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Norovirus recovery from floors and air after various decontamination protocols

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SUMMARY

Background: The dispersal of airborne norovirus (NoV) particles from the floor after contamination with faeces or vomit is a challenge for infection control, as this pathogen is infectious at low doses. Therefore, it is imperative to establish a safe protocol for floor decontamination.

Aim: To assess the presence of residual NoV-GII particles on floors and airborne particles following various floor decontamination procedures.

Methods: Two types of floor (vinyl and granite) were contaminated intentionally with 10% human faeces, positive for NoV-GII. Two decontamination protocols were implemented: cleaning followed by disinfection using 1% sodium hypochlorite, and cleaning followed by disinfection using a manual ultraviolet C (UV-C) light device. Swab samples were taken from the floors, and air samples were obtained using an air sampler. The TaqMan method for real-time reverse transcription-quantitative polymerase chain reaction was employed for analysis.

Findings: The disinfection protocol using 1% sodium hypochlorite after cleaning proved to be more effective than cleaning followed by UV-C light exposure ($P<0.001$). Viral particles were detected in 27 of 36 air samples after cleaning, with no significant difference between the two floor types. On average, 617 genome copies/sample were identified in air samples after cleaning, but the number decreased gradually after disinfection.

Conclusion: NoV-GII can be aerosolized during floor cleaning, and its particles may be inhaled and then swallowed or can settle on surfaces. Therefore, residual viral particles on floors must be fully eliminated. Cleaning followed by 10 min of 1% sodium hypochlorite disinfection proved to be the superior decontamination protocol.

Introduction

Norovirus (NoV) infection is one of the most common causes of gastroenteritis outbreaks, mainly due to exposure in semi-enclosed places. Contaminated food and water (including on cruise ships) [1], the persistence of NoV particles on high- and

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low-touch surfaces despite environmental hygiene measures [2,3], and the presence of NoV in the air in healthcare service areas where symptomatic and non-symptomatic patients are present have been described [3,4].

Sources of NoV include aerosol dispersal such as vomiting [5], toilet flushing, and agitation of bed linens and other fabrics [6–8]. However, to date, the hypothesis that NoV can be transmitted through airborne processes has been based solely on epidemiological outbreak reports that suggested indirect transmission (i.e. infected people who had not had direct contact with a source of infection) [9–11].

Contaminated surfaces can be touched by healthcare professionals, and when hand hygiene or glove removal precautions fail, the micro-organisms are spread to other surfaces and/or patients [12,13]. Considering the low infectious dose of NoV, even in healthy individuals (i.e. 10–100 particles) [14], the virus can persist in the environment for weeks [15], and its tolerance to disinfectant [16] raises concerns regarding the best and safest practices for eliminating NoV from the environment.

Moreover, it remains unclear whether NoV can be released into the air from floors. Thus, the aim of this study was to assess the presence of residual NoV-GII particles on floors and airborne particles after the application of various floor decontamination procedures.

Methods

In this experimental laboratory study, floor contamination was simulated using organic matter containing NoV particles, followed by the implementation of decontamination protocols. A contaminated faecal solution was poured directly on to the floor. This was subsequently cleaned and two different disinfection methods were used: 1% hypochlorite solution and a manual ultraviolet-C (UV-C) light device.

Faecal solution

Immediately before each stage of this study, a solution of 10% human faeces positive for NoV-GII was dissolved in phosphate-buffered saline (PBS) [17]. The faeces was obtained from clinical samples known to be positive for NoV-GII, assayed by real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and stored at -20°C. Before starting each experiment, 1000 μL of the solution was sampled and assayed by RT-qPCR to ensure that NoV particles would be present on the floor before starting the decontamination protocols; the solution was used as a positive control for each replicate.

Decontamination protocols

The experimental phase was performed in a closed room by two researchers who used personal protective equipment (i.e. N95 respirator, apron, gown, safety glasses and shoe covers). During the experiments, the temperature and relative humidity inside the room were controlled. After the end of each replicate, a UV-C lamp installed on the wall of the room was turned on for 30 min to decontaminate the room.

