

## Molecular identification, clinical assessment, characterization of SARS-CoV-2 RT-PCR results: Insights into twenty-one months of COVID-19 pandemic in a single center in Bangladesh

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### Abstract

**Introduction:** The pervasiveness of SARS-CoV-2 infection over millions of people renders a variety of symptoms among group of people and ages. This study aimed to appraise the clinical assessment, characterization, and comparative study on suspected SARS-CoV-2 infected patients and further illuminate its transmission dynamics.

**Methods:** It includes all the suspected SARS-CoV-2 infected cases from July 2020 to March 2022 in this retrospective single center study. All the cases confirmed by the golden method of detection (real-time RT-PCR) and analyzed for clinical and demographic features and laboratory data.

**Result:** Out of 35,630 cases, 11.4% cases confirmed with SARS-CoV-2 infection. With 91.4% new and 8.6% follow-up suspected cases in total, SARS-CoV-2 infection confirmed with 85.5% new cases and 14.5% from follow-up cases. 86.1% male suspected cases found where SARS-CoV-2 infection confirmed for 73.8% with this gender group. 56.7% SARS-CoV-2 infection confirmed for age range 20-39 and this age group counts 59.3% from the total suspected cases. Most confirmed cases (28.3%) counted from Uttara, Dhaka and this area covers 34.9% suspected cases from the total.

**Conclusion:** This study exhibited that, SARS-CoV-2 is more prone to male individual, individuals aged between 20-39 and occurs re infection hardly but not limited to. Symptoms observed for almost half of the confirmed cases.

**Keywords:** SARS-CoV-2; COVID-19 in Bangladesh; Pandemic; Clinical assessment, Characterization; Symptomatic; Asymptomatic; RT-PCR results

### 1. Introduction

The causative agent of the recent global pandemic of the COVID-19 (Coronavirus disease 2019) is SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), a pathogenic nanoparticle from the family *Coronaviridae* and the genus *Betacoronavirus*. SARS-CoV-2 ranked the seventh coronavirus to infect humans; initially spotlighted in Wuhan, China, in December 2019, and declared a pandemic on March 11, 2020<sup>1</sup>. Till now, SARS-CoV (severe acute respiratory syndrome coronavirus) and MERS-CoV (Middle East respiratory syndrome coronavirus), the two severe disease outbreaks reported to occur by coronavirus while the other four coronaviruses named HCoV-229E, HCoV-NL63, HCoV-

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OC43, and HCoV-HKU1 found to cause mild severity<sup>2</sup>. The number of cases is more than 1,951,911 in Bangladesh and 494,587,638 around 230 countries, areas, or territories as of April 08, 2022<sup>1</sup>. With many cases unreported, very few of the massive number of residents covered by COVID-19 surveillance. The first official COVID-19 case in Bangladesh reported on March 8, 2020, in Dhaka, the epicenter of COVID-19 with near about 20,000,000 residences, and the variant first sequenced on April 18, 2020, at Child Health Research Foundation, Dhaka, Bangladesh<sup>3-6</sup>. Till now all four major variants of concern (VOC) including Alpha, Beta, Delta, and Omicron have been confirmed by genome sequencing in Bangladesh<sup>5,7-10</sup>. So far, 5 major variants of concern (VOC) named Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), have been identified respectively in United Kingdom (Sep-2020), South Africa (May-2020), Brazil (Nov-2020), India (Oct-2020) and Multiple Countries (Nov-2021) along with fewer variants of interest (VOI) named Epsilon (B.1.427, B.1.429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37) and Mu (B.1.621) have been identified in USA (Mar-2020), Brazil (Apr-2020), Multiple Countries (Dec-2020), Philippines ((Jan 2021), USA (Nov-2020), India (Oct-2020), Peru (Dec-2020) and Colombia (Jan-2021), based on the changes in their

Except for the upper respiratory tract (Nasopharyngeal and Oropharyngeal), the nucleic acid of SARS-CoV-2 can also find in bronchoalveolar lavage fluid, sputum, faeces, urine, and serum samples of COVID-19 patients<sup>13-18</sup>. The gold standard for COVID-19 detection, real-time reverse transcription polymerase chain reaction (rRT-PCR) is the nucleic acid amplification test targeting N (Nucleocapsid), E (Envelope), S (Spike), and RdRp/ORF1ab (RNA-Dependent RNA Polymerase) genes with around 71 to 98% sensitivity and 95% specificity, which may vary upon mutation, disease stage, and viral multiplication<sup>18-21</sup>. Although it can show false-negative results upon low viral loads caused by mutation, PCR inhibitor, inappropriate handling and shipment, collection time, low viral RNA, or poor specimen quality<sup>18</sup>.

