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Analysis of surface contamination of severe acute respiratory syndrome coronavirus 2 in a health-care setting in the context of the coronavirus disease-2019 pandemic

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Abstract:

BACKGROUND: Hospital-onset coronavirus disease-2019 (COVID-19) infection has been reported and is probably linked to ineffective implementation of infection prevention and control measures. Contaminated surfaces and air are considered a key part of the transmission dynamics of severe acute respiratory syndrome, Middle East respiratory syndrome, influenza, and other organisms in hospitals. This study aimed to assess the extent and persistence of surface contamination with COVID-19.

MATERIALS AND METHODS: It was a hospital-based cross-sectional study conducted for a period for 2 weeks from December 03, 2020, to December 16, 2020, in Kathua district of J and K, India. The environmental samples were taken from the patient care area that included COVID isolation ward and intensive care unit (ICU) as per the guidelines of WHO Protocol "Surface sampling of COVID-19: A practical "how to" protocol for health care and public health professionals after seeking copyright permission from the WHO. Universal standard precautions were strictly followed. Descriptive analysis was done using the MS-Excel and expressed in numbers and percentages.

RESULTS: A total of 140 surface samples were taken, 70 each from the COVID ICU and isolation ward. The results of ten samples from the ICU turned out to be positive and 20 samples were positive from the isolation ward. Eleven (78.6%) out of the 14 samples taken from the corners of the ICU and isolation ward were found to be positive.

CONCLUSION: Our study revealed surface contamination in the hospital setting both in COVID ICU and isolation ward particularly from the corners of the COVID ICU and isolation ward followed by the samples taken from the linen. Strict adherence to COVID appropriate behavior, increased frequency of disinfection in high-risk areas, and sensitization of the staff are mandatory to minimize the infection risk.

Keywords:

Coronavirus disease, hospital based, infection control, severe acute respiratory syndrome coronavirus 2

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Introduction

The novel human coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been declared as a pandemic, causing an unprecedented change in the lifestyle of the global community. It causes a range of problems in humans, but the respiratory tract infection is the most prominent way it affects humans. The novel coronavirus spreads when an infected person releases respiratory droplets carrying the coronavirus, thereby contaminating his immediate surroundings and exposing a healthy person to this contaminated environment. The coronavirus has been associated with nosocomial outbreaks with

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the best contaminated environment for the virus to spread and mutate.[1] Respiratory droplets and aerosols released by an infected person not only contaminates the air but also the surfaces when these droplets settle down from the air. Although the common route through which these contaminated droplets enter our body is through nose and mouth, exposing the mucous membrane of eyes to this virus may also lead to infection. Hospital-onset COVID-19 infection (HOCI) has been associated with the spike in coronavirus disease (COVID) cases and could be a byproduct of the compromised infection prevention and control protocols adopted in the hospitals.[2,3] The transmission dynamics are still not fully understood in health-care environments and could be multi-factorial. Communicable diseases causing viruses and other microbes, such as SARS, Middle East respiratory syndrome, and influenza usually transmit through air and contaminated surfaces in hospitals.[4,5] Laboratory study suggests that the SARS-CoV-2 virus could easily survive in aerosols and on dry surfaces for days, especially on nonporous surfaces.[6,7] Besides, SARS-Cov-2 RNA has been predominantly detected in the environment that is most exposed to a COVID patient admitted in a COVID-dedicated hospital.[8] In this study, reverse transcriptase-polymerase chain reaction (RT-PCR) method was used to detect SARS-CoV-2, as part of the epidemiological investigation. The primary focus area was on the surfaces that come regularly in contact with an infected person admitted in the hospital facility to assess the extent of relation between the contaminated surfaces and HOCI. The ongoing pandemic demand an evaluation to assess the infection control practices pertaining to COVID-19 in patient care area and other areas of care. The study will be first of its kind in this part of the world. The study will help in improving and will guide to understand the role of environmental contamination in the transmission of COVID-19 and prevent further spread of COVID-19. The objective of our study was to assess the extent and persistence of surface contamination of COVID-19 and to identify any surface contamination of SARS-CoV 2 virus in the hospital setting. Till date, no similar study has been conducted in J and K. Based on study finding, it would further help in adopting vigorous Infection Control Policy in hospital setting and thereby preventing the spread of disease.

