Roney and Mohd Aluwi
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LETTER TO THE EDITOR

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Evolution of SARS-CoV-2 from BA.2.86 to JN.1 variations and detection in Bangladesh

Miah Roney[1,2*] and Mohd Fadhlizil Fasihi Mohd Aluwi[1,2]

To the Editor

The SARS-CoV-2 virus caused the COVID-19 pandemic and subsequent worldwide health issues because of the introduction of many subvariants, including Alpha, Beta, Gamma, Delta, Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda, Mu, and Omicron (Sheeza et al. 2024). Every variation has its own unique set of challenges. Moreover, the COVID-19 infection induces several cytokines, including TNF, IL-1β, and IL-6, as well as pro-inflammatory chemokines including CCL20, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL16 (Marlia et al. 2024). On the other hand, the SARS-CoV-2 saltation variation BA.2.86 has drawn attention across the world and was immediately identified as a variety under surveillance upon its appearance. BA.2.86 was first discovered in August 2023, is phylogenetically distinct from the circulating SARS-CoV-2 omicron XBB lineages, which include EG.5.1 and HK.3 (Satapathy et al. 2024). The spike protein of BA.2.86 has more than thirty mutations, it has a higher potential for immune evasion than XBB and BA.2. Furthermore, BA.2.86 showed a very high affinity for ACE2 binding but lacked a considerable growth advantage and humoral immune escape, when compared to current dominant variants like EG.5.1 and HK.3 (Yang et al. 2024). The development of BA.2.86 led to the appearance of JN.1 (BA.2.86.1.1) (Pirola) in late 2023 (Kaku et al. 2024; Satapathy et al. 2024). Therefore, natural immunity could be involved in warding off the illness. Herd immunity may be important if JN.1 spreads rapidly because some individuals have not yet gotten their vaccinations. Public awareness of social distance and other personal hygiene measures should also be reemphasized (Marlia et al. 2024).

A Variant of Interest (VOI) is a term used by the World Health Organization (WHO) to describe a SARS-CoV-2 variant with genetic changes that are known to affect how the virus behaves or its potential impact on human health (WHO 2023). JN.1 has been officially recognized as an autonomous VOI by the World Health Organization (WHO) (Looi 2023) and it is varied from BA2.86 by a mutation in spike protein (Leu455Phe) Which enhances transmissibility and immunological escape capability, is present in JN.1, HK.3, and other flip variations (Kaku et al. 2024). Furthermore, it features 26nt deletion in the 3′-UTR, 31ERS deletion in the N-protein, and 24LPP, 69HV, 145Y, 211N (208N in BA.2), and 483 V (480 V in BA.2) deletions in the spike. A study has identified other JN.1 spike mutations that may also be relevant, including 442N = H249N, 261D = A268D, 352 T = K360T, 400 K = R407K, 442H = P449H, 449W = L456W, 474 K = N485K, 480 K = A488K, and 566 V = A574V (CHAKRABORTY, 2024). Due to a mutation in a single spike protein, this variety could be able to evade immune system recognition. The capacity of neutralizing antibodies to recognize and neutralize the spike protein is hampered by its transformation into a whole new hazard (Roney et al. 2024). Since its initial discovery in September 2023 in the USA, it has quickly spread throughout the country and already makes up a sizable fraction of the circulating variations (CDC 2024).

*Correspondence:
Miah Roney
saroney35@gmail.com
[1] Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Kuantan, Pahang, Malaysia
[2] Centre for Bio-Aromatic Research, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Kuantan, Pahang, Malaysia

According to Forbes 2023 report, six mutations (T24I, V238L, G489S, K1155R, V1227I, and T1228I) have been found in the NSP3 protein of JN.1. NSP3 is a large non-structural protein which is essential for both immune evasion and viral proliferation. The function of the protein may be affected by these alterations, which may also improve the capacity to elude the host immune system or replicate more quickly of the virus. In addition to the spike protein, JN.1 also carries mutations in the nucleocapsid (N) protein, and the N-protein is required for the packaging and assembly of viral genomes. Modifications to this protein might affect the pathogenicity and capacity for replication of the virus. Beyond just the changes in the spike protein (Leu455Phe), mutations in non-spike proteins of JN.1, such NSP3 and N, may also be responsible for the increased transmissibility and immune evasion abilities of variant. These alterations may have an impact on the pathogenesis, replication, and capacity of the virus to elude host immune responses. To completely comprehend the effect of these non-spike protein mutations on the general traits of the JN.1 variation, more investigation is required (Forbes 2023).

