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Surface contamination by SARS-CoV2 RNA in dedicated COVID care area of a tertiary care hospital in North India

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Abstract Environmental surfaces are potential source of SARS-CoV2 transmission. The study assessed the efficacy of hospital disinfection policy and contamination of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) RNA in COVID management Hospital. Inanimate surfaces from both patient areas (n=70) and non-patient areas (n=39) were sampled through surface swabbing and subjected to Reverse transcriptase PCR. Out of the 70 samples collected from the COVID hospital, SARS-CoV2 RNA

positivity of 17.5% (7/40) and 6.7% (2/30) was seen in high risk and moderate risk area respectively. Samples from Non COVID related patient area such as CD ward and administrative block were assessed and the SARS CoV-2 RNA positivity was 0% and 10% respectively. Among the total 8 environmental surface samples positive for SARS-CoV2 RNA detected from the area surrounding the SARS-CoV2 infected patients, maximum positivity of 31.8% (7/22) was found among the environmental samples collected around

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the patients with < 20 Ct value in nasopharyngeal swab samples followed by 3.3% positivity (1/30) around patients with Ct value ranging from 20 to 25 whereas no SARS-CoV2 RNA (0/5) was detected around the patient with > 25 Ct value. Nearly 50% (2/4) of the surface samples came positive from the resident PPE and mobile of the treating doctors which largely elaborates the need for stringent doffing measurement and hand hygiene policy post doffing. The study emphasizes the necessity of frequent and aggressive disinfection policy to prevent nosocomial infection in such high risk areas within close vicinity of the patients.

Keywords Disinfection · Hospital infection control · Nosocomial infection · RT-PCR · Surface contamination

Introduction

The pandemic of Coronavirus disease 2019 (COVID-19) due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a novel β-coronavirus, has posed an unprecedented public health emergency all throughout the globe. As of May 22, 2022, there have been 521,920,560 confirmed cases of COVID-19, including 6,274,323 deaths, reported to World Health Organisation (WHO) in which India attributed to 43,131,822 confirmed cases [1]. Since its origin from Wuhan, China SARS-CoV2 person to person transmission observed to be the most common mode of transmission through respiratory droplets [2, 3]. Such a situation in nosocomial settings, would create huge public health impact in terms of collapsing the health care facility and might result in community transmission as well [4]. WHO estimation indicates 14% of COVID-19 cases occurring among the healthcare workers with an upsurge of 35% in some countries [5].

Environmental contamination by patients infected with SARS-CoV2 can occur through respiratory droplets and also through fecal shedding. This can be a potential source of hospital-acquired infection (HAI) of SARS-CoV2 virus posing health hazard for both patients and health-care professionals [6]. In fact, nosocomial outbreaks by coronaviruses in the form of hospital clusters have been previously reported for both SARS and Middle-East Respiratory Syndrome (MERS) coronaviruses [7]. Considering the virus viability for up to 28 days at 20 °C from surfaces such as glass and stainless steel the assessment of environmental surface contamination by SARS-CoV2 in hospital setup has become of paramount importance [8]. In view of the ongoing current SARS-CoV2 pandemic, the present study aimed to detect potential areas of surface contamination by SARS-CoV2 RNA in the environment around COVID-19 confirmed cases admitted for treatment in a dedicated COVID-19 management section of tertiary care hospital of North India catering to a wide geographical region. The findings helped in identifying the areas more prone to contamination so that those areas can be focused for more frequent and stricter adherence to disinfection procedures and hand hygiene practices.

Materials and methods

Study sites

The study was carried out in several areas of Nehru Hospital Extension (NHE) block of Post Graduate Institute of Medical Education and Research, Chandigarh, India which has been designated as COVID management hospital for the tricity (Chandigarh, Mohali and Panchkula). The NHE block of PGIMER comprised of COVID ICU, the high risk areas with critically ill patients, COVID wards as moderate risk area with mild to moderate disease cases and the administrative block in ground floor being the low risk area. In addition to the NHE, the environmental samples from Communicable disease (CD) ward which is located in main hospital (separate building) were also collected. The CD ward being designated for screening and sampling of Covid-19 suspected patients. The samples were collected between 1st May and 15th July, 2020. The study was approved by the Institute Ethics Review Committee (NK6769/study/125).