Slabs of vinyl and granite were used (60×80 cm) to mimic the floor of a typical healthcare service centre. Prior to the experiments, the slabs were cleaned and disinfected with 1% sodium hypochlorite for 10 min following the manufacturer's instructions (ProAction 1%, Grow, Indaiatuba, Brazil); the slabs were then rinsed three times with sterile distilled water and dried with sterile wipes. Following this initial cleaning, the floor slabs were contaminated with the faecal solution (500 mL), which was poured directly on to the slabs to prevent the formation of aerosols arising from the spill. The area of the slabs and volume of the solution were calculated based on visual contamination after simulating a projectile vomiting episode [18].

Ten minutes after the contamination event (i.e. the estimated amount of time between a surface contamination in a healthcare setting and a decontamination procedure), the standard decontamination protocols that were assessed in this study were commenced.

- Protocol 'H': cleaning by removing the excess organic matter with absorbent paper, sweeping with a microfibre mop that had previously been dampened in a neutral detergent and water solution, rinsing with tap water and disinfecting with 1% sodium hypochlorite for 10 min. The 1% sodium hypochlorite concentration was confirmed using a colorimetric test before starting disinfection.
- Protocol 'UV-C': validation of the appropriate UV-C light exposure time was performed using three slabs of granite (30×20 cm) that were intentionally contaminated with the faecal solution (250 mL) described above. Following the same cleaning steps described in Protocol 'H', the slabs were exposed to UV-C light using a manual device (irradiance of 13 mW/cm^2, wavelength 254 nm; Surface UV, MMOptics, São Carlos, Brazil) from a distance of 1 cm from the slab for 1-, 3- and 5-min exposure times. After swabbing the slabs with flocked nylon swabs (FloqSwabs, Copan, Brescia, Italy) and assaying by RT-qPCR, it was determined that NoV-GII was not present in the samples collected after 5 min of exposure. Therefore, Protocol 'UV-C' consisted of the same cleaning procedure as Protocol 'H' (removal of excess organic matter, friction with a disposable microfibre mop, water and neutral detergent, and rinsing), but included 5 min of disinfection with UV-C light (irradiance 3900 mJ/cm^2).

Each protocol was repeated three times for each floor type, one at a time to ensure disinfection of the room and to avoid cross-contamination, for a total of 12 replicates. The mop had a self-twisting system that was used to promote the rinsing and drying of the slabs. The mop was changed after each replicate.

Floor and air sampling

All samples were collected before contamination (negative control), after contamination (positive control), after cleaning and after disinfection.

Floor sampling was performed using sterile flocked nylon swabs (FloqSwabs). The swab was rotated and rubbed over the entire face of the slab, repeating the process in a zig-zag pattern. The swab tip was then placed in a tube with 1000 μL of PBS; when sodium hypochlorite was used, 195 μL of 1 N sodium thiosulphate solution was added [19]. This concentration proved to be effective in neutralizing the possible residual effect of the disinfectant on the surface, and was non-

toxic to NoV-GII (results not shown). After 30 min, the tube was vortexed for 20 s, and the tip was removed after being pressed against the wall of the tube [20]. The samples were centrifuged for 30 min at 5000 rpm at 4°C.

The Coriolis μ air sampler (Bertin Technologies, Paris, France), which was set at 300 L/min for 10 min (maximum period of time allowed for each sampling, following the manufacturer's instructions), was placed at two heights above the floor: 50 cm (h1; close to the floor) and 150 cm (h2; estimated height of the human mouth). Negative and positive control samples were collected at h2. For each replicate when decontamination protocols were performed, three samples were collected during and after cleaning (C1, C2 and C3) and three samples were collected after finishing disinfection (D1, D2 and D3). Samples C1 and D1 were collected at h1 and the others were collected at h2. The sterile cone coupled to the air sampler was prefilled with 15 mL of PBS. After finishing the air sampling, this volume was transferred to a centrifugal filter unit (Amicon Ultra15, porosity 30 kDa; Merck-Millipore, Darmstadt, Germany) and centrifuged for 10 min at 5000 rpm at 4°C.

