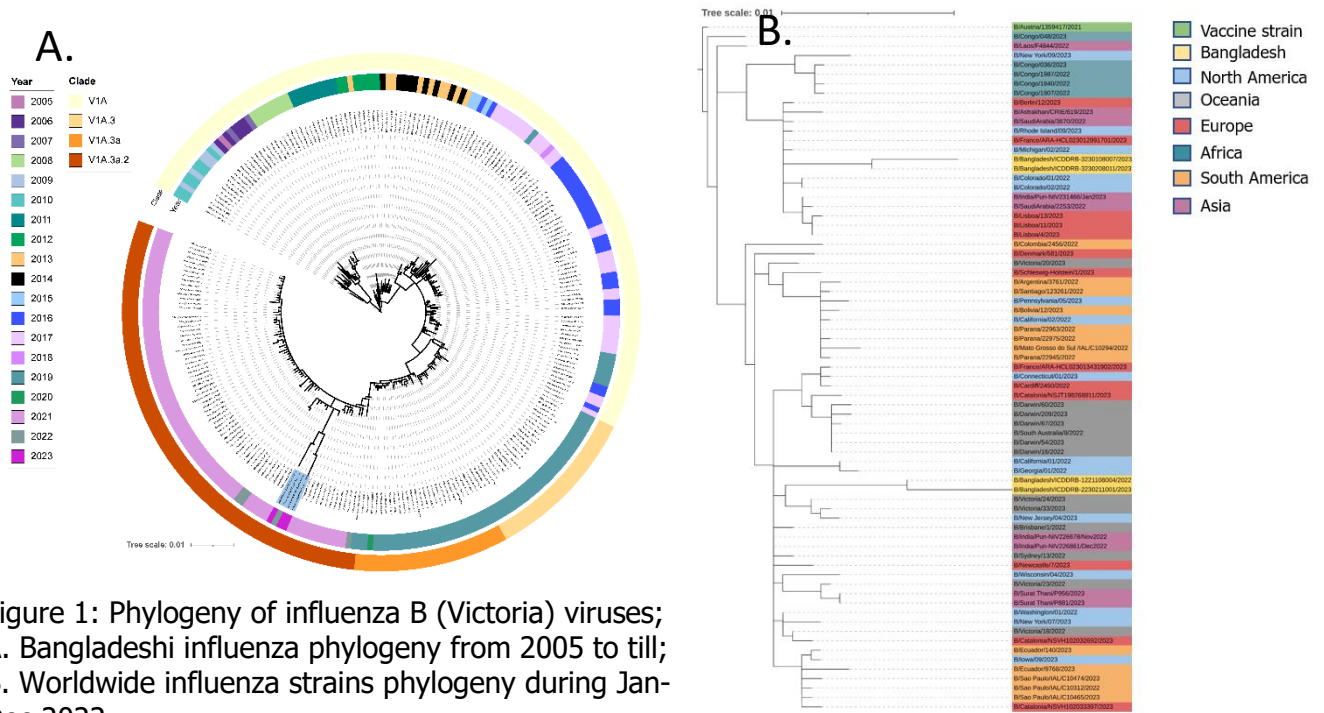


Figure 1: Phylogeny of influenza B (Victoria) viruses; A. Bangladeshi influenza phylogeny from 2005 to till; B. Worldwide influenza strains phylogeny during Jan-Dec 2022

Influenza virus phylogeny

- In Figure 1A, Bangladeshi influenza strains have been analyzed from 2005 to till date. All of our samples are V1A.3a.2 and they are clustered in between 2021 and 2022.
- For Figure 1B, EPI_ISL_1653999 (B/Austria/1350417/2021) was used as the reference sequence. Total 69 Global samples have been taken randomly during the study period (Jan-Dec 2022).
- Four of our Bangladesh samples clustered distinctly with different geographic regions.
- Two of our samples acquired many mutations that caused the high node distance from the vaccine strain.

RSV Phylogeny

- KT992094 (vaccine strain) was used as reference during the analysis.
- Total 21 Global samples have been taken randomly during study period (July-Dec 2022).
- 54 Bangladesh samples was used during this analysis.
- Based on F and G genes phylogeny, sequences were distinctly clustered according to the geographical regions (figure 2).
- Bangladesh sequences have created one cluster for F gene and two distinct clusters for G genes (figure 2).
- Total 115 SNPs have been identified in between all sequences.

