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Research paper

Assessment of basic reproduction number (R_0), spatial and temporal epidemiological determinants, and genetic characterization of SARS-CoV-2 in Bangladesh

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ABSTRACT

Epidemiological and molecular characterization of SARS-CoV-2 is essential for identifying the source of the virus and for effective control of the spread of local strains. We estimated case fatality rate, cumulative recovery number, basic reproduction number (R_0) and future incidence of COVID-19 in Bangladesh. We illustrated the spatial distribution of cases throughout the country. We performed phylogenetic and mutation analysis of SARS-CoV-2 sequences from Bangladesh. As of July 31, 2020, Bangladesh had a case fatality rate of 1.32%. The cases were initially clustered in Dhaka and its surrounding districts in March but spreads throughout the country over time. The R_0 calculated as 1.173 in Exponential Growth method. For the projection, a 20% change in R_0 with subsequent infection trend has been calculated. The genomic analysis of 292 Bangladeshi SARS-CoV-2 strains suggests diverse genomic clades L, O, S, G, GH, where predominant circulating clade was GR (83.9%; 245/292). The GR clades' phylogenetic analysis revealed distinct clusters (I to XIII) with intra-clade variations. The mutation analysis revealed 1634 mutations where 94.6% and 5.4% were non-synonymous and unique mutation, respectively. The Spike, Nucleocapsid, NSP2, and RdRP showed substantially high mutation but no mutation was recorded in NSP9 and NSP11 protein. In spike (S) protein, 355 predominant mutations were recorded, highest in D614G. Alternatively, I120F in NSP2 protein, R203K and G204R in nucleocapsid protein, and P323L in RdRp were more recurrent. The Bangladeshi genomes belonged to phylogenetic lineages A, B, B.1, B.1.1, B.1.1.23, B.1.1.25, B.1.113, and B.1.36, among which 50.0% sequences owned by the pangolin lineage B.1.1.25. The study illustrates the spatial distribution of SARS-CoV-2, and molecular epidemiology of Bangladeshi isolates. We recommend continuous monitoring of R_0 and genomic surveillance to understand the transmission dynamics and detect new variants of SARS-CoV-2 for proper control of the current pandemic and evaluate the effectiveness of vaccination globally.

1. Introduction

Novel coronavirus disease 2019 (COVID-19) emerged in the form of pneumonia cases with unknown etiology in Wuhan, China, in December 2019 and named as such by World Health Organization (WHO) in January 2020 (Chan et al., 2020; Guo et al., 2020). More than 18.0 million people infected and 0.69 million died by COVID-19 . The COVID-19 test can detect only clinically diseased individual, that might underestimate the actual number as the patient can transmit it during the incubation period (Singhal, 2020). The case fatality rate of COVID-19 was 2.3% in China (Novel, 2020), 2–3% in India (Singhal, 2020), and 7.2% in Italy (Onder et al., 2020), whereas the recovery rate was 46% in European countries (Karadag, 2020). Moreover, men were more affected than women in China (Yang et al., 2020) and some other countries of

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South Asia (Islam et al., 2020).

To reduce the viral spreading, various measures like social distancing, lockdown, closing of public institutes, travel ban, and border closing were taken throughout the world. Though Bangladesh, a densely populated developing country in South Asia (SA), took screening measure at airports, meanwhile the infection spread exponentially worldwide (Repici et al., 2020). In Bangladesh, 237,667 people have been infected, and 3118 have been died until July 31, 2020. Bangladesh's government also took some other control measures, but the evidence of success to reduce viral transmission is still obscure.

To understand any infectious diseases' transmission dynamics, estimating the basic reproduction number (R_0) is crucial. In the context of the current COVID-19 pandemic, the R_0 has been recognized as a critical parameter to characterize epidemic risk and predict the spread of COVID-19 (Anderson et al., 2020). R_0 is a measure to quantify the probability of new cases that result from an infected individual's active contact. If $R_0 < 1$, on average, an infectious individual infects less than one person, and spreading is expected to decrease. R_0 is determined not only by the inherent infectiousness of a pathogen but also by environmental conditions, host contact behaviors, and other factors that influence transmission. Therefore, it is unique for a specific population and region. According to WHO, R_0 of COVID-19 ranged from 1.4 to 2.5 (Viceconte and Petrosillo, 2020). An earlier study estimated the reproduction number of COVID-19 which varied between 1.4 and 6.49 across the world (Liu et al., 2020a). The R_0 is also computed in specific subpopulations, such as individuals aboard the Diamond Princess cruise ship and health care settings (Temime et al., 2020; Zhang et al., 2020). However, to the author's knowledge, there is no literature on the R_0 of COVID-19 in Bangladesh.

As the value of R_0 of COVID-19 found to be over 1 in almost all previous studies, we can understand that the disease is not going to subside easily in near future. Every time the virus transmits from one host to another, there are some changes in viral structure for better adaption. As there are community transmission in Bangladesh, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been changed a lot since its first detection on March 8, 2020. Because, the most important viral evolution mechanisms in nature being reported to be nucleotide substitution (Lauring and Andino, 2010). The rapid spread of the virus and its varying infectiousness and case fatality rate throughout the world raise questions about mutation in the viral genome. Analysis of 320 whole-genome sequences showed 483 unique variations among the genomes of SARS-CoV-2 (Lokman et al., 2020). SARS-CoV-2 strains have circulated under the two major lineages (Xiaolu et al., 2020). The Global Initiative on Sharing Avian Influenza Data (GISAID) categorized SARS-CoV-2 among six high-level phylogenetic groupings from the early split of S and L, to the further evolution of L into V and G and later of G into GH and GR, based on marker mutations (Shu and McCauley, 2017). Finally, Next strain's SARS-CoV-2 global genomic epidemiology analysis differentiated ten major clades for this virus (https://nextstrain.org/sars-cov-2/). The first full genome sequence of Bangladesh, a sub-strain of SARS-CoV-2, belongs to the A2a clade under G major clade and originated from the United Kingdom (UK) or other European countries (Ul Alam et al., 2020). In another earlier study, the genomic analysis of 95 full genome showed that the virus strains had 99.99% homology at both nucleotide and amino acid levels with a mutation rate of 30.53% and 29.47% respectively (Chan et al., 2020). Comparing the genetic diversity and trend of mutation of the Bangladeshi strains with other countries' strains are critical to know the introduction of new variant and effectiveness of vaccination in Bangladesh.

To address the knowledge gaps, the present study aimed to describe the epidemiological characteristics, spatial distribution, R_0, the genomic characteristics and mutation points of circulating strains of SARS-CoV-2 in Bangladesh.

2. Materials and methods

2.1. Data source/data extraction

We collected relevant data from the government officials (IEDCR, 2020), national web portal, which publicly reports COVID-19 related information (https://www.corona.gov.bd/). Since March 8, 2020, the Government of Bangladesh has been reporting the daily incidence of cases through media briefing. The regular case database is open for everyone to know the existing situation. We collected incidence data for 145 days (March 8 to July 31, 2020). As WHO and worldometer coronavirus databases are also being updated on a real-time basis for every parameter, we included data from these two websites from the 2nd week of March to the last week of July 2020 (WHO, 2020; Worldometers, 2020).

2.2. Epidemiological characteristics of COVID-19 in Bangladesh

We graphically illustrated the daily test frequency, case fatality rate, and the cumulative number of recovered. We estimated the daily case fatality rate as the proportion of the number of deaths over the number of cases per day and expressed as percentage. We estimated the recovery rate as the number of recovered over the number of infected and expressed as percentage. We calculated the age and sex specific case and death count. We also graphically showed the date wise change in the trend of infection and death count, control measures taken by the government, and different community level activities occurred during the study period.

2.3. Spatial distribution of COVID-19 in Bangladesh

We accumulated district wise monthly COVID-19 case data and collated into Microsoft excel spreadsheet. The datasheet was appended with the attribute table of the digitized shapefile of Bangladesh (Islam et al., 2021). We visualized the incidence of COVID-19 for each district by month using graduated color from the "symbology" menu in ArcGIS 10.4.1 (Sayeed et al., 2020).

2.4. R_0, comparative epidemic curve of COVID-19 and sensitivity analysis

An infection's generation time, the lag between primary and secondary cases, can be estimated by following up an infector to an infected. A mean generation time of 7.5 days with standard deviation of 3.4 days and "gamma" distribution were considered for R_0 estimation (Liu et al., 2020b). We used the R_0 package with the help of the "Modern Applied Statistics with S" library (https://CRAN.R-project.org/package=R0) in R Studio v1.2.5042. Though the calculation of R_0 at different points of the epidemic is usual; hence in this study, we computed the R_0 after around 12 weeks of the initially announced outbreak.

