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Environmental survey to assess viral contamination of air and surfaces in hospital settings

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SUMMARY

The presence of pathogenic viruses in healthcare settings represents a serious risk for both staff and patients. Direct viral detection in the environment poses significant technical problems and the indirect indicators currently in use suffer from serious limitations. The aim of this study was to monitor surfaces and air in hospital settings to reveal the presence of hepatitis C virus, human adenovirus, norovirus, human rotavirus and torque teno virus by nucleic acid assays, in parallel with measurements of total bacterial count and haemoglobin presence. In total, 114 surface and 62 air samples were collected. Bacterial contamination was very low (<1 cfu/cm^2) on surfaces, whereas the 'medium' detected value in air was 282 cfu/m^3. Overall, 19 (16.7%) surface samples tested positive for viral nucleic acids: one for norovirus, one for human adenovirus and 17 (14.9%) for torque teno virus (TTV). Only this latter virus was directly detected in 10 air samples (16.1%). Haemoglobin was found on two surfaces. No relationship was found between viral, biochemical or bacterial indicators. The data obtained confirm the difficulty of assessing viral contamination using bacterial indicators. The frequent detection of TTV suggests its possible use as an indicator for general viral contamination of the environment.

Introduction

The risk of viral diseases in healthcare settings has important implications for both patients and staff. There are innumerable agents, routes of transmission, procedures and conditions that can cause infections in people attending units such as transfusion centres, laboratories, haemodialysis, infectious disease clinics, intensive care, and surgery, not to mention during clinical waste collection. Blood-borne viruses (human immunodeficiency virus, hepatitis B virus and hepatitis C virus) are the most widely studied with regard to transmission in healthcare settings, and although epidemiological studies indicate accidents as the primary cause of exposure, there is a hypothetical although not proven possibility of viral transmission via droplets and surfaces.[1,2]

'Airborne' viruses, such as influenza virus, respiratory syncytial virus, adenovirus, rhinovirus, coronavirus, measles, rubella and mumps viruses and parvovirus B19, are easily spread by 'droplets' that can be inhaled directly or settle on surfaces. Moreover, viral agents transmitted via the faecal–oral route, such as rotavirus, human adenovirus 40 and 41 and norovirus, are frequently associated with healthcare setting infections spread by air, hand and surface contamination.[3]

To assess and control the risk of infection in healthcare settings, environmental microbial contamination monitoring can represent an invaluable tool for determining the means of transmission, testing the efficacy of preventive measures, conducting periodic checks of hygiene levels, and alerting staff to the need for preventive measures and ensuring their compliance.[4] However, such monitoring is generally limited to bacterial indicators (mainly total bacterial count) and specific studies on pathogenic agents have rarely taken viruses into account.[5,6]

The biological characteristics, resistance markers, epidemiology and ecology of viral agents are quite different from bacteria, hence the microbial indicators commonly used for environmental monitoring cannot truly represent viral contamination. This has been well documented with regard to water contamination, although, so far, data on healthcare settings remain few.[7] Thus, the aim of the present

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study was to assess viral contamination on hospital surfaces and in the air using biomolecular tests by contemporaneously testing for the presence of different viral agents. The viruses chosen are considered representative of nosocomial infections commonly transmitted through various routes: hepatitis C virus for the bloodborne route; norovirus and human rotavirus for faecal–oral transmission; and human adenovirus for the respiratory route. Apart from these agents of nosocomial infections, we also considered the torque teno virus (TTV), which, although not clearly associated with any specific disease, is widespread among the healthy population, irrespective of age, sex and any risk factors, reaching a prevalence of 100%.[8] Since TTV DNA has been found in peripheral blood mononuclear cells, liver, faeces, urine, saliva, cord blood, amniotic fluid, breast milk, semen, cervical secretions and sewage, it may be regarded as a possible indicator of general viral contamination.[9] Along with viral detection tests, surfaces and aerosols were also monitored for viable bacterial count (VBC). Although this parameter is not strictly associated with contamination, it is generally used to assess hygienic conditions in healthcare settings and the effectiveness of cleaning and disinfection procedures.[10] For this reason, it was chosen as a comparison with the data on viral presence. Other specific bacteria were also sought, but were found to be wholly absent from surfaces. In addition, their presence and mean counts in air were so variable that no hypotheses could be made over any association with viruses. Finally, haemoglobin (Hb) determinations were also made. These are commonly used in forensic settings to reveal bloodborne contamination of surfaces, but have also been applied to healthcare settings.[11]