JN.1 is phylogenetically different from the other SARS-CoV-2 mutations, and it has been identified as the most immune-evading variant. It has a substantially higher dissociation constant (KD) for the receptor-binding domain (RBD) compared to other variants, indicating weaker binding to the ACE2 receptor (Satapathy et al. 2024). Furthermore, L455S mutation likely contributes to the higher KD value and reduced ACE2 binding affinity observed for JN.1. Despite the weaker RBD-ACE2 interaction, the JN.1 variant has demonstrated significantly higher infectivity in pseudovirus experiments. This suggests that the enhanced infectivity of JN.1 is not solely dependent on ACE2 binding and may involve other factors, such as immune evasion or altered viral entry mechanisms (Yang et al. 2024). It is now the type with the fastest rate of development in countries including the US, France, Singapore, Canada, the UK, and Sweden, with documentation from more than 41 countries (Hemo and Islam 2024). Notably, JN.1 is primarily responsible for the increase in COVID-19 cases in Singapore, and its emergence in India has given rise to grave worries (Satapathy et al. 2024). Figure 1 provides an explanation of the genetic basis for how viruses undergo alterations in their genomes throughout time. These changes can have a substantial effect on the traits of the viruses as well as how they interact with humans and other host animals. Comprehending these systems is essential to formulating efficacious tactics against viral infections.

The COVID-19 pandemic had a devastating effect on Bangladesh and the first confirmed case was identified on March 8, 2020 (Islam et al. 2020). Moreover, the cases of all districts were reported by early of May, and the cumulative number of cases in Bangladesh surpassed that of China on June 13, (Rahman et al. 2021). Bangladesh had around 2,049,377 cases and 29,493 fatalities as of April 13, 2024 (Worldometers 2024). The government of Bangladesh launched a national COVID-19 vaccination campaign on February 7, 2021, using 7 million doses of the vaccine made by Oxford and AstraZeneca. Several more vaccines, including those made by Johnson & Johnson, Sinovac, Sinopharm, Pfizer-BioNTech, and Sputnik V, have now been approved for use by the government. As of February 26, 2024, 83.07% of the population in Bangladesh had received all recommended doses of the COVID-19 vaccination, and over 90% of the population had received at least one dose (Nazmunnahar et al. 2023).

The Institute of Epidemiology, Disease Control and Research (IEDCR) reported that JN.1 was found in samples from five individuals in Bangladesh, both from Dhaka and other locations, who had no history of recent travel (AA.com 2024; TheDailySTar 2024; TBNews 2024; DhakaTribune 2024; DBNews 2024). The health officials in Bangladesh advise the public not to panic because current vaccines will protect against life-threatening and severe illness caused by JN.1 and other COVID-19 variants that are circulating (TBNews 2024; DBNews 2024). The rise in morbidity prompted the Directorate General of Health Services (DGHS) to issue a notification directing the authorities to resume the first, second and booster doses of the COVID-19 vaccine program (TBNews 2024).

Nasopharyngeal swab specimens were requested from individuals across the country displaying COVID-19 symptoms as part of the regular SARS-CoV-2 diagnostic screening for the Respiratory Virus Genomic Surveillance in Bangladesh (Table 1). Real-time reverse transcription polymerase chain reaction (rRT-PCR) was utilized to target the new coronavirus (2019-nCoV) following the isolation of viral RNA using the QIAamp viral RNA micro kit (Qiagen, Germany). This was done using a nucleic acid test kit from Sansure Biotech in China. Eighteen and fourteen positive samples from routine surveillance with a cycle threshold (C_T) value of less than 27 for whole genome sequencing, in accordance with the amplicon-based SARS-CoV-2 sequencing protocol created by ARTIC Network (Tahsin et al. 2024). ARTIC v4.1 primer panels were used in the sequencing library preparation process. This method included multiplex PCR using Q5 high-fidelity DNA polymerase (New England Biolabs, USA) and the synthesis of viral cDNA using the LunaScript RT SuperMix Kit (New England Biolabs, USA). NEBNext Ultra II end repair/dA-tailing module (New England Biolabs, USA) was used to handle amplicons. Amplicons were combined into a single