Firstly, we used the exponential growth (EG) method. We plotted the EG rate using the method described by Wallinga and Lipsitch (2007). The EG rate, denoted by r, is the ratio of observing new cases per unit change of time where incidence data are integer and Poisson regression is directed for the estimation of the parameter as described by Boelle et al. (2009) and Hens et al. (2011). The basic reproduction number (R_0) is computed as $R_0 = 1/M^{(-r)}$, where M is the moment generating function estimated from the "gamma" distribution of moment generation time. EG is practical at the initial stage of an uncontrolled outbreak (de Picoli Junior et al., 2011; Hunt, 2014), which has been genuinely identified for COVID-19 in different countries (Courtemanche et al., 2020; Liu et al., 2020c; Remuzzi and Remuzzi, 2020; Yuan et al., 2020). A weekly trend in change of R_0 using EG methods has been illustrated.

Secondly, we calculated R_0 using Maximum likelihood (ML) estimation assuming that the number of secondary cases generated from a primary case following a Poisson distribution where the expected value

of R_0 is estimated by maximizing the log-likelihood (LL) (Forsberg White and Pagano, 2008). We used the following equation:

$$\mathrm{LL}(R_0) = \sum_{t=1}^{T} log\left(\frac{e^{-\mu t}\mu_t^{N_t}}{Nt}\right) \text{ where } \mu_t = \mathrm{R}_0 \sum_{i=1}^{t} N_t - {}_i w_i$$

Here, N_t is the number of cases for each time and w is the generation time distribution.

Thirdly, we used Sequential Bayesian (SB) method which relies on the SIR model-based statistical formula where incidence at $t+1$, $N(t+1)$ followed Poisson distribution with the mean value of $N(t)e^{(\gamma(R_0-1))}$ and $\frac{1}{\gamma}$ is the mean infectious period (Bettencourt and Ribeiro, 2008). The basic reproduction number (R_0) distribution framework for SB method is as follows:

$$P(R_0|N_0 \ldots . N_{t+1}) = \frac{P(N_{t+1}|R_0, N_0, \ldots, N_t)\ P(R_0|N_0, \ldots, N_t)}{P(N_0, \ldots, N_{t+1})}$$

Here, prior distribution of R_0 depends on the posterior distribution of R_0 of the previous day.

Finally, we calculated the time-dependent (TD) R_0 using the primary statistical formula described by Wallinga and Teunis (2004). We used 4 different methods in the statistical tools of R (Obadia et al., 2012) to calculate the basic reproduction number of COVID-19 in Bangladesh. We visualized the actual epidemic curve and model based epidemic curve using line graph. The reliability of each of the 4 model is highly affected by the data composition. If the data is available on a smaller time scale compared to the mean generation time, all methods are unbiased. But if there are zero values within the observations, the SB method fails to estimate the R_0. However, by aggregation of the data in periods up to twice of the mean generation time, only the EG method remains unbiased (Nishiura et al., 2010). We estimated R_0 using all four methods to understand the variation in R_0 values due to different estimation method (Obadia et al., 2012). However, in our dataset, there were some zero values indicating the absence of new cases at some point in time. For this reason, EG method was best fitted and we calculated the sensitivity of EG method based on changing the generation time distribution described by Obadia et al. (2012).

2.5. Future daily incidence prediction of COVID-19 through simulation

We performed the epidemic trajectories using the "projections" package with the aid of the "discrete" and "incidence" libraries in R studio (Jombart et al., 2017). We performed 1000 times simulation considering a serial interval of 7.5 days (Liu et al., 2020b) and used Poisson distribution function corresponding to R_0 values estimated by EG methods Wallinga and Lipsitch (2007). We utilized existing daily incidence numbers to predict the possible incidence and cumulative incidence for the next 60 days. We predicted a change of 20% in R_0 parameters on future incidence (Zhang et al., 2020).

2.6. Molecular characterization of SARS-CoV-2 in Bangladesh

2.6.1. Phylogenetic analysis

We retrieved 292 complete or near-complete genome sequences of SARS-CoV-2 from Bangladesh, deposited to The Global Initiative on Sharing Avian Influenza Data (GISAID) (Shu and McCauley, 2017) as of July 31, 2020.

We performed sequence alignment using a multiple sequence alignment program (MAFFT) command line (https://mafft.cbrc.jp/alignment/software/). To remove the ambiguity and low-quality sequences we checked aligned sequences with Molecular Evolutionary Genetics Analysis, across Computing Platforms (MEGA X) (Kumar et al., 2018). We selected all S, O, L, G, and GH clades and representative GR clades for the phylogenetic analysis. On the basis of complete information and sequence quality, we finally selected 124 sequences from 292 retrieved genome sequences. We selected the sequences based on random sample collection dates, age, gender, and the clade diversity until July 31, 2020. We provided the detail information related to accession ID, collection dates, gender, and locations of this subset sequences in Supporting Information S1 (Supplement S1). To create overall phylogenetic tree, we retrieved highly similar 61 identical (over 99.0%) global sequences based on the basic local alignment search tool (BLAST) hit. In case of GR clade phylogenetic tree, we used 56 highly similar sequences (identity over 99.0%) from other countries. We excluded genome without patient's metadata, partial genomes or genomes including gaps and sequences containing with legionary characters (N, R, X, and Y) other than A, T, G, and C from our analysis. We analyzed the sequences using the strain China/WHU01/2020/EPI_ISL_406716 as a reference genome for phylogenetic and mutation analysis. We developed a phylogenetic tree from the aligned sequences using the maximum likelihood methods to determine the genetic resemblance among isolates from Bangladesh and worldwide. We validated the tree structure by running the analysis on 1000 bootstraps replication datasets.

2.6.2. Mutation analysis in SARS-CoV-2 genome

We selected 292 Bangladeshi genome sequences for intensive analysis of unique and co-evolving mutations. We provided the detail mutation of the genomic segment of each virus in Supporting Information S2 (Supplement S2). We determined the frequencies of recurrent non-synonymous mutations and unique mutations with their specific amino acid level in the genomes using 'CoV server enabled by GISAID' in GISAID's EpiCoV database (https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/). We used the term unique mutation to elaborate those novel mutation which is detected only in Bangladeshi SARS-CoV-2 virus sequence in GISAID and not yet reported in other countries sequences during the time of the analysis. The server analyzed Bangladeshi SARS-CoV-2 sequences alongside all available genomic sequences including the Wuhan reference sequence deposited on GISAID until July 31, 2020.

2.6.3. Clade and lineage diversity of SARS-CoV-2

We determined the clade and lineage of SARS-CoV-2 using 'CoV server enabled by GISAID' in GISAID's EpiCoV database. We stored the data based on the sampling location and demography of the patients. We visualized the site-specific clade distribution in percentage using a graph in the area map produced in ArcGIS software. However, we presented the gender-based distribution of both clades and lineage using a bar diagram. We also presented the clade-specific distribution of lineage graphically.

3. Results

3.1. Epidemiological characteristics of COVID-19 in Bangladesh

Since the first case detection on March 8, Bangladesh has increased the daily test capacity, which reached around 18,498 on July 31 from approximately 84 different test points across the country (https://www.dghs.gov.bd/). The daily positive cases reached about 4019 and a cumulative number of 237,667 with 3118 death on July 31. Gradual improvement of test capacity presented in Fig. 1/A. On July 31, 2020, we calculated the overall case fatality rate as 1.32% of confirmed cases. The overall recovery rate has been estimated to be 56.85%. We illustrated the daily cumulative recovery number with the daily case fatality rate in Fig. 1/B, where there was an increased mortality trend initially, which was declined further with the increase of recovery number. Bangladesh detected the highest number of cases of 28.0% and 27.0% among people of 21–30 years and 31–40 years of age, respectively whereas the highest percentage of death (39.0%) was in >60 years of age group. Both infection and case fatality rate were higher among males than that of females (Table 1).

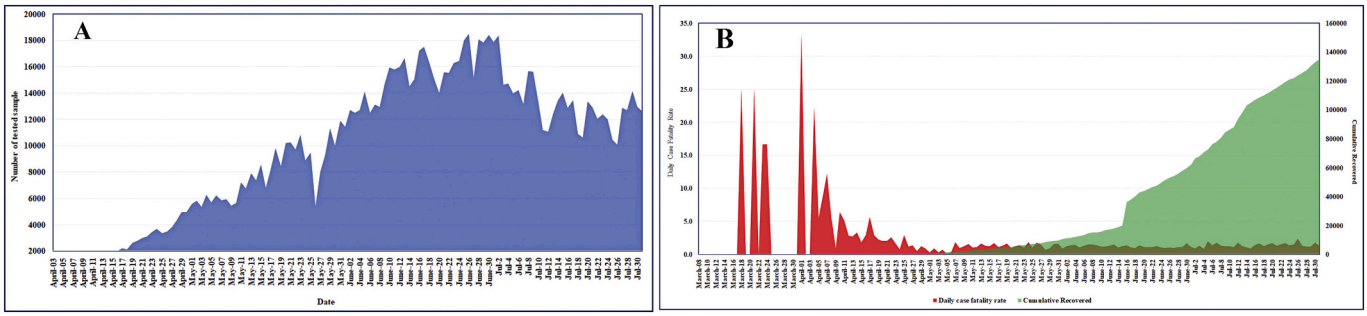

Fig. 1. Day-wise COVID-19 interface in Bangladesh. A) Blue colored area represents the frequency of RT-PCR based test performed per day B) Primary axis with the green colored area represents the cumulative number of daily recovered cases and secondary axis with the red colored area represents the daily case fatality rate (%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
COVID-19 cases and death by age and gender in Bangladesh.