Methods

Study setting

The study was carried out over two years during the following periods: January to August, and January to July, in the following units of Pisa University Hospital (1605 beds): sterilisation centre, dental clinic, burn unit, paediatrics, haematology, bronchial endoscopy, general surgery, paediatric oncohaematology, endocrinology, heart surgery, intensive care, neurology, neurological surgery, intensive cardiac care, ophthalmology, and psychiatry. In total 114 surface and 62 aerosol samples were collected. The sampling points were chosen to reflect the potential for contamination from healthcare procedures. Sampling was performed during working hours or shortly thereafter.

Samples were analysed for:

- both surface and air samples: hepatitis C virus (HCV), norovirus genogroups I and II (NoV GGI and NoV GGII), human rotavirus (HRV), human adenovirus (HAdV), torque teno virus (TTV) and viable bacterial count (VBC);
- only surface samples: haemoglobin (Hb).

Sampling procedures

Surfaces

Four adjacent 36 cm^2 squares were delineated by a polypropylene mask and sampled. For the detection of the RNA viruses (HCV, NoV GGI, NoV GGII, HRV), one square area was eluted with cotton swabs soaked in 1 mL of 3% beef extract at pH 9. The eluate was then neutralised with 1 N HCl and viral nucleic acids extracted using QIAamp RNA Mini Kit (Qiagen, Hilden, Germany). The RNA recovery efficiency of the technique has been estimated at 76% in a previous study, which also indicated a detection limit of about 10^2 genomic copies.[12] The DNA viruses (TTV and HAdV) were detected using a commercial kit (DNA IQ System, Promega Italia, Milan, Italy) designed for forensic use and modified for the detection of virus on surfaces. A previous study with artificially contaminated samples indicated an approximate DNA recovery efficiency of 73% and a detection limit of around 10^3 genomic copies.[13] In this preliminary study, for the method set up we included negative controls to confirm the absence of TTV on swabs, sterile surfaces and equipments.

For bacteriological analyses, the third adjacent surface delineated by the polypropylene mask was eluted by cotton swabs soaked in a 0.9% w/v NaCl solution. Swabs were then incubated in 2 mL of nutrient broth for 20 min at 37 °C. The whole solution was then seeded by inclusion on to plates containing Plate Count Agar and incubated for 48 h at 37 °C.

Finally, the fourth square was sampled for haemoglobin detection, using a commercial kit (OC Hemocard, Alfabiotech, Pomezia, Italy) used for blood detection in faeces and modified for environmental samples.[12]

Air samples

Air samples were collected with an impactor sampler (Microflow, Aquaria, Italy). For virus detection, 1000 L of air were sampled on Rodac plates containing Tryptone Soy Agar (TSA). The agar was then eluted in 3% beef extract at pH 9, and viral RNA and DNA were respectively extracted using a QIAamp RNA Mini Kit and a QIAamp DNA mini Kit (Qiagen, Hilden, Germany). In this case, the recovery efficiency has been estimated at 40.3% for viable virus with cell cultures and the detection limit was about 4.5×10^6 pfu.[14]

For bacterial counts, 180 L were sampled using an impactor sampler (Microflow), with Rodac plates containing Plate Count Agar (PCA). The plates were then incubated for 48 h at 37 °C. All bacterial and viral counts were normalised to a volume of 1 m^3.

Viral detection

The nucleic acids extracted from samples were analysed according to published protocols of nested (RT)–PCR to detect and distinguish the target viruses: the primers, virus genome regions and reaction conditions are reported in Table I.[15–20] For each sample series, negative and positive specific RNA or DNA controls were used.