Bangladesh has a history of becoming the hub for many transmissible diseases with its dense population (1,265 per square kilometer), where about 35.3% of its residents are below 14, about 60% are between 15 to 64 and just 4.7% are above 65 years of age<sup>22</sup>. It makes it more difficult to prevent or control, as SARS-CoV-2 has already thrived on several intermediates and final host before infecting Humans. Hence it dominates with higher transmissibility and infectivity. The infectivity, virulence, and severity of the outcome of SARS-CoV-2 may vary on factors such as age, sex, geographic locations, other chronic diseases, and so on<sup>22,23</sup>. Though SARS-CoV-2 varies no age, sex, or race; pregnant women, newborn, male (71%) individuals, and individuals over 20 years of age are prone to get infected by SARS-CoV-2<sup>2,24,25</sup>. Patients with SARS-CoV-2 infection may or may not develop any significant or chronic symptoms. About 18% to 31% of the SARS-CoV-2 infection observed asymptomatic with higher transmissibility and other patients found were minimally symptomatic, while they showed fever, cough, nasal congestion, anorexia, ageusia, dyspnea, rigors, pharyngeal erythema, malaise, sore throat, shortness of breath, headache, body ache, hemoptysis, fatigue, vomiting, diarrheal symptoms<sup>25-32</sup>. As SARS-CoV-2 utilizes the ACE2 (angiotensin-converting enzyme 2) receptor and latent CD147 (cluster of differentiation 147) to surplus invade towards cardiovascular, renal, endocrine, nervous, and gastrointestinal organ systems; associated viral invasion, dysregulated renin-angiotensin-aldosterone system (RAAS), hypoxia, hyperinflammation, cytokine storm, endotheliopathy, and thrombosis considered as a renowned pathophysiological mechanism of SARS-CoV-2 infection<sup>33</sup>. Immune response for confirmed SARS-CoV-2 infected patients is noticeable while mild to severe symptomatic patients develop significant IgM and IgA response, but antibody response of asymptomatic patients found less or unnoticed<sup>34</sup>.

This study focused on evaluating the clinical assessment, characterization of SARS-CoV-2 RT-PCR results on SARS-CoV-2 infected patients by molecular identification, logged on Novus Molecular Lab, Dhaka, Bangladesh. Following the guidance of the WHO digital documentation process of SARS-CoV-2 test results<sup>35</sup>, the availability of large disaggregated data such as sex, age, type of patients, the purpose of the test, geographical location, symptoms, etc stored on Novus database helped to reveal the obscured imbalances.

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## 2. Material and methods

### 2.1. Study design

#### 2.1.1. Development of a digital patient management system

The outbreak of corona virus disease-19 (COVID-19) is a public health emergency of international concern. During the COVID-19 outbreak Beximco Pharmaceuticals Ltd launched “BCD COVID-19 Medical Center” for the management of COVID-19 positive cases with symptoms. Any BCD cases and other beneficiaries (Permanent, Contractual, Temporary, Casual, Driver or DL) officially reports to the 24 hours HOTLINE number, if he/she or any of the following members residing with him/her shows any symptom and need any query related to COVID-19. After receiving a call, the Hotline doctor provides initial treatment and registers the patient on hotline software. If required, the Hotline doctor advises

the cases to do Covid-19 test as per patient condition and send the recommendation to the testing team. Depending on the condition, hotline doctors sometimes advise the cases for hospitalization and provide necessary support also.

Testing team accumulates all the test recommendations and registers them in Novus software in-coordination with Novus Molecular lab. Testing team also provides an appointment date and time and sends appointment details to the individual's phone number through SMS. After completing the test, an automatic SMS with the test result shall be sent to the individual's phone no. Testing teams accumulate all the reports and send it to all responsible authorities including all respective HR and admin and patient management teams.

After receiving the report, doctors of patient management team talked to COVID positive employees and provide him/her a prescription as per his/her condition and send the copy of the prescription to the responsible department to send the medicines to the patient to all the COVID positive cases. Doctor of patient management team follow-up the patients regularly as per requirement and advised the patient to do some additional investigation and/or hospitalization if necessary. In the course of disease, the doctors of patient management team provided all types of medical and mental support to the patients.