Variable	Category	Case (%)	Death (%)
Age	1–10	3.0	0.8
	11–20	7.0	1.5
	21–30	28.0	3.4
	31–40	27.0	8.3
	41–50	17.0	17.4
	51–60	11.0	29.6
	>60	7.0	39.0
Gender	Male	71.0	77.0
	Female	29.0	23.0

Fig. 2 illustrates the change in the number of cases and death in response to several government measures. The Government of Bangladesh banned all the international flights on March 15 except flight from the United Kingdom (UK). The government declared nationwide lockdown on March 24. Unfortunately, immediately after the lockdown declaration, people started leaving the capital city, Dhaka and travelled to their villages due to office closure. On April 2, 2020, the garment workers attempted to return to Dhaka, the identified epicenter of COVID-19. Again, within a short interval, the garment workers returned to the rural community, which might be responsible for the community transmission. Some unwanted situations and activities, including funeral gatherings, hitting of the cyclone, resuming the garments' activities, and mass transportation for Eid celebration with families, might be responsible for increasing the transmission of the virus across the country. Until July 31, the number of cases crossed 200,000. In Bangladesh, the case toll reached the first 100,000 after 103 days of initial infection, but another 100,000 tolls achieved within the next 29 days (Fig. 2).

3.2. Spatial distribution of COVID-19 in Bangladesh

Initially, in March, COVID-19 cases were distributed in Dhaka, the capital of Bangladesh, and surrounding few districts like Gazipur, Narayanganj, Narsingdi. Over the time, the infection spreads throughout the country. Still, Dhaka and its surrounding districts have a higher number of cases. Besides, Chattogram, a distantly located city from the capital, had also a higher number of cases than any other area in Bangladesh (Fig. 3).

3.3. R_0, comparative epidemic curve of COVID-19 and it's sensitivity analysis

3.3.1. R_0 of COVID-19

We estimated the R_0 of COVID-19 based on the government reported confirmed cases using four different methods. In EG, ML, SB and TD method, we found R_0 1.173 (95%CI: 1.172–1.174), 1.09 (95%CI: 1.08–1.10), 1.24 (95%CI: 1.18–1.29), and 1.07 (95%CI: 1.02–1.13) respectively. The overall basic reproduction number varied with time progression, which was highest in and around 20 to 25th days, estimated by SB and TD method (Fig. 4). The shade over the line in SB and TD (Fig. 4-B and D) is the indication of the subsequent confidence interval. We visualized the dynamics of R_0 in 7 days interval in Fig. 5. We observed the highest R_0 in the fourth week (29 March – 4 April) (R_0 = 4.86) and lowest R_0 in 20th week (19–25 July) (R_0 = 1.09) (Fig. 5).

3.3.2. Comparative epidemic curve of COVID-19 using different models

The epidemic curve of COVID -19 varied in different models. The exponential growth started from around 25th days after the first case. The SB model is equipped poorly with the data (Fig. 6). Again, there were some ups and downs trend of the number of actual cases in contrast

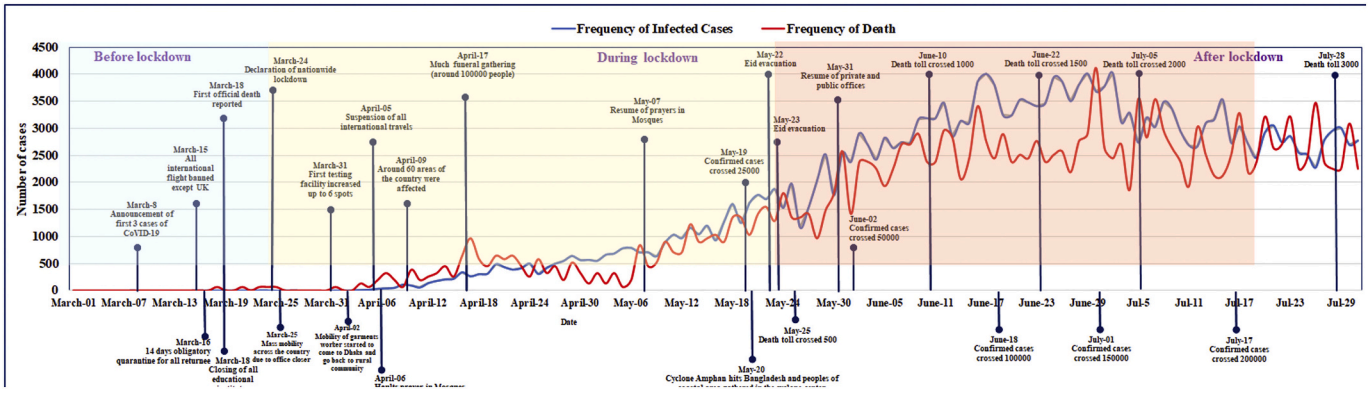

Fig. 2. COVID-19 cases and death trends over time with different Government initiatives and the occurrence of the varying incidence. Blue and the red-colored line is the presentation of the frequency of infected and death cases.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

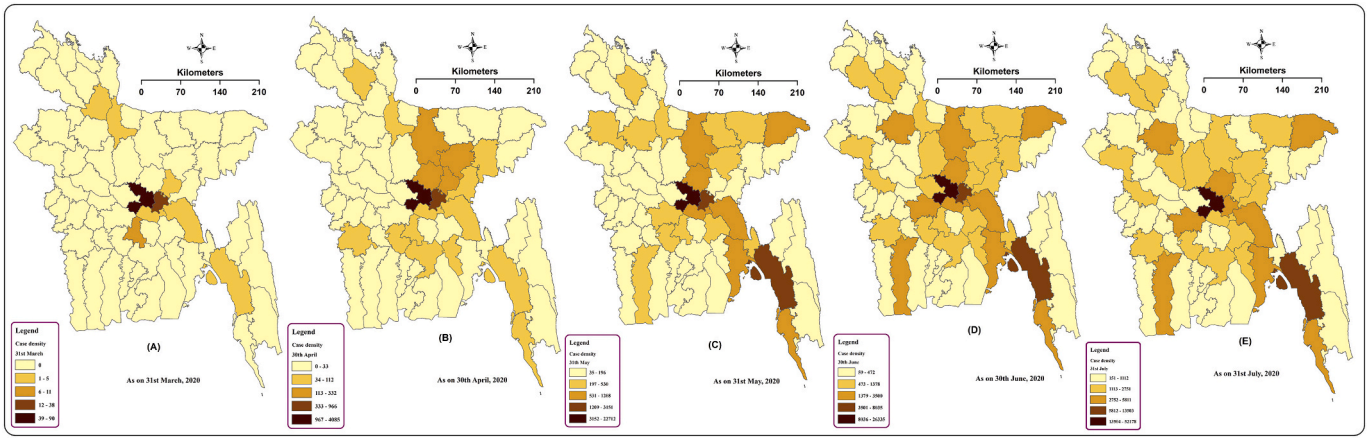

Fig. 3. Spatial distribution of COVID-19 cases in Bangladesh from Mar to July 2020.

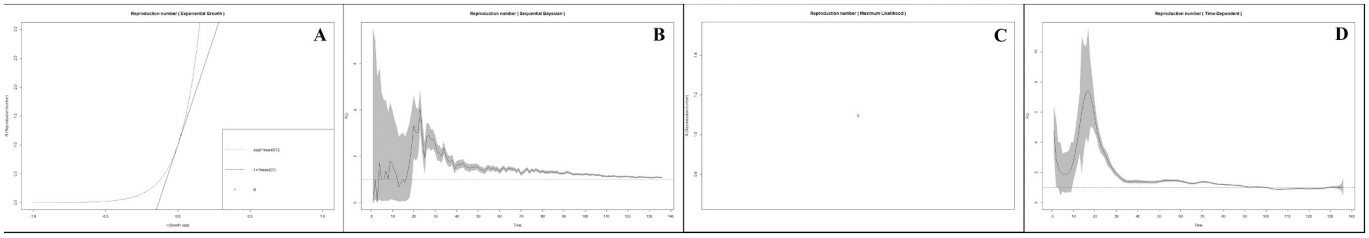

Fig. 4. Estimation of the reproduction number in different methods. A) Exponential Growth method, B) Sequential Bayesian method (x-axis is the individual day of each incidence occurrence denoted as time, and the y-axis is the reproduction number), C) Maximum Likelihood method, D) Time-Dependent method (x-axis is the individual day of each incidence occurrence denoted as time, and the y-axis is the reproduction number).