Table 1: Description of the samples taken

Sample code	Sample description
ES 1	Door knobs of main entrance
ES 2	Mobile phone of patients
ES 3	Stethoscope of consulting doctor
ES 4	Bed side table/wall
ES 5	Pulse oximeter used by patients
ES 6	Chair of hospital staff members dealing with patients
ES 7	Light switch
ES 8	Corners of ward floor
ES 9	Faucet of water tap
ES 10	Bed linen

ES= Environmental sample

Materials and Methods

Study design and setting

It was a hospital-based cross-sectional study conducted for a period for 2 weeks from December 03, 2020, to December 16, 2020.

Table 2: Reverse transcriptase-polymerase chain reaction results of environmental samples from intensive care unit for severe acute respiratory syndrome coronavirus-2

Days	Door knobs	Mobile phone of patient	Stetho - scope	Bed side table	Pulse oximeter	Chair	Light switch	Corners	Faucets of water tap	Bed linen
1	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
2	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
3	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
4	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
5	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
6	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative

Table 3: Reverse transcriptase-polymerase chain reaction results of environmental samples from isolation ward for severe acute respiratory syndrome coronavirus-2

Days	Door Knobs	Mobile phone of patient	Stetho - scope	Bed side table	Pulse oximeter	Chair	Light switch	Corners	Faucets of water tap	Bed linen
1	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Positive
2	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
3	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
4	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
5	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Negative	Positive
6	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive
7	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive

Sample collection

The environmental samples were taken from the patient care area that included COVID isolation ward and intensive care unit (ICU) as per the guidelines of WHO Protocol "Surface sampling of COVID-19: A practical "how to" protocol for health care and public health professionals after seeking copyright permission from the WHO. Surface samples were taken for a total of 14 days, 7 days each from the COVID ICU and isolation ward. Ten samples were taken on each day under the strict aseptic conditions.

Samples (Swabs in VTM vials) collected from the various areas in the environment of ICU and isolation wards were labeled ES one (ES1), ES two (ES2), ES three (ES3) up to ES ten (ES10) with date of collection and received in the RT-PCR laboratory for COVID-19 testing. These samples after getting designated by a laboratory ID were stored at −20°C till they subject to testing or as soon as possible.

RNA Extraction

RNASure Viral RNA Extraction kit (Genetix) was used for RNA Extraction.

Steps included were:

1. 560 µl of buffer VLB1-containing Carrier RNA was added to 150 µl of sample fluid and mixed thoroughly by vortex followed by incubated at the room temperature for 10 min
2. 560 µl of ethanol (96%–100%) was added and mixed thoroughly by pulse vortex (10–15s)
3. RNASure* Virus mini column was placed in collection tubes and 700 µl of lysate was loaded and centrifuged for one min at 8,000 × g. Fresh collection tubes were taken and loaded with residual lysis solution onto the column and centrifuged at 8,000 × g for 1 min. The flow through was discarded and the column was put in the fresh collection tube
4. 500 µl of buffer VWB1 was added to the column and centrifuged for 1 min at 8,000 × g. The flow through was discarded. This step removes contaminant PCR inhibitors
5. 750 µl of buffer VWB3 was added to the column and centrifuged for 1 min at 8,000 × g. The flow through was discarded along with the collection tube and the column transferred into a fresh collection tube
6. Step 5 was repeated for complete removal of wash buffer
7. The column was centrifuged at full speed (14,000 × g) for 1 min to dry it. The flow through was discarded along with the collection tube
8. The column was placed into elution tube and 50 µl of RNase free H_2O (preheated to 70°C) was added to it and incubated for 1–2 min. The content was centrifuged at 14,000 × g for 1 min
9. The viral RNA was stored at −70°C.