The decontamination protocols, sampling times and sample sizes are described in Figure 1.

NoV-GII detection in samples

Viral RNA was extracted from the samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA was stored at -70°C until analysis and was thawed only once during the study.

The analysis was performed using the TaqMan technique for RT-qPCR. A modified and adapted protocol, published previously by Kageyama *et al.* [21], was employed with the primers F-cog2F and R-cog2R and the probe Ring2. Using the Superscript II Platinum One-Step Quantitative RT-PCR System Kit with ROX reference dye, the reaction solution was prepared with 12.5 μL of the reaction mix + 4 μL of ultrapure water + 1 μL of each primer (400 nM) + 0.5 μL of probe (200 nM) + 0.5 μL of Superscript III Platinum + 0.5 μL of ROX + 0.5 μL of the sample, for a final total volume of 25 μL. RT-qPCR StepOne Plus (Applied Biosystems, Life Technologies, Singapore) was used under the following conditions: reverse transcription at 50°C for 10 min, TaqDNA polymerase activation at 95°C for 2 min, followed by 45 cycles of 10 s at 95°C for denaturation and annealing, and extension at 60°C for 1 min. Cycle threshold (Ct) values >40 were considered negative. For RT-qPCR, Ct values are inversely proportional to the amount of nucleic acid in the sample; that is, a Ct value close to 40 was weakly positive. For quantitative analysis, a standard curve was generated based on Norovirus GII Q Standard, CeeramTools (bioMérieux, La Chapelle Sur, France).

Data management

Statistical analysis was performed using the Tobit censored regression model and a linear mixed-effects model. $P<0.05$ was considered to indicate statistical significance.

Ethical considerations

This study was authorized by the Technical and Scientific Committee of the study institution (CTC 01-I/2016).

Results

NoV was not recovered from the negative control samples from the floor and air, whereas the positive control samples obtained from the faecal solution showed, on average, 14.27 x 10^6 genome copies/sample (average Ct=22.08). The differences between the number of NoV genome copies after cleaning (average $5.04x10^3$ genome copies/sample and Ct=37.35) and after cleaning plus disinfection (average $7.65x10^1$genome copies/sample and Ct=39.70) were significant, proving that disinfection after cleaning was more effective than cleaning alone ($P<0.001$). Protocol 'H' was significantly more effective than Protocol 'UV-C' ($P<0.001$). Indeed, when there were residual particles on the floor after cleaning, 1% sodium hypochlorite eliminated them from all samples. Furthermore, Protocol 'H' was equally efficient for both floor types ($P=0.99$). In contrast, cleaning followed by UV-C disinfection eliminated NoV particles on the floor for 46.15%