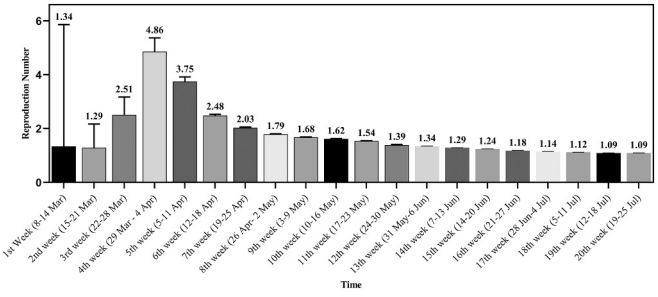

Fig. 5. Changing pattern of R_0 in 7 days interval by Exponential Growth (EG) Model.

to the predicted number of cases in the ML and the TD model (Fig. 6).

3.3.3. Sensitivity analysis of R_0 using EG model

The case reproduction number increased on EG-based estimation after changing the mean generation time. The reported reproduction number ranged from 1.04 to 1.19 in the EG method when the mean generation time varies from 1 to 8 days (Fig. 7).

3.4. Future daily incidence prediction of COVID-19 in Bangladesh through simulation

We have illustrated (Fig. 8) the median number and the cumulative number of cases for the next 60 days (until 1st week of September 2020) using $R_0 = 1.17$, estimated in this study (Fig. 8/A & B). However, a 20% increase in the value ($R_0 = 1.40$) showed an increase in daily incidence and cumulative incidence number in Fig. 8/C & D. Again, a 20% decrease of estimated values ($R_0 = 0.93$) showed a reduction of daily incidence and cumulative incidence in Fig. 8/E & F.

3.5. Molecular characterization of SARS-CoV-2 in Bangladesh

3.5.1. Phylogenetic analysis

The phylogenetic tree depicted that the viruses circulating in Bangladesh belong to six different clades- GH, GR, O, G, S, and L. Most of the sequences belong to GH and GR clade. Samples collected during June 2020 were under the clade GH and resembled strains from India and Oman. Moreover, one strain (EPI ISL 487368) from July, collected from Barisal district, is closely similar to Pakistan's strain. The strains collected during May to July, from various locations of Bangladesh clustered together under the GR clade and have nucleotide similarities with strain from Saudi Arabia. Isolates from Chattogram, Hobigonj, Nilphamari, and Rajshahi districts had the most common ancestors.

On the other hand, isolates from Mymensingh and Pabna has the same ancestral origin. Virus strains from Dhaka have clustered with isolates from almost every other district. We can also see that Bangladeshi strains have genetic relatedness with isolates from Italy and South Korea. Clade O is circulating only in Jhenaidah and Bagerhat districts, having similarity with Italian strain. Clade G contains only four strains from Bangladesh with ancestral relation to strains from UAE, Luxemburg, and Russia. Bangladesh detected the Clade L only in Dhaka during May, and it was the only clade nearest to the reference sequence from China. Five strains from May under S clade were detected only in Chattogram, which has nucleotide similarities with strains detected in Saudi Arabia (Fig. 9).

The current predominant clade circulating in Bangladesh is GR. We analyzed the intra-clade nucleotide sequences of GR among themselves. The differences among the isolates of clade GR have been illustrated in Fig. 10. The strains under GR clade make several distinct groups among themselves. We illustrated each separate cluster as the group denoted as I to XIII in the phylogenetic tree. Though all of them are under clade GR, they did not form a single cluster. Instead, they form many small clusters consist of 2–3 strains. It indicates several mutations in the sequences

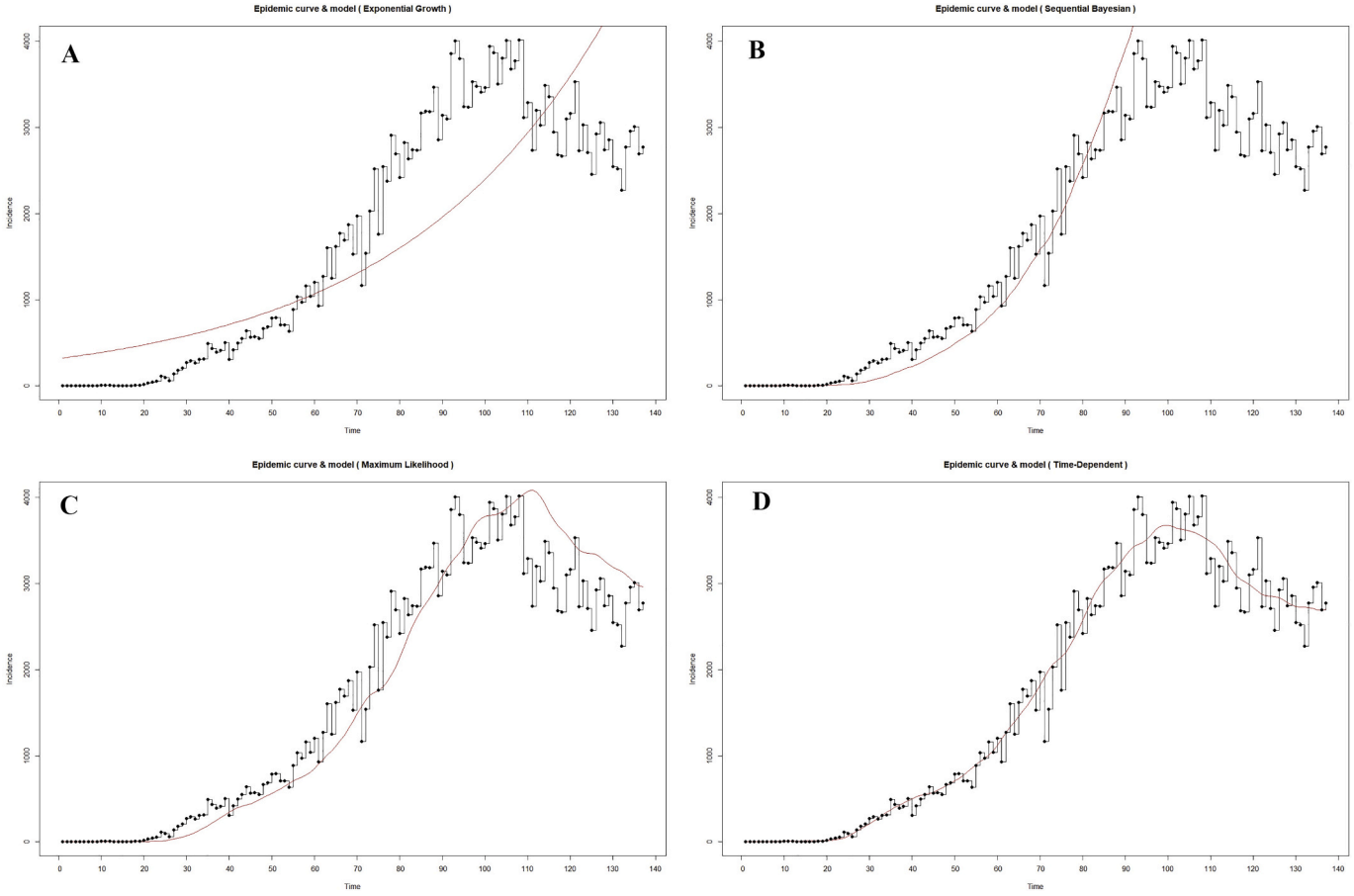

Fig. 6. Observed and predicted epidemic curve of COVID 19 (the node with the line is the observed case where the red dotted line is the expected case number in each model, the x-axis is the single day of each incidence occurrence denoted as time, and the y-axis is the number of incidence case). A) Epidemic curve in the exponential growth model, B) Epidemic curve in Sequential Bayesian model, C) Epidemic curve in Maximum Likelihood model, and D) Epidemic curve in Time-Dependent model.

