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Persistence of viable MS2 and Phi6 bacteriophages on carpet and dust

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Abstract

Resuspension of dust from flooring is a major source of human exposure to microbial contaminants, but the persistence of viruses on dust and carpet and the contribution to human exposure are often unknown. The goal of this work is to determine viability of MS2 and Phi6 bacteriophages on cut carpet, looped carpet, and house dust both over time and after cleaning. Bacteriophages were nebulized onto carpet or dust in artificial saliva. Viability was measured at 0, 1, 2, 3, 4, 24, and 48 h and after cleaning by vacuum, steam, hot water extraction, and disinfection. MS2 bacteriophages showed slower viability decay rates in dust (−0.11 hr^{-1}), cut carpet (−0.20 hr^{-1}), and looped carpet (−0.09 hr^{-1}) compared to Phi6 (−3.36 hr^{-1}, −1.57 hr^{-1}, and −0.20 hr^{-1}, respectively). Viable viral concentrations were reduced to below the detection limit for steam and disinfection for both MS2 and Phi6 ($p < 0.05$), while vacuuming and hot water extraction showed no significant changes in concentration from uncleaned carpet ($p > 0.05$). These results demonstrate that MS2 and Phi6 bacteriophages can remain viable in carpet and dust for several hours to days, and cleaning with heat and disinfectants may be more effective than standard vacuuming.

KEYWORDS

built environment, flooring, indoor environment, particulate matter, resuspension, virus

1 | INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in more than 160 million cases and 3.3 million deaths worldwide[1] since reaching the pandemic designation in March 2020. Spread of SARS-CoV-2 occurs predominantly in the indoor environment.[2] Transmission occurs primarily through droplets and aerosols, though fomite transmission may also contribute at a

lesser level.[3-8] Dust represents an intermediate material that can be conceptualized as either a fomite or an aerosol. In fact, other respiratory viruses can be transmitted via particulate matter and have been conceptualized as "aerosolized fomites".[9,10]

Viruses, including SARS-CoV-2, can persist on contaminated surfaces or materials.[5] In fact, SARS-CoV-2 remains viable on plastics and stainless steel, with a half-life on the order of hours.[4] A Norwalk-like virus outbreak in school children followed a contamination event from an infected individual who vomited in a concert hall on the previous day.[11] Typically, enveloped viruses (eg, SARS-CoV-2, influenza) decay more rapidly on surfaces than non-enveloped viruses (eg, Norwalk-like viruses, rotaviruses).[12] Environmental conditions such as relative humidity and the composition of carrier droplets also impact virus survival. Viruses typically remain viable the longest at low relative humidity levels, and viability may decrease as relative humidity increases or demonstrate a U-shaped pattern depending on droplet composition and presence of a viral envelope.[13] SARS-CoV-2 is also temperature sensitive, with viability time increasing as temperature decreases on a variety of surfaces from stainless steel to cotton cloth.[14]

RNA from SARS-CoV-2 and other viruses may be present at high levels in dust[15] as well as outdoor particulate matter,[16] and viruses on the floor are rapidly transported to hands and other surfaces.[17] Some evidence indicates that high particulate matter levels are associated with increased measles spread,[18,19] although higher exposure to particulate matter could influence susceptibility separately. In fact, transmission of influenza via dust has been demonstrated in guinea pigs,[9] and dust may contribute to spread of avian influenza.[10] However, viral persistence on indoor dust is not well understood.

The goal of this study is to determine persistence of two representative RNA viruses, Phi6 and MS2, on indoor dust and on carpet. Both viruses are bacteriophages and have widely been applied as surrogates for assessment of environmental fate of pathogenic viruses.[20-23] Phi6 (Φ6) has an enveloped capsid,[24] and MS2 is non-enveloped.[25] In this study, we assess viral viability over time and after four cleaning measures: disinfection, vacuuming, steam application, and hot water extraction with stain remover (often incorrectly referred to as "steam cleaning"). Persistence was assessed both by culture (ie, viability) and RT-qPCR (ie, RNA detection) methods. Results of this work have important implications for understanding viral transmission and monitoring in the indoor environment. This may also inform recommendations for cleaning practices following viral contamination.

Practical Implications

- Resuspension of particulate matter from flooring is an important source of human exposure to microbes, including viruses.
- Viral infectivity on dust and carpet decreases faster for enveloped Phi6 bacteriophage compared to non-enveloped MS2 bacteriophage, and cleaning with heat and disinfectant is more effective for virus removal than vacuuming.
- These results indicate that viruses can persist on flooring for hours to days depending on the virus and environmental conditions.
- On contaminated flooring, non-enveloped viruses should be cleaned with heat and/or a disinfectant to ensure sufficient inactivation, although in many cases the viral agent including the presence/absence of a viral envelope may be unknown.
- The risk of viral infection from flooring as a potential transmission route should be studied, especially for non-enveloped viruses.

2 | METHODS

2.1 | Overview

The persistence of viable virus in house dust and residential carpet was observed in this study by using viral surrogates, MS2 and Phi6 bacteriophages. MS2 infects *Escherichia coli*, does not have an envelope, and has single-stranded, positive-sense RNA. Phi6 infects *Pseudomonas syringae*, has an enveloped capsid, and has double-stranded RNA.

To simulate viral deposition, the viral surrogates were placed in an artificial saliva mixture and nebulized onto carpet and dust samples. The virus was extracted from each sample using a wash and filtration step. We also evaluated carpet cleaning methods including vacuuming, steam, hot water extraction with stain remover, and disinfection to examine the effectiveness for inactivation or removal of the viral surrogates. For all samples, an RNA extraction and RT-qPCR analysis were performed to determine RNA quantity for each virus. Plaque assays were performed to determine viability.

2.2 | Carpet and Dust Samples

Carpet samples were supplied by a major manufacturer with no antimicrobial, stain resistance, or soil resistance coatings. The carpets were composed of polyethylene terephthalate (PET) carpet fibers and a synthetic jute backing. PET was selected as its use in carpet has increased replacing nylon as the predominant carpet fiber construction material.[26,27] Two types of fiber construction processes were examined that included a cut pile (finished pile thickness 10 mm) and a looped pile (finished pile thickness 7.5 mm). Carpet samples consisted of a 5 cm × 5 cm square that contained a 3 cm × 1 cm cutout (which remained inside carpet square until the viral extraction step) in the center that was used for viral viability and viral RNA assays to avoid edge effects. Triplicate carpet samples were used for each fiber construction type at each time point.