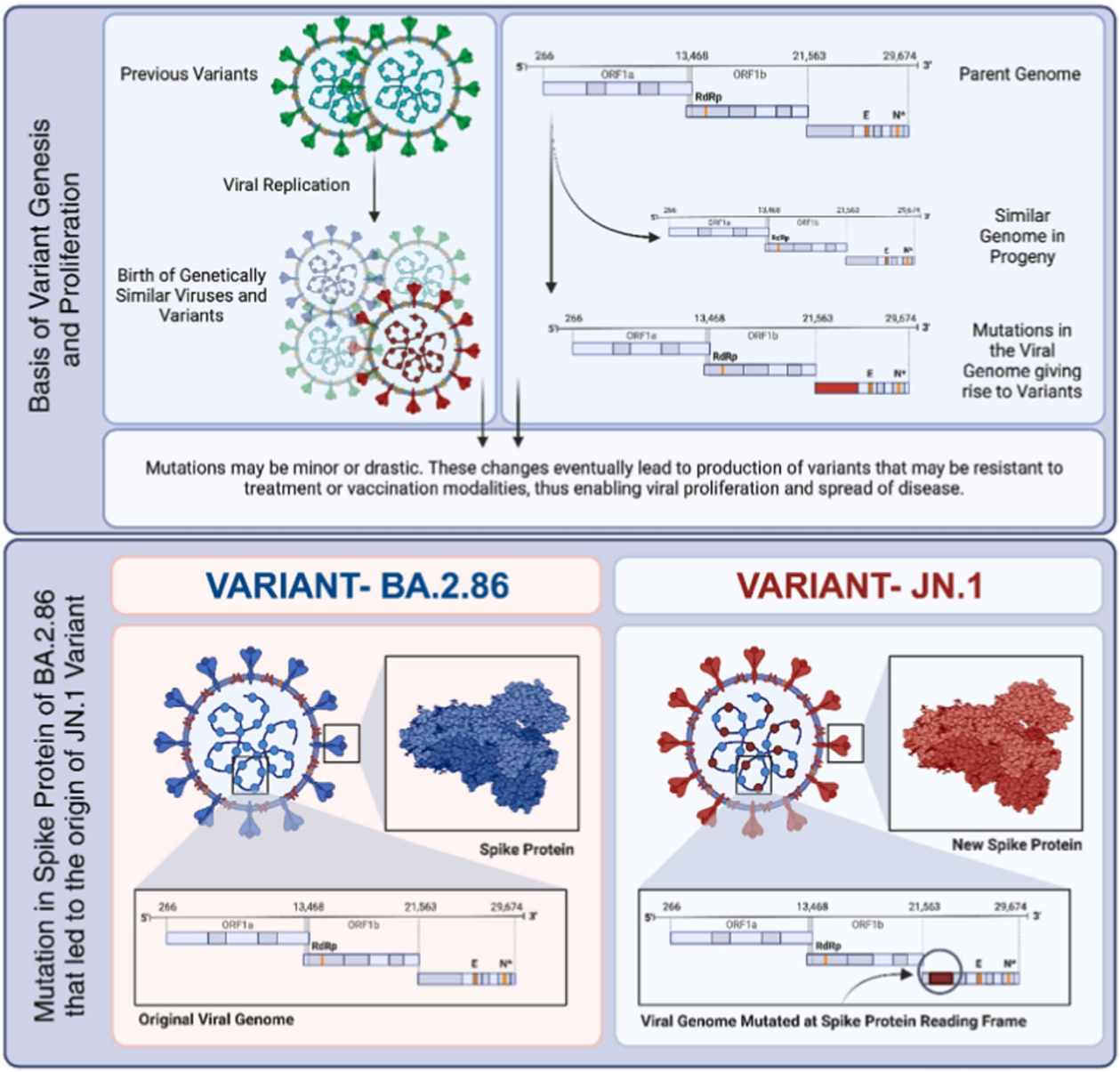

Fig. 1 Viral mutation mechanisms and the appearance of novel variations (Satapathy et al. 2024)