## 2.2. Study Population

The study enrolled patients suspected of getting SARS-CoV-2 infection between July 2020 to March 2022 from Novus Molecular Lab. The clinical information for each patient recorded during the registration process either over a phone call or in person. Participants provided their oral informed consent to perform the SARS-CoV-2 test with their specimens collected. Ethical clearance was taken from NILMRC ethical board.

## 2.3. Specimen Collection and Storage

The specimen of COVID-19 suspected patients collected on a 2 ml STM (STANDARD™ Transport Medium; Cat. No. 90VTM10D) by following CDC and WHO specimen collection guidelines<sup>36,37</sup>. Both nasopharyngeal (Both sides of the nasal opening) and an oropharyngeal swab simultaneously collected and immersed in the STM to ensure proper specimen collection. All the STM with patient's specimens marked with the Novus ID, individual's name, date, and age in a printed sticker. All specimens collected were immediately transported to Novus Molecular Lab after collection and kept in 2-8°C<sup>36,37</sup> before the test. Specimens tested within six hours of collection by RT-PCR method. All the specimen collection, lab testing, storage, and waste disposal followed the standard guidance, health, and safety by CDC and WHO<sup>36-39</sup>.

## 2.4. Sample preparation and RNA extraction

After vortexing, 200 µl of the specimen from each STM transferred to individual 1.5 ml microcentrifuge tubes for SARS-CoV-2 RNA extraction. The RNA extraction of SARS-CoV-2 carried out via STANDARD™ M SPIN-X Viral RNA Extraction Kit (Cat. No. 11SPN10). After transferring 200 µl of specimen, a multistep extraction was carried out following the kit manual. The volume of eluted RNA of SARS-CoV-2 collected was 50 µl.

## 2.5. RT-qPCR for detection of SARS-CoV-2

Using STANDARD™ M nCoV Real-Time Detection kit (Cat. No. 11NCO10), master mix was prepared for the detection of ORF1ab (RdRp) gene, E gene, and IC (Internal Control). The RT-PCR was carried out in Quant-Studio-5 Real-Time PCR System (Thermo Fisher Scientific). After completion of the successful amplification of RNA, results were assessed. Ct (Cycle threshold) value equal to or below 36 considered a cut-off value for COVID-19 positive patients, and Ct value above 36 considered as COVID-19 negative.

## 2.6. Statistical analysis

All the data were statistically analyzed using IBM SPSS Statistics 25.0 software (SPSS Inc Chicago, IL, USA). Descriptive statistics like frequency and percentage used.

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## 3. Results and discussion

Among total 35630 cases, positive cases were 4,075 (11.4%) and the rest 31,555 (88.6%) cases reported as COVID-19 negative (Table 1).

**Table 1** Demographic characteristics of study population

		Positive	Negative	Total	Percent	Chi-Square Value	df	Asymptotic Significance (2-sided) or p-value
Symptoms	Asymptomatic	2142	31555	33697	94.6	15878.896	1	P = <0.001 (Statistically Significant)
	Symptomatic	1933	0	1933	5.4			
	Total	4075	31555	35630	100.0			
Gender	Female	1068	3867	4935	13.9	589.204	1	P = <0.001 (Statistically Significant)
	Male	3006	27686	30692	86.1			
	Total	4074	31553	35627	100.0			
Test purpose	Local	3767	20841	24608	69.1	1176.854	1	P = <0.001 (Statistically Significant)
	Passenger	308	10714	11022	30.9			
	Total	4075	31555	35630	100.0			
Type of patient	New	3484	29095	32579	91.4	207.352	1	P = <0.001 (Statistically Significant)
	Follow-Up	591	2460	3051	8.6			
	Total	4075	31555	35630	100.0			
Age	0-9	27	135	162	0.5	331.767	9	P = <0.001 (Statistically Significant)
	10-19	212	1822	2034	5.7			
	20-29	1017	9591	10608	29.8			
	30-39	1291	9237	10528	29.5			
	40-49	741	6393	7134	20.0			
	50-59	486	3522	4008	11.2			
	60-69	192	634	826	2.3			
	70-79	83	186	269	0.8			
	80-89	23	32	55	0.2			
	90-100	3	3	6	0.0			
	Total	4075	31555	35630	100.0			
Area of patients	Dhanmondi	915	3154	4069	11.4	1230.439	6	P = <0.001 (Statistically Significant)
	Mirpur	416	1921	2337	6.6			
	Gulshan	943	10555	11498	32.3			
	Khilgaon	367	917	1284	3.6			
	Jatrabari	197	1977	2174	6.1			
	Uttara	1154	11286	12440	34.9			
	Outside Dhaka	83	1743	1826	5.1			
	Total	4075	31553	35628	100.0			

In the case of test requirements, among 35,630 cases, a total of 11,022 (30.9%) cases required by passengers (for immigration purposes) of which only 308 (7.6%) reported COVID-19 positive. (Table: 1). This might be the first ever study that revealed the type of passenger who performs COVID-19 screening test and clearly exhibits their condition

during the immigration. However, the rate of SARS-CoV-2 infection was noticeable and they might escape the attention if not screened as most of the cases come along with no symptoms.

