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Short Communication

First detection of SARS-CoV-2 genetic material in the vicinity of COVID-19 isolation Centre in Bangladesh: Variation along the sewer network

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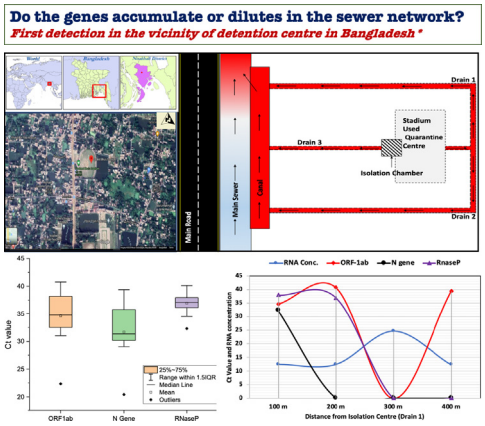
HIGHLIGHTS

- First detection report of SARS-CoV-2 RNA in the wastewaters of Bangladesh
- Probably first report on wastewater surveillance in the vicinity of COVID-19 isolation center
- Secondary/tertiary drains of sewer network exhibited RNA accumulation of SARS-CoV-2
- Distance in few meters from the excretion point has no significant influence on Ct-value.

GRAPHICAL ABSTRACT



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ABSTRACT

We made the first and successful attempt to detect SARS-CoV-2 genetic material in the vicinity wastewaters of an isolation centre i.e. Shaheed Bhulu Stadium, situated at Noakhali, Southeastern Bangladesh. Owing to the fact that isolation centre, in general, always contained a constant number of 200 COVID-19 patients, the prime objective of the study was to check if several drains carrying RNA of coronavirus are actually getting diluted or accumulated along with the sewage network. Our finding suggested that while the temporal variation of the genetic load decreased in small drains over the span of 50 days, the main sewer exhibited accumulation of SARS-CoV-2 RNA. Other interesting finding displays that probably distance of sampling location in meters is not likely to have a significant impact on the detected gene concentration, although the quantity of the RNA extracted in the downstream of the drain was higher. These findings are of immense value from the perspective of wastewater surveillance of COVID-19, as they largely imply that we do not need to monitor every wastewater system, and probably major drains monitoring may illustrate the city health. Perhaps, we are reporting the accumulation of

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Isolation Centre
COVID-19

SARS-CoV-2 genetic material along with the sewer network i.e. from primary to tertiary drains. The study sought further data collection in this line to simulate conditions prevailed in most of the developing countries and to shed further light on decay/accumulation processes of the genetic load of the SARS-COV-2.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes coronavirus disease 2019 (COVID-19), which are now being frequently reported in samples collected from the wastewater treatment plants around the world (Ahmed et al., 2020a; Haramoto et al., 2020; Kumar et al., 2020a, 2020b, 2020c; La Rosa et al., 2020; Lodder and de Roda Husman, 2020; Medema et al., 2020; Nemudryi et al., 2020; Barcelo, 2020a, 2020b, Bivins et al., 2020). However, the wastewater surveillance of COVID-19 (WWSoC-19) has mostly been reported from the wastewater treatment plants, and there is a dearth of SARS-CoV-2 RNA data in the ambient waters, and in the sewer system (Orive et al., 2020, Prevost et al., 2015; Randazzo et al., 2020; Rimoldi et al., 2020; Sherchan et al., 2020; Verbyla and Mihelcic, 2015; Wu et al., 2020; Wurtzer et al., 2020a, 2020b; Tang et al., 2020; Zhang et al., 2020). On the other hand, several developing countries like India, Bangladesh, Pakistan, and others do not have plenty of wastewater treatment plants and thus there requires a need of WBE validity and effectiveness to monitor a sewer system. The results may help the policymakers of these countries to make a decision pertaining to the national scale implementation of WWSoC-19.

Further, while the infectivity issues of SARS-CoV-2 RNA are not yet neglected or proved in the scholarly world, the public around the globe is sceptical about the wastewaters generating from the isolation centres. There have been some reports on the decay of genetic loading of SARS-CoV-2 (Ahmed et al., 2020b; Kumar et al., 2020b, 2021) in the wastewater systems, yet accumulation/decay needs to be still investigated in sewer networks. Overall, there is a lack of explicit understanding of the process of SARS-CoV-2 gene enrichment in the sewer systems along with the distance (downstream of the source/COVID-19 hotspot); and following the sewer network i.e. from small to larger drains; larger drains to the canal, and canals to the main sewer system (Kumar et al., 2021; Prevost et al., 2015; Tran, 2021).

