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Viability of Human Adenovirus from Hospital Fomites

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The monitoring of environmental microbial contamination in healthcare facilities may be a valuable tool to determine pathogens transmission in those settings; however, such procedure is limited to bacterial indicators. Viruses are found commonly in those environments and are rarely used for these procedures. The aim of this study was to assess distribution and viability of a human DNA virus on fomites in an Adult Intensive Care Unit of a private hospital in Rio de Janeiro, Brazil. Human adenoviruses (HAdV) were investigated in 141 fomites by scraping the surface area and screening by quantitative PCR (qPCR) using TaqMan® System (Carlsbad, CA). Ten positive samples were selected for virus isolation in A549 and/or HEp2c cell lines. A total of 63 samples (44.7%) were positive and presented viral load ranging from 2.48×10^1 to 2.1×10^3 genomic copies per millilitre (gc/ml). The viability was demonstrated by integrated cell culture/nested-PCR in 5 out of 10 samples. Nucleotide sequencing confirmed all samples as HAdV and characterized one of them as specie B, serotype 3 (HAdV-3). The results indicate the risk of nosocomial transmission via contaminated fomites and point out the use of HAdV as biomarkers of environmental contamination. ***J. Med. Virol. 86:2065–2069, 2014.***

KEY WORDS: adenovirus; viability; fomites; hospital; biomarker

INTRODUCTION

Studies performed in hospitals have described the transmission of pathogens through contact with contaminated fomites in these environments [Gallimore et al., 2004, 2005, 2006, 2008; Goodman et al., 2008; Carducci et al., 2011; Ganime et al., 2012]. Particularly, in the case of viruses, experimental, and epidemiological studies indicate that hospital surfaces could play an important role in the spreading of human adenoviruses (HAdV), group A rotaviruses (RVA), and noroviruses (NoV) suggesting that they might be responsible for 15–30% of nosocomial infections [Soule et al., 1999; Gallimore et al., 2004, 2005, 2006, 2008; Lopman et al., 2004; Rzezutka and Cook, 2004; Sattar, 2004; Goodman et al., 2008; Carducci et al., 2011; Ganime et al., 2012].

Procedures carried out in healthcare facilities could be one of the main sources of viral dissemination, causing considered impact on human health [Tuladar et al., 2012]. In those settings, blood-borne, air borne and viruses transmitted via the fecal–oral route could be easily transmitted by accidents with infected needles or sharp objects or spread by contamination of air, hands, and fomites [Aitken and Jeffries, 2001; Lopman et al., 2004; Davanzo et al., 2008].

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The monitoring of environmental microbial contamination in healthcare services could be a valuable tool to determine the means of transmission. However, this monitoring is usually limited to bacterial indicators and viruses are rarely used, even being common pathogens in hospitals and healthcare settings [Sattar, 2004; Creamer and Humphreys, 2008; Carducci et al., 2011; Ganime et al., 2012].

When compared with RNA viruses, DNA viruses are known to be more stable as they are considered good indicators of viral contamination in environmental samples [Puig et al., 1994; Horwitz, 1996; Pina et al., 1998; Bofill-Mas et al., 2000; Thurston-Enriquez et al., 2003; Carducci et al., 2011]. Particularly, human adenoviruses (HAdV) present several characteristics that qualify them as good viral indicators considering their resistance to environmental stressors, transmission by different routes, and also for not exhibiting seasonality [Myrmel et al., 2006; Haramoto et al., 2007a,b; Katayama et al., 2008]. Additionally, they could be replicated in several cell lines such as A549, HeLa, HEK 293, and HEp-2, among others enabling studies of infectivity and risk assessment [Leite et al., 1985; Rigotto et al., 2005; Filho et al., 2007; Cromeans et al., 2008]. Therefore, the aim of the present report was to investigate HAdV dissemination and viability in fomites obtained in a private hospital in Rio de Janeiro, Brazil. Results of previous investigations on rotavirus A (RVA) using the same samples were published elsewhere and were used to design this study [Ganime et al., 2012].