Sampling area and sample collection

High risk areas

Samples from COVID ICU were categorized as (1) samples within close vicinity of SARS-CoV2 confirmed patients (within three feet radius of patient bed) such as floor adjacent to the patients, bedrails (Side and below), table, High Flow Nasal Canula Oxygen (HFNCO) pipe, fluid bottle and monitor button (2) samples not within the close proximity of SARS-CoV2 confirmed patients included those collected from medicine table, medicine refrigerator handle, nursing table (horizontal and vertical), ICU mobile, ICU telephone, doffing area door knob and doffing area floor. Emergency section of NHE block through which SARS-CoV2 patients are being admitted was also considered as high risk area and sampling sites included the corridor, lift floor and lift switches.

Moderate risk area

Samples from COVID ward consisted of samples collected from bed rails, mobile, room floor, switches and restrooms utilized by SARS-CoV2 confirmed patients such as bathroom doorknob, wash basins and commodes. Sampling was done from the personal protective equipment (PPE), Vizor, shoes and mobiles of resident doctor immediately after attending the patients.



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Low risk areas

These samples were collected from the administrative areas and included lift floors, lift switches, clean area of donning room, mobiles of administrators and doctors, tables, door handles, floors of corridor as well as meeting room etc.

Non Covid patient area

From CD ward room table, mobile, main floor, sample collection room floor, nurse room floor, hospital attendant's room floor, switches, doffing area, PPE kit were also assessed for environmental contamination of SARS-CoV2 virus RNA.

The nylon flocked swabs were used for the study. The swabs were dipped in viral transport media (VTM) and were rubbed by moving the swab in two different directions while rotating sticks with gentle pressure over a recommended surface area of 25 cm² of the study surface and floor. The wet swabs were transferred immediately into a vial containing 1 ml. of viral transport medium with Tween 80 to neutralize the effect of residual disinfectant. The collected VTMs were wrapped in absorbent material and placed within the secondary receptacle with absorbent material and transported to the Department of Virology, Post Graduate Institute of Medical Education and Research, Chandigarh.

SARS CoV2 RNA detection by real time PCR

The samples were processed as per standard protocol following all infection control measures within a class II A2 biosafety cabinet. Approximately 140 µl of the sample was used for RNA extraction by Qiagen RNA extraction kit (Qiagen, Germany). The extracted RNAs were subjected for qualitative real time PCR for SARS-CoV2 RNA as per kit availability. Samples from COVID ward of NHE and CD ward of Nehru hospital were screened by RealStar SARS-CoV2 RT PCR kit (Altona, Germany) targeting E gene as screening gene and S gene as the confirmatory gene as per the manufacturers' instructions. Samples from the COVID ICU of NHE and Administrative ward of NHE (Ground floor) were tested in National Institute of Virology (NIV, Pune) SARS CoV2 assay where E gene was used as screening assay along with RNase P gene as internal control and ORF gene and RdRp genes as confirmatory assay.

Statistical analysis

Statistical analyses were performed using SPSS version 20.0 software (SPSS Inc.). The differences in the positive

rates were compared by x^2 test. A 2-sided α of less than 0.05 was considered to be statistically significance.

Results

A total of 70 samples from the environmental surfaces related to SARS-CoV2 patient area and 39 samples from Non SARS-CoV2 areas were screened. Out of the 70 samples collected from the COVID hospital, 57.1% (40/70) were collected from high risk areas and 42.9% (30/70) of samples were collected from moderate risk area. The SARS-CoV2 RNA was detected in 17.5% (7/40) of samples of high risk area and 6.7% (2/30) of samples from the moderate risk area. The samples collected from Non COVID related patient area included 19 samples from CD ward and 20 samples from the administrative block of NHE and the SARS CoV-2 RNA positivity was 0% and 10% (2/20) respectively (Fig. 1A).