Further, a broad observation is that most of the WWSoC-19 studies either correlated Ct-value or gene copies with the total infected person in the corresponding city or community. Uncertainties are high pertaining to the average amount of SARS-CoV-2 genes being shredded by an infected person, and its relationship with the number of genes detected during WWSoC-19. While we already know about the variations that exist in the length of viral shedding (Wu et al., 2020; Xu et al., 2020), the magnitude of the shedding keeps varying that ranges between 10^2 and 10^8 copies of RNA per gram of human waste (Lescure et al., 2020; Pan et al., 2020; Wölfel et al., 2020). The general trend has been to see the fluctuation in the Ct value and then estimate the corresponding increase or decrease of the COVID-19 patient in a given vicinity of the treatment plants. However, there has been a complete lack of studies on WWSoC-19, with known variation in infected symptomatic and asymptomatic individuals.

Accordingly, we conducted a preliminary detection survey of SARS-CoV-2 RNA in wastewater samples collected from the sewage network in the vicinity of the isolation centre at Noakhali, Bangladesh. The primary objective of the study was to understand the genetic load variation along with the sewer network in the vicinity of the isolation centre, under the preview of tracing the decay/accumulation processes of the SARS-CoV-2 RNA. We intended to contribute in policy decision regarding the WBE inclusion in developing countries by tracing the change among the primary, secondary and tertiary drains. The results are likely to appeal to the policymakers worldwide especially of the developing/low sanitation countries to adopt the wastewater surveillance for COVID-19 pandemic.

2. Material and methods

2.1. Sampling

We collected 16 samples between 10th July and 29th August 2020 from the drain, sewage, and toilets near Shaheed Bhulu Stadium Detention Centre (DC) for COVID-19 patient at Noakhali, Bangladesh. Wastewater samples were collected from the three different drains, i.e., coming out of Shaheed Bhulu Stadium at Noakhali, Bangladesh (22.8763° N, 91.0973°E), which connect to a canal (secondary drainage system) and eventually meets the main sewer system (tertiary drainage system) (Fig. 1). For this study, the sampling location was selected based on the fact that Shaheed Bhulu Stadium is the largest detention Centre for COVID-19 patients in the Noakhali district, Bangladesh. This facility has been established to accommodate more than two hundred COVID-19 positive patients for isolation purposes but kept around 200 patients all the time during the monitoring period. Hence, the prime source of SARS-CoV-2 RNA remains the isolation centre which we targeted to understand accumulation/dilution of it along with the drainage system. It is noteworthy that the main sewer is constructed as a drainage system for municipal sewage that connects the Maijdee city with ~0.1 million inhabitants in the upstream to the Bay of Bengal. In order to understand the weather during sampling, physico-chemical characteristics of sampled drains, canal and main sewer, and the number of patients being treated on the date of samples collected, information is given in supplementary data Table S1aa, b, and c.

This work is the part of a regular monitoring effort for SARS-COV-2 genes in the ambient water of isolation centre that was initiated in July 2020 following Kumar et al. (2020a) publication. Owing to several obstacles till a valid analyses and data quality, in this manuscript we included the data obtained during the one-month sampling period of August (7th to 29th August) with three main events i.e. on 7th August, 17–18th August and on 26th August 2020. Initially, we just focused on understanding the genetic decay/dilution/accumulation process along the sewer system and main interest was to find if various drains that connects to main sewer contribute to these processes, for which we had selected the distances of 100 m, 200 m, and 300 m (Table 1). However, further in order to add the discussion on the variation at 400 m and pre-condition of canal before main sewer we have re-analyzed two preserved samples (Table 1) collected in July i.e. canal samples collected on 10th July 2020 and Drain 3 at 400 (D3–4) on 26th July 2020. At these two occasions in July 2020 we did not have data at other locations, because we were checking our analytical capabilities with only two samples of drain and canal. It is worth mentioning that there were no significant changes in the rainfall, temperature, and humidity for each day during the duration of sampling (Supplementary Information-IA); as well as number of the patients in the isolation centre remains similar (Supplementary Information-IB).

Samples were aseptically collected in a 50 ml sterile falcon tube, transported in the laboratory keeping inside the ice-box, refrigerated at 4 °C during preparatory activities, and were analyzed on the same day. Sterile falcon tubes for sampling with identical blanks were analyzed to determine any possible contamination during the transport. All analyses were done at the Microbiology Laboratory of the