House dust was collected from a residential home vacuum bag in Ohio, USA. This dust was homogenized using a 300 μm sieve and confirmed to be negative for SARS-CoV-2 before use to comply with safety protocols, using the IDT SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay (Integrated DNA Technologies, Inc., Coralville, IA, USA) as described previously.[15] Dust was placed in sterilized glass dishes lined with tin foil that was previously baked (500°C for 12 h). Each dish contained two 50 mg aliquots, and a total of 3 dishes (6 dust piles) were used for each time point. One pile in each dish was used for viability assays and the other was used for RNA extraction and quantification. Carpet and dust samples were sterilized by autoclaving for 1 h at 121°C and then placed in a 100°C oven overnight (~12 h) to dry. Collection of dust for this study was approved by the Ohio State University Institutional Review Board (Study Number 2019B0457).

2.3 | Nebulization onto carpet and dust

Artificial saliva was created using a modified recipe[28] (Table S1). Porcine gastric mucin was obtained from Sigma-Aldrich (Type II, M2378).[29] We selected 10^8 plaque-forming units (PFU)/mL for both MS2 and Phi6 virus as a starting concentration to mimic the viral concentration of SARS-CoV-2 typically found in saliva, which ranged from 10^4 to 10^8 copies/mL one week after symptom onset.[30] Average viral concentrations in respiratory secretions and sputum range from 2.3×10^5 virions/mL to 1.9×10^7 virions/mL, depending on the time after symptom onset,[31,32] while peak viral load appeared 10 days after symptom onset.[31] This range of viral concentration in sputum was found to be similar in patients with both severe and mild SARS-CoV-2 symptoms.[33]

A total of 4 mL of the saliva with both MS2 and Phi6 (10^8 PFU/mL each) were nebulized into the chamber (3.8 L) with carpet or dust using a Medline Aeromist Compact Nebulizer. Nebulization of viruses onto samples were performed following a modified protocol that increased the nebulizing and settling time to 15 min.[34] A total of three nebulization runs were conducted for each time point. Each nebulization deposited the viral saliva solution onto triplicate cut carpet, looped carpet, and dust samples. The triplicate samples for each material type were then placed into separate incubation jars.

2.4 | Incubation

After nebulization, samples were placed into a 3.8 L autoclaved glass jar for incubation. A salt solution (500 mL DI water, 268 g $MgCl_2$) was made and placed in each jar to keep the equilibrium relative humidity (ERH) in the incubation chamber between 30% and 40%. Water activity of the salt solution was confirmed with an AquaLab 4TE (Meter Group, Pullman, WA). An Onset® HOBO® logger (Bourne, MA USA) was placed in the chamber to record temperature and ERH levels during the incubations. Incubation jars were covered with parafilm during each incubation period. Incubation time points that were sampled included time 0 (immediately after nebulization) followed by 1, 2, 3, 4, 24, and 48 h at which time the virus was extracted from each sample. All incubation jars were placed in a VWR incubator Model TFFU20F2QWA (Radnor, PA USA) at 25°C in the dark to avoid any potential ultraviolet (UV) light interactions. Actual temperatures recorded in the incubation jars ranged from 25 to 25.5°C for all jars.

2.5 | Carpet Cleaning

Several cleaning methods were evaluated for their effectiveness of inactivating Phi6 and MS2 bacteriophages. For these tests, cut carpet samples were nebulized with saliva/viral mixture as described above and cleaned immediately after nebulization (Time 0). Carpets were cleaned using vacuuming, hot water extraction with stain remover, steam, and application of a disinfectant solution. Each cleaning method was employed for 1 min, and then, viruses were extracted from each carpet sample. A standard canister vacuum (12-amp, 1440 W motor) was used for vacuuming, and a portable residential-grade hot water extraction carpet cleaner was used for hot water extraction. The carpet cleaner was equipped with a 3-inch cleaning attachment tool with bristles for water extraction. A soap solution of 500 mL of water and 75 mL of a commercially available carpet stain remover was heated to 60°C then added to the cleaning tank. Using the cleaning attachment tool, 3 sprays of the cleaning solution were applied to the carpet samples. The solution was allowed to sit for 10 s and was then removed using the attachment tool. For steam, water was boiled to 100°C in a 200 mL glass beaker. The carpet was placed upside down on top of the beaker so that the steam made contact with the carpet (Temperature on carpet backing measured 80°C). For disinfection, a disinfectant spray was created with active ingredients sodium troclosene (NaDCC) and hypochlorous acid (HOCl). This disinfection solution has been used to decontaminate indoor spaces such as COVID patient isolation rooms, not specifically on carpets, but could settle on to such materials in the process. The solution was diluted to a 1076 ppm available chlorine using 10 tablets in 946 mL of DI water as recommended by the manufacturer for emerging pathogens, including SARS-CoV-2. One spray (~2 mL) was applied to each carpet sample and allowed to sit for 1 min before viral extraction. The disinfectant could not be removed prior to washing, so disinfection may have continued in subsequent steps. All cleaning methods were compared to carpets that were not cleaned but were nebulized with the same saliva/viral solution over two experimental trials.

2.6 | Viral extraction

For carpet samples, the pre-cut 3 cm × 1 cm rectangles were pushed out and placed in a 50 mL plastic tube with 8 mL of phosphate-buffered saline (PBS) to wash the virus from the material similar to

previous extraction methods on fabric.[35] For all house dust samples, cups contained two separate 50 mg pre-weighed aliquots for each triplicate sample of which one was used for viability, and one was used for RNA analysis. Dust aliquots used for RNA analysis did not go through the PBS wash step due to potential inhibitory effects during the RNA extraction process. The carpet/dust and PBS were mixed by hand to gently wash the full surface areas of the materials and then vortexed. A total of 4 mL of each wash was extracted and placed in a Amicon® Ultra-4 Ultracel®-50k (Merck Millipore Ltd.) filter tube and centrifuged for 7 min at 7000 rpm. The filtrate collected on the top of the filter was collected into a 1.5 mL tube, and the volumes recorded for each sample. The process was repeated a second time using another 4 mL of PBS wash in order to collect enough for viability and RNA analyses.