High risk area (NHE Adult COVID ICU and Emergency)

Of the 35 samples collected, twenty seven were collected from environmental surface [SARS-CoV2 infected patient's surrounding floor, Bedrails Side, bedrails below, table, High Flow Nasal Canula Oxygen (HFNCO) pipe, fluid bottle and monitor button] from four admitted patients from COVID ICU where SARS-CoV2 RNA was detected among 22.2% (6/27) samples (Table 1). At this point of time, there were 4 patients admitted in the ICU and the nasopharyngeal swab samples from these patients labeled as Patient 'A', 'B', 'C', 'D' had Ct values of screening gene (E/N) of SARS-CoV2 were 20, 22, 18 and 32 respectively. Samples collected from fluid bottles of two patients showed the presence of Human RNase P gene though they were negative for SARS-COV2 RNA is suggestive of the presence of salivary droplets around the patients. No SARS-CoV2 RNA was found in COVID ICU environmental surfaces which were not within close vicinity of the admitted patients. Floor plan of COVID-19 ICU depicting a representative SARS-CoV2 confirmed sick patient and surrounding environment assessed for suspected SARS-CoV2 RNA contaminated surfaces is shown in Fig. 2. Among the sampling done from the emergency area of NHE situated in the ground floor (n=5), only emergency area corridor floor showed the presence of SARS-COV2 with positivity rate of 20% (1/5).

Moderate risk area (COVID isolation ward)

30 environmental surface samples were collected from various areas of cabin and restrooms used by 4 patients admitted there at the time of sample collection and



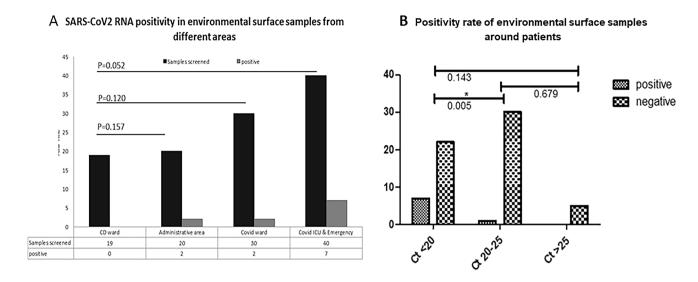


Fig. 1 A SARS CoV2 RNA positivity in environmental surface samples from different areas of a tertiary care hospital B Positivity rate of environmental surface samples around patients with lower to higher Ct values admitted in COVID ICU and Isolation ward

Table 1 Distribution of SARS-CoV2 RNA positivity from various environmental surfaces in high risk area consisting of adult COVID ICU and emergency area

Study site	Environmental sites	Samples tested	Samples positive for SARS-COV2 RNA	
		n=35	n=6	
COVID ICU				
Close vicinity of the patients [Patient	Floor next to the bed	4	1	
"A", "B", "C", "D"]	Bed rail side	3	1	
	Bed rail below	4	2	
	Table adjacent to bed	4	1	
	HFNCO pipe	4	1	
	Fluid bottle	4	0	
	Monitor switch	4	0	
Not within close vicinity of the patients	ICU telephone, ICU mobile, Doffing area door handle, Doffing area floor, Medicine table, Medicine refrigerator door handle, Nurse table horizontal, Nurse table vertical	8	0	
		N=5	N = 1	
COVID emergency				
	Floor near lift	1	0	
	Doffing area floor	1	0	
	Lift switch	1	0	
	Corridor area floor	1	1	
	Barrier area floor	1	0	

6.7% (2/30) samples were found to be SARS-CoV2 RNA positive (Table 2). The nasopharyngeal samples from Patient 'E', 'F', 'G', 'H' had Ct values of screening gene (E/N) of SARS-CoV2 were 16, 30, 29 and 32 respectively.