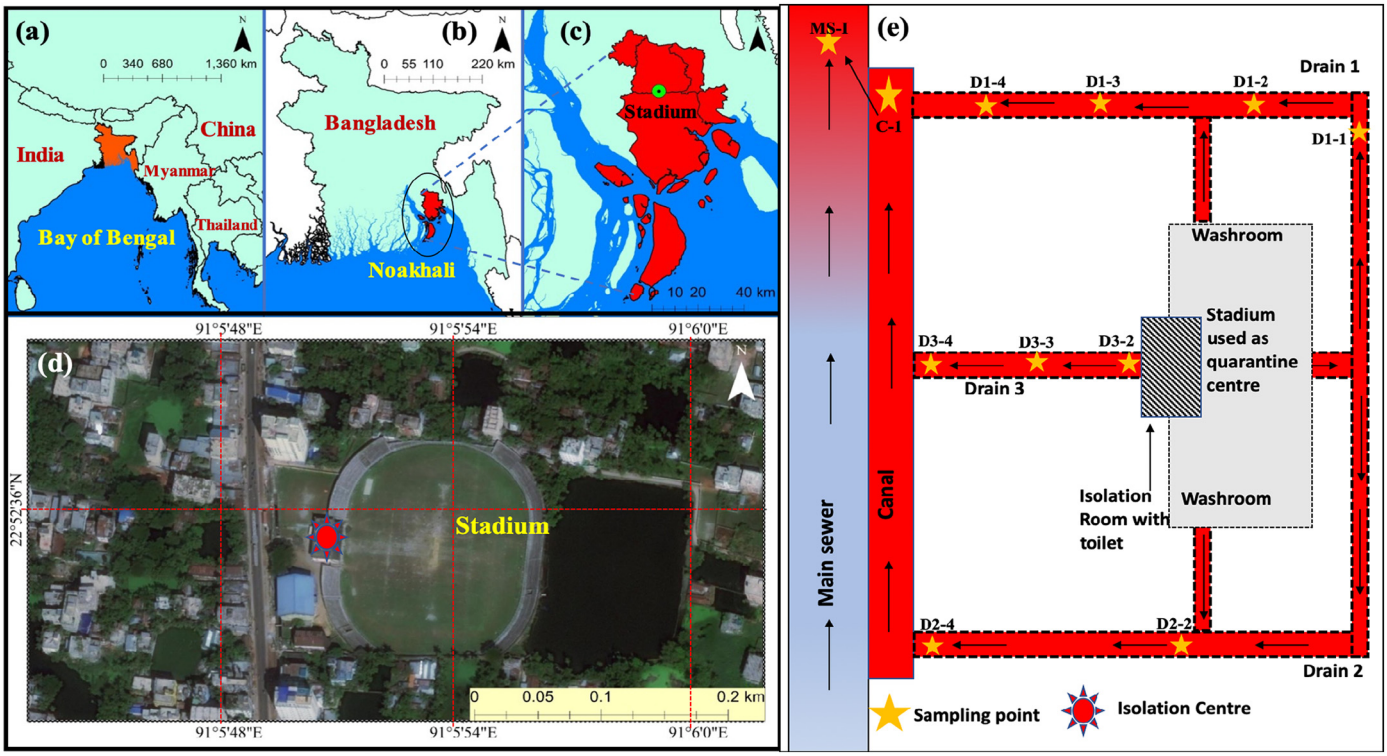

Fig. 1. Study area depicting, geographical locations of a) Bangladesh; b) Noakhali District in the Southern Bangladesh; c) Stadium location in the district; d) Stadium and isolation Centre established at the Shaheed Bhulu Stadium; and e) Schematic detail of the sampling location at various drains outlets, canal and main sewer line.

Department of Microbiology, Noakhali Science and Technology University (NSTU), Bangladesh. One argument is quite obvious that the main drain and canal may have some traces of SARS-CoV-2RNA before reaching to the vicinity of isolation centre and/or before the confluence with drains coming out of the isolation centre. However, we assume that owing to all identified patients being kept at the isolation centre, the genetic load prior to the confluence of the isolation centre's drain should remain negligible. Although, the presence of SARS-CoV-2 RNA traces cannot be nullified due to the presence of asymptomatic cases in the community.

2.2. Sample preparation, and procedure for the RNA extraction and concentration

We followed the same extraction procedure, as described by Kumar et al. (2020a). Briefly, sewage samples (50 ml) were centrifuged (Thermo Scientific) at 4500 ×g for 30 min followed by filtration of supernatant using 0.22-μm filters (Himedia). Further, each sewage filtrate was concentrated using the polyethylene glycol (PEG) method. In this method, PEG 6000 (80 g/l) and NaCl (17.5 g/l) were mixed in 25 ml filtrate, which was then incubated at 17 °C in 100 rpm shaking for overnight. The next day, the mixture was centrifuged at 13000 ×g for 90 min. The supernatant was discarded after centrifugation, and the pellet was resuspended in 300 μl RNase free water. This was further used as a sample for RNA isolation using a commercially available Favor Prep™ Viral Nucleic Acid Extraction Kit. In brief, PEG concentrated samples were transferred in a collection tube with a VNE-carrier RNA buffer. After the appropriate mixing of samples with proper incubation, a conventional column-based ethanol extraction procedure was followed using the VNE column. The RNase P (RP) primer and probe set was included with the commercial Sansure RT-PCR kit.