For the RNA viruses (HCV, NoV GGI, NoV GGII, HRV), a reverse transcription reaction was performed before the nested PCR: the extracted RNA was mixed with buffer containing 75 mM KCl, 3 mM $MgCl_2$, 50 mM Tris–HCl at pH 8.3, 10 mM DDT (Promega Italia, Milan, Italy), dNTPs, MuLV Reverse Transcriptase (Promega Italia, Milan, Italy) and the specific reverse primer for the virus target. They were then incubated under the conditions specified in Table I. For the first stage of the nested PCR, the obtained cDNA was mixed with reaction buffer containing 50 mM KCl, 0.1% Triton X-100, 10 mM Tris–HCl at pH 8.8, 2 μg/mL BSA, $MgCl_2$, dNTPs, 2 U of Taq Polymerase (Promega Italia, Milan, Italy) and specific target virus PCR primers. The samples were then incubated for amplification according to the specific protocol reported in Table I. In the second stage of the nested PCR, amplicons from the first step were mixed with primers and incubated according to the specific protocols reported in Table I.

For the DNA viruses (HAdV, TTV), in the first stage of the nested PCR the extracted DNA was mixed with reaction buffer containing 50 mM KCl, 0.1% Triton X-100, 10 mM Tris–HCl at pH 8.8, 2 μg/mL BSA, $MgCl_2$, dNTPs, 2 U of Taq Polymerase, and specific primers. The mix was incubated according to amplification protocols (Table I). In the second reaction, the amplicons from the first stage were amplified with the specific primers.

For each virus, the PCR products were detected under UV light after horizontal electrophoresis in 2% agarose gel. Positive PCR

Table I
Nested polymerase chain reaction (PCR) and reverse transcription (RT)–PCR for virus detection: target genomic region, primers and reaction conditions

Virus (region)	Primer: sequence 5′–3′	Protocol	Study
Norovirus GG1 (Capsid)	*SRI-1*: CCA ACC CAR CCA TTR TAC AT *SRI-2*: AAA TGA TGA TG G CGT CTA *SRI-3*: AAA AYR TCA CCG GGK GTA T	RT: 41 °C for 60 min and 95 °C for 5 min 1st step: 94 °C for 1 min, 25 cycles (94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), 72 °C for 1 min 2nd step: 94 °C for 1 min, 40 cycles (94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), 72 °C for 3 min	15
Norovirus GG2 (RNA pol.)	*SRII-1*: CGC CAT CTT CAT TCA CAA A *SRII-2*: TWC TCY TTY TAT GGT GAT GAT GA *SRII-3*: TTW CCA AAC CAA C CW GCT G	RT: 41 °C for 60 min and 95 °C for 5 min 1st step: 94 °C for 1 min, 25 cycles (94 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min), 72 °C for 1 min 2nd step: 94 °C for 1 min, 40 cycles (94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), 72 °C for 3 min	15
Rotavirus (Gene VP7)	*RV1*: GTC ACA TCA TAC AAT TCT AAT CTA AG *RV2*: CTT TAA AAG AGA GAA TTT CCG TCT G *RV3*: TGT ATG GTA TTG AAT ATA CCA C *RV4*: ACT GAT CCT GTT GGC CAW CC	Denaturation: 94 °C for 4 min with 0.5 μL DMSO RT: 41 °C for 60 min and 95 °C for 5 min 1st step: 94 °C for 1 min, 25 cycles (94 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min), 72 °C for 3 min 2nd step: 94 °C for 1 min, 40 cycles (94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), 72 °C for 3 min	15
HCV (5′ NCR)	*HCV1*: GAT GCA CGG TCT ACG AGA CCT C *HCV2*: AAC TAC TGT CTT CAC AGC CAG AA *HCV3*: GCG ACC CAA CAC TACTCG GCT *HCV4*: ATG GCG TTA GTA TGA GTG	RT: 42 °C for 60 min and 95 °C for 5 min 1st step: 94 °C for 5 min, 35 cycles (94 °C for 30 s, 45 °C for 1 min, 72 °C for 1 min 30 s), 72 °C for 7 min 2nd step: 94 °C for 5 min, 25 cycles (94 °C for 30 s, 45 °C for 1 min, 72 °C for 1 min 30 s), 72 °C for 7 min	16,17
Human adenovirus (Hexon)	*Hex1deg*: GCC SCA RTG GKC WTA CAT GCA CAT C *Hex2 deg*: CAG CAC SCC ICG RAT GTC AAA *Nehex3deg*: GCC CGY GCM ACI GAI ACS TAC TTC *Nehex4deg*: CCY ACR GCC AGI GTR WAI CGM RCY TTG TA	1st step: 94 °C for 3 min, 35 cycles (94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min), 72 °C for 5 min 2nd step: 94 °C for 3 min, 35 cycles (94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min), 72 °C for 5 min	18,19
TTV (UTR A)	*NG 133*: GTA AGT GCA CTT CCG AAT GGC TGA G AAC GCC *NG 147*: AGT CCC GAG CCC GAA TTG CC *NG 134*: AGT TTT CCA CGC CCG TCC GCA GC *NG 132*: AGC CCG AAT TGC CCC TTG AC	1st step: 94 °C for 9 min, 35 cycles (95 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s), 72 °C for 7 min 2nd step: 94 °C for 9 min, 25 cycles (95 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s), 72 °C for 7 min	20

DMSO, dimethylsulphoxide.

