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Evidence of the presence of SARS-CoV-2 virus in atmospheric air and surfaces of a dedicated COVID hospital

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Abstract

The present study was conducted from July 1, 2020 to September 25, 2020 in a dedicated coronavirus disease 2019 (COVID-19) hospital in Delhi, India to provide evidence for the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus in atmospheric air and surfaces of the hospital wards. Swabs from hospital surfaces (patient's bed, ward floor, and nursing stations area) and suspended particulate matter in ambient air were collected by a portable air sampler from the medicine ward, intensive care unit, and emergency ward admitting COVID-19 patients. By performing reverse-transcriptase polymerase chain reaction (RT-PCR) for E-gene and RdRp gene, SARS-CoV-2 virus was detected from hospital surfaces and particulate matters from the ambient air of various wards collected at 1 and 3-m distance from active COVID-19 patients. The presence of the virus in the air beyond a 1-m distance from the patients and surfaces of the hospital indicates that the SARS-CoV-2 virus has the potential to be transmitted by airborne and surface routes from COVID-19 patients to health-care workers working in COVID-19 dedicated hospital. This warrants that precautions against airborne and surface transmission of COVID-19 in the community should be taken when markets, industries, educational institutions, and so on, reopen for normal activities.

KEYWORDS
airborne, COVID-19, epidemiology, horizontal transmission, SARS-CoV-2, virus

1 | INTRODUCTION

Since the outbreak of coronavirus disease-19 (COVID–19) in Wuhan, China, humanity has been continuously engaged in a fight for controlling this pandemic. It is evident that we have become better at controlling the pandemic compared with previous pandemics. This is attributed to technological advancements. It took only about 2 weeks to identify and publish the gene sequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative coronavirus for COVID-19, and also to develop a diagnostic method for its detection using the reverse-transcriptase polymerase chain reaction (RT-PCR).[1–4] In addition, unprecedented progress has been made in other aspects of the disease. The roles of Angiotensinogen Converting Enzyme-2 (ACE-2) receptors and other molecules, cytokine storm, and coagulation dysregulation in its pathogenesis are established.[5–7] Serosurveillance by immunoassay of antibodies has been developed.[8–10] Many drug trials have been carried out that established the role of dexamethasone, anti-retroviral agents, hydroxychloroquine (HCQ), ivermectin, tocilizumab, curcumin, and so on, in the treatment of COVID-19 with some degree of success.[11–17]

Scientists have developed various vaccines against COVID-19 and these are under evaluation.[18–21] However, we do not have clear knowledge about the routes followed by the virus during transmission from one individual to others.[22,23] As of now, the general notion is that the virus gets transmitted either through droplets or through direct contact with an infected person or by touching the surfaces/ fomites where an infected person has shed the virus.[22–30] The virus enters the human body through the nose, mouth, or eyes. However, whether the virus spreads through air is far from clear, although some reports are present, which have suggested considering airborne precautions.[22,23] At a virtual workshop entitled "Airborne Transmission of SARS-CoV-2" conducted by the National Academies of Science, Engineering, and Medicine, various field experts reviewed the available data related to transmission routes of the virus along with its historical context.[31] The evidence for airborne transmission of SARS-CoV-2 is admittedly incomplete. They also stated that the evidence for it not being airborne is also incomplete. The evidence for transmission of this virus through air remains inconclusive. It is therefore imperative to address this issue and provide evidence of its being air-borne if it is so.