In addition, all the experiments were performed three times for confirmation of the results and accepted where variations were less than 10%. Covid-19 positive patient samples were used as an extraction control in each run. We employed qualitative measurement, and hence, increasing and decreasing viral load is measured based on the Ct value. RNA concentrations were measured by NanoDrop (Thermo Scientific™ NanoDrop 2000 and 2000c, BioRad) and were stored at −70 °C until further use.

2.3. RT-PCR analysis

RNAs were analyzed for the detection of SARS-CoV-2 by RT-PCR (CFX96, BioRad) using the Sansure RT-PCR kit (Sansure Biotech Inc., China). As described in the product manual, technical procedures carried out, and interpretations of results were made. In brief, we set the samples layout with RT-PCR protocol covering 45 cycles containing FAM fluorescence select for ORF1ab, ROX for N gene as well as CY5 for Internal control. As quality control measures, one positive control and one negative control were also run to validate the test procedure. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe set(s) is designed to detect RNA from SARS-CoV-2.

2.4. Methods for gene copies calculations

We used qualitative estimation of gene copies per unit of sample volume based on the correlation of the C_T value to copy numbers provided by the Sansure kit that was used for the gene detection. Correlation diagram was prepared using the principle of 3.3 C_T change equivalence to the 10-fold change in gene copies as described by Kumar et al. (2020a, 2020b). For relative quantification, we quantified extracted RNA of the SARS-Cov-2 positive samples and calculated the enrichment factor based on the least Ct value that has been described to contain 200 gene copies as per the Sansure kit protocol. A gradient with various dilutions was prepared to get linear regression lines

Table 1
Summary of the results of amplification cycles (Ct) of various sampled wastewater along with the distance of sampling in the vicinity of isolation center with 50 days temporal resolution i.e. in between 10th July and 29th August 2020.

Source	Distance (m) and Sample ID	Date of Sample collection	RNA Quantity		Ct Value (Sansure Kit)			Gene Copies (gc/ml)		
			Conc. (ng/μl)	Ratio (260/280)	FAM ORF1ab	ROX N gene	Cy5 RnaseP	FAM ORF1ab	ROX N gene	Cy5 RnaseP
Drain 1	100 (D1-1)	18-08-2020	12.42	1.97	34.69	32.25	37.91	524	1,385	725
		29-08-2020	25.5	1.95	ND	ND	36.42	ND	ND	1747
	200(D1-2)	17-08-2020	12.37	1.99	40.72	ND	36.95	14	ND	1277
	300(D1-3)	07-08-2020	24.68	1.92	ND	ND	ND	ND	ND	ND
	400(D1-4)	26-08-2020	12.23	2	39.43	ND	ND	31	ND	ND
Drain 2	200 (D2-2)	18-08-2020	13.18	1.92	36.94	35.78	37.57	137	153	886
	400 (D2-4)	26-08-2020	14.37	2.01	ND	ND	36.14	ND	ND	2060
Drain 3	200 (D3-2)	17-08-2020	17.47	1.97	36.78	35.82	39.08	151	149	363
		18-08-2020	22.61	1.98	22.35	20.42	32.3	827,624	2,225,489	19863
	300 (D3-3)	17-08-2020	15.97	1.92	31.03	29.03	37.08	4,655	10,330	11863
		26-08-2020	22.16	1.99	32.85	30.47	40.13	1,571	4,206	196
	400 (D3-4)	26-07-2020	15.31	1.95	32.84	30.35	34.52	1,581	4,533	5359
Canal	NTS	10-07-2020	27.31	1.97	40.77	39.38	38.79	14	16	431
		26-08-2020	18.14	2	32.18	30.18	35.32	2,344	5,040	3343
Main Sewer	NTS	07-08-2020	24.63	1.96	ND	ND	ND	ND	ND	ND
		18-08-2020	16	1.94	34.85	33.24	37.94	476	747	712

NTS = Distance not to scale; ND: Not Detected.

between Ct values and gene copies, and then each obtained Ct values from RT-PCR were converted into gene copies per ml of the sample.

3. Results and discussion

A summary of the results of amplification cycles (Ct) of various sampled water along with the distance of sampling in the vicinity of isolation centre with 50 days temporal resolution i.e. in between 10th July and 29th August 2020 has been presented in Table 1 and amplification plots obtained through RT-PCR illustrating temporal variation through Ct value in various sampling drains, (a) Drain 1 (b) Drain 2 (c) Drain 3, and (d) Main sewer is presented in Fig. 2.

