Original Article

Viral load could be an important determinant for fomites based transmission of viral infections

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Abstract

Background and Objective: Fomites are common sources of transmission of certain infections. Infectious pathogens, such as viruses known to cause respiratory tract infections, are common examples of being transmitted by fomites. However, the load of the particular pathogen on these inanimate surfaces is a crucial factor for the transmission. The current study aims at investigating the load of one such viral pathogen on the surfaces of commonly used materials. **Methods:** Based on the cycle threshold (Ct) values in the diagnostic system using gene amplification for the considered viral pathogen, we categorized the positive samples for high (17 to < 24), moderate (24 to < 31), or mild (31 to < 38) viral load. Five randomly selected samples from each of these category were smeared on commonly used cardboard surface (absorbent surface) and stainless steel (non-absorbent surface). After an observation duration of 90 min, samples from the surfaces were analyzed again for gene amplification using RT-PCR. **Results:** Viral load/titter positively correlated with the viral material on either of these investigated surfaces post-observation duration. Higher viral load (low Ct) samples exhibited higher probability of being detected on the surfaces than those samples with lower/moderate (high Ct) viral load. **Interpretation and Conclusion:** Common inanimate surfaces are potential source of the viral transmission, however the viral load on these surfaces are key determinant of such transmission.

Keywords: Cycle threshold, fomite viral transmission, gene amplification, RT-PCR

Introduction

Viral pathogens are responsible for the majority of infectious diseases among humans[1] and respiratory infections account for majority of them.[2] Microbial risk assessment of the viral pathogen is well-recognized entity in the transmission dynamics of infectious diseases.

Some of the viral respiratory infectious diseases, that were previously regarded to be transmitted by direct contact with the aerosols produced by the cough/sneeze of infected individual, are significantly being transmitted via inanimate/fomite surfaces. Viral respiratory pathogens such as influenza, rhinovirus, and coronavirus are well documented for their potential fomite based transmission.[3-8]

Viral load plays a crucial role in the disease transmission, by significantly contributing to the longer survival on the inanimate surfaces. A recent study highlighted the importance of viral load and disease transmission of viral respiratory infectious disease.[9]

Reverse transcriptase–polymerase chain reaction (RT-PCR) test is among the popular specific tests for identification of the viral gene. The cycle threshold (Ct) value is the number of replication

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cycle required for producing a fluorescent signal, which is an indirect measure of the viral nucleic acid copies. Samples with high viral copies would reveal fluorescent signal in relatively shorter duration (fewer cycles) as compared to those with low viral copies, hence samples with high viral RNA copies will exhibit low Ct and vice versa.[9] The cycle threshold (Ct) values ranging between < 38 are regarded as positive for the infectious viral pathogen. Current study explored the detectability of the viral nucleic acid on surfaces of commonly used materials under controlled conditions.

Methods

Collection of environmental samples

Samples from the floors and walls of the hospital ward, toilet, and other common utility areas were collected using viral transport media moistened swabs. The hospital was primarily involved in the management of infectious respiratory disease of viral origin. Samples with Ct values 17 – <24, 24 – < 31, and 31– <38 of the particular viral pathogen were primarily used and categorized as high (H), moderate (M), and low (L) viral load samples respectively. Five (5) samples from each of the categories were used for further experiments. The study received necessary institutional permission from the regulatory bodies including the ethical committee as required.

Preparation of infected surfaces

Current study considered 2 commonly used materials, i.e., cardboard (commonly used for packing) & stainless steel, for exploring the detectability of viral nucleic acid on absorbent surface & non-absorbent surfaces respectively. Fifteen (15) square-shaped cardboard pieces measuring 6 centimeters on each side were sterilized using 70% Isopropyl alcohol (IPA) in addition to ultraviolet treatment for 30 min. Similarly, 15 stainless steel surfaces of the biosafety cabinet of similar dimensions of the cardboard pieces were marked and sterilized with IPA & 30-min UV irradiation. Swab samples from the sterilized surfaces were collected to ensure sterility. The sterility of the surfaces (Cardboard and stainless steel) was confirmed by demonstrating undetectable viral RNA (i.e., Ct values > 40 or undetermined) on these surfaces.

Five samples each from low, moderate, and high viral load were smeared on the sterilized cardboard pieces and stainless steel surfaces within the bio-safety laboratory level – 2. The samples were allowed for 90 min under controlled conditions. Swab samples from these surfaces were again collected after the 90-min observation period.