In Novus Molecular lab, 32,579 (91.4%) cases were new with no history of SARS-CoV-2 infection before, of which 3,484 (85.5%) reported COVID-19 positive. A total of 3,051 (8.6%) cases were received for follow-up with confirmed COVID-19 history and 591 (14.5%) of them reported confirmed COVID-19 positive (Table 1). Some of the follow-up cases found with COVID-19 for a longer period, found for more than 30 days.

Most cases found were 21,136 (59.3%) with ages ranging from 20 to 39 with 2,308 (56.7%) COVID-19 positive cases. Following them, 1,227 (30.1%) COVID-19 positive cases recorded out of 11,142 (31.2%) suspected cases with ages ranging from 40 to 59 (Table 1). Cases representing ages less than 20 and more than 60 are negligible in count and might indicate the laxity of infection by SARS-CoV-2.

Overall, a total of 33,697 (94.6%) asymptomatic cases were found. Only 1,933 (5.4%) symptomatic cases were found that were noticeable in positive cases (47.4%) (Table 1)

The abundance of male cases was 30,692 (86.1%) with 3,006 (73.8%) COVID-19 positive cases. There were 1068 (26.2%) COVID-19 positive cases for females out of 4,935 (13.9%) suspected cases (figure 1). Chi square test was done and found significant.

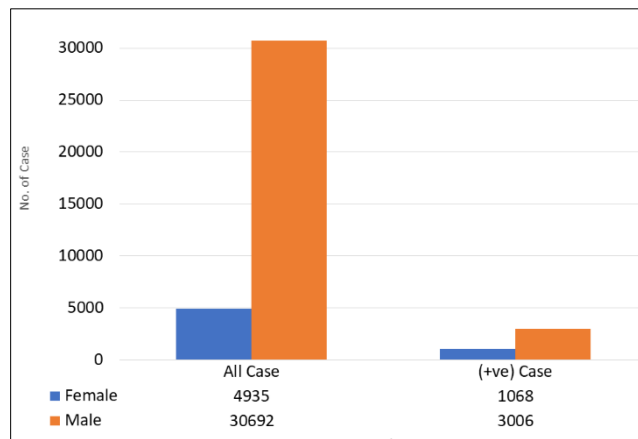


Figure 1 Male and Female ratio and rate of positivity

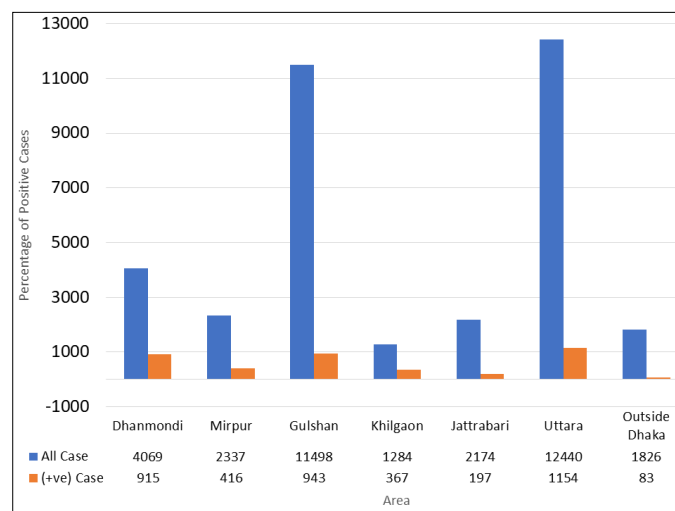
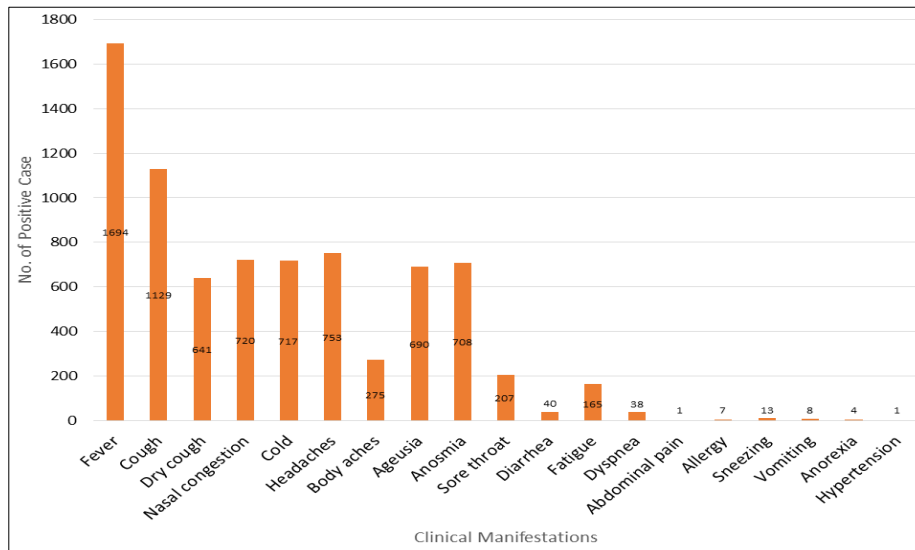