Table 1 summarizes the Ct values obtained during the wastewater monitoring which ranged between 20.42 and 39.38 for N genes which correspond to 10,000 gc/ml to not detected, and 22.35 (8620 gc/ml) to 40.72 (ND) for ORF1ab genes, implying a huge variation in gene copies

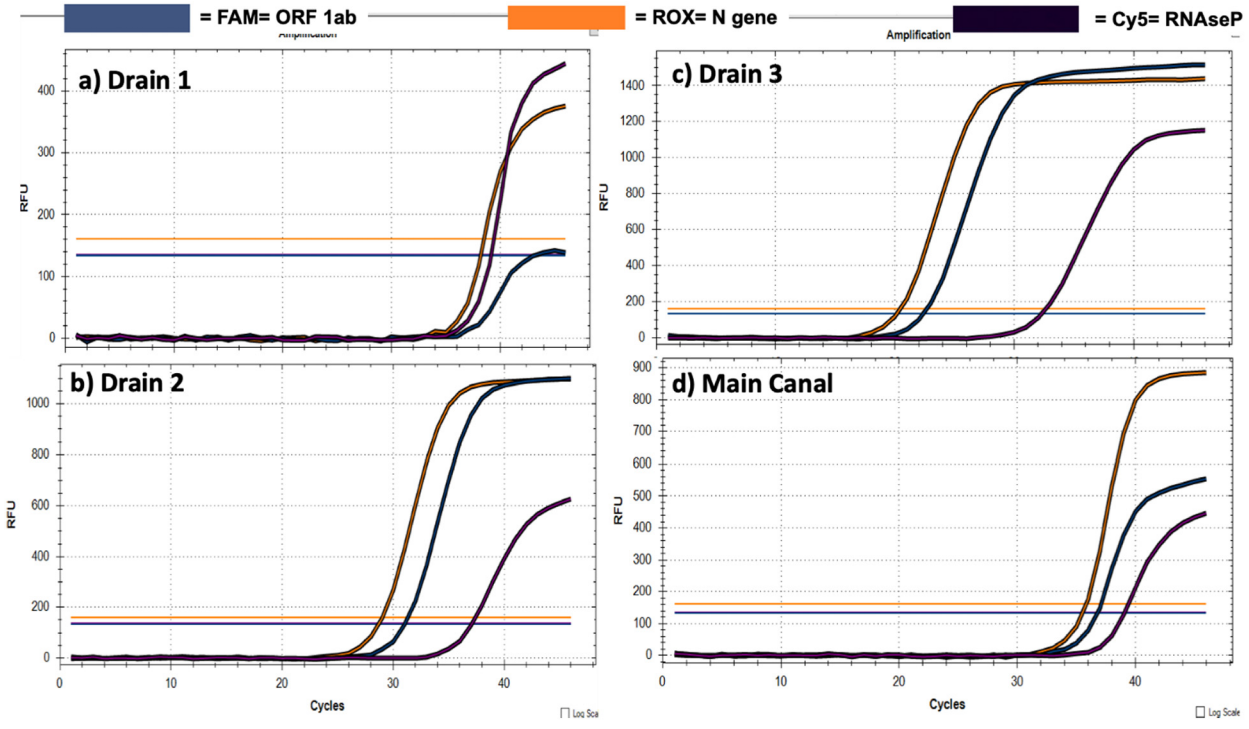

Fig. 2. Amplification plots obtained through RT-PCR in the samples from the drains and the main canal.

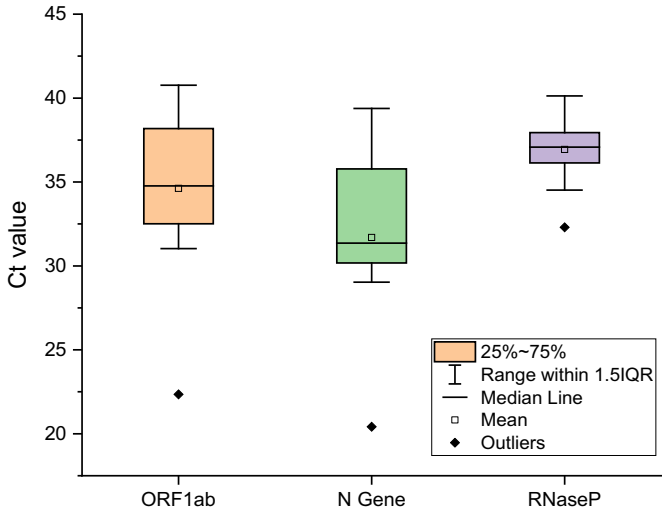

Fig. 3. Box-whisker plot illustrating the Ct-value variations of three genes for the entire monitoring period along with the outliers.

of SARS-CoV-2. Interestingly as their lowest and highest values belong to corresponding samples of the same date i.e. 17th and 26th August, it seems sewer systems played a critical factor in WWSoC-19. Other than this anomaly, 17th August samples exhibited higher loading of genetic material than 29th August 2020, while the number of patients in the containment remained the same during the monitoring period. This emphasizes the fact that just becoming COVID-19 positive is not a measure of the viruses shedding by the infected person, but perhaps the state of infection matters. It is easy to speculate that with each day passing owing to aggressive testing and capacity building of carrying out the tests, early detections of COVID-19 positive people were happening, and thus probably it is reflecting on the genetic load. Moreover, temporal environmental variations due to huge rainfall with temperature and humidity fluctuations along with inadequate sewage treatment might have an insignificant impact on the quantitative variations of SARS-CoV-2 viral genetic material.