Total viral RNA from the samples was extracted using QiAmp Viral RNA isolation kits (Cat# 52906, Qiagen, GmbH, Hilden, Germany) by following the manufacturer's protocol. Using predefined protocols for the viral detection in real-time PCR by amplification of ORF1ab gene (BGI Real Time Fluorescent RT-PCR kit, BGI Biotechnology Co. Ltd, China).

Results

All samples used for smearing the surfaces exhibited Ct value <38 with sigmoidal curve amplification, ensuring positive for viral RNA as prescribed by the manufacturer (BGI Real Time Fluorescent RT-PCR Kit, BGI Biotechnology Co. Ltd, China). The viral RNA was recovered from a fraction of the smeared surfaces after the observation period. The surfaces smeared with low viral load had relatively fewer recovery rates as compared to those smeared with samples of high viral load. The exact number of surfaces, positive for viral RNA after the 90-minute observation period is described in Table 1, Figure 1a and 2a The results were consistent with both absorbent (cardboard) and non-absorbent (stainless steel) surfaces.

The patterns of recovery of the viral nucleic acid from the surfaces after 90-min observation period resembled the patterns of those used for smearing the surfaces, i.e., surfaces smeared with low, moderate, and high viral load samples exhibited a similar trend of low, intermediate, and high viral load post-90-min observation period. Spearman Correlation exploring the relationship between viral load of samples used for smearing, and viral load recovered from the surfaces (after observation period) was statistically significant and positive for both cardboard and stainless steel surfaces. However, the magnitude of correlation was higher among the cardboard surface ($r = 0.8$) as compared to the stainless steel ($r = 0.6$) [Figures 1b and 2b].

Discussion

Survival of the virus over inanimate objects (fomites) and chance of transmission of the viral respiratory infection from them is possibly dependent on a number of factors such as the presence of viral load, underlying surface's absorbability, moisture content of environmental air, time since contamination of the surface, individual behavior facilitating entry of virus in body, etc.[8] The present study has generated evidence in support of the possible role of viral load (based on cycle threshold) and absorbability of underlying surface towards viral persistence (i.e., transmissibility of infection).[10]

Current results are suggestive of the presence of the viral RNA on common surfaces such as packaging cardboard and stainless steel surfaces even after a while. The viral load is a key factor for the presence of the infectious virus on the surfaces and possibly contributing to its transmission, even after a considerable duration. The viral RNA has higher chances of being identified post-90-min observation period on surfaces contaminated with higher viral load, thereby surfaces with higher viral load are potentially contagious for longer period as compared to those with lower viral load.

The study identified a positive relationship between the viral load of samples used for contaminating the surface and viral load of the surfaces post-90-min observation period. The relation was stronger among cardboard surfaces than stainless steel surfaces.

Table 1: Percentage of samples identified positive after the observation period

Viral load (Ct values)	Cardboard surface (% positive after observation period)	Stainless steel surface (% positive after observation period)
5 samples with low viral load (31-<38)	1 (20%)	1 (20%)
5 samples of moderate viral load (24-<31)	3 (60%)	3 (60%)
5 samples of high viral load (17-<24)	5 (100%)	4 (80%)

Table describes the number (%) of surfaces (among various categories of viral load) identified positive for viral RNA after the 90-min observation period.

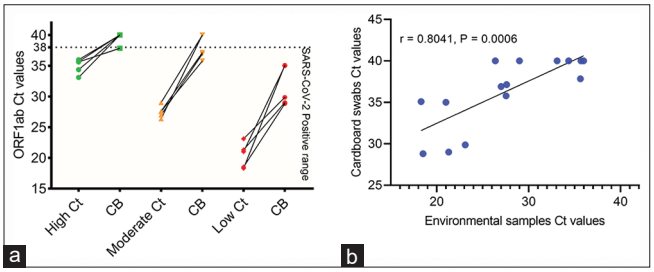

Figure 1: Effect of viral load on its detectability on the common absorbent surface (cardboard surface). (a) Scatter plot (before-after type) of Ct values (for ORF1ab gene of the viral pathogen) of the samples used for smearing and their corresponding Ct values from the surfaces after the observation period. High Ct values (i.e., 31–<38) represented in green indicate low viral load, while low Ct values (i.e., 17–<24) represented in red indicate high viral load, and the moderate Ct values (24–<31) represented in orange indicate moderate viral load. (b) Spearman's correlation between Ct values of samples (X-axis, environmental samples) used for smearing and those of cardboard surfaces (y-axis, cardboard swabs). Exact *P* value (two-tailed) were reported for assessment of statistical significance. CB = cardboard, Ct = Cycle threshold