Figure 2 SARS-CoV-2 infection rates in all areas of Dhaka city

About 1,154 (28.3%) confirmed COVID-19 positive cases recorded highest out of 12,440 (34.9%) suspected cases in Uttara. Gulshan and Dhanmondi were walking behind with 943 (23.1%) and 915 (22.5%) COVID-19 positive cases out of 11,498 (32.3%) and 4,069 (11.4) suspected cases respectively (Figure 2). But considering the ratio, Khilgaon exhibits a higher SARS-CoV-2 infection rate compared to other area. Chi square test was done and p value was <0.001 which is highly significant.

In terms of clinical significance, fever and cough dominated among 1,694 (87.6%) and 1,129 (58.4%) cases respectively, where headache, nasal congestion, cold, anosmia, ageusia, and cough trailed them by severing among 753 (39.0%), 720 (37.2%), 717 (37.1%), 708 (36.6%), 690 (35.7%) and 641 (33.2%) respectively. Other symptoms arise were less severe compared to others (Figure 3)



**Figure 3** Predominant symptoms among study population

As the male (67.7-73%) individuals are more prone to the SARS-CoV-2 viral infection than female (32.3-27%) in Bangladesh <sup>40-42</sup>, current study manifests no exceptions. However, there is no clear gender bias of SARS-CoV-2 infection found from across the world, shows the data available so far. Even so, ages from all groups are prone to SARS-CoV-2 infection as the highest infection reported for middle-aged people ranging from 30-60 years according to Siam <sup>22</sup>. Approximately more than 50% cases recorded with SARS-CoV-2 infection are aged between 21 and 40 in Bangladesh <sup>2,41,43</sup> which is a clear indication of a commendatory population for SARS-CoV-2.

As, SARS-CoV-2 found to transmitted through a patient with symptoms or no symptoms at all during its incubation period, it is more likely to found several associated clinical manifestations during the COVID-19 disease condition. In the contingency table of clinical manifestations (Table 2), out of 1,933 symptomatic confirmed SARS-CoV-2 infected case, cough (1,129) featured highest (1009) association with fever (1,694). Other most associated (571-711) manifestations were cough, nasal congestion, cold, headache, ageusia and anosmia. SARS-CoV-2 infected cases also featured with body ache, sore throat, diarrhea, fatigue, dyspnea, abdominal pain, allergy, sneezing, vomiting, anorexia, and hypertension that associated along with fever, cough etc and are the most frequent features to happen. Research revealed that, ageusia (5.6-88%), anosmia (5.1-85.6%), fever (45.4-88.7%), headache (1.3-70.3%), anorexia (28.17-78.64%), cough (45.07-72.2%) diarrhea (2.0-38.1%) are the most cutaneous manifestations observed, however symptoms like sore throat (30.1%), abdominal pain (4%), body ache (13-31.1%), loss of appetite (37.9%), fatigue (70%), dyspnea etc present were noticeable but exceptional <sup>2,22,41,44-47</sup>. Compared to other studies that exhibited some rare symptoms such as allergies, constipation, red eye etc, present study also found clinical features likewise. A growing body of research relates trademarks the clinical manifestations and relevant responses during the period of SARS-CoV-2 infection, though a valuable number of cases found are asymptomatic. Cases found with SARS-CoV-2 exposers may develop symptoms within 2-14 days and the symptoms might last for one to three weeks prior to the onset of COVID-19 disease<sup>37</sup>. Clinical and epidemiological disparity of SARS-CoV-2 infection across various geographical locations have also seen.