reaction tube after native barcode (EXP-NBD104 and EXP-NBD114) ligation (Oxford Nanopore Technologies, UK) using blunt/TA DNA ligase (New England Biolabs, USA). The completed DNA library was loaded into a FLO-MIN106D flow cell (R9.4.1) after adaptor ligation with NEBNext Quick T4 DNA ligase (New England Biolabs, USA). For a minimum of six hours, sequencing was done using a MinION Mk1C sequencing apparatus using the factory quality score filter (Q-score = 8). Using the Fast base-calling model on MinKNOW v22.12.5 (bionic), demultiplexing and base calling of raw readings were done. The readings that were processed were assembled using the ARTIC guppyplex code script. On ARTIC EPI2ME desktop agent v3.7.3, consensus sequences in fasta format were created using Medaka v1.4 (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). Moreover, the phylogenetic tree was depicted in Fig. 2 for forty-three (43) viral genomes including research strains.

JN.1 developed an extra mutation in spike protein (L455S) and six mutations (T24I, V238L, G489S, K1155R, V1227I, and T1228I) in NP3 non-spike protein to its parent BA.2.86 variant as compared to the reference Wuhan Hu-1 SARS-CoV-2 genome (GenBank accession

Table 1 Information about the Omicron JN.1 sub-lineage of Bangladesh SARS-CoV-2

Sample ID	Date (DD/MM/YR)	Age	Sex	Location	GenBank accession no	GISAID accession no	SRA[b] accession no	Number of raw reads	Sequence length (bp)	Read length N50	Reads depth	GC (Guanine-cytosine) content (%)
IEDCR9848794	19/12/2023	58	M	Dhaka	PP301387	EPI_ISL_18787957	SRR27909868	90,263	29,721	509	344x	39.26
IEDCROIS23050	21/12/2023	12	M	Gazipur	PP301398	EPI_ISL_18851606	SRR27909879	69,016	29,721	514	356x	39.65
SIRS_3231203017	22/12/2023	1	F	Kishoreganj	PP231959	EPI_ISL_18839342	SRR27797267	175,510	29,709	485	3060.75	37.91
IEDCR1802057	30/12/2023	12	M	Gazipur	PP301386	EPI_ISL_18787955	SRR27909867	430,999	29,721	515	449x	39.71
IEDCR1802649	30/12/2023	18	M	Gazipur	PP301388	EPI_ISL_18787958	SRR27909869	257,355	29,721	503	443x	39.14
SIRS_2240113003	3/1/2024	48	M	Chattogram	PP231962	EPI_ISL_18839347	SRR27797253	147,422	29,709	473	2537.17	37.89
SIRS_2240113004	3/1/2024	40	F	Chattogram	PP231965	EPI_ISL_18839348	SRR27797252	136,389	29,709	474	2328.47	37.88
SIRS_2240103007	6/1/2024	37	M	Kishoreganj	PP231961	EPI_ISL_18839343	SRR27797257	141,683	29,709	473	2431.25	37.91
SIRS_3240111007	6/1/2024	2 m, 15 d	M	Chattogram	PP231968	EPI_ISL_18839352	SRR27797265	64,018	29,709	473	1088.62	37.87