Environmental surface positivity around patients with high viral load (low Ct value)

Among the total 8 environmental surface samples positive for SARS-CoV2 RNA detected from the area surrounding



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Diagrammatic representation of environmental sampling sites inside COVID ICU

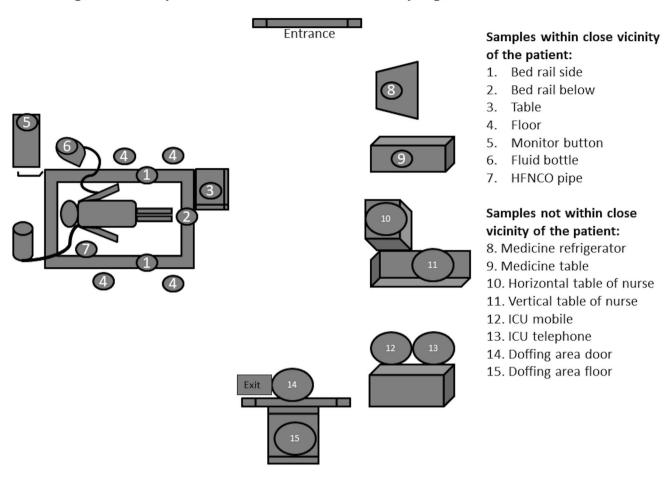


Fig. 2 Floor plan of COVID 19 ICU depicting a representative SARS-CoV2 confirmed sick patient and surrounding environment assessed for suspected SARS-CoV2 RNA contaminated surfaces

Table 2 Distribution of SARS-CoV2 RNA positivity from various environmental surfaces in moderate risk area consisting of COVID Isolation ward

Study site	Environmental sites	Samples screened	Positive for SARS-CoV2 RNA
		n=30	n=2
COVID ward			
From environmental surfaces of Room and restroom used patients ["E", "F", "G" & "H"]	Floor of the room	4	0
	Bedrails side#	3	1
	Bedrails below	4	0
	Mobile ^{\$}	3	1
	Electric switch	4	0
	Bathroom doorknob	4	0
	Washbasin	4	0
	Commode	4	0

^{*}Patient "H" did not have side bedrails



^{\$}Patient "F" did not have mobile

the SARS-CoV2 infected patients, 6 were detected from the high risk area (COVID ICU) and 2 were detected from the COVID isolation ward. Maximum positivity of 31.8% (7/22) was found among the environmental samples collected around the patients with < 20 Ct value in nasopharyngeal swab samples followed by 3.3% positivity (1/30) around patients with Ct value ranging from 20 to 25 whereas no SARS-CoV2 RNA (0/5) was detected around the patient with > 25 Ct value (Fig. 1B).

Low risk area

From the ground floor of NHE, PGIMER which is used as administrative area 20 environmental surface samples were collected with a positivity of 10% (2/20). The distribution of the samples screened and detection of SARS-CoV2 RNA are shown in the Table 3.

SARS-CoV2 in CD ward samples

None of the environmental surface samples (n=19) collected from the CD ward patient area which is used as COVID sampling from suspected patients showed the presence of SARS-CoV2 RNA. The samples included floors of various rooms (n=8), furniture (n=2), PPE kit (n=2),

Table surface (n=1), mobile of doctor (n=1), door knob (n=2), bed rail (n=1), Switch (n=2).

Sampling from the resident doctor

Samples were collected from resident doctor post examination of SARS-CoV2 confirmed patients in COVID isolation ward. Resident shoes and vizor were not contaminated with SARS-CoV2 however surface swab samples collected from Residents PPE and mobile showed the positivity of SARS-CoV2 RNA.