For viability analysis, a dilution series of 9 was made for each sample using 100 μL of the collected sample and 900 μL of PBS. More details are below. Phi6 and MS2 were extracted from samples of nebulized carpet utilizing the QIAamp DSP Viral RNA Mini Kit (Qiagen, Germantown, MD) and from samples of dust utilizing the RNeasy PowerMicrobiome Kit (Qiagen, Germantown, MD), respectively. 140 uL of the homogenous mixture of virus and PBS was placed in the lysis tube from the QIAamp DSP Viral RNA, and the extraction protocol given in the kit was followed. To extract from the dust samples, 50 mg of nebulized dust was utilized following a modified RNeasy PowerMicrobiome Kit protocol using 10x the procedure recommended 2-mercaptoethanol and phenol chloroform-based lysis.[15] All RNA extractions performed included a processing blank that was confirmed negative by real-time quantitative polymerase chain reaction (RT-qPCR).

2.7 | Viral propagation and enumeration

Phi6 Bacteriophage.

Pseudomonas syringae (Phi6 bacteriophage host) was grown from a frozen stock (supplied by Dr. Karen Kormuth at Bethany College) on 1.5% Luria-Bertani (LB) agar plates (20 g/L Difco™ Miller LB Broth, 10 g/L Bacto™ Agar) for 48 h at 25°C. *P. syringae* was then transferred to an LB Broth (1 L DI water, 20 g Difco™ Miller LB Broth); one colony was used per 8 mL of LB Broth. This liquid culture was incubated for 16 h at 25°C while shaking at 180 rpm. Phi6 bacteriophage (supplied by Dr. Karen Kormuth at Bethany College) was propagated using an enhanced $MgCl_2$ solution (50 mL DI water, 50 mL LB, 1.25 mL of 1 M $MgCl_2$, 5 mL of *P. syringae* overnight culture, and 20 μL of stock Phi6) incubated for 24 h at 25°C. The enhanced solution was centrifuged for 30 min at 4000 rpm and filtered through a 0.22 μm filter. The high-titer Phi6 solution was made into a 40% glycerol solution and stored at −80°C until use. For enumeration of Phi6 bacteriophage, a 0.75% LBA (20 g/L Difco™ Miller LB Broth, 7.5 g/L Bacto™ Agar) was made and when cooled to 48°C was infused with *P. syringae* overnight culture (1 mL *P. syringae* per 10 mL of soft LBA). A total of 10 mL of the infused soft agar was pipetted into each culture plate. After the agar cooled, spot plating of each sample dilution was performed by using six 10 μL drops and was incubated for 24 h at 25°C. Plaques were counted with a Darkfield Quebec® Colony Counter (Reichart, Inc. Depew, NY, USA), and PFU/mL was calculated. This value was converted to PFU per square centimeter of carpet and milligram of dust.

MS2 Bacteriophage.

Escherichia coli F_{amp} (MS2 bacteriophage host) cultures were incubated in LB liquid media from a frozen stock (supplied by Dr. Karl Linden and Dr. Ben Ma at University of Colorado Boulder) on a shaker table at 180 rpm and 36°C for 16 h. After incubation, 1.467 mL of this overnight culture was transferred to 200 mL of LB and incubated at 225 rpm for an additional 2.5 h. For propagation of MS2 bacteriophage, 10 mL of this culture was transferred to a new flask where 1.267 mL of 1 M $MgCl_2$ and 633 μL of frozen MS2 stock (supplied by Dr. Karl Linden and Dr. Ben Ma at University of Colorado Boulder) were added. The solution was gently mixed and allowed to sit for 25 min before resuming incubation at 36°C at 185 rpm for another 2.5 h. After propagation, cultures were centrifuged at 7000 rpm at 10°C for 15 min. The supernatant was aliquoted into 1 mL stocks and stored at −80°C until use. For MS2 enumeration, a 0.75% LBA was made and when cooled *E. coli* F_{amp} from the 2.5-hour incubation was added (200 μL *E. coli* F_{amp} per 10 mL of 0.75% LBA). The samples were spotted onto the plates using the same method as the Phi6 bacteriophage and were incubated at 36°C for 24 h at which time PFU/mL was counted and calculated in the same manner as Phi6 bacteriophage. Viability detection limits for both MS2 and Phi6 bacteriophages on carpet, house dust, and plaque assays were 6.0 PFU/cm^2, 0.5 PFU/mg, and 16.7 PFU/mL, respectively.

2.8 | RT-qPCR

In preparation for cDNA synthesis, heat shock treatments were performed to denature the dsRNA segments in the Phi6 genome. For heat treatment, 5 uL of sample was held at 100°C for 5 min followed by 5 min on ice as recommended by Gendron.36 A heat shock treatment was not used for MS2 genomes. cDNA was reverse transcribed from RNA samples using the iScript cDNA Synthesis Kit (Biorad, Hercules, CA) according to the recommended reaction protocol on the ProFlex PCR System (Applied Biosystems, Forest City, CA). The cDNA was stored at −80°C. MS2 and Phi6-specific primers and probes were used to determine RNA concentrations. The MS2 forward primer (5'-GTCCATACCTTAGATGCGTTAGC-3'), reverse primer (5'-CCGTTAGCGAAGTTGCTTGG-3'), and probe (5'-/56-FAM/ACGTCGCCAGTTCCGCCATTGTCG/3BH) and the Phi6 forward primer (5'-TGGCGGCGGTCAAGAGC-3'), reverse primer (5'-GGATGATTCTCCAGAAGCTGCTG-3') and probe (5'-/5FAM/CGGTCGTCGCAGGTCTGACACTCGC/3BH) were used in the PCR reactions.[36] The PCR final reaction mixture contained 1X TaqMan® master mix (Applied Biosystems™), 1 μM of forward and reverse primers, 150 nM of MS2 probe or 300 nM of Phi6 probe, and 2 μl of cDNA template, and the volume was adjusted with sterile water to 25 μL.[36]