In India, the index case was detected in Thrissur, Kerala on January 30, 2020 and by the end of October 2020, the total number of confirmed cases crossed 81 million.[32] Various measures are being taken here to manage this pandemic. Mild cases are isolated at home or in isolation centers created for this purpose. Few hospitals with intensive care unit (ICU) care facilities are dedicated to treating moderate and severe cases of COVID-19 patients only. Lok Nayak Hospital at New Delhi, India, where the study has been conducted, is one among those hospitals. Despite the best precautions, many health-care workers working in these hospitals got infected and a few of them died.[33–35] Air-borne transmission or transmission by touching surfaces is

suspected in the transmission of COVID-19 in the hospital set up.[36–38] Hence, this study was conducted to collect the data from this COVID-19 dedicated hospital to find out whether the SARS-CoV-2 virus spreads beyond 1 m from COVID-19 patients through air and if the viral concentration in air decreases with increase in distance from the active patients. The study also explored the presence of SARS-CoV-2 viral RNA on different surfaces of wards admitting COVID-19 patients.

2 | METHODOLOGY

2.1 | Study sites

The collaborative study was conducted by the Departments of Biochemistry, Microbiology, Maulana Azad Medical College in collaboration with the Departments of Medicine and Anaesthesia, Lok Nayak Hospital (LNH), and CSIR-National Physical Laboratory, New Delhi, India from July 1, 2020 to September 25, 2020. Delhi, the capital city of India having more than 20 million population is listed as one of the most populated megacities of the world. Delhi according to India Meteorological Department (IMD) has four different seasons, that is, winter (January–February), summer or pre-monsoon (March–May), monsoon (June–September), and post-monsoon (October–December). Winters are chilly (temperature can drop to ~ 2°C) and observe intense fog and haze events. Summers are generally very hot and dry (temperature can go up to ~ 47°C) and with frequent dust storms. Swabs and particulate matter from the air were collected from (a) emergency ward, (b) medicine ward, and ICU of LNH which was declared as a dedicated COVID-19 hospital.

2.2 | Study design

It was an in vitro study.

2.3 | Ethical approval

Written informed consent was obtained from the participants ≥18 years of age before they were recruited for the study. The study protocol was approved [no: F.1/IEC/MAMC/(77/05/2020/No.143) dated June 19, 2020] by the Institutional Ethics Committee (IEC) of Maulana Azad Medical College (MAMC) and associated Lok Nayak Hospital (LNH), University of Delhi, New Delhi, India.

2.4 | Selection of cases

Six patients per day who were suffering from COVID-19 (RT-PCR confirmed from their nasopharyngeal and nasal swab) with moderate illness were selected randomly among the COVID-19 patients

admitted to the medicine ward within the last 48 h. This protocol was repeated on 6 different days not necessarily consecutive. Moderate illness was evidenced by the presence of lower respiratory disease on clinical examination and/or imaging (X-Ray or computed tomography (CT) scan of chest) during this period. These patients had high-grade fever, cough and sneezing, and SpO_2 levels above 94% in room air at the time of sample collection. They were asked to breathe out, talk, cough, or sneeze on a 47 mm diameter polyvinylidene difluoride (PVDF) membrane filter having 100 nm pore size (M/s. Millipore Corp) fixed on a petri dish by always keeping the petri dish within one foot from his mouth for 15 min. Immediately, the filter was placed with the help of forceps into a 15 ml centrifuge tube containing 5 ml of viral transport media (VTM) and then transported in an icebox to the laboratory for detection of SARS-CoV-2 by RT-PCR. The positive report confirmed that these patients were releasing the SARS-CoV-2 virus from their respiratory tract and was defined as active cases. A swab from the bed and floor and particulate matter from the air nearby the area of the patient were collected only if the patient turned out to be an active case.

Similarly, on 6 different days, six different patients suffering from RT-PCR confirmed COVID-19 with severe illness requiring admission to ICU within the last 48 h for high flow oxygen therapy or invasive ventilator support were selected randomly among the COVID-19 patients admitted to ICU. As these patients were very sick and on noninvasive or invasive ventilator support to perform the above-mentioned activity, it was not checked if they were active cases or not. In the emergency ward, the COVID-19 patients were getting clinically evaluated, receiving immediate management, and were sent to either the medicine ward or ICU depending on the treatment need. So the patients were in transit and were staying for few minutes to few hours in the emergency ward. Whether these cases were active or not was not checked when particulate matter from the air was collected from the emergency ward.