METHODS

Sampling Procedures

Samples were obtained from fomites available on seven rooms of an Adult Intensive Care Unit of a private hospital in Rio de Janeiro with the capacity to treat any medical–surgical condition. Sampling was carried out between January and June 2009. The Adult Intensive Care Unit provides privacy and surveillance in seven individual rooms, each one with individual ventilation and air filtration systems.

All hospital cleaning protocols are performed twice a day routinely, emphasizing that after discharge of the patient's, the room undergoes a more rigorous process of disinfection. Weekly, the hospital performs track of colonization of mechanically ventilated patients, by surveying methicillin resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* (MDR), and gram-negative *bacillus* (BGN) using tracheal and rectal swabs.

The sites were chosen to represent areas commonly in contact with hands. At least 50% of fomite areas (e.g., companion chairs) were scraped with swabs and dipped in Dulbecco's modified eagle's medium (DMEM—Gibco®, Grand Island, NY), pH 7.2, as described previously [Ganime et al., 2012]. HAdV were investigated in 141 samples, 73 previously positive for RVA, and 68 negative for RVA that were selected randomly [Ganime et al., 2012].

Controls

HAdV type 2 and DMEM were used as positive and negative controls, respectively, for all methodologies used [Leite et al., 1985]. To avoid cross-contamination, all molecular and cell cultures procedures were performed in different rooms, with UV decontamination.

Nucleic Acids Extraction of Swab

For nucleic acid extraction, guanidinium thiocyanate–silica method was adapted as described previously [Boom et al., 1990; Gallimore et al., 2004].

Human Adenovirus Detection and Quantification

Nucleic acids extracted from swabs were quantified using TaqMan® qPCR, targeting the conserved region segment of the first HAdV hexon gene part [Hernroth et al., 2002].

Virus Viability Assay

Samples presenting a high genomic quantification by TaqMan® qPCR cycle threshold (Ct) <38 were selected for investigation of viral viability using the integrated cell culture/nested-PCR (ICC/nPCR) assay. HAdV strains were propagated into 80–90% confluent A549 and HEp2c cell lines supplemented with 2% FBS as described previously [Cromeans et al., 2008]. Nested-PCR was performed targeting a segment from the conserved region of the HAdV hexon gene to confirm HAdV isolation [Allard et al., 2001].

Nucleotide Sequencing

Purified DNA obtained from nested-PCR products were sequenced in both directions using Nehex3deg and Nehex4deg primers [Allard et al., 2001]. Generation of contiguous sequences and pairwise alignments of the HAdV hexon gene 171 bp inter-primer region were performed at the Platform for DNA Sequencing (PDTIS; Oswaldo Cruz Foundation, Rio de Janeiro, Brazil).

Statistical Analysis

Statistical analysis was performed using the chi-square test as implemented by Epi info® software, version 3.5.1, and the two-tailed Fisher's exact test. The threshold for statistical significance was $P < 0.05$.

RESULTS

Sixty-three out of 141 (44.7%) fomites were positive for HAdV using TaqMan® qPCR. The viral load ranged from 2.48×10^1 to 2.1×10^3 genomic copies per millilitre (gc/ml). The Chlorhexidine® dispensers presented the highest viral load (2.1×10^3 gc/ml),

although the most frequently contaminated surface was the common trash bin covers followed by the TV remote control.

The analysis of fomites from rooms showed that HAdV was detected in all seven rooms of the Adult Intensive Care Unit, with a detection percentage ranging from 12.7 to 19%. The room that presented the highest percentage of detection (19%) was located farther from the room occupied by the medical staff.

Table I summarize the results obtained in the present study compared with RVA previous detection showing a mixed contamination in 31.2% (44/141) of analyzed samples. The statistical analysis demonstrated that the probability of a sample to be HAdV positive is 2.16 times ($P < 0.05$) higher when it is also RVA positive.

The rate of HAdV detection in the first months of the study (January [25/45, 55.6%] and February [32/34, 94.1%]) was significantly higher than in the following months: March (1/16, 6.3%), April (2/12, 16.7%), May (1/20, 5%), and June (2/14, 14.3%) ($P < 0.05$).