IEDCR9837394	8/1/2024	56	M	Dhaka	PP301385	EPI_ISL_18787954	SRR27909866	314,310	29,721	511	452x	39.67
SIRS_2240111009	8/1/2024	5	F	Sylhet	PP231964	EPI_ISL_18839346	SRR27797254	170,325	29,709	476	2947.39	37.87
SIRS_2240113010	8/1/2024	4y 1 m	F	Chattogram	PP231967	EPI_ISL_18839349	SRR27797251	105,880	29,709	477	1837.19	37.88
SIRS_3240113010	8/1/2024	1 m 8d	F	Chattogram	PP231970	EPI_ISL_18839353	SRR27797264	98,371	29,709	475	1698.62	37.88
SIRS_1240108008	9/1/2024	7	F	Comilla	PP231960	EPI_ISL_18839344	SRR27797256	117,614	29,709	477	2011.9	37.88
SIRS_2240114009	9/1/2024	18	M	Dinajpur	PP231969	EPI_ISL_18839351	SRR27797266	134,461	29,709	470	2286.05	37.88
SIRS_1240110006	10/1/2024	38	F	Jashore	PP231963	EPI_ISL_18839345	SRR27797255	168,461	29,709	472	2887.35	37.84
SIRS_3240113013	10/1/2024	1y5m	F	Chattogram	PP231971	EPI_ISL_18839354	SRR27797263	128,548	29,709	474	2204.56	37.88
SIRS_2240113014	10/1/2024	45	F	Chattogram	PP231966	EPI_ISL_18839350	SRR27797250	167,464	29,709	475	2862.94	37.89
SIRS_3240113015	11/1/2024	4m5d	M	Chattogram	PP231973	EPI_ISL_18839355	SRR27797262	132,742	29,709	476	2305.08	37.87
IEDCR9850602	12/1/2024	52	F	Dhaka	PP301389	EPI_ISL_18851597	SRR27909870	302,710	29,721	515	452x	39.55
IEDCR9850683	12/1/2024	57	M	Dhaka	PP301390	EPI_ISL_18851598	SRR27909871	126,879	29,721	516	317x	41.25
IEDCR2201113	13/1/2024	50	F	Dhaka	PP301391	EPI_ISL_18851599	SRR27909872	358,693	29,721	507	454x	40.69
IEDCR1625069	14/1/2024	49	F	Dhaka	PP301392	EPI_ISL_18851600	SRR27909873	128,654	29,721	515	371x	42.58
IEDCR1625075	14/1/2024	64	M	Dhaka	PP301393	EPI_ISL_18851601	SRR27909874	45,187	29,721	516	345x	40.25
IEDCR1450955	15/1/2024	24	F	Dhaka	PP301394	EPI_ISL_18851602	SRR27909875	111,939	29,721	501	420x	39.12
SIRS_2240111020	15/1/2024	1y3m	F	Sylhet	PP231975	EPI_ISL_18839357	SRR27797260	44,085	29,709	467	692.74	37.83
IEDCR9848717	16/1/2024	65	F	Chandpur	PP301395	EPI_ISL_18851603	SRR27909876	198,273	29,721	517	377x	39.56
IEDCR1450962	16/1/2024	62	F	Dhaka	PP301396	EPI_ISL_18851604	SRR27909877	70,507	29,721	515	381x	40.14
SIRS_3240113019	1/16/2024	3m15d	M	Chattogram	PP231976	EPI_ISL_18839359	SRR27797258	81,437	29,709	470	1375.79	37.88
SIRS_1240108019	1/20/2024	15	F	Comilla	PP231972	EPI_ISL_18839356	SRR27797261	35,228	29,709	468	547.55	37.75
SIRS_3240113025	1/20/2024	8m26d	M	Chattogram	PP231974	EPI_ISL_18839358	SRR27797259	105,253	29,709	473	1806.47	37.88
IEDCROIS23049	21/1/2024	56	M	Dhaka	PP301397	EPI_ISL_18851605	SRR27909878	72,169	29,721	510	390x	41.20

