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Major article

Environmental viral contamination in a pediatric hospital outpatient waiting area: Implications for infection control

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Background: Nosocomial outbreaks of viral etiology are costly and can have a major impact on patient care. Many viruses are known to persist in the inanimate environment and may pose a risk to patients and health care workers. We investigate the frequency of environmental contamination with common health care–associated viruses and explore the use of torque-teno virus as a marker of environmental contamination.

Methods: Environmental screening for a variety of clinically relevant viruses was carried out over 3 months in a UK pediatric hospital using air sampling and surface swabbing. Swabs were tested for the presence of virus nucleic acid by quantitative polymerase chain reactions.

Results: Viral nucleic acid was found on surfaces and in the air throughout the screening period, with adenovirus DNA being the most frequent. Door handles were frequently contaminated. Torque-teno virus was also found at numerous sites.

Conclusion: Evidence of environmental contamination with viral pathogens is present in health care environments and may be indicative of an infectious virus being present. Screening for viruses should be included in infection control strategies. Torque-teno virus may provide a better marker of contamination and reduce time and cost of screening for individual viruses.

Outbreaks of viral infection are common in health care environments. This is particularly true of pediatric hospitals because children are often admitted with infections related to community epidemics (eg, those caused by respiratory or gastrointestinal viruses).[1-5] Viral disease may not be the reason for admission, but patients may asymptomatically carry the virus and shed infectious particles for lengthened periods of time.[6-10] Viral infections can be fatal in immunocompromised patients, and nosocomial outbreaks of viral disease have significant financial impacts. Norovirus (NV) outbreaks, for example, are estimated to cost the UK National Health Service >£100 million in high incidence years.[11,12] In addition to financial concerns, virus outbreaks can affect staffing levels on wards and have substantial impacts on clinical outcome, recovery time, and psychologic health of patients.[13,14]

Identification and analysis of microorganisms in indoor environments have grown in popularity over recent years, linked to the development of next-generation sequencing technologies. Environmental microbiomes are being mapped to enable the understanding of what species of microorganisms we share our world with.[15-18] Environmental virus investigation is currently largely focused on samples such as sewage, water, and soil.[19] The lack of data investigating the indoor virome is likely caused by the absence of a universal gene target analogous to the 16S ribosomal RNA gene of bacteria and Archaea, the more limited scope of public sequence databases for viruses, and the comparatively low numbers of viruses expected to be found in the indoor environment. The identification of RNA viruses and viruses with very small genomes also poses technical challenges. However, the investigation of viruses in indoor environments is still important because of the potential risk to vulnerable patients.

Many viruses that are of concern in health care environments have been shown to survive on inanimate objects or in the air, often for extended periods of time; therefore, these are considered to be important routes of transmission of infection.[20,21] Viruses may also be resistant to disinfectants and alcohol hand gels often used in infection control practices.[22-24] For example, NV is stable in the environment and relatively resistant to commonly used disinfectants[25]; therefore,

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incidences of infection can lead to substantial environmental contamination.[20,25] Once excreted into the environment, adenovirus (AV) can remain infectious for up to 35 days.[26] Respiratory syncytial virus (RSV) infection is the most common cause of hospitalization of children with respiratory illness, and between 53,000 and 199,000 deaths are reported globally per year.[27] RSV is spread by contact, and the virus has been shown to survive well in the environment.[28] It has also been suggested that contact transmission, either by hands or fomites,[29] occurs for human metapneumovirus (hMPV). Rotavirus (RV) infection is the most common cause of diarrhea-related hospitalization in younger children, and an estimated 570,000 children die each year from RV-associated dehydration.[4] Approximately 100−1,000 virions can be shed per milliliter of stool during diarrhea episodes.[4] Given that the infectious dose of RV is between 10 and 100 virions, it is unsurprising that outbreaks occur rapidly with heavy contamination of the environment.[4]

Given the prolonged environmental survival, high infection rates and high transmissibility of viruses, and the large number of susceptible hosts in pediatric health care environments, assessments of viral contamination are appropriate. Doors, work surfaces, and high hand-touch sites have been previously shown in other work to be contaminated with virus nucleic acids.[30,31] This study aims to determine if nucleic acids from potentially pathogenic viruses can be recovered from environmental surfaces and air in our location. It has been suggested that the use of bacterial counts for surface monitoring is an inappropriate marker for viral contamination because of distinct properties and survival characteristics of viruses and bacteria.[30] We, therefore, investigate the use of a highly ubiquitous virus, torque-teno virus (TTV), as a potential marker for virus contamination.