HAdV were isolated from 5 out of 10 investigated samples using A549 and/or HEp2c cell lines. Two samples, collected from telephone handles and common trash bin covers, demonstrated viability by ICC/nPCR using A549 cell line. The samples collected from the common trash bin covers could be isolated in both cell lines (A549 and HEp2c). Samples collected from alcohol gel dispensers (n = 2) and Chlorhexidine® dispensers (n = 1) were isolated in HEp2c cell line.

All isolates were confirmed as HAdV by nt sequencing presenting blast homology ranging from 96% to 98%, one of which characterized as specie B, serotype 3 (HAdV-3).

DISCUSSION

The percentage and concentration of HAdV detected on fomites are consistent with previous data concerning their stability in environmental samples [Rutala et al., 2006; Carducci et al., 2011]. HAdV have been described as resistant to environmental stressors as disinfectants when deposited on fomites, recovered from dry inanimate surfaces from 7 days to 3 months [Kramer et al., 2006; Rutala et al., 2006].

In this study, environmental mixed contamination with HAdV and RVA was demonstrated corroborating data that shows mixed infection on hospital surfaces with gastroenteric viruses [Gallimore et al., 2006]. Virus survival on surfaces depends on several conditions although the most important contribution so far is the type of material/fluid in which it is discharged [Sattar, 2004]. Additionally, the type of surface can also influence the detection of these viruses. Abad et al. [1994] show that RVA persists longer than enteric HAdV when dried on porous and non-porous fomites. That information was not evaluated in this study, but may explain the presence of RVA in HAdV negative samples.

A significant decrease in the percentage of HAdV detection ($P < 0.05$) from March onwards was similar to the one previously observed for RVA [Ganime et al., 2012], although the detection of HAdV was significantly higher ($P < 0.05$) than the RVA in the first 2 months of the investigation (January and February). That reduction resulted from changing of both cleaning and disinfection procedures after finding RVA spread in hospital settings [Ganime et al., 2012]. The change of strategy in cleaning procedures resulted not only in the decrease of viral contamination, but also in the control of hospital infections by *Acinetobacter spp*. (data not shown). Those data emphasize the importance of preventive and corrective measures to reduce both direct and indirect transmission of microorganisms. Wilhelmi et al. [2003] support that hand hygiene before and after contact with patients or with objects that may be contaminated, as essential to disinfect contaminated surfaces. Training techniques of hand washing, for healthcare professionals, relatives, and visitors should be frequently provided especially those who attend any Intensive Care Unit [Soule et al., 1999]. This finding is supported by Ansari et al. [1988] who stated that the hands can donate or receive virus during occasional contact with animate and inanimate surfaces.

The negligence in cleaning procedures and the lack of compliance in hand hygiene was noted in this study. HAdV was detected in all rooms investigated; however, the fact that the most frequently contaminated room were the ones located away from the medical staff revealing non-compliance with effective cleaning protocols caught our attention. This kind of human behavior could explain why the common trash bin covers were the most frequently contaminated surfaces with HAdV. Such observation led us to confirm the incorrect use of the trash bin by relatives, visitors, and health professionals, when making the use of the hands to open the trash bin covers instead of the pedal. The qPCR results also revealed that Chlorhexidine® dispensers presented the highest viral load, corroborating data that suggest that health professionals are not compliant with proper hand washing using soap and water [Soule et al., 1999; Gallimore et al., 2005, 2008; Kramer et al., 2006; Ganime et al., 2012].

The isolation of HAdV and RVA from fomites indicate that those viruses remain infectious and should be useful to monitor environmental contamination. The higher percentage of HAdV detection together with the availability of cells for their isolation, suggests the use of HAdV as an indicator of viral contamination of fomites. Despite the isolation of enteric HAdV (specie F [40 and 41]) is considered a difficult procedure to be carried out when compared to other HAdV species [Rigotto et al., 2011], G293 and A549 cell lines have been described for isolating and propagating those viruses [Cromeans et al., 2008]. RVA is known as a fastidious virus and its isolation

TABLE I. Human Adenovirus Detection in Fomites Obtained in an Adult Intensive Care Unit