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, California, USA). The results were genotyped through the 'Basic Local Alignment Search Tool' (BLAST). Sequence analyses were carried out using the National Center for Biotechnology Information Genebank.

Results

Surfaces

The results of the environmental surface analyses are reported in Figure 1. The VBCs were consistently very low (<1 cfu/cm^2), probably due to frequent disinfection procedures in use. Nevertheless, haemoglobin was found in two samples from the dental clinic, and 19 samples (16.7%) tested positive for viral nucleic acids: one (0.8%) for HAdV DNA, one (0.8%) for NoV RNA, both from sites in the general surgery unit, and 17 (14.9%) for TTV DNA, from the sterilisation centre (5), paediatrics (2), haematology (3), bronchial endoscopy (2), endocrinology (1), cardiac care (1), neurology (1), ophthalmology (1) and psychiatry (1) (Table II). Sequencing of positive samples revealed that the HAdV belonged to type 2, NoV to the GGII, and TTV to genogroups I and III, with a higher percentage (65%) for genogroup III (Figure 1).

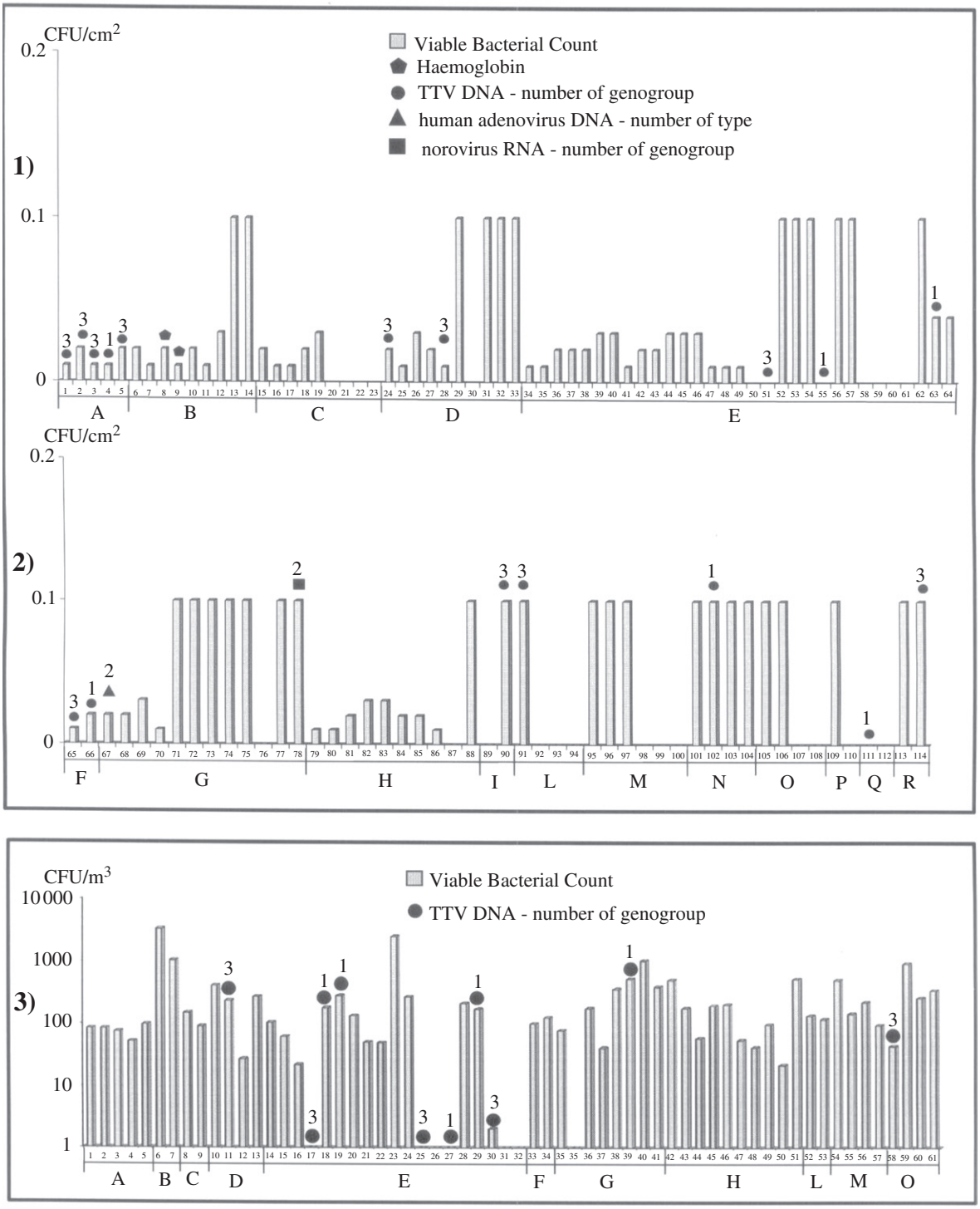
Air samples

Air samples (Figure 1) yielded an average VBC value of 282 cfu/m^3 (SD ± 535), corresponding to a 'medium' contamination level according to the European Commission (1993) (<500 cfu/m^3).[21] At eight (12.9%) sampling points, however, the load was 'very low', corresponding to 2 cfu/m^3 for a sterile room (no. 30) and below the detection limit for the other seven points. These last sampling points were located in a corridor (no. 27), an operating room (no. 36) and a further five sterile rooms, where the air is filtered by high efficiency particulate air (HEPA) filters (nos. 17, 25, 26, 31, 32). Of the viruses, only TTV DNA was