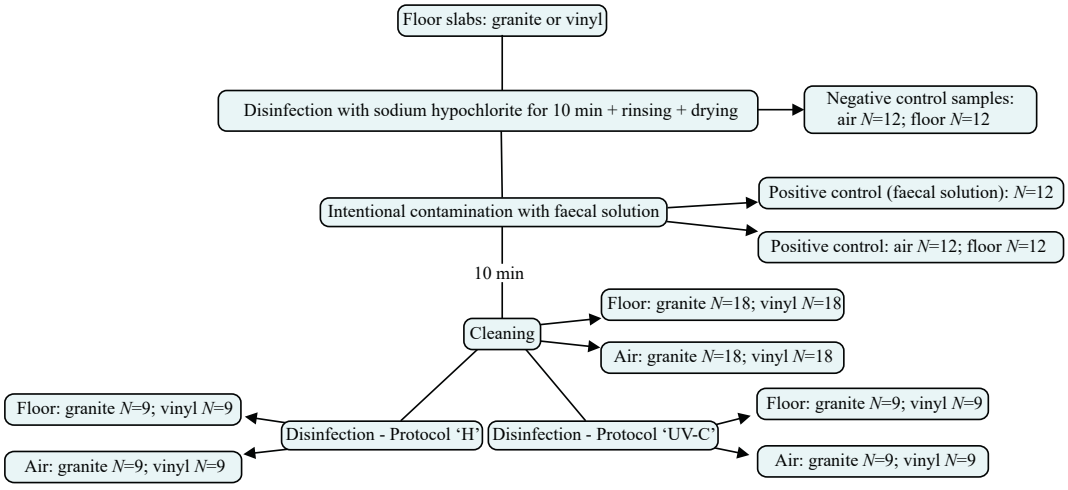

Figure 1. Flowchart with the decontamination protocols, sampling times and sample sizes. Protocol 'H', cleaning followed by disinfection with 1% sodium hypochlorite; Protocol 'UV-C', cleaning followed by 5 min of disinfection with ultraviolet-C light.

(6/13) of the samples. There was a residual average of 278.28 genome copies/sample on granite (Ct=38.75) and 28.1 genome copies/sample on vinyl (Ct=39.80). Protocol 'UV-C' was more effective for the vinyl floor compared with the granite floor ($P<0.001$) (Figure 2).

Immediately after contamination of the slabs, NoV particles were recovered from air in one of the 12 samples (Ct=38.38); 27 of the 36 (75%) samples collected after cleaning were positive, and Ct ranged from 31.55 to 40.00, meaning an average of 617 genome copies/sample. As three samples were collected after cleaning and three samples were sequentially collected after disinfection, it was possible to observe that the number of genome copies/sample reduced starting from the third sample after cleaning (C3, Figure 3). The presence of NoV particles in the air after cleaning was significantly higher compared with that after disinfection, independent of the disinfection method ($P<0.001$). In relation to the level of air contamination after decontamination, there was no significant difference between the two floor types.

In all 12 replicates in this study, the temperature and relative humidity inside the room increased (mean initial temperature of 22.7°C, mean final temperature of 23.1°C; initial relative humidity of 59.5%, final relative humidity of 69%). However, there was no evidence of a significant association between these variables and the results.

Discussion

To the authors' knowledge, this is the first study to show that NoV can be aerosolized from the floor during wet mopping, which may play a role in the transmission of airborne NoV. This evidence confirms the need to implement a safe decontamination protocol for use on floors that have been contaminated with organic matter. Furthermore, this research emphasizes the role of cleaning before disinfecting, even on floors where organic matter is present. This study found that cleaning with water and detergent followed by disinfection with 1% sodium hypochlorite for 10 min was effective for complete elimination of NoV from the floor; this result differed from that after disinfection with UV-C light.

The role of contaminated floors in the disease transmission model and the need to use disinfection protocols remain controversial. The main methods of pathogen transmission from floors are direct human contact or dispersal or re-dispersal of particles during human activities, such as walking, mopping and traffic flow [22]. Although it is difficult to confirm that a person can be infected through aerosolization of pathogens from the floor, dissemination of a non-pathogenic bacteriophage virus inoculated on to multiple floor surfaces suggests that floors contribute to microbial transmission [23].

Despite the known heavy contamination of floors in healthcare settings [22,24,25], re-contamination occurs rapidly even after cleaning and disinfecting [26]. Considering the low infectious dose of NoV [14], its prolonged persistence on surfaces [15] and the findings of the present study regarding aerosolization, NoV must be completely eliminated from floors and from high-touch surfaces. During a suspected or confirmed gastroenteritis case or outbreaks caused by NoV, floor cleaning followed by disinfection must be performed.

The decontamination protocol assessed in this study proves that cleaning, when conducted systematically, is able to eliminate all or most NoV particles from the surface evaluated. However, it is known that standard decontamination protocols have different results because they depend on human behaviours [27,28], which justifies the use of a safe and cost-effective disinfectant to complement the cleaning of large areas, such as floors. In this study, sodium hypochlorite proved to be superior compared with UV-C light, with the former eliminating NoV particles from all vinyl and granite samples. This result confirms the previously described efficacy of sodium hypochlorite against NoV [17,29]. Overall, this study found great variability after cleaning and disinfecting with UV-C light, with a residual average of 153.18 genome copies/sample, independent of floor type.