Discussion and conclusion

Stability of SARS-CoV2 on environmental surfaces attribute to hospital acquired infection by SARS-CoV2. Surveillance of environmental surfaces to prevent transmission from contaminated areas is required for effective intervention to prevent hospital acquired infection. In our study, we aimed to detect SARS-CoV2 RNA in high to low risk area surfaces surrounding patients infected with SARS-CoV2 and outcome measured in terms of number of contaminated surfaces and the number of contaminated PPE. Of 113 screened samples, 10.9% (11/109) of patient care area found to be infected

Table 3 Distribution of SARS-CoV2 RNA positivity from various environmental surface samples in low risk area not frequented by patient

Study site	Environmental sites	Samples screened	Positive for SARS-COV2 RNA
		n=20	n=2
Ground floor of NHE			
Administrator's mobile and mask (0/4)	Mobile	3	0
	Mask	1	0
Surface of the floor (0/5)	Endocrinology doffing room clean area floor#	1	0
	Committee room floor	1	0
	Corridor floor near committee room	1	0
	HDU doffing area floor clean area#	1	0
	ICU doffing area floor clean area#	1	0
Surface of the table (0/3)	Committee room table	1	0
	Committee room entrance table	1	0
	Isolation area entry table	1	0
Lift (0/2)	Lift switch	1	0
	Lift floor	1	0
(Arterial blood gas analysis) ABG area (1/4)	ABG machine	1	0
	ABG table surface	1	0
	ABG ramp floor	1	1
	ABG ramp table	1	0
Others (1/2)	Donning area locker	1	1
	Committee room washroom handle	1	0

^{*}Located at the second floor of NHE building



demonstrates a satisfactory hospital disinfection policy in our hospital and 72.7% (8/11) positive sample were identified in the close vicinity of the patients which emphasizes a need of frequent disinfection of areas and equipment surrounded by patients. Targeted measures such as creation of physical barriers between patients and treating doctors may be recommended to prevent nosocomial infection. The top most site in terms of infection rate was found to be the bed railings surrounding the patients so special emphasis should be given for frequent disinfection of the surface of bed rails by infection control personnel. One unique finding in the study was the presence of housekeeping genes in the surface areas surrounding the patients which is possibly suggestive of substantial aerosol and viral dispersion through coughing and endotracheal suction in critically ill patients. This necessitates frequent and aggressive disinfection policy to prevent nosocomial infection in such high risk areas within close vicinity of the patients. Similar study carried out in a hospital of South Korea reported SARS-CoV-2 positivity in 10 of 57 (17.5%) samples from areas surrounding the patients including the Ambu bag and infusion pump [4]. Ong et al. demonstrated that resident shoes could be a source of SARS-CoV2 transmission [6] though in the current study such observation was not found however 50% (2/4) surface samples came positive from the resident PPE and mobile after treating patients which largely elaborates the need for stringent doffing measurement and hand hygiene policy post doffing.

The ground floor of the NHE is being used as an administrative area and environmental positivity in the locker handle and ABG ramp floor is of concern. The locker situated here is used by the staff to store their personal belongings and clothes before changing into surgical scrubs and PPE. Subsequently the health care staffs go to the COVID areas situated from the 1st to 3rd floor of the same building. The doffing areas are situated on these floors from where the personnel come back to the ground floor after PPE doffing and hand hygiene. The detection of SARS CoV-2 RNA on the locker handle indicates possible improper hand hygiene post doffing. The current study is a cross-sectional observational study in nature. The study was carried out at a time when the bed occupancy of COVID dedicated hospital was high. Even though the number of observation was less, it still represents about the nature of surface contamination that can occur in and around the SARS-CoV2 positive patients as well as areas of the COVID dedicated hospital which are not frequented by SARS-CoV2 positive patients. Samples collected from surfaces like floor adjacent to patient bed, bed rails, fluid bottle, mobile, door knobs in the COVID ICU, COVID emergency and COVID ward representative of the surfaces with increased chances of SARS-CoV2 exposure and/or contamination and comparison was done in this study with surfaces of non-COVID areas as the effort was made to evaluate surface contamination of SARS-CoV2 from majority of the surfaces of the COVID hospital. However, the current study could not assess the surface contamination of the those areas in repeated manner since access to such high risk areas was only for limited time period and limited individual as per hospital policy of the study period. Still for further validation of the surface contamination due to SARS-CoV2, large sample size would be required for SARS-CoV2 as well as for other respiratory viruses.