Master mixing and reverse transcriptase-polymerase chain reaction

PCR Workstation JCA-09A (Microteknik) was ultraviolet irradiated for 10 min and then alcohol sterilized before use. Meril COVID-19 One Step RT-PCR Kit was used for detection of COVID-19 using the real-time PCR.

The first step involved homogenization of the lyophilized Enzyme mix with enzyme mix buffer and RNase free H_2O in the fixed volume ratio of 4:5, respectively, and then kept for 30 min for stabilization. The Master Mix was prepared by mixing re-suspended enzyme mix with COVID-19 Primer Probe mix and RNase free H_2O in the fixed volume ratio of 9:1:5, respectively, and multiplied by the number of tests to attain the final volume. The Master Mix so prepared for 96 wells PCR plate was distributed in the fixed volume of 15 µl in each well. The extracted RNA (5 µl each) was mixed with master mix (15 µl) in each well designated for individual or pooled samples sparing the first well for negative control (DEPC treated H_2O) and the last well for positive control (Viral RNA) provided in the kit. The PCR plate with reactions was then sealed properly and carefully with the transparent sealer followed by spinning the plate with horizontal vortex (REMI) for settling PCR reactions mix without air bubbles at the well's bottoms.

The plate was then loaded onto the plate chamber of Real-Time PCR System 7500 Fast Dx of Applied Biosystems by Thermo Fischer Scientific. The test panel was setup according to the positions of positive control, negative control and RNA samples in the settings of the program. The real time PCR program was adopted for targeting the specific conserved sequence encoding the ORF1ab gene and the nucleoprotein N gene. The PCR amplification program included two stages and the reactions swing between the two.

Stage 1 involves reverse transcription of extracted RNA at 50°C for 15 min followed by cDNA initial denaturation at 95°C for 3 min for one repetition. Stage 2 involves short denaturation period at 95°C of 15 s followed by annealing, extension and fluorescence measurement at 55°C for 40 s up to the forty repetitions of this cycle followed by cooling at 25°C for 10 s.

The positive and negative results depended both on the amplification curves and Ct Value (cycle threshold) of the two genes (ORF1ab and N-gene) with respect to positive control. For positive samples, amplification curves of both genes were comparable to Positive control and Ct value below ≤ 35 for both the genes were considered.

Ethical consideration

Permission was sought from the Institutional Ethics committee of the GMC Kathua (Ethical code number-IEC/GMCK/49/Pharma dated 27.08.2020).

Statistical analysis

The collected data was entered in Microsoft Excel, coding of the variables was done and thereby interpretation and analysis of the collected data was done using numbers and percentages.

Results

A total of 140 samples were taken over a period of 2 weeks, 70 each from the COVID isolation ward and ICU. 10 samples were taken each day and were labeled as ES1, ES2, ES3 up to ES10 [Table 1]. Among them, 30 samples (10 samples from ICU and 20 samples from isolation ward) were found to be positive for SARS-COV 2 virus. Majority of the samples reported positive from ICU were taken from the corners of the ICU floor [Table 2]. In case of COVID isolation ward with approximately double the number of positive samples, the rate of positivity was found highest among samples taken from corners of the floor and bed linen (71.42% each) [Table 3]. The samples taken from the door knobs and chairs of the hospital staff were found negative in both environments. All samples from bed side table, pulse oximeter and bed linen were found negative in ICU ward. The samples taken from stethoscope were also found negative in both the areas except on a single occasion from ICU.