2.5 | Collection of particulate matters from environmental airì

Total suspended particulate (TSP) air sampler, (M/s. Vayuvodhan, Okhla Industrial Area, New Delhi) which was calibrated as per national standards by CSIR-NPL, India was used for collecting suspended particulate matter from the air. The air sampler used in this study was portable and handy with three sample collection passages for which flow rate was fixed at 1.5, 16.7, and 27 litre per minute (LPM). The air sampler consisted of an anodized aluminum body fitted with a vacuum motor that pulled the ambient air onto the 47 mm diameter PVDF membrane filters having 100 nm pore size (M/s. Millipore Corp) placed on sample collection passages.

Particulate matters from the ambient air of the medicine ward were collected by using the above-mentioned air sampler keeping it at a distance of 3 m from the head end of the selected active COVID cases. Then the process was repeated by keeping the air sampler at 1-m distance from the selected patients. The air sampler was placed on a table to cover up the height of the patients' bed.

From ICU, suspended particulate matters were collected similarly by keeping the air sampler at 1- and 3-m distance from the selected cases who were on noninvasive ventilator support.

In the emergency ward, particulate matter from the air was collected by keeping the air sampler at the center of the ward. The TSP samples were collected for a 1-h period at every instance.

After a collection period of 1 h, PVDF membranes were removed from the air sampler, placed with forceps into 15 ml centrifuge tubes containing 5 ml VTM and sent similarly for RT-PCR.

For collection of negative control samples ($n = 3$), particulate matter from the air was collected from the green zone (area without any known COVID-19 patients) of Maulana Azad Medical College, New Delhi.

2.6 | Collection of swabs from surfaces

Swabs were collected from various surfaces to check the presence of SARS-CoV-2. On the surfaces of ward and ICU. The swab samples were collected randomly from 2.0 square feet area of the selected patient's bed, ward floors (within 1 m from selected patients' bed), and tables placed in the nursing working station of the medicine ward and ICU. The collected swabs were placed in 5 ml of VTM and transported to the laboratory in an icebox for RT-PCR testing.

For negative controls, we collected swab samples ($n = 3$ each) from floors and tables placed in the green zone of Maulana Azad Medical College, New Delhi.

2.7 | Detection of SARS-CoV-2 virus by RT-PCR

The PVDF membranes and swabs added to VTM were sent to the microbiology laboratory for the detection of the SARS-CoV-2 virus by using RT-PCR. A tube containing swab/membrane was mixed thoroughly for 3 min by using vortex mixture, centrifuged at 3000 rpm in a clinical centrifuge for 3 min and 200 µl of supernatant VTM was used for extraction of RNA by using a fully automated nucleic acid extraction system (Magna Pure, Roche) utilizing Magna Pure 96 viral RNA Large Volume Kit (Roche) according to the manufacturer's instructions. The RT-PCR technique, which specifically targets E-gene for common coronavirus and RNA-dependent RNA polymerase (RdRp) gene for SARS-CoV-2 was used for the detection of SARS-CoV-2 (SD Biosciences). The details of primers and probes used were as described by Afzal[2] are depicted in Table 1. This STANDARD M nCoV Real-Time Detection kit was clinically evaluated by the manufacturer and independently for SARS-CoV-2 detection. The performance of the kit in terms of positive predictive value (PPV) and negative predictive value (NPV) are 100% (95% confidence interval [CI]: 88.65%–100%) and 100% (95% CI: 88.65%–100%) by the manufacturer and 100% (95% CI: 93.0%–100%) 99% (95% CI: 95.0%–100%) independently.[2,38]

Briefly, 25 µl reaction was set up which contained 4 µl of the template RNA, 14 µl of a primer and probe mixture (2019-nCoV

TABLE 1 The details of the target genes and their primers, probes, and sequences used for detection of the SARS-CoV-2 virus