METHODS

Study design

Samples (n = 78) were taken from fixed, high-touch sites in an outpatient waiting area (Table 1) once a month over 3 winter months (November-January) at a UK pediatric hospital. Approximately 30 patients were seen per day at the clinic, and each patient had at least 1 parent or guardian present. Virus species investigated were chosen on the assumption that they may be present in the environment during the sampling period and for their clinical significance: NV, AV, RSV types A and B, RV, and hMPV. Cytomegalovirus (CMV) was included as a potential marker of mucosal contamination and as a possible reference for recent contamination. TTV was included to investigate its use as a marker of contamination.

Surface sampling

To investigate the presence of virus nucleic acids on surfaces, wet cotton swabs were run over each 10-cm^2 swab site horizontally, vertically, and diagonally before snapping off into 1-mL RNA*later* stabilization buffer (Qiagen, Manchester, UK). To assess the bacteria present on surfaces, Tryptone Soy Agar contact plates (Oxoid, Baisingstoke, UK) with a 5.5-cm diameter were pressed onto surfaces with an even pressure for 10 seconds and incubated at 37°C for 48 hours prior to counting.

Air sampling

A Burkard C90M cyclone sampler (Burkard, Rickmansworth, UK) was placed in a location where it would not be tampered with by visitors (the nurse's station). It was set at a flow rate of 16.6 L/min and left to run for 10 hours, starting 1 hour prior to the area being open to patients and ending 1 hour after the final patient vacated the area, with 1-mL RNA*later* as the collection buffer.

Table 1
Virus screening sites in an outpatient area

Sample number	Sample site
OP1	Chair
OP2	Heater
OP3	Reception desk
OP4	Trolley top
OP5	Bin lid
OP6	Nurse desk
OP7	Nurse chair arm
OP8	Chair arm
OP9	Chair top
OP10	Small table
OP11	Large table
OP12	Book
OP13	Toy cooker (top)
OP14	Toy cooker (base)
OP15	Plastic toy
OP16	Floor by toilet
OP17	Floor by room 9
OP18	Top of television
H1	Door handle water closet
H2	Door handle room 5
H3	Door handle room 6
H4	Door handle room 7
H5	Door handle room 8
H6	Door handle room 9
H7	Door handle room 10
Air	Nurse's station

H, handle; *OP*, outpatient.

Virus quantitative polymerase chain reactions

DNA and RNA were coextracted using the AllPrep Mini Kit (Qiagen, Manchester, UK), and quantitative polymerase chain reaction (qPCR) was carried out according to in-house standard operating procedures. DNA assays were prepared by adding 12.5 μL QuantiFast Mastermix (Qiagen, Manchester, UK) per reaction, and RNA assays were prepared by adding 12.5 μL QuantiFast RT Mastermix (Qiagen, Manchester, UK) and 0.2 μL reverse transcriptase enzyme (Qiagen, Manchester, UK) per reaction. Primer and probe mixes (MWG Eurofins, Ebersberg, Germany) were added at a final concentration of 10 pmol/μL, and cycling conditions were carried out according to Qiagen protocols on an Applied Biosystems 7500 Real-Time PCR system (Life Technologies, Glasgow, UK). Standard curves were made for AV and CMV, and plasmid standards were used for RV, hMPV, NV genogroups GI and GII and RSV types A and B. Amplification curves were viewed and analyzed with Applied Biosystems Sequence Detection Software version 4.1 (Life Technologies, Glasgow, UK).

RESULTS

qPCR and total viable counts

Figure 1 shows schematics of the sampled area for each month. Viral nucleic acid was detected on a variety of surfaces and in the air. Of all sites sampled, 42% (n = 33) were positive for the presence of viral nucleic acid. Of these, 19% (n = 8) were positive for >1 virus, with AV and TTV most commonly being identified at the same site. More frequent detection of viral nucleic acid occurred in December, with 60% (n = 15) of sites being positive compared with 56% (n = 13) in November and 20% in January (n = 5) (Fig 1). Of the 78 samples taken, 29 (37%) were positive for the presence of AV DNA. AV DNA was also isolated from the air at the November time point (Fig 1, *A*).