Discussion

Contamination of frequent touch surfaces in healthcare settings are potential source of viral transmission. SARS-CoV-2 RNA was detected frequently from surfaces across the COVID ICU and ward we tested, and was detected more frequently among samples taken from corners of the floor and bed linen. 30 samples out of the total 140 samples taken were found to be positive in our study. Rate of positivity was found to be double in the isolation ward in comparison to the ICU. This may be attributed to enhanced and effective surface disinfection in the ICU. All the samples taken from the door knobs were found to be free from the virus contamination which suggested the effectiveness of the high hand hygiene and also the frequent disinfection of these high touch surface areas. Our findings are supported by the study of Ong *et al*. who demonstrated the survival of coronaviruses on the surfaces of a patient ward toilet and hand basin;[9] Ye *et al*. demonstrated the survival of coronaviruses on the surfaces of isolation ward door handles and used gloves. It was found that 14% of 626 surface samples were positive for viral RNA, with a higher proportion of surface samples positive in the ICU (32% of 69 samples) in a hospital caring for patients with COVID-19 in Wuhan, China.[8] Similarly, Kampf *et al*. showed that the new coronavirus can survive on inanimate surfaces for a certain period of time (glass or plastic for up to 9 days).[10] These studies show that contaminated objects may become a new source of infection, increasing the risk of cross-infection in the hospital. The positive rate were found to be 25% and 37.5% for the general isolation ward ICU, respectively in a study conducted by Wu *et al*. in a designated hospital for COVIDs.[11] In contradiction, study conducted by Wang *et al*. identified very little or no surface or air contamination where none of SARS-COV-2 RNA was detected among the 36 objects surface samples and 9 staffs PPE samples in isolation ward.[12] Several studies have noted a higher contamination rate in the ICU s compared to other departments aimed at combating COVID-19 as observed in study conducted by Andrie *et al*. where 65% of the surface samples taken from the ICU were found to be positive whereas only 32% of the surface samples were found positive in respiratory infection department.[13] This can be explained by various factors. First, the efficiency of the active ventilation system in the ICU can be insufficient. Second, significant contamination of SARS-CoV-2 RNA may be associated with cleaning regimes in the hospital, as well as with the spread by medical personnel themselves.[14] The WHO recommends "to ensure that environmental cleaning and disinfection procedures are followed consistently and correctly.[15] In conclusion, the SARS-COV-2 RNA monitoring results of the hospital isolation ward and ICU demonstrated that the frequency of disinfection was not as per the recommendations. The reason being the lack of human resources (sweepers) as per the IPHS guidelines interrupted supply of disinfectants and lack of trained workforce. Thorough cleaning of environmental surfaces with water and detergent and applying commonly used hospital-level disinfectants (such as sodium hypochlorite) are effective and sufficient procedures for infection control.This was a single center observational study, therefore the results obtained may not be generalizable to the other health care facilities. To prevent surface and other contaminations, strict adherence to COVID appropriate behavior and standard operating procedures (SOPs) of behavior regarding sanitation and hygiene, induction training of new health-care workers involved in sanitation and re-orientation training of the existing sanitation staff and vigorous monitoring by sanitation in charge is required. At the same time, logistics pertaining to sanitation should be available in the ample quantity.

Limitation and recommendation

RTPCR tests do not indicate the viability of virus. Due to resource constraint, viral culture could not be done and only COVID ICU and isolation ward were included

in the study and recommendation for this study and future study.

1. Strict adherence to COVID appropriate behavior and SOPs of behavior regarding sanitation and hygiene
2. Induction training of new health-care workers involved in sanitation and re-orientation training of the existing sanitation staff needs to be emphasized
3. Vigorous monitoring by sanitation in charge
4. Availability of ample supply of logistics pertaining to sanitation
5. Maintenance of all the records pertaining to sanitation.

Conclusion

Our study revealed surface contamination with SARS-CoV-2 RNA was found in the hospital setting, particularly from the corners of the COVID ICU and isolation ward followed by the samples taken from the linen which is a potential source of transmission of disease. Strict adherence to COVID appropriate behavior, increased frequency of disinfection in high-risk areas, and sensitization of the staff are mandatory to minimize the infection risk.

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Conflicts of interest

There are no conflicts of interest.

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