Target gene	Size and position of amplicon	Primers and probes (Dye)	Sequence (5'–3')
ORF 1ab (RdRp) gene	101 bp	ORF gene-forward primers	5'GTGARATGGTCATGTGTGGCGG3'
	(15,431–15,330)	ORF gene-reverse primers	5'CARATGTTAAASACACTATTAGCATA3'
		ORF gene probe (FAM)	5'CAGGTGGAACCTCATCAGGAGATGC3'
E-gene	112 bp	E - gene-forward primers	5'ACAGGTACGTTAATAGTTAATAGCGT3'
	(26,269–26,381)	E gene-reverse primers	5'ATATTGCAGCAGTACGCACACA3'
		E - gene probe {JOE (VIC or HEX)}	5'ACACTAGCCATCCTTACTGCGCCTTCG3'

Abbreviations: ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Reaction Solution) containing "a mixture of Taq polymerase, deoxyribonucleotide triphosphates (dNTPs), and magnesium chloride", 6 µl of RTase mix, 0.5 µl of carboxy-X-rhodamine used as reference dye and 0.5 µl of internal control A. The thermal cycling profile consisted of one cycle of 50°C for reverse transcription for 15 min, one cycle of 95°C for initial denaturation for 3 min and five cycles of preamplification consisted of 95°C for 5 s and 60°C for 40 s. The final amplification profile consisted of forty (40) cycles of denaturation at 95°C for 5 s, 60°C for 40 s. The results of RT-PCR were expressed as cycle threshold (C_t) values. A cycle threshold less than 35 was interpreted as positive for SARS-CoV-2. If RT-PCR gave a C_t value of <35 for both the E-gene and RdRp gene, the sampling was considered positive for SARS-CoV-2.

2.8 | Statistical analysis

Collection of particulate matter from the air was carried out on 6 different days using different patients and similarly, swabs were collected from surfaces on three different occasions. Quantitative data (C_t value) was presented as mean and standard deviation. Qualitative data (+ve RT-PCR) were presented as a percentage. C_t values were compared by unpaired t test or one-way analysis of variance followed by Tukey's post hoc test. A $p < .05$ was considered statistically significant.

3 | RESULTS AND DISCUSSION

As shown in Table 2, the RNAs of SARS-CoV-2 were detected in atmospheric air at a distance of 1 and 3 m from the active COVID-19 patients of the medicine ward and ICU and as well as in the emergency ward of the dedicated COVID-19 hospital. RT-PCR positivity rate was higher when particulate matter was collected at a 1-m distance than that collected at a 3-m distance from COVID-19 patients. (Table 3).

Viral load, in terms of cycle threshold (C_t) values for both the RdRp gene and E-gene of SARS-CoV-2 varied inversely with distance from the patients (Figure 1). It indicates that SARS-CoV-2 is present in atmospheric air of hospitals treating COVID-19 patients and its concentration decreases with the increase in distance from the cases. Droplets produced during breathing, coughing, sneezing, or talking usually get settled within 1 m.[22,23] However, the presence of SARS-CoV-2 RNA at a 3-m distance from the patients indicates that air-borne transmission of virus, probably through micro-droplets is possible, at least in the hospital environment, although viral load decreases with distance possibly due to (a) settlement of droplets within 1 m and (b) dilutional effect on micro-droplets (that can move beyond 1 m) on their migration to distant places, which is now affirmed by World Health Organization.[22]

As shown in Table 4 and Figure 1, PCR positivity rate and the C_t values of E-gene and RdRp gene at 1 and 3-m distances from COVID-19 patients in ICU did not differ from those at the medicine ward. It indicates that viral concentration in air of ICU does not differ from that in the medicine ward despite various medical procedures (intubation, suction, etc.) that are conducted in ICU more frequently producing lots of aerosols.[23,39] However, one factor that might contribute to this observation is that number of patients and patient: ward area ratio in the ICU were always lower than that in the medicine ward.

