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Major article

Viral contamination of aerosol and surfaces through toilet use in health care and other settings

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Background: The airborne spreading of enteric viruses can occur through the aerosol and droplets produced by toilet flushing. These can contaminate the surrounding environment, but few data exist to estimate the risk of exposure and infection. For this reason environmental monitoring of air and selected surfaces was carried out in 2 toilets of an office building and in 3 toilets of a hospital before and after cleaning operations.

Methods: To reveal the presence of norovirus, enterovirus, rhinovirus, human rotavirus, and Torque teno virus and to quantify human adenovirus and bacteria counts, molecular and cultural methods were used.

Results: On the whole, viruses were detected on 78% of surfaces and in 81% of aerosol. Among the researched viruses, only human adenovirus and Torque teno virus were found in both surface and air samples. In several cases the same adenovirus strain was concurrently found in all matrices. Bacterial counts were unrelated to viral presence and cleaning did not seem to substantially reduce contamination.

Conclusions: The data collected in our study confirm that toilets are an important source of viral contamination, mainly in health care settings, where disinfection can have a crucial role in preventing virus spread.

Surfaces such as door handles, banisters, flush handles on toilets, toys, telephones, drinking cups, and fabrics have all been implicated in the transmission of enteric viruses,[1] namely norovirus, rotavirus, and rhinovirus,[2] in localized cases and outbreaks. These surfaces can be contaminated directly by contact with infected material or indirectly by dirty hands or settling of large aerosol droplets. Evidence for the role of air and surfaces for viral transmission can be drawn from studies regarding air and fomite contamination, studies of the transfer and survival of viruses, from experimental trials in human volunteers, from epidemiologic data, and from disinfection studies.[3]

Hygiene and disinfection intervention studies have demonstrated that proper cleaning of hands and efficient disinfection of fomites decreases surface contamination and may interrupt the spread of disease caused by norovirus, rotavirus, and coronavirus.[4]

The role of airborne viral spreading is not well defined because of the difficulties in identifying this transmission route for single cases or outbreaks. Nevertheless, person-to-person transmission could also be due to environmental contamination by suspended or settled aerosol droplets. For example, during the severe acute respiratory syndrome epidemic transmission was believed to occur primarily by direct physical contact with ill persons or by large-droplet spread; however, several clusters of infection were difficult to explain by these routes and many health care workers were infected despite compliance with World Health Organization infection control guidelines.[5,6] The role of aerosol and surface contamination in the transmission of viral infection in hospitals is generally recognized,[7,8] but its sources and dimensions are not deeply studied because of the difficulties in measuring environmental virus contamination and its relation to specific clinical cases.[9,10]

In addition to respiratory droplets, toilets should be considered as a possible source of indoor air and surface viral contamination. In fact, consistent microbial contamination of the indoor environment typically occurs after a toilet flush, and this can be an important source of diffusion, not only for enteric viruses, but also for respiratory ones, which are also often eliminated by the fecal route. A toilet flush generates a large number of droplets of different dimensions: the largest droplets settle rapidly on the surrounding surfaces, whereas the smallest can be inhaled or remain airborne for a long time.[11,12] Despite the long-standing evidence of toilet flush as an important source of infective aerosol, a systematic study of toilet

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flush contamination as it affects risk assessment has not yet been completed, especially regarding viral contamination. Nevertheless, from the perspective of risk management, the quantitative assessment of this putative method of exposure would be of utmost importance to select control measures and define the points where they can be successfully applied. To this aim, environmental monitoring was carried out in toilets of a hospital unit (nephrology) and of an office building. Aerosol and surface samples were collected and analyzed for total bacterial count (TVBC), assessing hygienic conditions and the effectiveness of cleaning procedures, and the presence of human viruses. These were chosen taking into account the results of our previous study[9] and to represent different mechanisms of diffusion: norovirus, genogroups I and II (NoV GI and GII) and human rotavirus (HRV) for the fecal–oral route, rhinovirus (RV) and TTV for the respiratory route, and human adenovirus (HAdV) and enterovirus (EV) for both.

MATERIALS AND METHODS

Study setting and sampling

The study was carried out from December 2009 to April 2010, examining 5 toilets of the nephrology ward of Leghorn Hospital and 2 toilets in an office building, in which roughly 30 persons were usually present during working hours. Among the hospital toilets, 1 was dedicated to health care personnel and the other 4 were adjacent to patient rooms (3 from 2-bed rooms and 1 from a 4-bed room). For each toilet, at least 5 replicate sampling campaigns were conducted. In each campaign, 2 sets of samples were collected: 1 before and 1 after the application of a cleaning procedure. In each toilet and for each set of samples, 1 aerosol and 4 surface samples were collected. The surfaces were chosen to reflect the potential for hand contamination: the toilet seat and its cover, the flushing handle/button, and the internal door handle. In addition to air and surfaces, the water inside each toilet was also sampled as the possible source of the environment contamination. In total, 172 surfaces (108 and 64 for hospital and offices, respectively), 43 air (27 and 16 for hospital and offices, respectively), and 19 water (4 and 15 for hospital and offices, respectively) samples were collected.