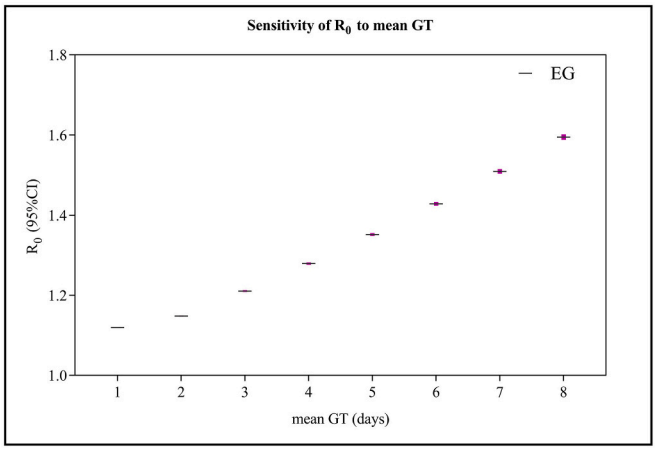

Fig. 7. Sensitivity analysis of reproduction number in contrast to mean generation time using EG model.

over the time. The virus detected mostly from May to July 2020 was under the group I to XII. Group I to XI have no genetic relation with virus global strains. However, the group XII had the most recent common ancestor with a virus strain from the USA. Group XIII is related to virus strain, mostly from abroad (Fig. 10).

3.5.2. *Mutation analysis of SARS-CoV-2 genome*

The mutation analysis revealed 1634 mutations across the entire set of studied genomes compared to the reference strain, Wuhan-Hu-1 (Accession NC_045512). Our study revealed 1546 (94.6%) synonymous and 88 (5.4%) unique mutations in the entire studied genome. The highest percentage of unique mutation estimated in Spike (23.9%; $n = 21$) followed by NSP3 (14.8%; $n = 13$) protein. The lowest percentage of unique mutation estimated in NSP4 and NSP16 both (1.1%; n = 1) (Fig. 11) (Supplementary Table 1). All the mutation of the studied COVID-19 genome supplied in supplementary file 1. The Nucleocapsid (N) and Spike (S) proteins appear to have the highest mutation rates. NSP2, NSP3, RNA dependent RNA polymerase (RdRp), ORF3a also showed substantially large number of mutations. Interestingly, some proteins such as NSP9, NSP11 had no mutation over the study period. Additionally, NSP7, Envelope (E), NSP7a, ORF6, NSP16, and ORF7a, ORF7b had the lowest mutational frequency. Besides, we detected only four mutations in 5′-UTR region.

In case of S protein, we found 20 predominant mutation sites, among which 13 were in N-terminal domain (NTD) fragment (T95I, Q14H, S13I, T75I, H49Y, N211Y, D138H, V127F, P26, G75V, S255, Y248H, and S95F). The remaining other sites were located in different regions within the protein, including L5F, D614G, G769V, E516Q, T791I, L518I. The fusion peptide region, S′ including heptad repeats HR1 and HR2 regions contains 3 (G769V, T791I, A783S), 2 (S939Y D936Y), 1 (K1191N) mutated regions, respectively. We observed the most common amino acid substitutions D614G in S protein and I120F in NSP2. We detected only two mutations sites (E516Q, L518I) in RdRp conserved peptide region. On the other hand, we found the unique aa substitutions at

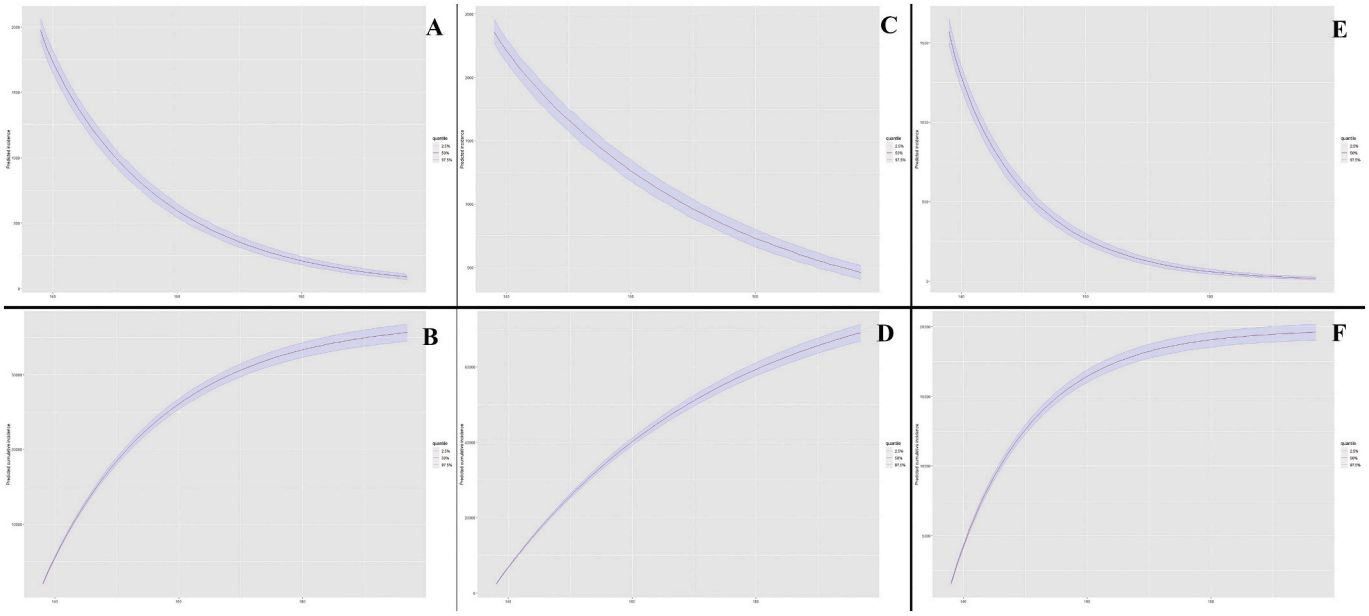

Fig. 8. Future daily incidence prediction of COVID-19 over 60 days (up to 1st week of September 2020) in Bangladesh through simulation. A) Median incidence and cumulative incidence prediction number with 1st and 3rd quantile values (R0 = 1.17), C &D) Median incidence and cumulative incidence prediction number with 1st and 3rd quantile values (R0 = 1.40), E&F) Median incidence and cumulative incidence prediction number with 1st and 3rd quantile values (R0 = 0.93).

position 518 (L> > I) and some unusual mutations like L5F, D138, G594S, and S98F in the S protein. We observed three types of aa (S4F, H125Y, A2V) changes in the membrane (M) protein followed by three changes (A99V, L21F, F20L) in the E protein. Besides all other essential protein, another most abundant changes were observed in the N protein (R203K, G204R), which is mainly targeted in the diagnostics purposes.

3.5.3. Clade and lineage diversity of SARS-CoV-2 in Bangladesh

The highest number of samples were sequenced from Dhaka division, followed by Chattogram, Rajshahi, Rangpur, Barisal, Khulna, Sylhet, and Mymensingh. The current predominant clade circulating in Bangladesh is GR (83.9%; 245/292) followed by GH (5.8%), G (4.8%), O (3.4%), S (1.7%) and L (0.3%). In Dhaka division, all the clades except 'S' had been identified. In Chattogram division, the 'GR' clade was dominant, followed by other clades except 'L'. Only 'GR' clade was identified in Mymensingh division. In Barisal and Rangpur division, 3 different clades including 'G,' 'GH' and 'GR' had been identified whereas, in addition to 'GR,' and 'GH', clade 'O' was identified in Khulna. In Sylhet and Rajshahi division, only 'GH' and 'GR' clade had been identified (Fig. 12). Clade GR was most prevalent both in males (85.9%) and in females (83.3%). In females, only 1% strain was under Clade L, but it was not found in males. The Bangladeshi strains were under 8 lineages, assigned by Pangolin lineage (A-1.7%, B-0.34%, B.1–4.8%, B.1.1–23.6%, B.1.1.23–12%, B.1.1.25–50.0%, B.1.113–5.8%, and B.1.36–0.3%). The highest percentage of B.1.1 (97.1%), B.1.1.23 (97.1%) and B.1.1.25 (98.6%) lineages into GR clade where B.1.36 (100.0%) lineage fell into GH clade only (Fig. 12). The highest percentage of strains was under lineage B.1.1.25 in both males and females (Fig. 13). Highest lineage variability estimated in Dhaka where B, B.1, B.1.1, B.1.1.23, B.1.1.25 lineages were at highest percentage in contrast to other locations of the country. However, B.1.36 and B.1.113 lineage was more common in Rajshahi, and Chattogram, respectively (Fig. 14).

4. Discussion

4.1. Epidemiological characteristics and spatial distribution of COVID-19 in Bangladesh

At the beginning of the pandemic, a limited number of test facilities at a few test points were available in Dhaka. The number of RT-PCR based test has been increased up to around 18,500 per day at 60 different test points across the country. Still, this number is insufficient in contrast to the density of the population of this country. However, it is of utmost relief that our estimated case fatality rate (13,423 per million/ 1.5%) was much less than other countries in the world. China reported the case fatality rate as 2.3% (Novel, 2020), whereas India reported as 2–3% (Singhal, 2020), and Italy as 7.2% (Onder et al., 2020). The lower-case fatality rate of COVID-19 in Bangladesh may have resulted from fewer confirmatory tests done in the country. The recovery rate (56.85%) of COVID-19 in Bangladesh was much higher than the European countries (46%) (Karadag, 2020). Moreover, the people of 21–30 years age groups were infected more than other age groups, which may due to their involvement in different activities and regular transportation and movement. Males were more affected and died than that of females. Similar gender trends were seen in patients from China (Yang et al., 2020).