Based on the present results and the results of others [30], there is insufficient evidence to support the implementation of UV-C light to eliminate NoV in healthcare or other settings. Some limitations related to such efficacy reduction include distance, shading, organic matter load, type of surface, exposure time and pathogen type [31,32]. Surface UV (MMOptics, São Carlos, Brazil), the manual device used, presents additional

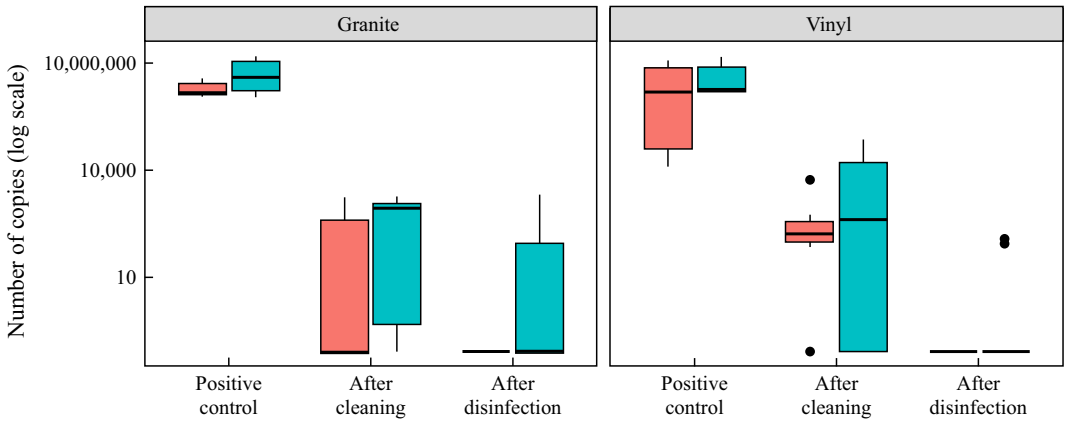

Figure 2. Average number of genome copies/sample from different phases of sample collection from the floor for different decontamination protocols and different floor types [i.e. after contamination (positive control), after cleaning and after disinfection]. Red bars, Protocol 'H' (cleaning followed by disinfection with 1% sodium hypochlorite); blue bars, Protocol 'UV-C' (cleaning followed by 5 min of disinfection with ultraviolet-C light).

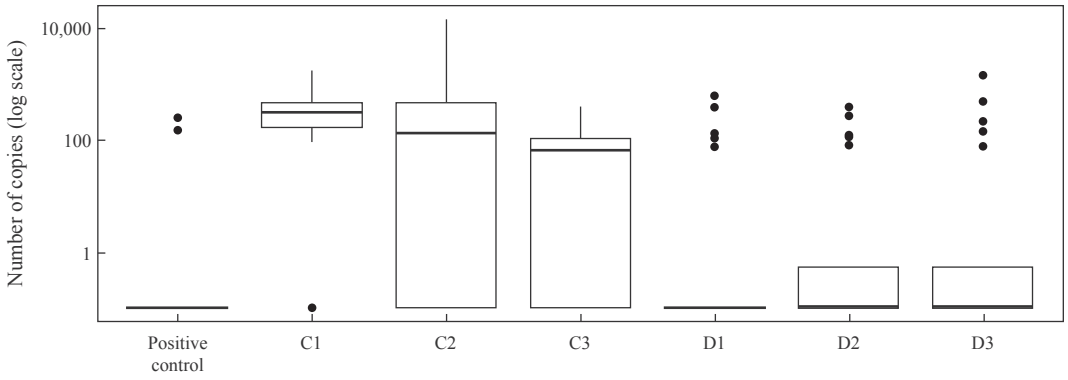

Figure 3. Average number of genome copies/sample of the samples collected from air following the two decontamination protocols at the following times: after contamination (positive control), after starting cleaning (C1, C2 and C3) and after disinfection (D1, D2 and D3) of the two floor types.

limitations such as difficulty in keeping the distance of 1 cm for a prolonged period, disinfection of large areas being hindered by a small area of UV-C light irradiation, and the risk of occupational exposure to aerosols among those handling the device. Therefore, the cost-effective relationship should be considered prior to the implementation of UV-C technology in hospitals or other settings where multiple pathogen contamination occurs on surfaces and different types of surfaces are present.