Quantitative polymerase chain reaction (qPCR) was completed on a QuantStudio™ 6 Flex System (Applied Biosystems™) with samples prepared on a 384-well (0.2 mL) plate in triplicate. Amplification protocol consisted of 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 94°C for 15 s and 60°C for 1 min. All RT-qPCR runs consisted of a non-template control (NTC) as well as Phi6 and MS2 bacteriophage standards as a positive control. High-titer Phi6 and MS2 pure culture solutions were used to create qPCR calibration standards. The pure cultures were extracted using the QIAamp DSP Viral RNA Mini Kit (Qiagen, Germantown, MD). The RNA standards were then quantified as previously described[15] using the QuantStudio 3D Digital (QS3D) PCR System (Applied Biosystems, Forest City, CA). Non-template controls were included with every run and were confirmed negative. We designated any value outside of the qPCR calibration standards for MS2 and Phi6 as non-detectable. All amplified values were well above these detection limits, and all non-detects reported were not amplified on qPCR. Detection limits for RNA persistence as measured by qPCR for carpet were 5111 gene copies/cm^2 for MS2 and 1920 gene copies/cm^2 for Phi6. In house dust, these detection limits were 307 genome copies/mg for MS2 and 115 genome copies/mg for Phi6.

2.9 | Statistical analysis

We evaluated decay of viral viability and RNA persistence over time on carpet and dust as well as the reduction in viral viability after use of cleaning techniques. The decay curves from all experiments were fit to a first-order decay model. The first-order decay rate constant, k (h^{-1}), can be calculated as the slope of the line ln (C_t/C_0) versus time where C_t is the concentration of the virus at any time t and C_0 is the initial concentration of the virus at time zero. The mean of triplicate measurements was calculated at each time point and used in the simple linear regression analysis to calculate the first-order decay rate constant. All decay rate constants, regression coefficients, and confidence intervals were calculated using GraphPad PRISM ver. 9 (GraphPad, San Diego, CA) fixing the y-intercept to zero. Confidence bands represent the boundary for all possible lines and were determined and plotted using GraphPad PRISM ver. 9. Statistical correlation between the decay of MS2 and Phi6 bacteriophages in the two different types of carpet (cut and loop) and dust was determined using analysis of covariance (ANCOVA). This analysis was also performed to determine correlation between the decay of the two different types of bacteriophages (MS2 and Phi6) in dust and artificial saliva. Comparison between untreated cut fiber carpet and carpet cleaned by vacuum, steam, hot water extraction, and disinfection was done for Phi6 and MS2 bacteriophage using Kruskal-Wallis test followed by Dunn's multiple comparisons test. Values below the detection limit were assumed to be half of the detection limit for the Kruskal-Wallis test.

3 | RESULTS

3.1 | Viral decay over time

Understanding decay viral viability over time in house dust and carpet is an important first step to understanding potential exposure for resuspended dust in the indoor air and contact with carpet materials. In house dust, Phi6 had a faster viability decay rate (−3.36 hr^{-1}) compared to MS2 bacteriophage (−0.11 hr^{-1}) (Figure 1, Table 1), and the difference was statistically significant (ANCOVA, $p = 0.0001$). Phi6 had faster viability decay than MS2 in both looped and cut carpet type, and the difference was statistically significant for cut carpet (ANCOVA $p = 0.0018$ for cut and $p = 0.20$ for looped).

Phi6 bacteriophage viability decay in saliva alone was not statistically different from zero whereas MS2 decay in saliva had a small growth rate that was statistically different from zero but much less than the rates for decay on carpet/dust ($p = 0.001$ for MS2, $p = 0.098$ for Phi6) (Figure S1, Table S2). Only single samples for both MS2 and Phi6 bacteriophages in saliva were run at each time point.

For both Phi6 and MS2 bacteriophages, viability decay occurred faster in cut carpet fibers (−1.57 and −0.20 hr^{-1}) compared to looped carpet fibers (−0.20 and −0.09 hr^{-1}), and the difference was statistically significant for Phi6 (ANCOVA $p = 0.0011$ for Phi6, $p = 0.35$ for MS2). Additional data including viability values and viable concentration plots can be found in the supporting information (Figures S2-S4, Tables S3-S5).

Decay of RNA was much slower compared to decay of viability in both carpet types and in dust for both Phi6 and MS2 (Figure 2, Table 2). Decay of Phi6 RNA was not significantly different from decay of MS2 RNA in looped and cut carpet but was significantly different in dust (ANCOVA $p = 0.67$ for loop, $p = 0.91$ for cut, and $p = 0.042$ for dust). MS2 RNA decay was also not statistically different from zero in dust, looped, and cut carpet (ANCOVA $p = 0.11$ for loop, $p = 0.12$ for cut, and $p = 0.31$ for dust). Phi6 RNA decay in dust and cut carpet was statistically different from zero whereas decay in looped carpet was not statistically different from zero (ANCOVA $p = 0.01$ for dust, $p = 0.03$ for cut, and $p = 0.06$ for looped). There was also no statistical difference in Phi6 RNA decay between dust, looped, and cut carpet types (ANCOVA $p = 0.07$ for looped/cut, $p = 0.19$ dust/cut, and $p = 0.57$ dust/loop). Additional data including raw qPCR values and RNA concentration plots can be found in the supporting information (Figures S5-S7, Tables S6-S8). Actual ERH measurements varied depending on the sample with cut carpet, looped carpet, and house dust all having different peak ERH and a different amount of time that it took to reach equilibrium (Figure S8).