The air in the emergency ward was also found to be contaminated with SARS-CoV-2 virus (Table 2). Athough the patients stay here for very short duration, yet the viral load in air was similar to that in atmospheric air of ICU and medicine ward (as found at 3-m distance from the patients). Hence, we presume that chance of acquiring COVID-19 by air-borne route from the emergency ward is not less than that of ICU and medicine ward. Work stations in medicine ward and ICU had a glass separation from the area where patients were admitted and in the air of those work station areas, SARS-CoV-2 RNA was not detected (Table 4). Therefore, airborne transmission of COVID-19 is not probable from these walled nursing work-station areas. But in the emergency ward, the workstation did not have any wall or glass barrier to keep it separate from the environment where patients were admitted. Hence, the chance of air-borne transmission for health-care workers from work-station at emergency ward might be present.

Patients' bed and floor of the wards but not the tables in the work stations of nurses in medicine ward and ICU were found to

TABLE 2 Results of RT-PCR for E-gene and RdRp gene of SARS-CoV-2 virus performed with suspended particulate matter obtained from atmospheric air of medicine ward, ICU, and emergency ward of a dedicated COVID hospital

Location	Distance from the COVID + ve patient	Different days	Flow rate in TSP air sampler in liter/minute	E-gene		RdRp-gene	
				+/−	C_t value	+/−	C_t value
Medicine ward admitting ~ 50 mild to moderate COVID-19 patients. (area 1000 × 600 Feet²)	One meter from the head end of the patients	Day 1	1.5	Positive	32.14	Positive	29.6
			16.7	Positive	23.12	Positive	26.5
			27	Positive	26.11	**Positive**	**16.11**
		Day 2	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	27.51	Positive	29.1
			27	Positive	27.2	Positive	23.12
		Day 3	1.5	Positive	28.69	Positive	31
			16.7	Positive	22.08	Positive	26.3
			27	Positive	24.65	**Positive**	**22.41**
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	25.3	Negative	Negative
			27	Positive	23.6	Positive	18.2
		Day 5	1.5	Positive	31	Negative	Negative
			16.7	Positive	23.2	Positive	24.09
			27	Positive	18.32	Positive	21.82
		Day 6	1.5	Positive	32.08	Positive	29.4
			16.7	Positive	26	Positive	25.9
			27	Positive	16.11	Positive	19.3
	Three meters from the head end of the patients	Day 1	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 2	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Positive	28.1	Positive	31.8
		Day 3	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	21.11	Positive	29.85
			27	Positive	29.7	Positive	34.1
		Day 5	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 6	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative

(Continues)

TABLE 2 (Continued)

Location	Distance from the COVID + ve patient	Different days	Flow rate in TSP air sampler in liter/minute	E-gene		RdRp-gene	
				+/−	C_t value	+/−	C_t value
ICU admitting ~20 patients with severe COVID-19. (area: 200 × 50 Feet²)	One meter from the head end of the patients	Day 1	1.5	Positive	30.1	Positive	29.81
			16.7	Positive	29.32	Positive	27.51
			27	Positive	23	Positive	18.41
		Day 2	1.5	Positive	26.3	Negative	Negative
			16.7	Positive	29.2	Positive	24.15
			27	**Positive**	**24.3**	Positive	17.26
		Day 3	1.5	Positive	27.5	Positive	30.2
			16.7	**Positive**	**30.2**	**Positive**	**26.87**
			27	Positive	21.68	Positive	19.47
		Day 4	1.5	Positive	26.7	Positive	30.26
			16.7	Positive	19.8	Positive	25.51
			27	Positive	19.11	Positive	22.4
		Day 5	1.5	Positive	30.7	Positive	29.11
			16.7	Positive	25.3	Positive	24.98
			27	Positive	20.3	Positive	16.84
		Day 6	1.5	Positive	30.4	Negative	Negative
			16.7	Positive	27.02	Positive	24.52
			27	Positive	21.66	Positive	20.1
	Three meters from the head end of the patients	Day 1	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Positive	31	Negative	Negative
		Day 2	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 3	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	30.2	Positive	32.89
			27	Positive	32.5	Positive	30.5
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	**Positive**	**29.9**	**Positive**	**33.67**
		Day 5	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Positive	31.9	Positive	33.25
		Day 6	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
Emergency Ward with variable number of patients ~3-4/H.	Air sampler placed at the center of Emergency ward	Day 1	1.5	Positive	29.5	Negative	Negative
			16.7	Positive	27.03	Positive	33.69