As far as different characteristics of sampled drains are concerned, as depicted in Fig. 2, drain 3 seems to carry the greater RNA load of SARS-CoV-2 followed by drain 2 and drain 1. Although dissimilarities observed between the primary drainage line (drain samples) and secondary drainage system (canal) and tertiary drainage system (main sewer), however, a trend of higher genetic material loading in the secondary and tertiary system was also found. This is a unique finding where gene accumulation has been observed instead of the general expectation of dilution in the larger sewer system. **The probable reasoning, other than the accumulation of loading from various drains of the isolation centre, in support of this observation can be the additional contribution of RNA excreted from the asymptomatic patient as well as yet to be diagnosed people into the sewer system.**

The Ct values of different genes are presented as Box and Whisker plots in Fig. 3. The average calculated for different genes viz. ORF1ab, N genes, and RNase P were found to be 34.62, 31.69 and 36.93, respectively. N genes showed the lowest Ct values, followed by ORF1 ab and RNase P. Also, 50% Ct values (Q1 and Q2) were ranged between 29.03 and 31.36 for N genes and 31.03 to 34.78 for ORF1ab. We employed the distance factor in our sampling strategies and the results are presented in Fig. 4. While drain 3, in general showed the increased concentration of ORF1ab and N genes, drain 1 did not follow a consistent trend in genetic material loading of SARS-CoV-2 along with the distance. We tend to propose that probably distance in meters is not likely of a critical factor capable of producing a trend. However, it will be interesting to investigate the variation in results. If wastewater travels downstream more than 400 m in open drain or if sewer pipes are extended beyond 400 m.

Table 2 shows a comparative analysis of the monitoring period, percentage of positive samples detected, and range of Ct-value along with their reference (Ahmed et al., 2020a; Balboa et al., 2020; Kocamemi et al., 2020; Kumar et al., 2020a, 2020b, 2020c; Medema et al., 2020; Rimoldi et al., 2020; Wu et al., 2020; Wurtzer et al., 2020a). One finding stands out on this comparison is the Ct value of 20.42 which corresponds to much higher genetic material loading of SARS-CoV-2 than any other study reported. This may be because we sampled in the vicinity of the isolation centre, while other studies compared in Table 2 have reported the values from the wastewater treatment plant. Overall, we successfully detected ORF1ab and N protein genes from the wastewater samples of Bangladesh, which is for the first time, reported the data from the containment centre. Our study provides an opportunity to produce a realistic coefficient in the future for the conversion of genetic material loading with the number of infected people in the community.

Further, referring to the limitations of the present study, surrogate samples were sent to other laboratories to check how precisely the Sansure kit data matches with others. We also tested the filtration methods to check the efficiency of the PEG method used in this study. Although, Hata and Honda (2020) reported a high efficiency of the PEG method in wastewater collected from Japan, and the same has been found true by Kumar et al. (2020a) while testing the sample concentration efficiency between PEG and filtration. While lack of supply always poses critical challenges in any research, during the lockdown we could not find a supply of MS2, as used by Kumar et al. (2020a) or could perform the whole process control (WPC) together with MPC as recommended by Haramoto et al. (2020). We had to use a swab sample of a symptomatic person as our control as indicated by CY-5 as quality control of our analyses, which makes sense and provides a low-cost control for such analyses, yet it may be controversial, less precise and sometimes comes negative as the case in a few samples in our case (drain 1 samples at 300 and 400 m and main sewer sample on 07th and 26th August). **By applying fluorescence quantitative RT-PCR technology, this test utilized the novel Covid-19 ORF1ab and the specific conserved sequence of coding nucleocapsid protein N gene as the target regions, which were designed for conserved sequences of the double target genes to achieve detection of wastewater samples RNA through fluorescent signal changes**. The PCR detection system used the positive internal control, which monitors the presence of PCR inhibitors in test samples by detecting whether the internal control signal is normal, to avoid a false negative result when used for human RT-PCR experiments. In our experiments with wastewater, few samples were negative for CY5, indicating that the human gene RNase P gene was missing in the samples. Hence it has been noted that the human RNase P gene is more vulnerable to degradation than Covid-19 viral genes.