3.2 | Removal after cleaning treatments

We compared concentrations of viable MS2 and Phi6 bacteriophage as well as concentrations of RNA from MS2 and Phi6 bacteriophage on untreated (no cleaning), vacuumed, steam treated,

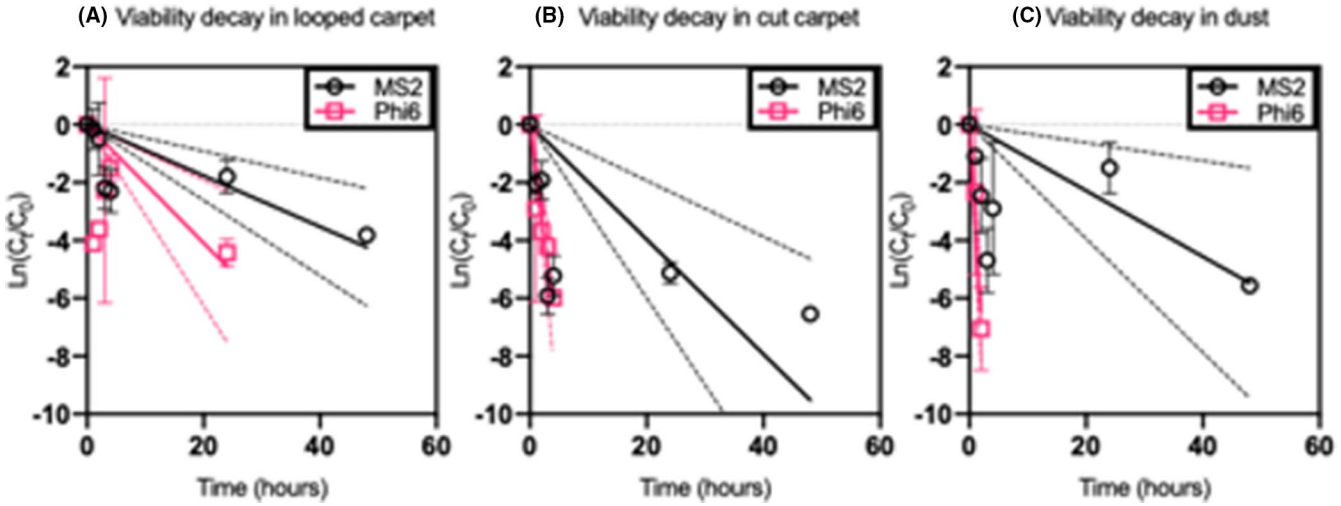

FIGURE 1 Decay of MS2 and Phi6 bacteriophage viable virus in loop (A) and cut (B) carpet types and dust (C). Each data point represents the average of experimental triplicate measurements from each sample type (cut carpet, looped carpet, and dust) at each time point. Each point represents mean sample concentration and error bars represent the standard deviation for each sample. Viable Phi6 was detectable for 24 h for looped carpet, 4 h for cut carpet, and 2 h in dust. Viable MS2 was detectable for the full duration of the experiment (48 h) in all conditions. Dashed lines represent 95% confidence bands for regression lines

TABLE 1 Viability first-order decay rate constants for Phi6 and MS2 bacteriophage in house dust, cut and loop carpet types.

Phage Viability	Conditions	k (hr^{-1})	CI	R^2	T_{90} (hrs)	T_{99} (hrs)
Phi6	Loop carpet	−0.20	−0.31 to −0.10	0.49	11.5	23.0
	Cut carpet	−1.57	−2.03 to −1.11	0.97	1.47	2.93
	Dust	−3.36	−4.27 to −2.44	0.98	0.69	1.37
MS2	Loop carpet	−0.09	−0.13 to −0.05	0.72	26.1	52.3
	Cut carpet	−0.20	−0.30 to −0.10	0.57	11.6	23.2
	Dust	−0.11	−0.20 to −0.03	0.54	20.2	40.4

disinfected, and hot water extracted carpet (Figure 3). These cleaning methods were tested on cut carpet fibers only. Viability concentrations were below the detection limit for cut carpet cleaned with steam and disinfectant for both Phi6 and MS2, and concentrations of Phi6 (Kruskal-Wallis, $p = 0.014$ for steam and 0.030 for disinfectant) and MS2 (Kruskal-Wallis, $p = 0.015$ for steam and $p = 0.028$ for disinfectant) bacteriophage were statistically different on these treated carpets compared to untreated carpet. Hot water extraction with stain remover and vacuuming left measurable viable MS2 and Phi6 bacteriophage on the carpet fibers, and concentrations of MS2 (Kruskal-Wallis, $p = 0.27$ for hot water extraction and $p = 0.095$ for vacuuming) and Phi6 (Kruskal-Wallis, $p = 0.28$ for hot water extraction and $p = 0.62$ for vacuuming) were not statistically different on these treated carpets compared to untreated carpet (Figure 3A).

Concentration of RNA in the untreated samples was not statistically different compared to vacuum cleaned or steam cleaned for Phi6 (Kruskal-Wallis $p = 0.29$ for vacuum and $p = 0.75$ for steam). Concentration of RNA from carpets cleaned with hot water extraction and disinfectant were statistically lower than the untreated samples for Phi6 (Kruskal-Wallis $p = 0.0007$ for hot water extraction and $p = 0.0023$ for disinfectant). Utilizing a vacuum or steam did not influence the RNA concentration of MS2 when compared to the untreated samples (Kruskal-Wallis $p = 0.28$ for vacuum and $p = 0.15$ for steam). However, RNA concentrations of MS2 were statistically lower when utilizing hot water extraction or a disinfectant (Kruskal-Wallis $p = 0.016$ for hot water extraction and $p = 0.0001$ for disinfectant). Additional raw data values for cleaning method viral viability and RNA are found in Tables S9-S10.

4 | DISCUSSION

Carpet and dust are potentially important reservoirs for microbial exposure to humans in the built environment because they serve as both a sink and a source for bacteria, fungi, and viruses.[37] Our work demonstrates that viruses can remain infective in dust for hours to days, and that the presence of a viral envelope may be an important factor in determining persistence time. Additionally, cleaning methods have a range of removal efficiencies, with methods that employ heat or disinfectants being more effective than vacuuming. RNA persisted on carpet and dust longer than viable viruses.