TABLE 2 (Continued)

Location	Distance from the COVID + ve patient	Different days	Flow rate in TSP air sampler in liter/minute	E-gene		RdRp-gene	
				+/−	C_t value	+/−	C_t value
(area: 150 × 100 Feet²)			27	Positive	26.66	Positive	31.89
		Day 2	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	30	Positive	34.01
			27	Positive	27.07	Positive	29.9
		Day 3	1.5	Positive	29.91	Negative	Negative
			16.7	Positive	26.33	Positive	27
			27	Positive	28.11	Positive	24.09
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Positive	26.66	Positive	30
			27	Positive	28.21	Positive	26.3
		Day 5	1.5	Negative	Negative	Negative	Negative
			16.7	**Positive**	**28**	**Positive**	**29.01**
			27	Positive	30.23	Positive	32.04
		Day 6	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
Nursing station area of medicine ward separated from the patients by glass wall. (area: 20 × 15 Feet²)		Day 1	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 2	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 3	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 5	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 6	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
Nursing station area of ICU separated from the patients by glass wall. (area: 20 × 20 Feet²).		Day 1	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 2	1.5	Negative	Negative	Negative	Negative

(Continues)

TABLE 2 (Continued)

Location	Distance from the COVID + ve patient	Different days	Flow rate in TSP air sampler in liter/minute	E-gene		RdRp-gene	
				+/−	C_t value	+/−	C_t value
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 3	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 4	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 5	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative
		Day 6	1.5	Negative	Negative	Negative	Negative
			16.7	Negative	Negative	Negative	Negative
			27	Negative	Negative	Negative	Negative

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; RT-PCR, reverse-transcriptase polymerase chain reaction; TSP, Total suspended particulate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 3 Frequency of SARS-CoV-2 detection in air samples collected at 01 and 03- m distance from COVID-19 patients in medicine ward and ICU

Site of sample collection	Distance from the patients	No. sample collected	SARS-CoV-2 positivity rate (%)	C_t Value (RdRp gene)
Medicine ward	1 m	06	6/6 (100%)	25.31
				27.43
				24.56
				23.12
				28.92
				27.56
	3 m	06	02/06 (33%)	34.32
				33.09
ICU	1 m	06	06/06 (100%)	22.32
				23.12
				21.32
				25.79
				19.18
				19.23
	3 m	06	03/06 (50%)	24.54
				23.00
				21.76

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

contain RNA of SARS-CoV-2 (Table 4) indicating possible surface transmission of COVID-19 through contaminated surfaces from patients' beds and ward floors. It is a known mode of transmission of COVID-19.[22] So, we surmise that hospital-acquired COVID-19 infection by health- care workers may occur through these contaminated surfaces.

As shown in Figure 2, it was found that chance of detecting SARS-CoV-2 viral RNA is less when flow rate of air sampler is fixed at 1.5 LMP for 1 h and that probability increased with increase in flow rate to 16.7 and 27 LMP. Therefore, we recommend that TSP collection should be done for 1 h at flow rate of 16.7 and/or 27 LPM for capturing SARS-CoV-2 from air for such studies.