Both surface and air samples were analyzed for HAdV, NoV GI, NoV GII, EV, RV, HRV, TTV, and TVBC. Water samples were analyzed only for HAdV.

Sampling procedures

Surfaces

Three adjacent 36 cm^2 squares were sampled.[9] The first, for the detection of the RNA viruses (NoV GI, NoV GII, RV, HRV, and EV), was swabbed with cotton swabs soaked in 1 mL 3% beef extract at pH 9. The eluate was then neutralized with 1 M hydrogen chloride and 140 μL of it was used for viral nucleic acids extraction using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).[13] The second square area was sampled for the detection of DNA viruses (TTV and HAdV), using a commercial kit (DNAIQ System, Promega, Fitchburg, Wis) designed for forensic use and modified for the detection of virus on surfaces.[14] For bacteriologic analyses, the third square surface was eluted with cotton swabs soaked in a 0.9% w/v sodium chloride solution. Swabs were then incubated in 2 mL nutrient broth for 20 minutes at 37°C. The whole solution was then seeded onto plates containing plate count agar and incubated for 48 hours at 37°C.

Air

Air samples were collected with an impactor sampler (Microflow, Aquaria, Italy).[9] For virus detection, 1,000 L air was sampled on replicate organism detection and counting (Rodac) plates containing tryptone soy agar. The agar was then eluted in 3% beef extract at pH 9, and viral RNA and DNA were isolated using a QIAamp RNA Mini Kit and a QIAamp DNA mini Kit (Qiagen, Hilden, Germany), respectively.[15]

For bacterial counts, 180 L were sampled using an impactor sampler (Microflow), with Rodac plates containing plate count agar. The plates were then incubated for 48 hours at 37°C.

Water

Water was withdrawn directly from the toilet in a 50-mL plastic tube. Water samples were directly analyzed by isolating DNA with QIAamp DNA mini Kit from 200 μL.

Virus detection

For NoV GI and NoV GII, EV, RV, HRV, and TTV, the isolated nucleic acids were analyzed using nested reverse transcription polymerase chain reaction (RT-PCR) according to previous protocols.[9-16] For each virus, the PCR products were detected under ultraviolet light after horizontal electrophoresis in 2% agarose gel.

HAdV was detected and its genomic concentration was quantified using real time quantitative PCR according to published protocols.[17] The samples tested were analyzed in 96-well optical plates and read with an ABI 7300 sequence detector system (Applied Biosystems by Life Technologies Corporation, Monza, Italy). The genome copy numbers of HAdV in tested nucleic acid extracts were extrapolated from the equation of the standard curve that was generated from the dilution series (range, 10^2-10^7) of known amounts of nucleic acids. The standard curves were constructed by cloning the entire hexon region of Ad41 into pBR322.[18]

For each series of samples both for RT-PCR than for quantitative PCR, neat and a 10-fold dilution of the RNA or DNA suspensions were run in duplicate; for quantitative PCR each dilution of standard DNA suspensions was run in triplicate. Standard precautions were applied in all assays, including separate areas for the different steps of the protocol and addition of nontemplate control and nonamplification control to each run. The presence of enzymatic inhibitors was evaluated by adding target DNA or RNA as an external control to a separate tube that was assayed with the same protocol condition of extracted nucleic acids.

Virus identification

Positive PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and confirmed by sequencing with an ABI PRISM 373 DNA Sequencer (Applied Biosystems by Life Technologies Corporation, Monza, Italy). The results were analyzed using the Basic Local Alignment Search Tool (BLAST; www.blast.ncbi.nlm.nih.gov) and the sequence analyses were carried out using the National Center for Biotechnology Information GeneBank.

HAdV infectivity test

Positive HAdV samples were assessed to reveal infectivity using cell cultures. The samples, after decontamination with chloroform, were cultivated on the A549 cell line (European Collection of Cell Cultures, Public Health England, Porton Down, Salisbury, UK) in Dulbecco's Modified Eagle's Medium (Euroclone, Milan, Italy) with 2% of fetal bovine serum (Euroclone, Milan, Italy). The culture was incubated at 37°C and observed daily by optical microscope for 2 weeks until typical cytopathic effects were detected. The first culture was followed by 2 subsequent confirmation steps.[18]