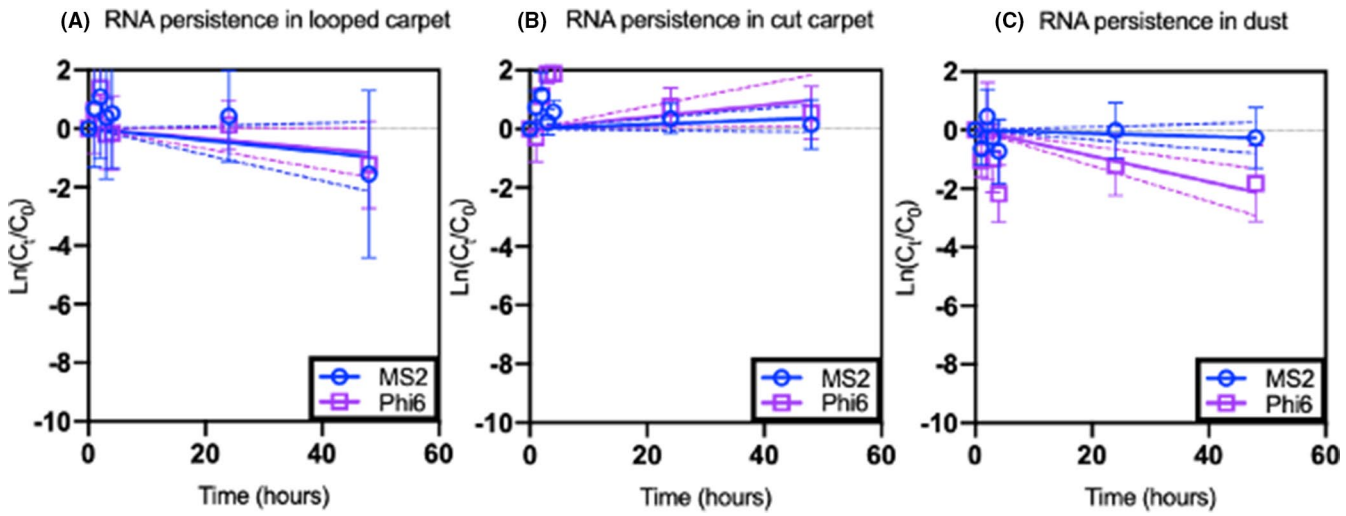

FIGURE 2 Decay of MS2 and Phi6 bacteriophage RNA in loop (A) and cut (B) carpet types and dust (C). Each data point represents the average of triplicate measurements. Each point represents mean sample concentration and error bars represent the standard deviation for each sample. MS2 and Phi6 RNA were detectable for the full duration of the experiment (48 h) in all conditions. Dashed lines represent 95% confidence bands for regression lines

TABLE 2 RNA first-order decay rate constants for Phi6 and MS2 bacteriophage in house dust, cut and loop carpet types

Phage RNA	Conditions	k (hr^{-1})	CI	R^2	T_{90} (hrs)	T_{99} (hrs)
Phi6	Loop carpet	−0.02	−0.04 to 0.00	0.56	115	230
	Cut carpet	0.02	0.00 to 0.04	0.14	N/A	
	Dust	−0.04	−0.06 to −0.03	0.50	52.1	104
MS2	Loop carpet	−0.02	−0.05 to 0.01	0.36	135	271
	Cut carpet	0.01	0.00 to 0.02	0.13	N/A	
	Dust	−0.01	−0.02 to 0.01	0.10	414	827

Note: N/A = Not applicable because k is positive so there is no observable decay

4.1 | Role of flooring in viral resuspension

Transmission of respiratory viruses occurs predominantly in the indoor environment, and it is critical to understand viral viability on flooring and dust because these are an important source of human exposure.[38,39] Exposure to virus in flooring will be influenced predominantly by two factors: 1) presence of the virus, influenced by deposition and viability decay, and 2) resuspension into the breathing zone. Regarding the former, nucleotides from SARS-CoV-2 and other viruses, like influenza, are detectable in dust and air samples, but often viability is not measured.[15,40-43] In our study, PET carpet fibers with a synthetic jute backing material were utilized and the fiber construction varied between looped pile and cut pile. Both viruses were viable longer on the loop carpet compared to the cut carpet. This may be attributed to the differences in construction between cut and loop pile products. Specifically, the cut pile consisted of 2 soft PET and 1 standard PET yarns piled together, while the loop pile only used the standard PET. In addition, the cut pile had a much larger surface area compared to the loop since the soft PET contains 3 times more filaments than the standard PET (private communication with the carpet manufacturer). The filament type and increased surface area may also have made it more difficult to extract viable MS2 and Phi6 bacteriophages from the carpet. Further studies are needed to understand these construction effects on viruses.

Resuspension of dust due to walking is an important contributor to human exposure, particularly in carpeted areas.[39] The resuspension of dust in flooring, however, is also likely impacted by type of flooring material such as looped carpet, cut pile carpet, and hard flooring.[44,45] A recent study posited that a possible source of aerosolized SARS-CoV-2 is due to the resuspension of floor dust from walking in areas with confirmed positive patients.[46] Dust particles contaminated with influenza virus may be resuspended and serve as aerosolized fomites of viable influenza virus.[9,47] In fact, the concentration of the resuspended influenza virus was 40% higher at one meter than two meters, such that shorter people and children may be more likely to come in contact with these particles.[47] Understanding the resuspension of SARS-CoV-2 and other viruses in carpet will be important in measuring exposure route, and resuspension models are needed to determine the rate of resuspension of SARS-COV-2 in varying carpet types.

