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Major article

Control of the spread of viruses in a long-term care facility using hygiene protocols

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Background: Approximately 50% of norovirus cases in the United States occur in long-term care facilities; many incidences of rotavirus, sapovirus, and adenovirus also occur. The primary objectives of this study were to demonstrate movement of pathogenic viruses through a long-term care facility and to determine the impact of a hygiene intervention on viral transmission.

Methods: The coliphage MS-2 was seeded onto a staff member's hands, and samples were collected after 4 hours from fomites and hands. After 3 consecutive days of sample collection, a 14-day hygiene intervention was implemented. Hand sanitizers, hand and face wipes, antiviral tissues, and a disinfectant spray were distributed to employees and residents. Seeding and sampling were repeated postintervention.

Results: Analysis of the pre- and postintervention data was performed using a Wilcoxon signed-rank test. Significant reductions in the spread of MS-2 on hands ($P = .0002$) and fomites ($P = .04$) were observed postintervention, with a >99% average reduction of virus recovered from both hands and fomites.

Conclusion: Although MS-2 spread readily from hands to fomites and vice versa, the intervention reduced average MS-2 concentrations recovered from hands and fomites by up to 4 logs and also reduced the incidence of MS-2 recovery.

Within the last 2 decades, contaminated fomites have become recognized as one of the primary mechanisms for the spread of health care–associated infections (HAIs). In many community and health care facility outbreaks, environmental surfaces have been identified as the primary reservoirs for pathogens, including various enteric viruses, such as norovirus and rotavirus. Viruses are often transferred throughout a health care facility on the hands of health care workers.[1,2] Pathogenic organisms are transmitted to workers either directly from colonized or infected patients or from contaminated patient surfaces. Many health care–associated pathogens have the ability to survive on hands or gloves from 2 minutes-1 hour.[3]

Once settled on a surface, viruses can remain in the environment for prolonged periods of time. Studies have shown that norovirus is frequently transferred from contaminated surfaces to fingertips and then to other surfaces, such as toilet lids, door handles, and telephones.[4] In addition to norovirus, other enteric viruses have also been identified as being transferred from fomite to fomite.[5] Under optimal conditions of pH, relative humidity, and temperature, a virus can remain virulent on a surface for several days.[2,5,6] Although inactivation and desiccation do occur, human exposure to even low doses of most viruses (10^1-10^2 virus particles) can cause infection.[7,8]

In previous studies, surrogate organisms have been used to model movement of pathogens through different environments. In a

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study design by Beamer et al, a surrogate organism was inoculated onto a door handle in an office work place. After a period of time, surfaces in the office and a group of volunteers were sampled for the tracer. The study then implemented a healthy workplace initiative in efforts to reduce the spread of pathogenic viruses throughout office settings.[9] Similarly, Sifuentes et al, inoculated 2 distinct tracer organisms, MS-2 and ϕx-174, into a hotel environment. The tracers were allowed to spread, and then after the given time period, they were found throughout the hotel facility. Because the tracers were found in areas outside of the inoculation site, the spread was attributed to contact with both housekeeping staff and guests of the hotel.[10] Tracer studies using surrogate organisms are an efficacious way to learn more about the dynamics of viral dispersion in different environments where the extent of pathogen spread and the associated risks of exposure may not be easily determined.[9,10]

Numerous pathogens have been identified on fomites in health care settings. In 2010, Weber et al,[2] defined the general characteristics of a pathogen that increase transmission and risk in a health care facility. The characteristics are as follows: prolonged survival on surfaces (days to months), retained virulence, frequent contamination of environmental surfaces, transient ability to colonize health care workers' hands, and transmission via health care worker hands.[2]

The spread of HAIs can be particularly detrimental in long-term care facilities (LTCFs) because of the vulnerable nature of the population. In the United States, approximately 12 million individuals rely on some form of long-term care (LTC) service, with older adults (≥65 years) comprising just over 50% of this group at >1.5 million residing in LTCFs.[11] Unfortunately, hand hygiene compliance among workers in these facilities is often inadequate. Studies have reported hand hygiene compliance rates as low as 14.7%[12] and 17.5%[13] in LTCFs. Between 1.6 and 3.8 million infections are reported in U.S. LTCFs every year.[14] Although the Centers for Disease Control and Prevention and the World Health Organization published protocols for hand hygiene designed for application in all health care facilities, LTC workers face different challenges and exposure scenarios than workers in typical hospital or acute care settings.