		Fomites											
RVA	TFB	CCH	BC	TRC	CST	BDH	IDR	AGS	CD	KIP	TE	TBC	Total
HAdV (+)													
(−)	1/13 (7.7)[a]	2/19 (10.5)	2/11 (18.2)	3/7 (42.8)	0/9	0/8	1/13 (7.7)	3/17 (17.6)	0/14	3/8 (37.5)	2/10 (20.0)	2/12 (16.7)	19/141 (13.5)
(+)	3/13 (23.1)	3/19 (15.8)	0/11	2/7 (28.6)	2/9 (22.2)	4/8 (50.0)	4/13 (30.8)	7/17 (41.2)	8/14 (57.1)	2/8 (25.0)	2/10 (20.0)	7/12 (58.3)	44/141 (31.2)
Total	4/13 (30.8)	5/19 (26.3)	2/11 (18.2)	5/7 (71.4)	2/9 (22.2)	4/8 (50.0)	5/13 (38.5)	10/17 (58.8)	8/14 (57.1)	5/8 (62.5)	4/10 (40.0)	9/12 (75.0)	63/141 (44.7)
HAdV (−)													
(−)	3/13 (23.1)[b]	9/19 (47.4)	4/11 (36.4)	1/7 (14.3)	6/9 (66.7)	3/8 (37.5)	6/13 (46.1)	3/17 (17.7)	6/14 (42.9)	1/8 (12.5)	5/10 (50.0)	2/12 (16.7)	49/141 (34.7)
(+)	6/13 (46.1)	5/19 (26.3)	5/11 (45.4)	1/7 (14.3)	1/9 (11.1)	1/8 (12.5)	2/13 (15.4)	4/17 (23.5)	0/14	2/8 (25.0)	1/10 (10.0)	1/12 (8.3)	29/141 (20.6)
Total	9/13 (69.2)	14/19 (73.7)	9/11 (81.8)	2/7 (28.6)	7/9 (77.8)	4/8 (50.0)	8/13 (61.5)	7/17 (41.2)	6/14 (42.9)	3/8 (37.5)	6/10 (60.0)	3/12 (25.0)	78/141 (55.3)

RVA, group A rotaviruses; HAdV, human adenoviruses; TFB, toilet flush buttons; CCH, companion chairs; BC, bed controls; TRC, TV remote controls; CST, coffee support tables; BDH, exterior bathroom door handles; IDR, interior door handles; AGS, alcohol gel supports; CD, Chlorhexidine® dispensers; KIP, keyboard infusion pumps; TE, telephones; TBC, common trash bin covers; (+), positive sample; (−), negative sample.

[a]Positive/total samples (%).

[b]Negative/total samples (%).

requires long-time procedures [Kapikian et al., 2001; Ganime et al., 2012].

Nucleotide sequencing confirms HAdV isolation and sorting serotype 3 by molecular analysis. HAdV-3 is often associated with eye infections and severe cases of bronchiolitis and pneumonia [Tian et al., 2011]. Molecular technologies have been described as useful tools for viruses' detection mainly due to their rapidity and easiness when compared to classic detection of virus isolation in cell culture, although unable to distinguish between infectious and non-infectious viral particles [Parashar et al., 1998; Haramoto et al., 2007a,b]. RVA and HAdV isolation from fomites trialled previously by molecular techniques highlights the use of those methods for monitoring viruses' contamination of hospital fomites allowing inferring infectivity for risk assessment studies.

HAdV is associated with various diseases in adults and children, such as pneumonia, acute gastroenteritis, and epidemic keratoconjunctivitis, and despite different transmission paths, all serotypes are eliminated in the feces fostering fomites contamination by several species [Greening, 2006; Kramer et al., 2006; Rutala et al., 2006; Matsushima et al., 2012; Rodríguez-Lázaro et al., 2012]. Its investigation is particularly important in hospitals that admit immunocompromised patients and neonatal Intensive Care Unit as described by Pham et al. [2003] showing that severe and fatal HAdV infections are not infrequent, particularly among the immunocompromised population. Kelley [2010] and Henquell et al. [2009] reported fatal cases of pre-term infants with disseminated HAdV infection.

CONCLUSION

Considering the detection and isolation of HAdV and the totality of information, HAdV could be inferred as biomarkers for hospital fomites, as they can also be valuable to test the effectiveness of preventive measures and hygiene levels from hospital fomites, in order to alert healthcare professionals to the need of prevention measures and to ensure their compliance.

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