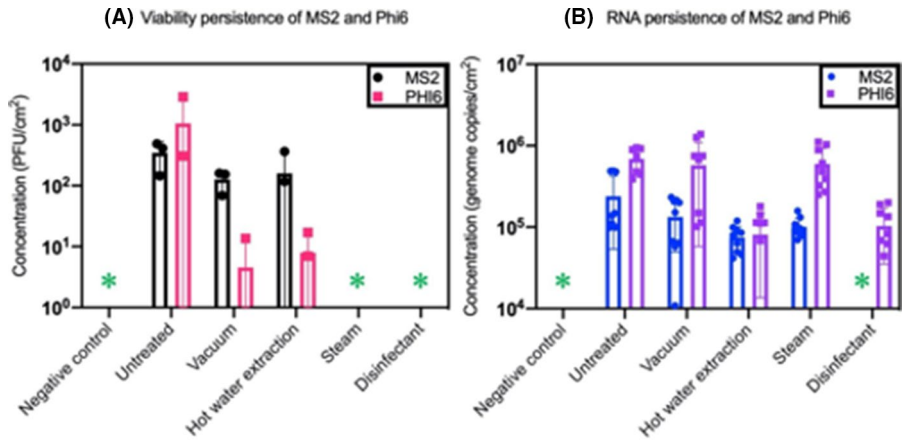

FIGURE 3 Concentrations of viable MS2 (black) and PHI6 bacteriophage (pink) on untreated (MS2 detected 3/3, PHI6 detected 3/3), vacuumed (MS2 detected 3/3, PHI6 detected 1/3), hot water extraction treated (MS2 detected 2/3, PHI6 detected 2/3), steam treated (MS2 detected 0/3, PHI6 detected 0/3) and disinfection treated (MS2 detected 0/3, PHI6 detected 0/3) cut carpets (A). Concentrations of RNA from MS2 (blue) and PHI6 (purple) bacteriophage on untreated, vacuumed, hot water extraction, steam treated, and disinfection treated cut carpets (B). Points represent concentration in each sample and error bars represent standard deviation. *Represents values below the detection limit. Viability detection limit for carpets is 6.0 PFU/cm^2. RNA Persistence detection limits for carpet were 5111 gene copies/cm^2 for MS2 and 1920 gene copies/cm2 for Phi6

4.2 | Viral envelope may impact persistence on flooring and dust

The presence of carpets as the primary flooring material indoors has been linked to viral infections, especially for non-enveloped viruses. In one instance, carpet fibers still contained Norwalk-like viruses 13 days after the last infection.[48] Outbreaks of the non-enveloped human Norwalk-like virus have also been linked to viral transfer of surfaces such as cotton and polyester.[49] In one study, human Norwalk-like virus surrogates were determined to survive for ~15 days on carpet fibers depending on relative humidity condition and fiber type.[50] Non-enveloped viruses, such as rotavirus and poliovirus, persist for up to two months on surfaces[51,52] while enveloped respiratory viruses, such as H1N1, human coronaviruses, and SARS-CoV, persist for several hours to days.[53,54]

In our study, the non-enveloped viral surrogate MS2 persisted as viable in carpet longer than enveloped Phi6. This is consistent with previous studies that found non-enveloped viruses (minute mouse virus and coxsackievirus B4) remained viable weeks longer than enveloped viruses (influenza type A and herpes simplex virus type 1) on plastic surfaces as measured using cell lines.[12] In general, viral envelopes are fragile and loss of function could result in faster loss of viability for enveloped viruses such as Phi6 bacteriophages compared to non-enveloped viruses like MS2.[55] As SARS-CoV-2 is an enveloped virus, it may persist in carpet more similarly to that of Phi6, and this can be evaluated in future studies. We used a first-order equation to model viral viability decay. Model fit may have been influenced by variability inherent in sample processing, which may be due to differences in nebulization, carpet wash steps, and viral plaque assays in general. However, the trend was clear that Phi6 viability decays more quickly than MS2. RNA from both bacteriophages persisted longer than viability. In this paper, we found that viral RNA was stable in carpet and dust, contrary to perceptions of RNA instability. The mechanism behind the extended RNA persistence from viruses on dust and carpet is an important, interesting topic for a future study. Bacteriophage RNA stability has important implications for using dust to monitor for viral outbreaks in buildings with high-risk populations.[15]

In all cases, the type of material does impact the persistence of the virus. Here, we saw different viability decay rates based on carpet fiber type and on carpet compared to dust. Additional work is necessary to understand specific mechanisms of viral inactivation in dust and carpet. SARS-CoV-1 survives on metal, wool, paper, glass, and plastic for 4–5 days.[56-58] In other cases, on non-porous surfaces such as glass, stainless steel, and vinyl, infectious SARS-CoV-2 was detected after 28 days; however, it was not detected on the porous material cotton cloth after 14 days at 20°C potentially due to an immediate absorption effect.[14] Persistence of SARS-CoV-2 on plastic (eg, PET, which is the same material of our carpet) lasts for up to 72 h and in one study had the longest viability of materials tested.[4]

4.3 | Relative humidity and virus viability

Airborne viruses are sensitive to humidity conditions. Viruses remain viable the longest at low relative humidity, and viability may decrease as relative humidity increases or demonstrate a U-shaped pattern (high-low-high) depending on droplet composition and presence of a viral envelope.[13] SARS-CoV-2 is most stable at low relative humidity (<40%) and stability decreases as relative humidity is increased between 50 and 90%.[59] Virus viability rapidly decays at high temperatures (38°C) and high relative humidity (>95%).[57] Other studies have determined that saturated humidity conditions may facilitate the spread of the SARS-CoV-2 via cluster spread.[60] At

elevated humidity conditions between 65% and 100%, aerosolized SARS-CoV-2 may be viable for up to 3 h.[4] In this study the relative humidity was maintained ~30% to mimic realistic home environment conditions; however, due to incubation limitations, this humidity level varied between 40% and 90% early in the incubation depending on the sample (Figure S1). The variation between carpet fiber structures could change the moisture retention properties and thus impact the decay rates for MS2 and Phi6 bacteriophages. However, this variation in carpet fiber construction is realistic in real-world building environments and should be investigated further to examine its effect on viral saliva droplet deposition and evaporation. Elevated humidity conditions in carpet dust are known to influence fungal growth,[61,62] and this humidity may influence the viability and stability of viruses in carpet dust. Continued work is needed to fully understand how elevated humidity in carpet may impact the viability as well as the stability of SARS-CoV-2 and other viruses in dust.