Although RT-PCR is the most sensitive and reliable diagnostic method for NoV, this method does not provide information about the infectious capacity of the detected residual NoV genomes when decontamination protocols are compared [16,33]. That is, it is not clear whether the NoV genomes identified on the floor and in the air in this study would be able to infect a person. Recent studies have analysed the effect of enzymatic pre-RT-PCR treatments to distinguish NoV infectious particles, although the findings are controversial [33,34]. Nonetheless, there have been new advances in cultivating NoV particles in human intestinal enteroids, which may improve analysis of NoV control measures [35,36].

To the authors' knowledge, this is the first study to evaluate clinical NoV particles in a controlled environment, and the study design enabled estimation of the aerosolization effect. Paton *et al.* [37] found that the number of *Bacillus atrophaeus* particles re-aerosolized from floors was higher after heavy walking traffic and at heights closer to the floor. In the present study, the highest number of positive samples were identified in air when the first samples were collected at 50 cm from the floor and during mopping. These NoV aerosols may be displaced by air flows or a route explained by Brownian theory, whereby billions of collisions promote the movement of small particles that can ultimately come into contact with high-touch surfaces or be inhaled, moved to the pharynx and swallowed [7].

The route of NoV transmission was not clearly determined, yet controlling transmission can be a challenge in semi-enclosed places, especially for immunocompromised patients who exhibit severe levels of disease [38]. Other at-risk groups are healthcare professionals and cleaning professionals, who are at the frontline of contamination, often before a diagnosis has been confirmed. In addition, these professionals are exposed to the NoV particles that spread to air after a vomiting episode or by toilet flushing, and there is a possibility that these professionals may become contaminated while wiping floors or other surfaces. The present findings suggest that a N95 respirator must be available to healthcare workers and cleaning personnel when symptomatic patients are present, and that these workers must receive instruction and training about the possibility of air contamination.

Additionally, other interventions can be implemented to avoid or minimize NoV airborne transmission. If a surface is contaminated with organic matter, decontamination should be performed as quickly as possible, and excessive organic matter should be removed with absorbent disposable paper; these measures constitute an important step that reduces the probability of aerosolization during wiping. The presence of ventilation and air conditioning systems in healthcare services with adequate exhaust air [39], or the promotion of natural ventilation when possible [8,40] can enhance the dilution of air and dissipation of the virus. Keeping windows open with a safely and carefully planned design has advantages, such as low cost and low maintenance, in addition to sustainability, for preventing airborne transmission in semi-enclosed places [8,41,42]. Finally, methods for air decontamination should be further investigated.

This study has strengths and limitations. In terms of strengths, this was an experimental study so variables could be controlled; this would not be possible in a healthcare setting. Large slabs were used instead of carriers to evaluate the efficacy of decontamination protocols that mimic the cleaning procedures used in healthcare settings. Additionally, the faecal solution used was prepared using clinical samples from human NoV infection and not an NoV surrogate. The impossibility of distinguishing between infectious and non-infectious NoV particles should be mentioned as a limitation.

In conclusion, NoV-GII is aerosolized even during the wet mopping of floors, regardless of floor type, and viral particles may be inhaled and then swallowed or settle on to surfaces, meaning that they must be fully eliminated. Cleaning (consisting of mopping with detergent and tap water) followed by 10 min of disinfection with 1% sodium hypochlorite disinfection proved to be superior to cleaning followed by disinfection with UV-C light.

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Conflict of interest statement
None declared.

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