As a part of hospital disinfection policy, spraying 1% sodium hypochlorite is being implemented although spraying disinfectants might not cover the corners of the floor so wiping in "S" shaped manner might yield a better result [9]. Strict adherence to the hospital infection control policy is a key to prevent any nosocomial transmission of SARS-CoV2 among health care workers. The study explored the viral RNA contaminated surfaces which will be the key focus area for disinfection on priority in resource limited settings. Current study elucidated the viral presence in terms of RNA and build up a disinfection policy with respect to the surfaces that are priority for surface disinfection. The frequency of surface disinfection in viral RNA negative surfaces which in term means absence of virus can be done with lesser frequency as compared to areas where RNA has been found and are more prone for harbouring infectious agents. In our study, virus viability of the contaminated SARS-CoV2 virus particles in the form of culture was not done which is the limitation of the study as the presence of viral RNA on surfaces does not equate to the presence of infectious viral particles [10]. However, the current study could highlight the areas with presence of RNA as representative of areas with increased chances of having viral particles. Study conducted in Hong Kong by Cheng et al. found 7.7% (1/13) positivity of SARS-COV2 in environmental samples and the authors described that through their escalated infection control policy they managed to prevent nosocomial transmission of SARS-CoV2 among 413 health care workers caring for the SARS-CoV2 laboratory confirmed patients [11]. Similar study conducted in tertiary care referral hospital in northern Italy collected sixteen swabs from inanimate surface samples located at high risk containment area inside the wards and found all inanimate surface samples were free from SARS-CoV2 RNA contamination [12]. Maximum positivity rate of environmental contamination (39.3%; 44/112) was documented in a study conducted by Li Wei et al. where 4 environmental samples were found positive in an area occupied by asymptomatic COVID positive patients [13]. Wu et al. elucidated overall positivity of 19% among 200 environmental samples studied with a positivity rate in isolation ward and ICU of 25% and 37.5% respectively [9]. In our study, positivity rate of 6.7% (2/30) in COVID isolation ward and 17.1% (6/35) in COVID ICU were found among environmental surface samples studied.



Ong et al. conducted environmental surface samples around one patient with Ct value of 25.6 indicating higher viral shedding and surface samples collected around the patient had a positivity rate of 80% (16/20) [6]. Our study also corroborated the finding and detected statistically significant number of environmental surface samples collected from COVID ICU and COVID isolation ward i.e. 31.8% (7/22) around the patients with <20 Ct value whereas 3.3% positivity (1/30) around patients with Ct value ranging from 20 to 25. Environmental samples were also collected surrounding two other patients with Ct value of 31.3 and 35.3 and none were found to be positive. The current study also failed to detect any positivity in environmental surface samples collected from the surrounding of the patient with Ct value of > 25 in nasopharyngeal swabs. The role of fomites in transmission of SARS-CoV-2 has been indicated in surface survival studies which show a 99% reduction in infectious SARS-CoV-2 and other coronaviruses under typical indoor environmental conditions within 3 days (72 h) on common non-porous surfaces [14]. The current study aimed to detect the surfaces more prone to surface contamination by SARS-CoV-2 in different patient care areas considering the role of fomites in virus transmission. Risk of surface contamination mediated SARS-CoV-2 transmission through fomites is albeit low but still present during the first 72 h of a person with suspected or confirmed COVID-19 being introduced to an indoor space. With continuous influx of positive cases in the indoor patient care facilities, we felt that there was need to reduce the surface contamination as far as practicable by regular disinfection as suggested by CDC [14].

Thus, the study highlighted the increasing chances of environmental positivity and potential source of transmission when the patient is harbouring the virus with a Ct value < 25, however a large sample size as well as sampling at various intervals post disinfection would have been more informative on the dynamics of the virus shedding and efficacy of disinfection procedures followed in the COVID hospital set up.

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Declarations

Conflict of interest Authors declare that they have no conflict of interest.

Consent for publication All authors have read the manuscript and approved the final manuscript.

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