Government of Bangladesh imposed nationwide lockdown on March 26, 2020, after 19 days of the first case identification. After announcing the lockdown, more than 11 million people left Dhaka city and travelled to their home districts. They commenced the risk of COVID-19 infection to the entire 64 districts of Bangladesh. Even within the lockdown, some offices were open, and people could travel from one place to another. Moreover, as the major Muslim festival Eid-Ul-Fitr approached, mass people gathered in shopping centers. They moved across the country to celebrate the festival with their family members in the villages, despite the risk of spreading the transmission. When the nationwide lockdown was lifted on May 30, there was a surge of people toward Dhaka city (Cowley et al., 2021). Besides, around 10.3 million nationals of Bangladesh are working in different countries worldwide (www.old.bmet.gov.bd), including China, Italy, the UK, the USA, Spain, Singapore, and Canada. Due to the COVID-19 pandemic, many expatriates came back to Bangladesh and spread the virus throughout the country, which might be one of the potential COVID-19 transmissions factors.

Dhaka city, located at the country's center, is the 9th densely populated city in the world (UN, 2018). This megacity has 46 thousand people living per square kilometer and accommodating more than 1.1 million slum dwellers. Most of these slum dwellers are illiterate and are living in densely populated houses. It is tough to inform them about the

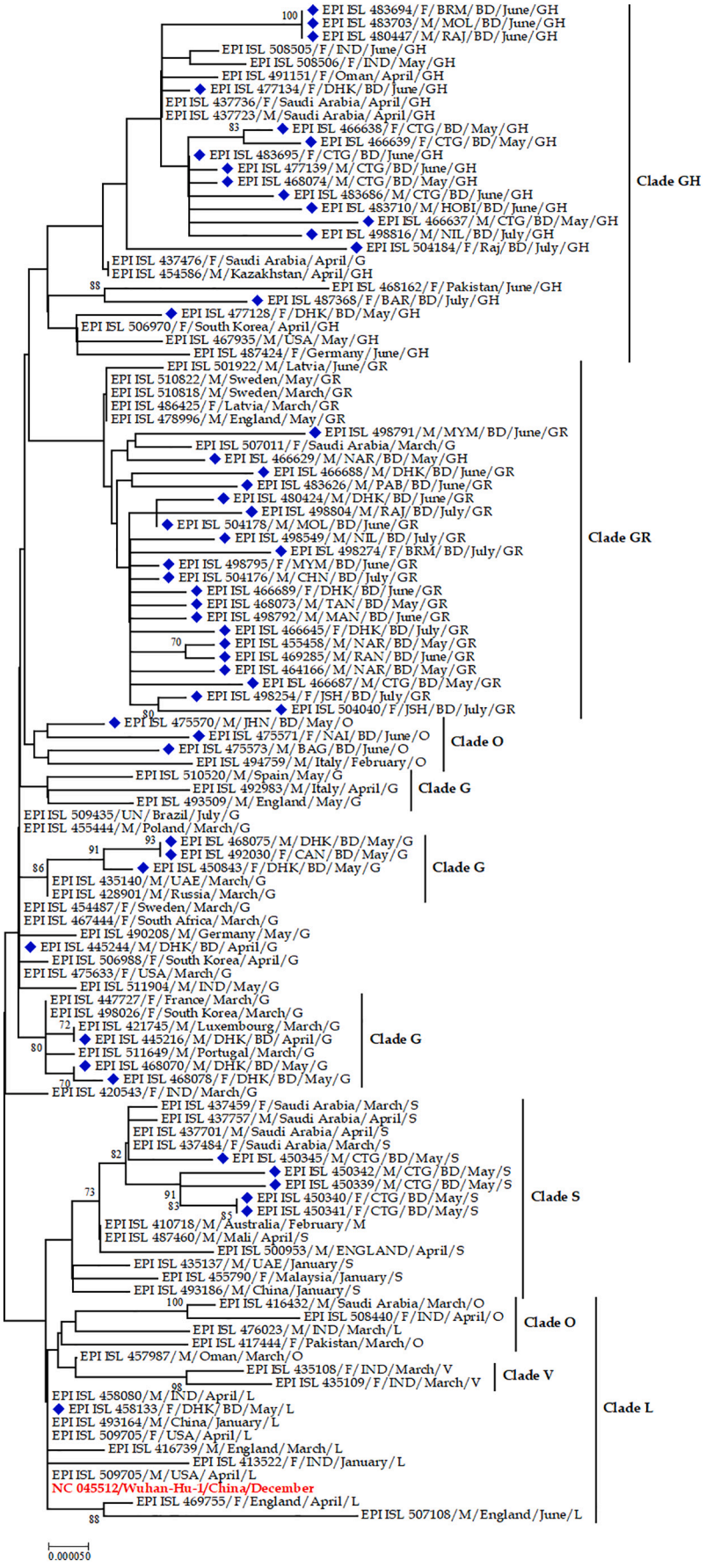

Fig. 9. Phylogenetic analysis of SARS-CoV-2 strains from Bangladesh.

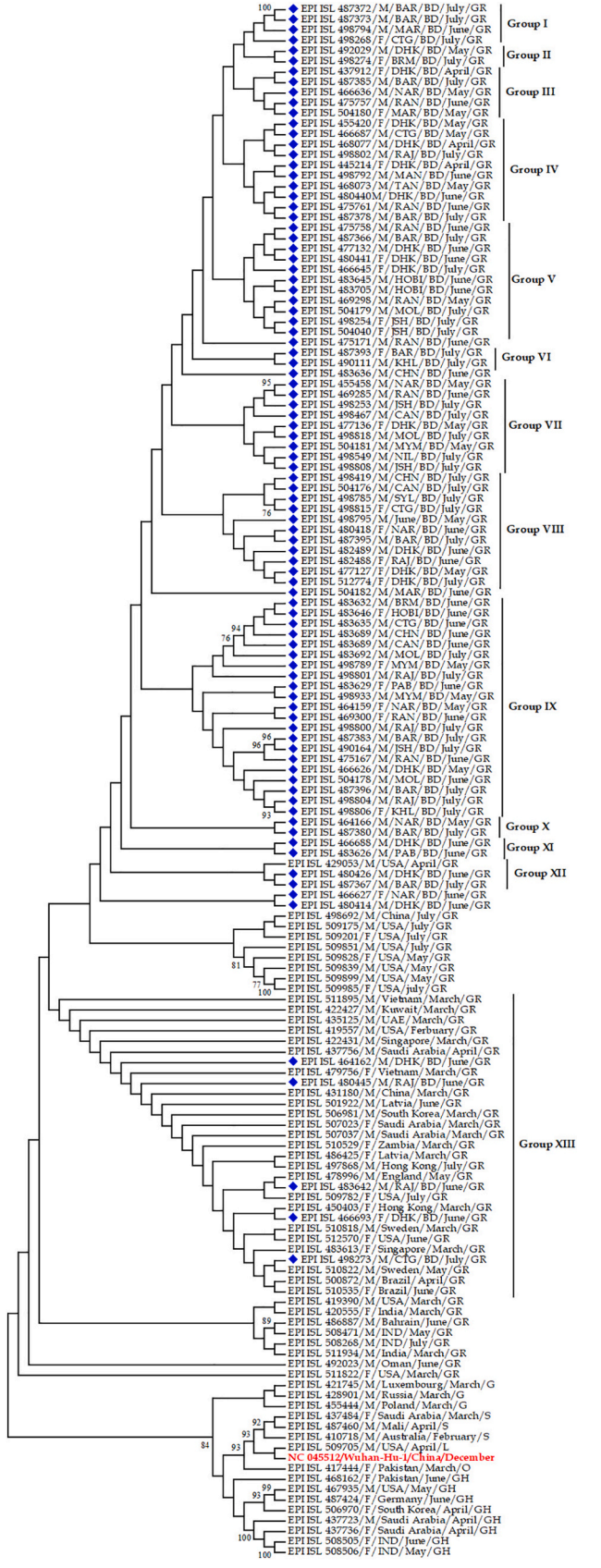

Fig. 10. Phylogenetic analysis of SARS-CoV-2 strains from Bangladesh under GR clade.

COVID-19 pandemic threat and impossible to maintain social distancing in their dwelling areas (BBS, 2011). Therefore, there was a high chance of community transmission in contrast to another town of Bangladesh (Islam et al., 2020). Additionally, almost 80–90% of air travelers use the airport in Dhaka for home and abroad travel. Many susceptible travelers denied institutional quarantine and travelled to their home city. It may be a cause of enhancing the COVID-19 transmission in Dhaka city (Islam et al., 2020). It is believed that community transmission in the rural community resulted from the free movement of infected persons. People exposed to infected persons returned from abroad or from Dhaka or nearby cities. The number of cases were increasing day by day, along with the number of deaths. Still there are almost 23–24 death per day. Now Bangladesh is within the top 30 affected country in the world.