Schweon and Kirk outline various moments of unexpected patient contact that occur in LTCFs because of their home-like environments, which limit the practice of hand hygiene prior to contact with patients.[15] In some cases, a worker could experience a moment of patient contact without the opportunity to wash their hands after direct contact with a previous patient. LTC workers frequently experience unexpected moments of patient contact, such as hugging, kissing, and handholding. Other events that require immediate staff attention include emergency situations, such as safety alarms that need to be addressed quickly, body alignment or readjustments, and fixing clothing.[15]

The combination of unique contact moments and lower-than-average hand hygiene compliance by LTC workers and high infection rates suggests the need for adjustments to hygiene routines in LTCFs. Sustainable improvements in patient and staff hygiene behaviors and attitudes are expected to decrease pathogen exposures and infection risks in LTCFs.[15] In this study, staff and patient hygiene practices in a Southwestern United States LTCF were modified through a hygiene intervention consisting of product addition and replacement (hand sanitizers, gloves, face and hand wipes, disposable clothes, tissues, and disinfectant) and personnel education. The primary objectives of this study were to characterize movement of pathogenic viruses (via MS-2) throughout an LTCF and to quantitatively determine how a hygiene intervention impacts on the spread of these viruses. The bacteriophage MS-2 was chosen as a surrogate because it is of similar size and shape of multiple nonenveloped, human enteric viruses of clinical importance in LTCFs. MS-2 is also environmentally stable.[16]

Table 1
Sample location sites

Location	Items sampled
Entryway-lobby	Elevator button
	Hand railing—entry
	Hand railing—hallway
	Medicine cart 1
	Medicine cart 2
	Medicine room door handle
Dining room	Coffee table
	Door handle
	Chair 1
	Chair 2
Nurses' station	Large table
	Small table
	Records binder
	Medical chart
	Desk
	Stapler
	Phone
Team room	Door handle
	Table
	Chair 1
	Light switch
Patient rooms	Door handle
	Dresser
	Bedside table
	Bathroom door handle
	Remote call button
Activity room	Staff refrigerator door handle
	Faucet handle
	Food tray table
	Chair
	Game table
Shower room	Door handle—inside
	Door handle—outside
	Faucet handle
	Hand rails

METHODS

Sampling site selection

The study was performed in an LTCF skilled nursing unit with a maximum capacity of 67 patients. The unit was composed of primarily semiprivate rooms (2 beds), with some private and isolation rooms. The facility also had various shared community rooms, including a craft and activity room, therapy room, and dining room. In this ward, there were 3 staff-only rooms, 5 offices, 2 patient shower rooms, and a storage room.

A total of 37 fomites (Table 1) were chosen for sampling over the duration of the study. The site selection was based on observed staff member touch frequency, patient movement, and visitor movement over a period of 2 hours. In addition to sampling of fomites, 10 nursing and administrative staff volunteers were selected for hand contamination monitoring. Housekeeping staff were not included in the study because their direct contact with patients, visitors, and other staff was limited.

Study design

Sampling was conducted during pre- and postintervention periods consisting of 3 consecutive sampling days that served as replicates. During the preintervention phase, the spread of the viral surrogate throughout the facility was evaluated before the intervention. After the preintervention sampling, the 14-day intervention was implemented and immediately followed by 3 consecutive days of postintervention sampling.

In a single-blinded design, the hands of 1 volunteer were seeded with 100 μL of MS-2 (starting concentration 10^{12} plaque forming

Table 2
Hygiene intervention products, placement, and education

Product description	Product placement				Education training topics
	Nursing staff	Administrative staff	Housekeeping	Residents-communal	
Kleenex Ultra Moisturizing Hand Sanitizer Stands (Kimberly Clark Professional, Roswell, GA)	N	N	N	Y	1, 2, 4, 5
Kleenex Splash 'N Go! Moist Wipes (Kleenex, Neenah, WI)	Y	Y	N	Y	1, 2, 4, 5
Kleenex Ultra-Moisturizing Hand Sanitizer: personal and large pumps (Kimberly Clark Professional, Roswell, GA)	Y	Y	Y	Y	1, 2, 4, 5
Kleenex Anti-Viral Tissues (Kleenex, Neenah, WI)	Y	Y	N	Y	1, 2
SCOTT Disinfectant Spray (Kimberly Clark Professional, Roswell, GA)	N	N	Y	N	1, 2, 3, 4, 5
Kimberly-Clark Nitrile Exam Gloves (Kimberly Clark Professional, Roswell, GA)	Y	N	N	N	4, 5
KIMTECH PREP* KIMTEX* Wipers (disposable)	N	N	Y	N	4, 5