In the present study, we also abstained from putting any semi-quantitative calculation of gene copies due to lack of enough replicates, kit intended for the human sample, as well as uncertainties involving RT-PCR (Stuart et al., 2014). Nevertheless, the bottom line is that the patterns of obtained Ct values suggest successful detection of SARS-CoV-2 RNA from the wastewater samples in Bangladesh. Also, their increasing abundance in tertiary drain demonstrated that it is not difficult to employ the COVID-19 surveillance through wastewater in the sewer systems to know the community health as we probably do not need extensive and rigorous sampling. However, the major drains may be enough to use the capability of wastewater-based epidemiology in south-Asian settings.

Pertaining to the limitation of this study, the sampling design strategies, influences of environmental factors, the contribution of viral loads other than the symptomatic/asymptomatic patients at the isolation centre, as well as the condition of sewer (canals/drains) system are not explicitly characterised. It may also be argued that the assumption that the discharge from the isolation centre is constant due to the constant number of COVID-19 patients, can introduce a bias in the interpretation. This biasness discussion is far-fetching as WBE capabilities are

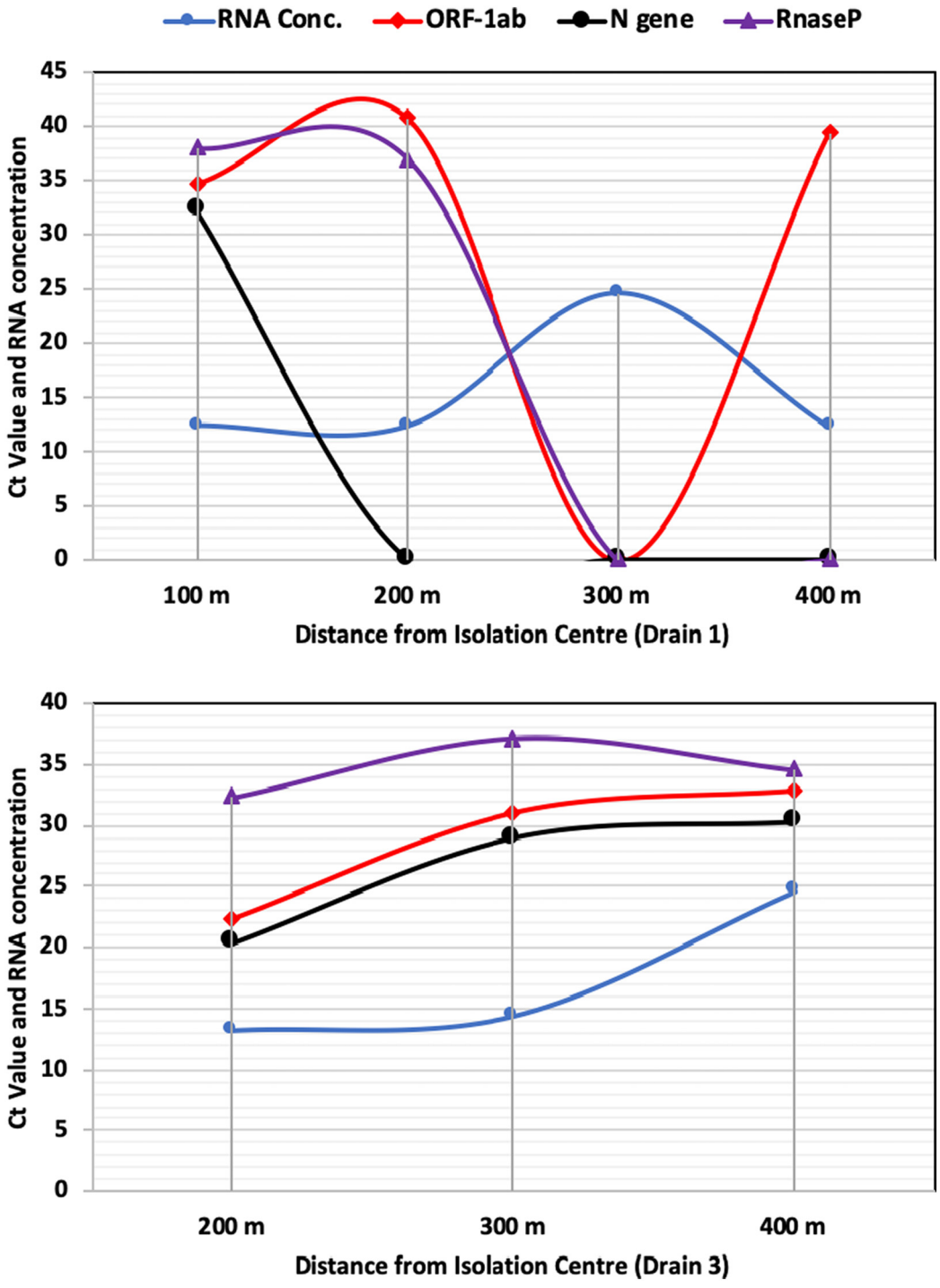

Fig. 4. Trend in genetic material loading of SARS-CoV-2 along with the distance.