**Table 2** Cross tabulation of symptoms

Symptoms	Fever	Cough	Nasal con.	Congestion	Headaches	Body aches	Ageusia	Anosmia	Sore throat	Diarrhea	Fatigue	Dyspnea	Abdominal P	Allergy	Sneezing	Vomiting	Anorexia	Hypertensi	Total
Fever	1694	1009	681	676	711	240	649	655	156	34	128	28	1	6	9	6	3	0	1694
Cough	1009	1129	645	621	634	115	616	618	108	17	74	25	1	4	9	3	3	0	1129
Nasal con.	681	645	720	571	590	38	584	580	30	5	19	9	1	1	0	2	0	0	720
Headaches	711	634	590	586	753	69	583	581	33	6	36	9	1	3	2	4	2	1	753
Body aches	240	115	38	17	69	275	25	28	48	16	45	13	0	2	2	4	2	0	275
Ageusia	649	616	584	574	583	25	690	651	17	7	25	8	1	1	0	0	0	0	690
Anosmia	655	618	580	584	581	28	651	708	26	7	24	7	0	1	3	0	0	0	708
Sore throat	156	108	30	22	33	48	17	26	207	2	27	8	0	0	1	1	0	0	207
Diarrhea	34	17	5	4	6	16	7	7	2	40	5	1	0	0	1	0	1	0	40
Fatigue	128	74	19	12	36	45	25	24	27	5	165	5	0	1	2	3	1	0	165
Dyspnea	28	25	9	4	9	13	8	7	8	1	5	38	0	1	1	0	0	0	38
Abdominal P	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1
Allergy	6	4	1	2	3	2	1	1	0	0	1	1	0	7	0	0	0	0	7
Sneezing	9	9	0	4	2	2	0	3	1	1	2	1	0	0	13	0	0	0	13
Vomiting	6	3	2	0	4	4	0	0	1	0	3	0	0	0	0	8	1	0	8
Anorexia	3	3	0	2	2	2	0	0	0	1	1	0	0	0	0	1	4	0	4
Hypertension	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	1694	1129	720	717	753	275	690	708	207	40	165	38	1	7	13	8	4	1	1933

### 3.1. Limitation and future scope of the study

In this study we also had some limitations as the collection and inclusion of Ct value data was a bit tough for this massive number of cases. We could not collect the vaccination history. Collection and analysis of COVID-19 associated biochemical data could enrich the potency of this research. We also could not obtain the genomic sequence of the confirmed SARS-CoV-2 specimen because of its massive sample size, but addition of sequenced data and its correlations could give us clearer etiology of SARS-CoV-2 found. Addition of vaccination or immunization history with quantitative immune protection could also make the research clearer in terms of understanding associated clinical manifestations.

## 4. Conclusion

This study encloses the situations of SARS-CoV-2 infection recorded in Novus Molecular Lab and only represents a portion of the country. It basically reflects the population that are more prone to getting SARS-CoV-2 infections living in capital Dhaka, a core of the country. Along with clinical and demographic data, this study provides us with a precise and clear vision of infection dynamics of SARS-CoV-2 during the period of twenty-one months. Similarities in prospective SARS-CoV-2 infected community with age range, gender, compared to the country, the study also reveals some unique finding such as the rate of infections towards the passengers and locals, follow-up and new cases, and the demographic overview in a monthly basis. For understanding the enormity, clinical spectrum, natural progression and to take proper control measure in case of SARS-CoV-2 infection all-round the Bangladesh or over any south Asian countries point of view, epidemiologist, virologists, or any other scientific professionals can get a sharp-edged apprehension. Foremost essence of this paper is an imbalanced risk on the extremely endangered population striving

on SARS-CoV-2 infection and encouraging to confront SARS-CoV-2 outbreak with a better understanding and preparations.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No competing interests exist by the authors. This manuscript has not submitted to, nor is under review at another journal or other publishing venue.

### *Statement of ethical approval*

This study received ethical clearance from NILMRC ethical committee.

### *Statement of informed consent*

As we collected data retrospectively from our database, so it was not necessary to take Informed consent from all participants.

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