RT-PCR test results were negative with all negative air and swab control samples from green zone. This implies that SARS-CoV-2 virus detected by RT-PCR from air suspended particulate matters and swabs from the wards of COVID-19-dedicated hospital samples are less likely to be due to false-positive result. Three limitations of this study are: (a) detection of virus was done by RT-PCR (which is a sensitive but less specific method than when accompanied with sequencing of PCR products) and not by viral culture as these facilities are not available in our set up. So, we cannot comment if the RNAs detected are from live virus or from dead ones, (b) we did not determine the corona species for the samples that showed positive test for corona virus (E-gene) but negative test for SARS-CoV-2 (RdRp-gene), and (c) the study was conducted in a hospital and not in the community, hence we need to be cautious while extrapolating these data to claim airborne transmission of COVID-19 in the community.
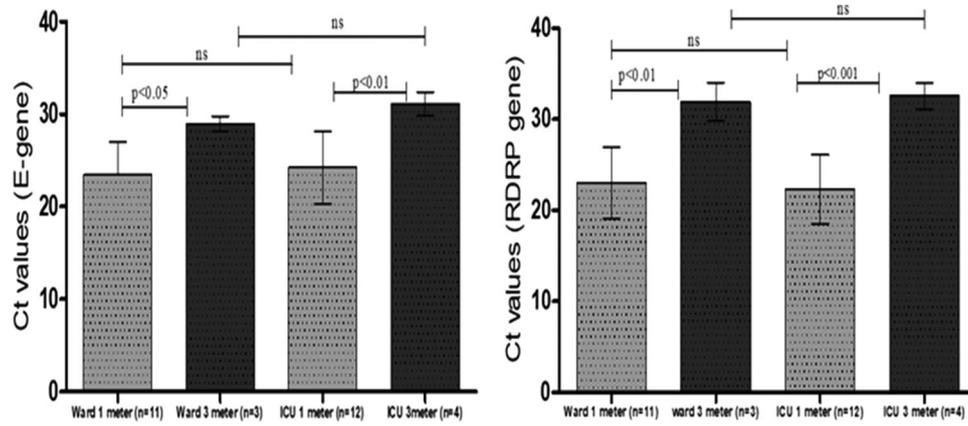
FIGURE 1 Bar diagram showing mean and standard deviation (*SD*) of cycle threshold (C_t) values of RT-PCR test for E-gene and RdRp gene of SARS-CoV-2 virus conducted with particulate matter obtained from air at distance of 1 and 3-m from COVID-19 patients at medicine wards and ICU. Mean and *SD* were calculated from the samples, which were positive for both E-gene and RdRp gene and collected through channels of air sampler that was set at air flow rate of 16.7 and 27 liters per minute. COVID-19, coronavirus disease 2019; ICU, intensive care unit; RT-PCR, reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

**TABLE 4** Results of RT-PCR for E-gene and RdRp-gene of SARS-CoV-2 virus performed with swabs collected from patients' beds, floor, and nursing working stations at the medicine ward and ICU of a COVID-dedicated hospital

Different days	Location	Site of sample collection	CORONA (E-gene)		COVID (RDRP gene)	
			+/−	C_t value	+/−	C_t value
Day 1	ICU	Patients' bed	Positive	19.58	Positive	18.61
	ICU	Floor	Positive	30.6	Positive	27.36
	ICU	Nursing station	Negative	Negative	Negative	Negative
	Medicine ward	Patient bed	Positive	28.9	Positive	22.52
	Medicine ward	Floor	Positive	33.8	Negative	Negative
	Medicine ward	Nursing station	Negative	Negative	Negative	Negative
Day 2	ICU	Patients' bed	Positive	28.76	Positive	19.54
	ICU	Floor	Positive	28.9	Positive	23.33
	ICU	Nursing station	Positive	34.02	Negative	Negative
	Medicine ward	Patient bed	Positive	29	Positive	19.55
	Medicine ward	Floor	Positive	32	Positive	23.43
	Medicine ward	Nursing station	Negative	Negative	Negative	Negative
Day 3	ICU	Patients' bed	Positive	21.69	Positive	18.55
	ICU	Floor	Positive	28.81	Negative	Negative
	ICU	Nursing station	Positive	30.12	Negative	Negative
	Medicine ward	Patient bed	Positive	26.72	Positive	22.13
	Medicine ward	Floor	Positive	32.54	Negative	Negative
	Medicine ward	Nursing station	Negative	Negative	Negative	Negative