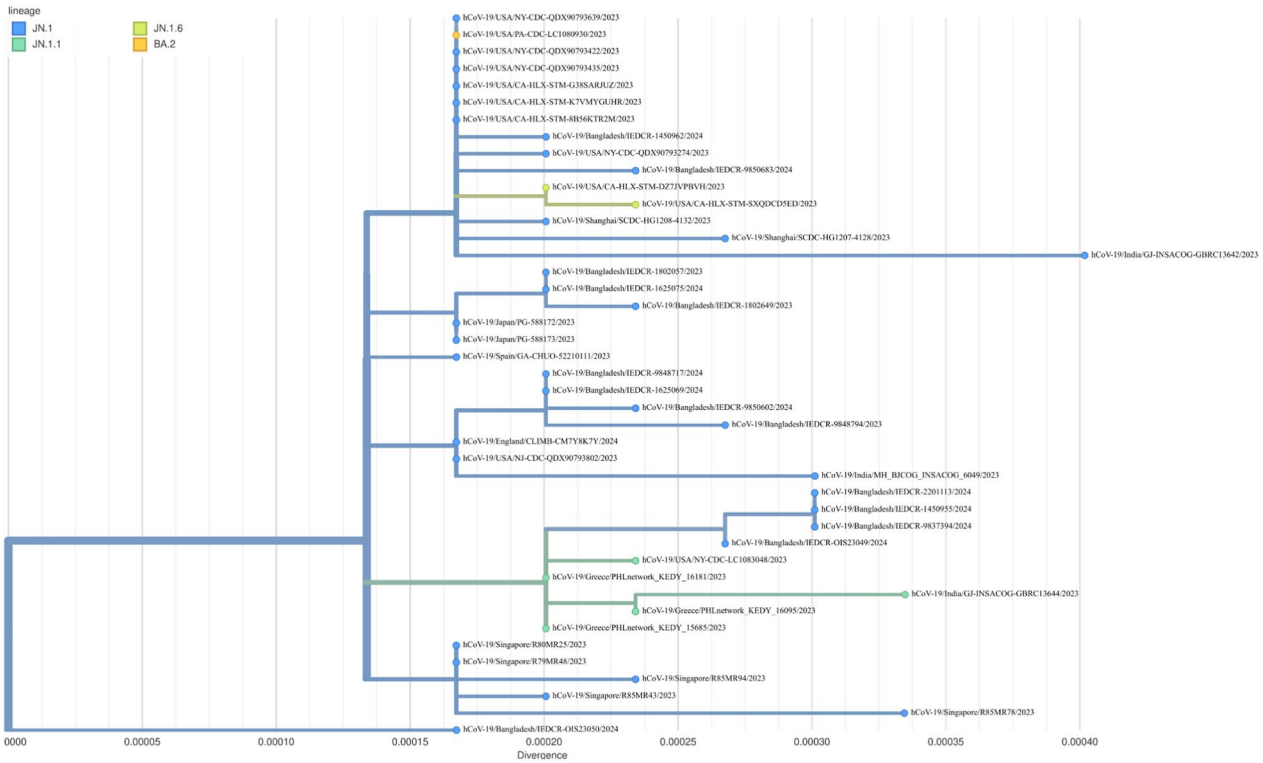

Fig. 2 Molecular evolution of coding-complete Genomes of the Omicron sub-lineage JN.1 of SARS-CoV-2. Forty-three (43) viral genomes in all, including research strains, are shown. Augur was used to construct and annotate the tree. The color of a tip is determined by ancestry. Divergence indicates the length of the branch (Jony et al. 2024)

NC_045512.2). As a result, JN.1 is presently responsible for the greatest incidence of SARS-CoV-2 infection globally (Kaku et al. 2024; Khan et al. 2024; Marlia et al. 2024). Furthermore, BA.2.86 shows significant evolutionary drift and more than 30 spike-glycoprotein changes compared to its parent Omicron BA.2, suggesting a great potential for immune evasion (Tahsin et al. 2024). The descending JN.1.4 and JN.1.11, which are derived from JN.1, have distinct differences in the amino acids of their ORF1a-protein (T1701I) and spike protein (V1104L), respectively. The rapid spread of these mutations across Bangladesh may suggest that they were carried there by unaffiliated parties. Therefore, continuous genomic monitoring is required to monitor newly emerging SARS-CoV-2 strains in order to facilitate effective public health planning and implementation.