N, no; *Y*, yes; *1*, active ingredients; *2*, safety precautions; *3*, effective contact times; *4*, recommended times to use product; *5*, recommended methods for use of product.

Table 3
Percent of fomites positive for MS-2 pre- and postintervention

Experiment day	Preintervention	Postintervention
1	47.2 (17/36)	48.6 (17/35)
2	50.0 (18/36)	28.6 (10/35)
3	50.0 (17/34)	20.0 (7/35)
Overall	49.1 (52/106)	32.2 (34/105)

NOTE. Values are % positive (no. of positive samples/no. of total samples).

units) using a micropipette (Eppendorf, Hamburg, Germany), and 9 others were seeded with 100 μL of sterile letheen broth. The 10 volunteers were instructed to rub their hands together until dry. During the preintervention period, all staff were advised to continue their workday without deviation from typical activities, including washing hands, using hand sanitizer, and disinfecting personal items. Samples were collected approximately 4 hours after seeding of the volunteer's hands with the surrogate. Samples were collected from 100-cm^2 areas of each fomite and from the palms and fingers of both hands for each volunteer ($n = 10$) using a sponge stick (3M Brand, St. Paul, MN) moistened with 10 mL of letheen broth.

Intervention

After the 3-day preintervention phase, a 14-day hygiene intervention was implemented that consisted of 2 components: training and product placement. The products, placement, and education-training topics are listed in Table 2. Representatives from Kimberly-Clark administered the training component to the nursing, administrative, and housekeeping staffs. Training sessions were completed during staff breaks and lasted 15-30 minutes, depending on the group size. The nursing and administrative staffs were trained during the same sessions, and the housekeeping staff was trained separately. The main training topics covered were active ingredients, safety precautions, effective contact times, recommended times to use the product, and recommended methods for product use. Facility residents were given information on how to use the products by University of Arizona researchers when the products were distributed.

Preparation of stock culture

MS-2 (ATCC 15597-B1) was propagated using the bacterial host *Escherichia coli* (ATCC 15597). A pure colony isolated using a sterile loop was added to 150 mL of sterile Tryptic Soy Broth (TSB) (BD, East Rutherford, NJ) and incubated at 37°C with agitation for 4 hours. A 500 μL volume of host culture in log phase growth and 100 μL of phage were added to 5 mL of melted top agar and swirled gently to mix. The tubes were poured onto sterile Tryptic Soy Agar (TSA) (BD, East Rutherford, NJ) plates, solidified at room temperature (25°C), and inverted and incubated at 37°C for 24 hours. Six double-layer agar overlay plates were prepared using this method on the first day of propagation.[15]

After incubation, 6 mL of sterile 1X phosphate buffered saline (PBS; pH 7.4) was added to each plate and agitated every 30 minutes for 2 hours to elute the coliphage. After 2 hours, the eluent was collected using an electric pipette and transferred to a 50-mL conical tube. The tubes were centrifuged for 20 minutes in a tabletop centrifuge at 1,090 × *g* to remove bacterial debris. The supernatant was then filtered using a 0.22-μm filter (BD, East Rutherford, NJ) prewetted with 3 mL of 3% beef extract (BD, East Rutherford, NJ) (pH 9.1), and the bacteriophage concentration in the filtrate stock was determined via 10-fold serial dilutions with PBS plated using the double-layer agar method as previously described.[15] The propagation and purification of the coliphage were done in accordance with the ATCC recommendations and guidelines for MS-2 when used with *E coli* (ATCC 15597).