detected in air samples. Six of the 10 positive samples demonstrated medium-level bacterial contamination and four, very low level. Of these latter air samples, three came from the six above-mentioned sterile rooms (nos. 17, 25, 30). The genome sequencing of the positive PCR samples confirmed positive results for TTV belonging to genogroups I and III (Figure 1).

Discussion

Due to the inherent difficulty in finding a correlation between environmental contamination and cases of infection, microbiological monitoring of the environment in healthcare settings is generally considered of limited utility.[22] It is more often associated with safety control rather than risk assessment. Although this is particularly true for viruses, where detection on surfaces and in air is very difficult, the low reliability of bacterial counts as indicators of viral contamination, suggests studying alternative parameters for assessing virological safety.[7]

In the present study, monitoring revealed the presence of viral nucleic acid in 29 out of 176 field samples; adenovirus DNA and norovirus RNA were found only once each and only on surfaces. TTV was detected frequently, with positive findings in 14.9% of surface samples and 16.1% of air samples, indicating environmental



Samples

1) Surfaces monitoring (sample 1-64)
2) Surfaces monitoring (sample 1-114)
3) Aerosol monitoring (sample 1-62)

Hospital Unit

A: Sterilization Center
B: Dental Clinic
C: Burn Unit
D: Paediatrics
E: Haematology
F: Bronchial Endoscopy
G: General Surgery
H: Paediatric Oncohaematology
I: Endocrinology
L: Heart Surgery
M: Intensive Care
N: Neurology
O: Neurologic Surgery
P: Cardiac Care
Q: Ophthalmology
R: Psychiatry

Figure 1. Results of surface (samples nos. 1-114) and aerosol (samples nos. 1-62) monitoring.