Based on the cases in different area, the Government divided different regions into the Red, Green, and Yellow zone from June 9, 2020. Their plan was turning the red zones to green gradually by reducing the number of cases. However, our estimation suggested comprehensive zoning of hotspot where there might be a possible chance of infection transmission to other districts. Lockdown of a specific area rather than complete red zoning might be useful in hotspot areas.

4.2. R_0 and prediction of COVID-19 cases through simulation

The data-driven mortality rate per million people guided to estimate the infectiousness and transmissibility in this study. Calculating R_0 had been an imperative focus of epidemiological work to understand the transmission dynamics and pandemic trajectory of COVID-19. The calculation of transmissibility determines the disease spreading, which depends on the infection generation time. Nevertheless, in a highly infectious disease like COVID-19, it is almost impossible to determine the generation time by observing infected patients' movement in a densely populated country like Bangladesh.

The basic reproduction number is one of the critical values predicting whether the disease will spread into a population or die out. We estimated the basic reproduction number were varying from 1.07 to 1.17 in different models but, still within the range of WHO-recommended value of 1.4 to 2.5 (Viceconte and Petrosillo, 2020). Almost similar R_0 was reported in South Korea (1.5; 95% CI: 1.4–1.6) (Shim et al., 2020). But our estimated value still was below the calculated value of 2.68 to 6.4 by Wu et al. (2020), and 2.2 by (Association, 2020). The projected values were highest at 25th days of reported infection and then declined slightly, which may due to the government's strict isolation and quarantine rules. Although the value is within the WHO limit, it is still significantly higher than 1, indicating the virus's rapid transmissibility. However, changing R_0 over the different periods indicates government initiatives' positive effects on COVID-19. From the economic point of view, it is hard to maintain strict lockdown for a long time. So, the Government is still searching the way to reduce the transmissibility within a considerable limit ($R_0 < 1$).

Bangladesh is a small but densely populated country, making it difficult to cope with the

situation by maintaining strict lockdown rules. The R_0 forecasted that a considerable proportion of secondary cases are practically emerging before a person is being tested COVID-19 positive. However, the highest estimated values of R_0 (1.17) suggested tracing at least 50% of sufficient contact is needed to decrease the R_0 (Hellewell et al., 2020). Different equations were used to calculate the R_0 by various studies around the globe, but it is impossible for us to include all the methods of calculation in this study. This might introduce bias in the present study. The study was conducted for helping visualization of new situation, and the authors have misled no direction.

The future prediction of simulated model indicated a decrease in the number of daily incidences of COVID-19 near about 100 after 60 days with the estimated R_0's persistence. Meanwhile, 70,000 patients will be added with the current COVID-19 infected number predicted till July 31,

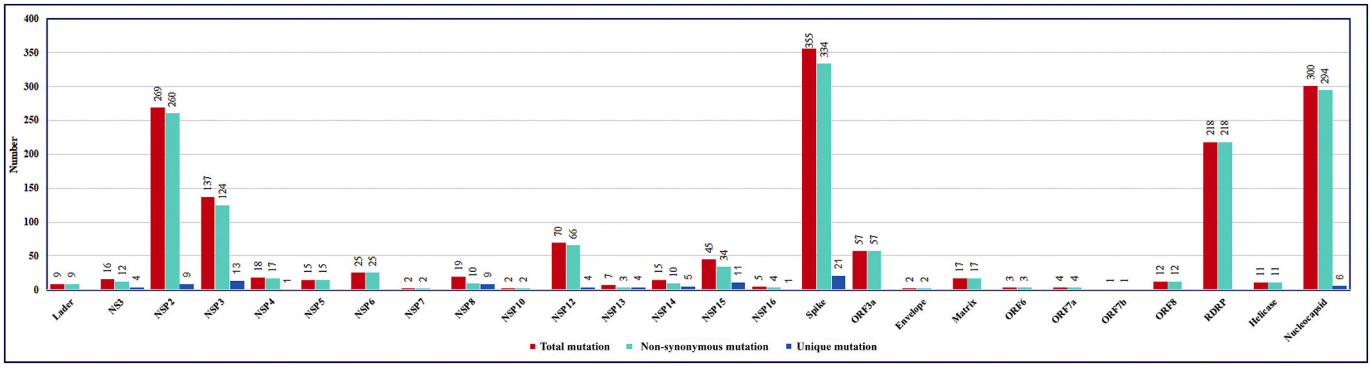

Fig. 11. Non-Synonymous and unique mutation of SARS-CoV-2 virus genome in Bangladesh.

2020. Nevertheless, if we assume that the present R_0 will increase up to 20%, then there will be around 500 cases daily from the 60th day. Yet, if we can trap the transmission up to 20% of the current R_0, then we can hope that the daily incidence will decline to less than 100 cases with an addition of around 20,000 cases within next 60 days. Our model predicted that if we can ensure proper personal hygiene with strict lockdown measures in the affected region, the infection might be under control in October 2020.

4.3. Molecular characterization of SARS-CoV-2

The presence of G clade in Bangladesh indicates that the virus might be originated from a European country (Fuertes et al., 2020). In Chattogram, the sequences were identified as S clade at the earlier stage, might be resulted from the returning of expatriates from the USA. But later, the S clade was diminished from Bangladesh. Most of the isolates from Bangladesh belonged to clade GH and GR. Clade GH's distribution was limited to Dhaka and later spread to other districts like Rajshahi, Brahmanbaria, Chattogram, Habigonj, and Barisal. The massive transportation of people may help spreading the virus throughout the pandemic. Clade O was limited to outer districts than Dhaka city. However, GR clade was the most prevalent across the country. Some intra-clade differences were observed in clade GR. Probably there are mutation in the strains over the time. Our study found 13 major groups of strains circulating in Bangladesh by studying protein non-synonymous mutations. In S protein, the D614G mutation was also prevalent in Europe and North America. Over time, there are some changes in the nucleotide sequences of the isolates under this clade. As a result, the isolates formed several small clusters of 3–4 isolates rather than creating a large cluster by grouping together.

We also saw the variation in the lineage detected at different districts in Bangladesh. But the most common (50.0%) lineage was B.1.1.25 which falls under pangolin lineage. Other two pangolin lineage B.1.36 and B.1.1 were found as 0.3% and 23.6%, respectively. Lineage B.1.1.25 were found in the United Kingdom and Australia. Initially, the clade GR and lineage B.1.1.25 were not prevalent but over the time, these clade and lineage's rise suggests considerable amount of mutation. Furthermore, Bangladesh reported emerging UK variant in 45 patients and South Africa variants in 174 patients (www.gisaid.org) (Hossain et al., 2021) until April 22, 2021, which signifies the continuing the robust SARS-CoV-2 virus sequencing to monitor the new variants. However, the mink variants (Hammer et al., 2020) and other emerging variants of SARS-CoV-2 have not been detected yet in Bangladesh.

Though it is hard to identify the actual player for infection transmission in the community in Bangladesh, the predicted factors cannot be underestimated. Therefore, the accounting for the R_0 and prediction is needed to restructure the planning to halt the spreading of COVID-19 in Bangladesh.

This study is not beyond limitation. A long list of values of generation time were available but we calculated R_0 considering a specific generation time. It was not possible to consider all the available values of generation time. Therefore, the inflation of generation time may produce a deviation of the output. Moreover, asymptomatic cases were not considered for the present study, as the directorate general of health services, Bangladesh has not recorded those cases. Again, we recommended to conduct a future study including multiscale model to understand how genome evolution at individual level influence the transmission dynamics of virus in population level. However, the study is unique and comprehensive in its own way to combine the epidemiological characteristics, specially R_0, with molecular data. The R_0 and the epidemiological measures can forecast about the future case generation whereas the genomic surveillance data is important to know about the new variants that have been introduced in Bangladesh and to evaluate the effectiveness of vaccination in the specific country.

5. Conclusion

This study highlighted the spatiotemporal distribution, R_0, and predicted the future epidemic size of COVID-19 in Bangladesh. Moreover, we also evaluated the genomic epidemiology of SARS-CoV-2 circulating in Bangladesh. These information are crucial to control and mitigates the pandemic situation in any country or territories, including in Bangladesh. This study can inform health policymakers to successfully take appropriate preventive measures and interventions to break the transmission chain and control the epidemic. Insight into the phylodynamic and clade and lineage diversity of the virus will help to develop a potential vaccine for the Bangladesh context. Finally, we strongly recommend continuous genomic surveillance to understand the strains' diversity and detect new variants of SARS-CoV-2 for proper control of current pandemic and design effective vaccine globally.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2021.104884.