Sample analysis

Samples were transported on ice and processed within 24 hours. The sponges were eluted by application of manual pressure, the volume recovered from each sample was recorded, and the eluent was assayed using the double-layer agar overlay technique.[15] Only the samples from the seeded volunteer were diluted before plating using 10-fold serial dilutions with 1x PBS (pH 7.4). Sterile top agar tubes were used to mix the host organism and the sample before plating onto sterile TSA. The host (*E coli* ATCC 15597) was propagated in 125 mL of sterile TSB at 37°C with agitation to achieve exponential phase bacterial growth (approximately 4 hours).

A 500 μL volume of the host culture and 1 mL of the sample eluent were added to a melted top agar tube (approximately 50°C). The tube was swirled gently to mix and then poured over a sterile TSA plate, which was then incubated for 24 hours at 37°C. After the incubation period, plaques on each plate were enumerated and the data recorded. The concentration per 1 mL of sample was determined, and the concentration per total sample was calculated. These processing methods were used for all samples collected during the pre- and postintervention phases.

Data analysis

To assess the efficacy of the hygiene intervention, pre- and postintervention MS-2 concentrations and frequencies were compared using a signed-rank test, a nonparametric test that allows data to be analyzed based on an equality of populations rank. This test was chosen because the data contain pairs of observations

Table 4
Mean MS-2 concentrations isolated pre- and postintervention

	Preintervention					Postintervention				
Surface	n	Mean*	SD*	95% Confidence interval*		n	Mean*	SD*	95% Confidence interval*	
Fomites	106	1.10 E+06	1.13 E+07	<1	3.28 E+06	105	8.22 E+02	4.10 E+03	47.9	1.6 E+03
Hands	28	1.74 E+02	3.60 E+02	3.43 E+01	3.13 E+02	29	1.8 E+00	6.27 E+00	<1	4.19 E+00

*Units: fomites = PFU/100 cm^2; hands = PFU/sample.

Table 5
Arithmetic mean PFU per 100 cm^2 of MS-2 recovered from contaminated fomites

	Preintervention		Postintervention	
	All sites		All sites	
Experiment day	n	Mean	n	Mean
1	36	2.8 E+03	35	9.8 E+02
2	36	3.3 E+06	35	1.2 E+03
3	34	1.5 E+04	35	2.8 E+02
Overall	106	1.1 E+06	105	8.2 E+02

PFU, plaque forming units.

Table 6
Arithmetic mean PFU per sample of MS-2 for contaminated hands

	Preintervention		Postintervention	
	All hands		All hands	
Experiment day	n	Mean	n	Mean
1	9	1.3 E+02	10	5.2 E+02
2	10	1.1 E+02	10	<.01
3	9	2.9 E+02	9	<.01
Overall	28	1.5 E+03	29	1.8 E+00

PFU, plaque forming units.

and included many values that were below the limit of detection (1 PFU/site).[17] Separate signed-rank tests were performed for the fomites and hands sampling categories. The qualitative data (±MS-2 recovery) were also tested for marginal homogeneity using a McNemar test. Data analyses were performed using Stata 13 (StataCorp, College Station, TX) software.

RESULTS

Pre- and postintervention comparison

MS-2 was isolated from an average of 49.1% (52/105) of fomites sampled during the preintervention phase and 32.4% (34/106) of fomites after the intervention Table 3. The mean PFU of MS-2 recovered during the postintervention phase was reduced by 4 logs for fomites and 3 logs on the volunteers' hands versus the pre-intervention phase (Table 3 and Table 4). MS-2 was most commonly recovered from the large table located in the nurse's station, which was contaminated on 5 out of 6 total days (pre- and post-intervention). The average concentration of the 5 positive samples was 4,200 ± 842 PFU.

Data analysis

The results of the signed-rank test demonstrated a significant reduction in recovered MS-2 on sampled fomites ($P = .04$) and volunteers' hands ($P = .0002$). A McNemar test performed on the collective qualitative (±) results (N = 211) showed a statistically significant ($P = .0253$) decrease in the number of sites testing positive for MS-2 from the preintervention phase versus the post-intervention phase.