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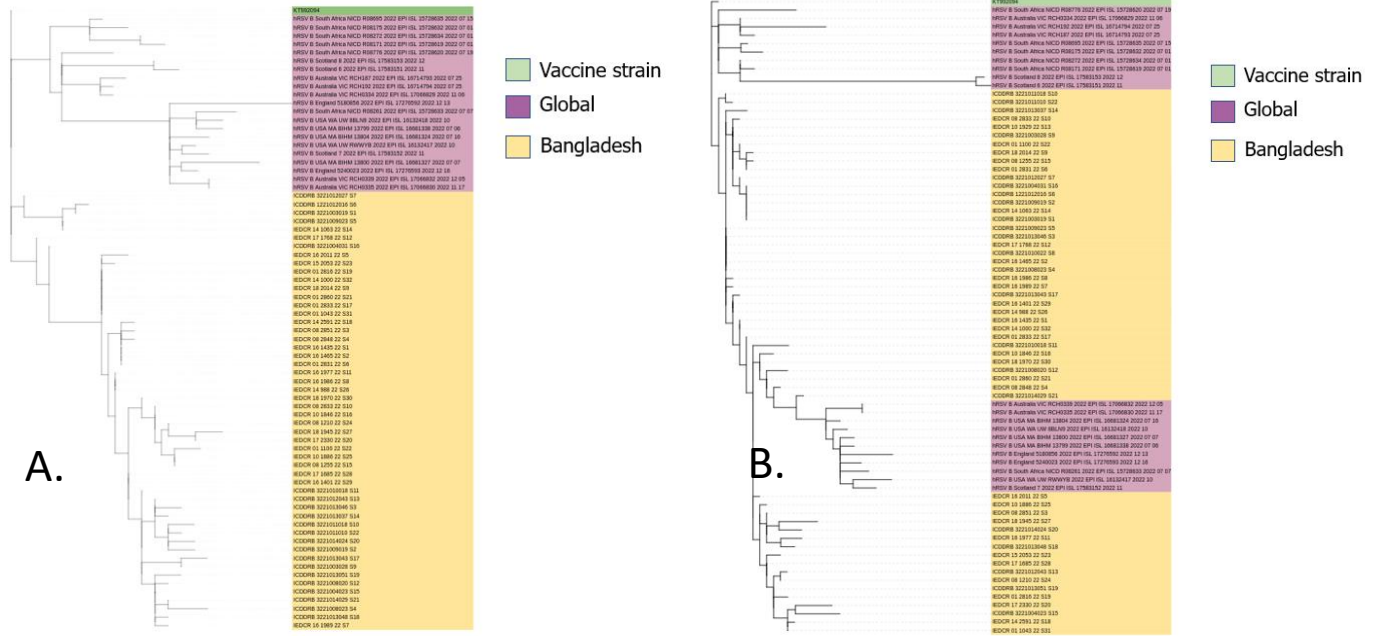
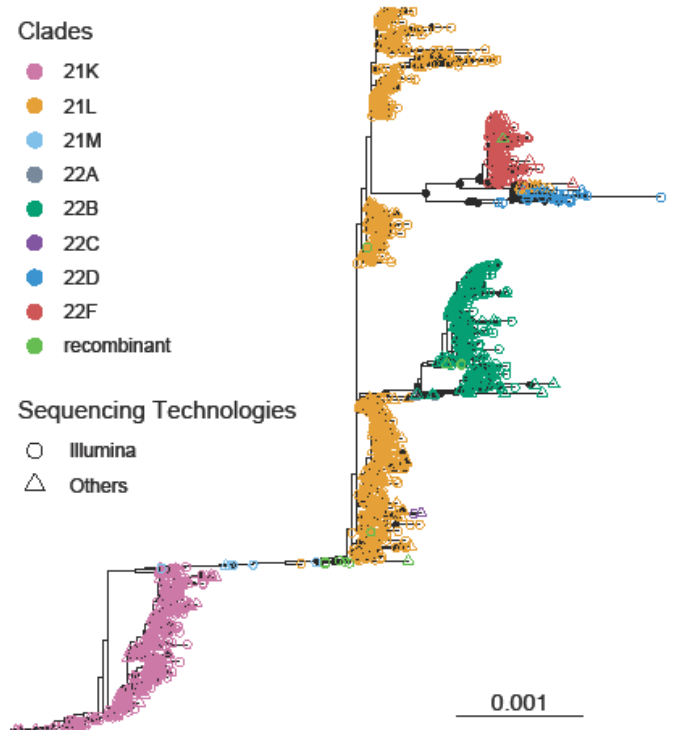


Figure 2: Phylogeny of RSV B viruses from July-December 2022; A. based on F-gene; and B. based on G-gene

SARS-CoV-2 phylogeny

- Represents all Omicron sequences (1711) reported from Bangladesh, dated 2nd of April, 2023.
- It is an unrooted maximum likelihood tree calculated with 1000 bootstraps in IQtree.
- The BA.1, BA.1.1, BA.2, BA.2.10, BA.2.10.1, BE.4.1 and XBB.1 lineages represent more than 80 percent of the total 1711.
- Branch support >75% are represented in black dots.
- The XBBs belong to clade 22F and the BM.1.1.1 belongs to clade 22D



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