Ethical approval and consent to participate

Approval was not required.

Availability of data and materials

All data and materials used in this paper are publicly available.

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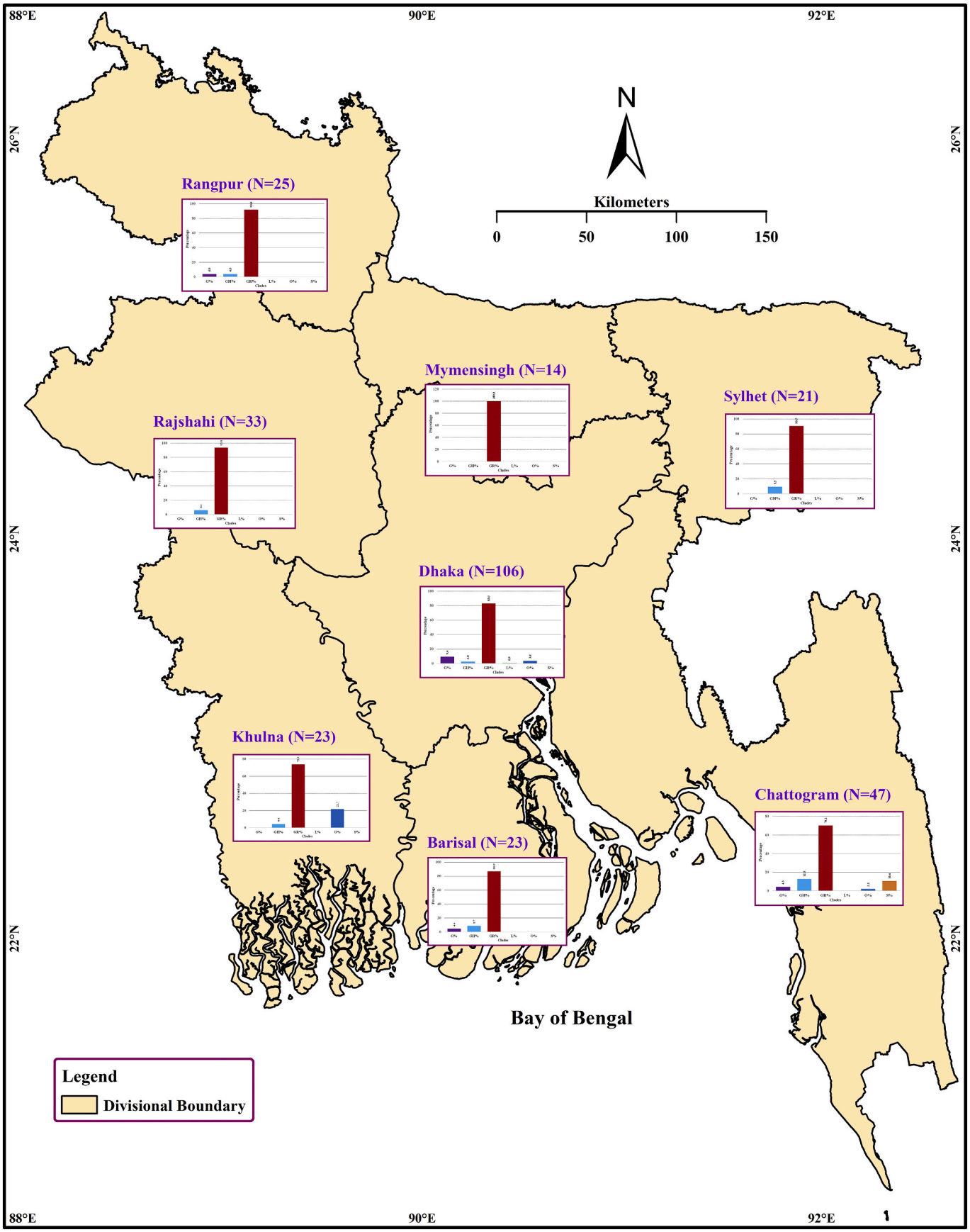

Fig. 12. Geographic distribution of different clades of SARS-CoV-2 in Bangladesh.

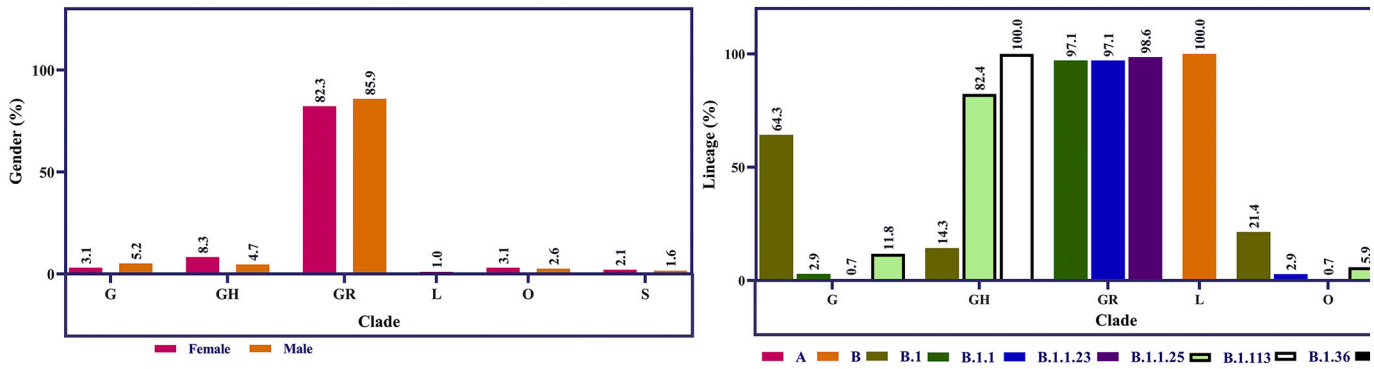

Fig. 13. Clade and lineage diversity of SARS-CoV-2 in Bangladesh. A) Sex-specific distribution of different clades, B) Lineage diversity of SARS-CoV-2 in Bangladesh.

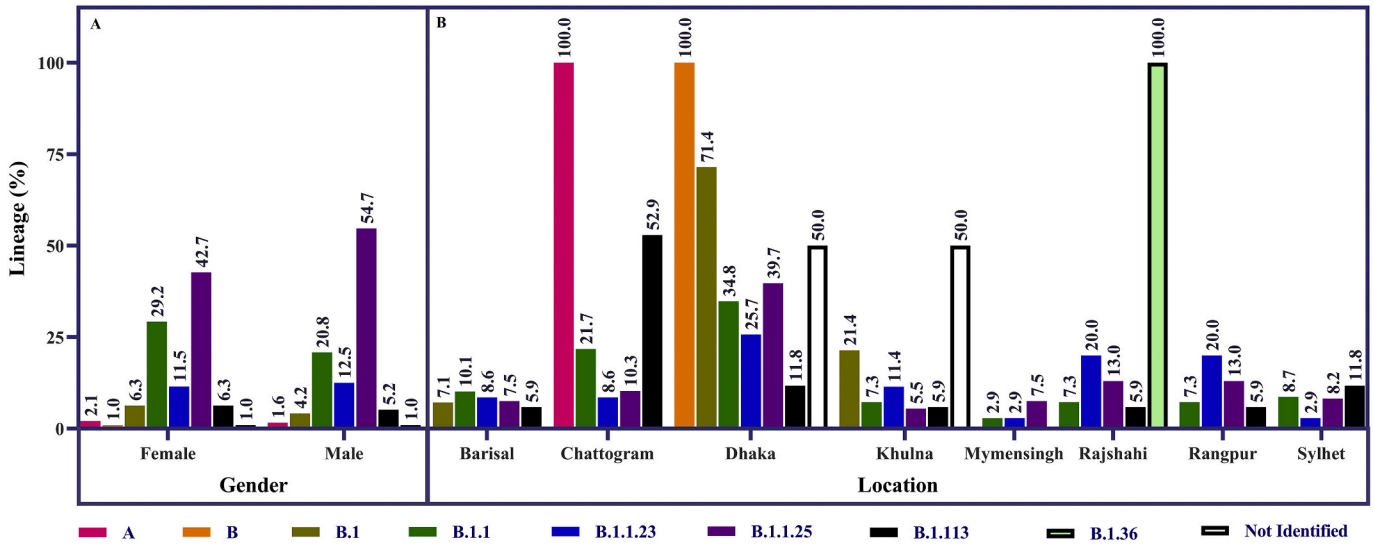

Fig. 14. Lineage diversity of SARS-CoV-2 in Bangladesh. A) Sex-specific distribution of different lineage, B) Location specific lineage diversity of SARS-CoV-2 in Bangladesh.

Declaration of Competing Interest

The authors of the correspondence do not have any conflict of interest.

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