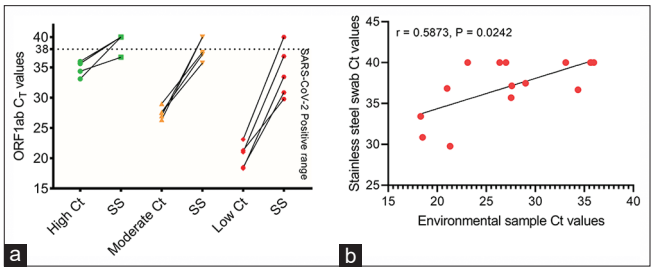

Figure 2: Effect of viral load on its detectability on the common non-absorbent surface (stainless steel surface). (a) Scatter plot (before-after type) of Ct values (for ORF1ab gene of the viral pathogen) of the samples used for smearing and their corresponding Ct values from the surfaces after observation period. High Ct values (i.e., 31 – <38) represented in green indicate low viral load, while low Ct values (i.e., 17–<24) represented in red indicate high viral load and the moderate Ct values (24–<31) represented in orange indicate moderate viral load. (b) Spearman's correlation between Ct values of samples (X-axis, environmental samples) used for smearing and those of stainless steel surfaces (y-axis, stainless steel swabs). Exact *P* value (two-tailed) were reported for the assessment of statistical significance. SS = stainless steel, Ct = Cycle threshold

A probable explanation can be acquired from the fact that the moistened surface on an absorbing cardboard could provide a better harboring site for viral particles than a total nonabsorbent surface such as stainless steel. The results however partly corroborate with a similar study, where the cultured viral titer was measured over a duration on various surfaces.[11] Our study does not suggest the viability of viral particles on the surface but it only assesses the presence of amplifiable viral RNA for specific genes (ORF1ab, in this case). The mentioned study, suggest the viability of these viral particles for over 3 days on these surfaces.[11]

Present study is perhaps the earliest from India, to document the relationship between viral load and their detectability on common surfaces. In addition, surfaces with contaminated with relatively higher viral load and with higher absorbability (cardboard) are independently associated with higher risk of COVID-19 retention and transmission. Considering the rapidly evolving literature and experimental procedures, our study is limited by a single sample collection (post 90-min observation duration) and does not indicate the viability of viral particle/virion. Further, the results may be extended to emphasize the need for sterilizing such fomite surfaces to prevent viral transmission. Considering the positive relation between viral load and the disease contagiousness,[12-14] the sources (spreader/positive subjects) with high viral load should be treated with great care, i.e., health care facility with possibly high viral load should adopt maximum precautionary measures.

The results indicate fomites could play role in the disease spread in addition to human contact, particularly at COVID-19 care facilities. Awareness on fomite-based COVID-19 transmission and the persistence of virion on these surfaces among the health care workers could reduce their risk of contracting COVID-19. Viral load on fomites and the potential role in disease transmission have potential implications in limiting transmission of the recent viral infectious respiratory disease.

Above has far-reaching public health implications for educating the public for adopting safer behaviors to avoid transmission through fomites. If secretion contains a high viral load, the infectivity will remain for prolonged time which needs to be studied further in-depth. About a fraction of the infected population harbor high viral load and designated as super spreader which is a matter of great concern.[15] Apart from person to person transmission, the above population would also spread the infection at a much higher rate through fomites unless effective public health controls are undertaken. Similarly, infected cases with moderate vial load would spread the said infection at a moderate rate both by person to person as well as fomites. Consequently, there should be an effective mass awareness programme using suitable mass awareness education tools by experienced health care workers. This is more important in places like business areas, shopping malls, tours and traveling, etc., where large gathering occurs with high population mobility and there is every possibility of transmission through fomites apart from person to person

spread. So, respective authorities must pay adequate attention to minimize the spread in the above areas as mentioned already.

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Conflicts of interest

There are no conflicts of interest.

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