Table 2
Comparative positive sample and the range of Ct value reported of ORF-lab genes of SARS-CoV-2 in the wastewater of various countries.

Country	Period of examination	Positive sample from total samples	Ct	Reference
Italy	14/04/2020–22/04/2020	4(12)	–	(Rimoldi et al., 2020)
Spain	06/04/2020–21/04/2020	7(7)	33.61–39.60	(Balboa et al., 2020)
Turkey	21/04/2020–25/04/2020	7(9)	34.67–39.54	(Kocamemi et al., 2020)
Netherlands	05/02/2020–16/03/2020	10(24)	–	(Medema et al., 2020)
France	05/03/2020–23/04/2020	100% of samples	–	(Wurtzer et al., 2020a)
Australia	20/03/2020–01/04/2020	2(9)	37.5–39	(Ahmed et al., 2020a)
U.S.A.	08/01/2020–25/03/2020	10(12)	33.87–38.39	(Wu et al., 2020)
India	04/05/2020–12/06/2020	100% of samples	32.65–34.18	(Kumar et al., 2020a)
Israel	10/03/2020–21/04/2020	10(26)	32.76–38.5	
Bangladesh	**10/07/2020–29/08/2020**	**12 (16)**	**20.42–40.77**	**(Present study, 2020)**

too limited to determine the number of viruses being shredded from the patients (before, during and after COVID-19 infection). Thus the observation still holds fairly well with or without that assumption.

Further, since the sampling of different parts of the network were not sampled on the same day, another argument is quite possible about the validity of RNA loading comparisons among different dates. However, the focus of the present study was to understand the distance impact on SARS-CoV-2 gene loading along with the sewage network under the preview of possible decay/accumulation of the same in a given sewer network. We put effort to generate information on the pertinent question related to the minimum number of samples required in a given sewer network of the community. Such information will be vital for the promotion of WBE study for COVID-19. To the best of our knowledge, this is the first- ever study aimed at understanding the accumulation and decay of SARS-CoV-2 in a sewer/ drain system that can be helpful in triggering further research, and comparisons on decay/accumulation along with the drainage system, which is vital for ascertaining monitoring locations for COVID-19.

4. Conclusions

While the wastewater surveillance of COVID-19 has been focused on wastewater treatment plants worldwide, we have opted for drain water monitoring in the vicinity of the isolation centre, which is the first of its kind. Apart from this is the first detection report of SARS-CoV-2 RNA in the wastewaters of Bangladesh. Further, the uniqueness of the study has been the tracing of the genetic load in the vicinity of the isolation centre that contains almost the constant number with 200 COVID-19 patients, which takes the variable of the number of infected persons out of the equation. This has been the key feature of this study as most of the study reported worldwide has either reported total infected person in the city or country. There has been a complete lack of infected person information contributing to the total genetic load to the sampled wastewater. We have found about 75% of our sampled water positive based on the absence or presence of ORF1ab gene. However, the critical observation has been the temporal variation where small drains showed an easing of the genetic load, while the bigger canal, and main sewer exhibited temporal accumulation of SARS-CoV-2 RNA genetic materials. On the other hand, the distance of sampling location appears to be insignificant from the perspective of wastewater surveillance of COVID-19. We intend to analyze further samples taken in June, July, and August and preserved to shed further light on the temporal variation and decay/accumulation processes of the genetic load of the SARS-CoV-2.

Ethics statement

The work did not involve any human subject and animal experiments.

CRediT authorship contribution statement

Firoz Ahmed: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Md. Aminul Islam:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Manish Kumar:** Conceptualization, Visualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Maqsud Hossain:** Conceptualization, Formal analysis, Resources. **Prosun Bhattacharya:** Data curation, Methodology, Project administration. **Md. Tahmidul Islam:** Project administration, Funding acquisition. **Foysal Hossen:** Data curation, Investigation, Visualization. **Md. Shahadat Hossain:** Data curation. **Md. Sydul Islam:** Data curation, Software, Visualization, Writing – review & editing. **Md. Main Uddin:** Investigation, Validation, Writing – original draft. **Md. Nur Islam:** Data curation, Investigation, Software. **Newaz Mohammed Bahadur:** Funding acquisition, Writing – review & editing. **Md. Didar-ul-Alam:** Writing – review & editing. **Hasan Mahmud Reza:** Writing – review & editing. **Md. Jakariya:** Conceptualization, Funding acquisition, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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