Table II
Samples positive for virus detection

Code letter (see Figure 1)	Hospital unit	Surface sampling points (positive: type of virus/total no.)	Air sampling points (positive: type of virus/total no.)
A	Sterilisation centre	Work benches (5: TTV/5)	Rooms (0/5)
B	Dental clinic	Walls (0/2), instruments (0/7)	Rooms (0/2)
C	Burn unit	Wall tanks (0/6), walls (0/1), instruments (0/1), bedside table (0/1)	Passage (0/1), emergency room (0/1)
D	Paediatrics	Walls (0/3), work benches (2: TTV/7)	Medical centre (1: TTV/3), operating room (0/1), room (0/1)
E	Haematology	Walls (3: TTV/13), work benches (0/6), trolley (0/5), instruments (0/7)	Medical room (0/3), passage (3/5), patient rooms (1: TTV/4), rooms with HEPA-filtered air (3: TTV/6), stock (0/1)
F	Bronchial endoscopy	Work benches (2: TTV/2)	Rooms (0/2)
G	General surgery	Walls (0/1), instruments (0/3), cupboards (0/2), others (1: HAdV, 1: NV/6)	Intensive care room (1: TTV/2), operating room (0/1), emergency room (0/1), medical centre (0/2), patient rooms (0/2)
H	Paediatric oncohaematology	Walls (0/1), instruments (0/3), trolleys (0/2), others (0/4)	Passage (0/1), patient rooms (0/8), laboratory (0/1)
I	Endocrinology	Walls (1: TTV/2)	–
L	Heart surgery	Door (1: TTV/1), work bench (0/1), trolley (0/1), cupboard (0/1)	Patient rooms (0/2)
M	Intensive care	Trolleys (0/2), wall (0/1), work bench (0/1), instrument (0/2)	Operating room (0/4)
N	Neurology	Walls (1: TTV/3), trolley (0/1)	
O	Neurosurgery	Walls (0/3), cupboard (0/1)	Patient rooms (1: TTV/2), operating rooms (0/2)
P	Cardiac care	Trolley (0/1), wall (0/1)	–
Q	Ophthalmology	Trolley (1: TTV/1), wall (0/1)	–
R	Psychiatry	Trolley (1: TTV/1), sink (0/1)	–
	Total	19/114	10/62

contamination consistent with the high prevalence of TTV carriage. Furthermore, the detected strains belonged to the genogroups most widespread in the general population.[23] This study is the first in which TTV has been sought and detected in a hospital setting, hence no comparisons with other studies are possible. The finding of different ratios of positive samples in different hospital units indicates that some areas may be more prone to viral contamination than others. The highest rates of positive results for surfaces were found in the sterilisation centre and bronchoscopy unit, and in the haematology units for air samples.

The parallel monitoring for viruses and two indicators (VBC and haemoglobin) failed to reveal any associations; on surfaces where VBC was consistently $\leq$0.1 cfu/cm^2, TTV DNA was more frequently detected than other viruses or haemoglobin. No differences in VBC were observed between virus-positive and -negative samples in air samples.

Although biomolecular methods for virus detection do not enable infectivity assessment, positive results indicate previous viral contamination. Accordingly, such an interpretation seems compatible with the present findings. Since TTV is non-cultivable, environmental longevity has been assessed only through DNA persistence. On surfaces where effective disinfection procedures have been applied (as confirmed by a very low bacterial load), detected TTV could have been inactivated, and its presence indicative only of previous contamination. Moreover, although the antibacterial action of the disinfectant used (1000–5000 ppm of chlorine) has been clearly established as per guidelines for hospital surfaces (5 $\log_{10}$ reduction), no such data are available for its effect on viruses. This is the case for virion inactivation and degradation of nucleic acids, so that any possible effects on PCR reaction are unknown.[24] In the air, which is not routinely disinfected (as indicated by the mean bacterial counts), TTV could presumably remain viable and possibly lead to airborne transmission. Where the air is filtered, as in sterile rooms, the presence of TTV DNA could indicate that air filters fail to retain the virus. However, the actual significance of its presence cannot be clearly understood without an evaluation of virus viability. Some experimental data (not shown) on TTV DNA persistence on artificially contaminated surfaces following different disinfection procedures have shown that after 2 h treatment with 2.9 ppm chlorine, 64.6% of the initial genome copies were still present. Virus infectivity remained indeterminable because TTV cannot be cultured. The same test, conducted in parallel with HAdV (culturable) revealed the persistence of 23.7% of genome copies and 5.8% viable virus. These data suggest the possible survival of infective TTV on surfaces, though to a lesser extent than suggested by its nucleic acid stability.

In conclusion, although total bacterial count revealed only a low-to-medium degree of contamination of the hospital environment, viral DNA was found to be widespread, both on disinfected surfaces and in filtered air. Since the spread of viral infections in healthcare settings is still an unresolved, and possibly underestimated issue, greater and more specific attention should be devoted to viral contamination, especially in areas where airborne or surface viral transmission could potentially lead to infections.

Conflict of interest statement
None declared.

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