The spike protein of JN.1 carries about 30 mutations, including a new mutation (L455S), which makes it harder for antibodies to attach to the virus and stop infection (Khan et al. 2024; Marlia et al. 2024). This could lead to increased immune evasion compared to previous variants (Khan et al. 2024). Bangladesh faces several challenges that could exacerbate the spread of JN.1. The country has a high population density and an inadequate water surveillance system, raising the possibility of rapid transmission. The medical infrastructure and shortage of healthcare professionals of Bangladesh may also struggle to handle a severe outbreak (Marlia et al. 2024). Additionally, public awareness about COVID-19 prevention measures has decreased since the WHO ended the public health emergency declaration in May 2023. Around 10 million individuals in Bangladesh are still without their second vaccine dose, leaving them vulnerable. The vaccines used previously may also provide limited protection against the mutated JN.1 strain. To mitigate the risks, Bangladesh should focus on improving hospital facilities, conducting investigations on the continued validity of previous pandemic response measures, and promoting adjuvant antiviral and vaccination therapies. Raising public awareness about personal hygiene and social distancing will also be crucial to combat the potential spread of the JN.1 variant (Marlia et al. 2024). The following are the key recommendations for Bangladeshi public health authorities about the COVID-19 JN.1 variant:

Enhance surveillance and testing

1. Increase COVID-19 testing capacity, especially in high-risk areas, to rapidly identify any JN.1 cases.
2. Conduct genomic sequencing on a representative sample of positive cases to track the spread and evolution of the JN.1 variant.
3. Strengthen disease surveillance systems to quickly detect any significant increases in COVID-19 transmission.

Strengthen healthcare preparedness

1. Ensure hospitals and healthcare facilities have sufficient medical supplies, equipment, and staffing to handle a potential surge in COVID-19 patients.
2. Implement contingency plans to rapidly scale up healthcare capacity if needed, such as setting up additional treatment centers.
3. Prioritize vaccination efforts, especially for high-risk populations, to reduce severe illness and hospitalizations.

Enhance public health messaging

1. Communicate clearly with the public about the JN.1 variant, its potential risks, and the importance of continued preventive measures.
2. Encourage adherence to COVID-19 safety protocols such as masking, distancing, and testing among the general population.

Furthermore, the government of Bangladesh has implemented many strategies to curb the proliferation of the JN.1 COVID-19 variant:

1. In response to an upsurge in diseases, the Directorate General of Health Services (DGHS) has sent a notification asking authorities to begin the COVID-19 vaccination programme for first, second, and booster doses (TBNews 2024; AA.com 2024).
2. Health officials have advised the public not to worry since JN.1 and other circulating COVID-19 variations will not cause serious illness or death if current vaccinations are administered (TBNews 2024; TheDailyStar 2024).
3. Five people from Dhaka and other areas who had no history of recent travel were tested for JN.1, and the Institute of Epidemiology, Disease Control and Research (IEDCR) verified this finding (Marlia et al. 2024; TBNews 2024; TheDailyStar 2024).
4. Despite being less severe, JN.1 has been designated as a variation of interest by WHO because of its potential for rapid dissemination (DhakaTribune 2024; TBNews 2024).
5. Nearly 30,000 people have perished and over 2 million out of 170 million people have contracted COVID-19 since the virus's first cases were verified in Bangladesh in March 2020 (DhakaTribune 2024; AA.com 2024).

In Bangladesh, where COVID-19 was first identified in March 2020, about 2 million of the country's 170 million inhabitants have fallen ill. Approximately 30,000 people have died from the virus. Overall, the detection of JN.1 in Bangladesh highlights the need for continued vigilance and public awareness to combat the spread of the virus and ensure the effectiveness of vaccination efforts.

Abbreviations

CDC	The Centers for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
DGHS	The Directorate General of Health Services
IEDCR	The Institute of Epidemiology, Disease Control and Research
RBD	Receptor-binding Domain
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
UK	United Kingdom
US	United States
VOI	Variant of interest
WHO	World Health Organization

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Author contributions
MR conceptualized, performed the data curation, analysis, write the original draft. MFFMA reviewed and edited. All authors have read and approved the manuscript.

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Not relevant.

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