4.4 | Differential viral removal efficacy of cleaning methods

Inactivation (loss of viability) or removal (physical removal) of viruses from house dust or residential carpets is an important tool that can be used to reduce viral transmission in indoor environments. Inactivation was determined by measuring the decay of viability of MS2 and Phi6 bacteriophages (PFU/(mg dust or cm^2 carpet)), while removal was the measurement of RNA collected from these samples (Genome copies/(mg dust or cm^2 carpet)). Vacuuming is a common housekeeping routine used to remove accumulated soils from carpet. This method reduced viable viruses on our carpet samples when compared to carpet samples that were not cleaned but was not statistically significant ($p > 0.05$) and viable virus was still detectable in the carpet afterward. However, vacuuming, and hot water extraction removed viable Phi6 (enveloped) more effectively compared to MS2 (non-enveloped). Vacuuming could also resuspend viral particles into the air.[63] Similarly, hot water extraction is often used to maximize physical removal of soils in carpet and is readily available for use in homes. Hot water extraction is also often referred to incorrectly as "steam cleaning," although no steam is used. This cleaning method reduced viable virus on carpet samples, but viruses were still detected after cleaning and the difference was not statistically significant. In this study, we were unable to determine whether reduction of viable Phi6 and MS2 bacteriophages after vacuuming and hot water extraction was due to physical removal of the virus or inactivation. Applying steam to carpet samples reduced virus viability to below detectable limits but is not necessarily realistic for in-home decontamination of carpets. The application of a disinfectant to the carpet samples also reduced viruses to below detectable limits, although disinfectant may have continued to contact the virus during the wash step of our experiment. Disinfectants are an easy and realistic method building occupants may use to inactivate viruses on carpet following viral illness. The contact time for disinfection on the carpet fibers in this study was short and not completely saturated as recommended.[64] However, the particular use case for the disinfectant used in this study was not meant for direct disinfection of carpet but would settle on carpet fibers in the process. These factors are likely to be most important in commercial/public, high-risk areas such as hospitals, and may only be relevant in residential settings under unique circumstances. In addition, improper use of disinfectants could have potential adverse health effects on indoor occupants and increased discharge of chemicals into the environment.[65]

5 | LIMITATIONS

All carpet and dust samples were autoclaved before nebulization of viruses. However, due to the complexity of these samples, it is likely that bacterial and fungal quantities were substantially reduced but not completely sterilized.[66] A salt solution was used to attempt to maintain ERH in the incubation chambers at 30%–40%. However, each sample type (dust, cut, and looped carpet) showed different absorption of nebulized saliva, which affected the peak ERH and the time in which it took to lower to under 50% ERH. Cut carpet peaked close to 90%, looped carpet approximately 80%, and dust at 60% ERH while taking 12, 6, and 3 h, respectively, to reach 50% ERH. These differences in ERH and duration may have affected the decay rates of each viral surrogate in this study, although carpet structure might also reflect moisture retention in carpets in buildings. No chemical analyses were conducted on the house dust used in this study; therefore, it is possible its contents, such as surfactants, could potentially affect viability rates for enveloped and non-enveloped viruses. This may have also resulted in suboptimal first-order model fit, in addition to other factors that contributed to variation including variability in nebulization, carpet wash steps, and viral plaque assay limitations. Other models, such as biphasic decay, require more data points for accurate curve fit and could be evaluated in future studies. During aerosolization and deposition of the viral surrogates onto these samples, phage aggregates may have formed. This could create an uneven distribution among samples leading to an underestimation of the viable virus observed on carpet and in dust.[36] Different laboratory personnel performed the cleaning methods and time series viability testing but the same laboratory member within each experiment; therefore, user differences in the nebulization, carpet/dust extraction, and plaque assay protocols may have been introduced. For the disinfection cleaning method, we were unable to determine whether viral viability was lost from contact on the carpet or while it was mixed during the viral wash extraction protocol.

This study used bacteriophage viral surrogates, which are different from human pathogenic viruses. Bacteriophages are considered good surrogates to study airborne viruses. These viruses can be produced in large quantities, pose little hazard for laboratory workers, and do not require specialized containment protocols.[21] The bacterial viruses MS2 and Phi6 were chosen for this work because of their similarities with known pathogenic viruses, including SARS-CoV-2.[20,21,67,68] However, these are different viruses, and real pathogens may behave differently.

6 | CONCLUSIONS

These results suggest that MS2 and Phi6 bacteriophages can persist on dust and carpet for hours to days depending on viral structure and environmental conditions, but more information is needed to understand risk. RNA persists longer than viable viruses and may be useful for surveillance methods.[15] This current study was based on bacteriophages, and additional research is needed using human viruses in the appropriate biosafety facilities to confirm the results, followed by risk modeling. Additionally, many enveloped viruses (including SARS-CoV-2 and others) may only remain infectious in dust for a very brief period of time (on the order of hours post-deposition). Thus, transmission may only be possible shortly after deposition, when expelled respiratory aerosols may also continue to be a transmission risk in the same space. Cleaning of such spaces could be delayed by several hours to reduce infection risk to maintenance staff. An important aspect of this study showed that vacuuming may not be effective for removing viable MS2 and Phi6 bacteriophages from carpeted floors. Non-enveloped viruses (Norwalk-like viruses and others) may be more easily transmitted via flooring over longer periods of time, and appropriate cleaning techniques using heat and/or disinfectants may be more critical to reduce infection risk. Unfortunately, in many cases, the virus causing infection may not have been identified prior to the need for an environment to be cleaned, so it may be prudent to both wait some time if the room can be vacated and then employ heat-based or disinfection-based methods, when possible, to clean contaminated flooring.

Ultimately, future research can improve our understanding of dust as a potential transmission route for viral infection. Risk modeling should follow this analysis. This may have important implications for reducing viral spread in the general population, and also for custodial and cleaning staff who may be working closely to clean contaminated flooring. A more nuanced recognition of this potential exposure pathway can help contribute to the fight against viral illnesses such as influenza and COVID-19.

AUTHOR CONTRIBUTION STATEMENT

KD and NN with the assistance of NH and KB designed the experiment. NN wrote the manuscript with input, writing, and review from all authors. NN, SC, and JP performed all incubation and viral viability assays. AB and SC conducted cleaning experiments. NR, AB, and SH performed RNA extractions and quantification with qPCR. JG compiled data, made figures, and performed statistical analyses with input from KB. NB and KS assisted with material and sample preparations as well as viral inoculations. All authors approved the final version of the manuscript.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available in article supplementary material.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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