Abbreviations: COVID, coronavirus disease; ICU, intensive care unit; RT-PCR, reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
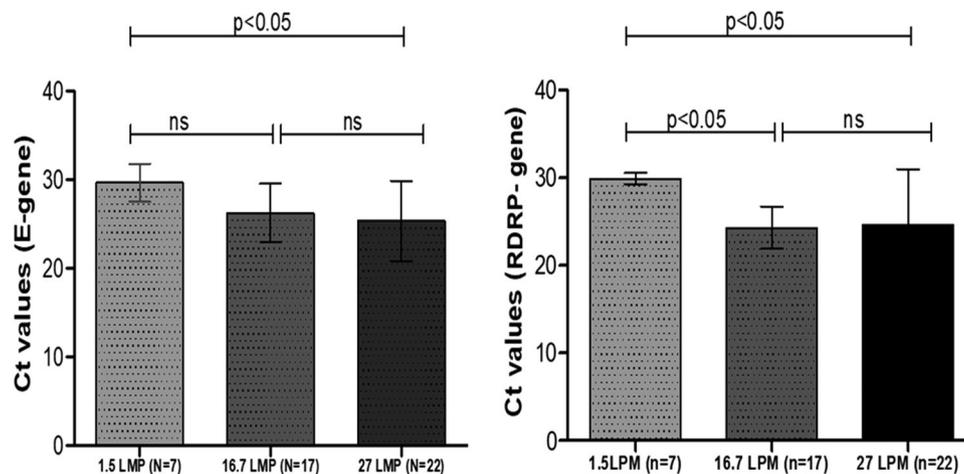
FIGURE 2 Bar diagram showing mean and standard deviation (*SD*) of cycle threshold (C_t) values of RT-PCR test for E-gene and RdRp gene of SARS-CoV-2 virus conducted with particulate matter obtained from air of different wards admitting COVID-19 patients when the flow rate of air sampler was adjusted to 1.5, 16.7, and 27 liter per minute (LPM). Mean and *SD* were calculated from the samples those were positive for both E-gene and RdRp gene irrespective of distance from the patient at which the air sampler was placed. COVID-19, coronavirus disease 2019; RT-PCR, reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

4 | CONCLUSIONS

Environmental air and surfaces of the hospitals treating COVID-19 patients are contaminated with SARS-CoV-2 virus indicating potential airborne and surface transmission of SARS-CoV-2 from the patients. The viral load was similar in the atmospheric air of ICU and medicine ward indicating a similar chance of transmission at both the places. Similar kind of experimental data in localities having COVID-19 patients would be useful for further policy making to prevent the spread of COVID-19 infection in the community.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHORS CONTRIBUTIONS

Conceived and designed the experiments: Abhishek Dubey, Subash C. Sonkar, Vijay K. Singh, Tuhin K. Mandal, Bidhan C. Koner, and Binita Goswami. *Carried out the experiments*: Abhishek Dubey, Subash C. Sonkar, Vijay K. Singh, Garima Kotnala, Aastha Bansal, Monica Irungbam, Farah Husain, and Sameer A. Guru. *Collection of clinical samples*: Abhishek Dubey, Subash C. Sonkar, Vijay K. Singh, Monica Irungbam, Aastha Bansal, Garima Kotnala, and Sameer A. Guru. *Performed the analysis*: Binita Goswami, Sonal Saxena, Farah Husain, Sudhir K. Sharma, Ravindra K. Kotnala, Tuhin K. Mandal, Chhemendra Sharma, Kirti N. Saxena, Dinesh K. Aswal, Suresh Kumar, Vikas Manchanda, and Bidhan

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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