DISCUSSION

Bacteriophage movement

By 2050, the number of individuals using paid LTC services in any setting (eg, at home, residential care such as assisted living, skilled nursing facilities) is expected to double from the 13 million using services in 2000 to 27 million people, primarily because of an increase in the elderly population.[18] Little is known about the rate of nosocomial infections or the spread of pathogens in the unique environments of LTC. Because the LTCFs' nursing staff has a high level of direct contact with residents and patient environmental surfaces, they are presumed to be at a great risk of acquiring pathogens and spreading infection.[6] Although the use of gloves was frequently observed in the facility, previous studies have indicated that organisms can be transferred from gloves to bare hands during removal,[19] increasing the likelihood that nursing staff could unknowingly spread pathogens throughout the facility, including to fomites and other staff members.

This study evaluated the spread of a virus tracer during routine LTCF practices. MS-2 was recovered from various fomites throughout the facility, surviving the interval between seeding and sampling (approximately 4 hours) at concentrations of up to 10^7 on fomites and 10^3 on hands. These concentrations exceed the dose required for infection of 50% of the individual exposed (ID_{50}) of most enteric viruses, which typically range from 10-100 virus particles.[7] In addition, previous studies have determined that an average of 23% of the starting viral concentration on a fomite is transferred to the finger pads on contact.[20,21] Based on the average recovery of MS-2 from fomites during the preintervention phase, residents and staff could be at risk for exposure to infective levels of enteric pathogens.

Intervention

Postintervention sampling indicated a 16.7% reduction in the number of sites from which MS-2 was recovered. The average MS-2 concentration isolated from fomites was reduced by up to 4 logs. Based on the observed phage recovery, the intervention successfully reduced phage concentrations to levels below the ID_{50} (10-100 viral particles) for enteric viruses. However, the average phage concentration recovered from volunteers' hands still exceeded the ID_{50} at 820 PFU/sample. The intervention reduced the average concentration of phage recovered from the seeded volunteer's hands by 9 logs and from the hands of the other volunteers by 3 logs.

SDs and confidence intervals were calculated for each sampling phase (Table 5 and Table 6). The lower bounds of the confidence intervals were adjusted to <1 PFU/sample based on the limit of detection. The observed SDs were very high (10^2-10^6) because of the extreme variability of the data, with some concentrations being extremely low (<1 PFU/sample) and others much higher (10^7 PFU/sample). A different sampling

strategy could have been implemented to account for this variability, such as only sampling sites where direct contact was observed. However, this project was intended to quantify the movement of the surrogate virus throughout the entire facility, not limited to surfaces that were known to be contaminated. The confidence intervals were also widely varied, likely for the same reason.

During the intervention, all previously used hand sanitizers, gloves, tissues, and hand and face wipes were removed. The most commonly removed hand sanitizers included 70% ethanol and <5.0% isopropanol as the active ingredients. These sanitizers were sparsely distributed throughout the facility ($n = 5$) and on medicine carts ($n = 4$). Because staff members were not provided with individual bottles, many did not carry or have easy access to hand sanitizer. The distribution of hand sanitizers as part of the intervention had a substantial impact, reducing both incidence of MS-2 recovery and concentrations recovered. The distribution of >100 individual hand sanitizers allowed each staff member easy access before and after patient contacts (including direct contact and contact with patient surfaces). The placement of the hand sanitizer stands ($n = 6$) in the entryway and halls of the facility also provided many opportunities for visitors and residents to sanitize their hands. This study demonstrates use of hygiene products increases when these items are readily available for staff. It is likely that the same effect would be seen with residents and visitors; however, this was not evaluated in this study.

In addition to the accessibility of the hand sanitizing products, the educational training component was integral to the success of the intervention. In the training, staff members were reminded of appropriate times to use hand sanitizer as opposed to traditional soap and water handwashing. Each training session also educated staff about how easily microorganisms can pass from person to person, surface to person, and person to surface. During the post-intervention phase, researchers observed that staff members seemed to be more knowledgeable about microbial spread and movement and appeared more conscious of appropriate times for sanitizing hands and personal desk items.

Limitations

The impact of the other products used during the intervention phase is unclear. In both phases of the study, contamination of the resident areas was very uncommon ($n = 8$). Resident rooms were the primary areas where the housekeeping staff used the intervention cleaning products and the nursing staff used gloves. Based on the transient nature of staff members throughout the facility and because housekeeping schedules were not tracked, it was impossible to determine whether the bacteriophage was spread and then inactivated during the interval between seeding and sampling.

This study was conducted in a 67-bed facility; it is considered to be a smaller facility, as defined by the American Healthcare Association. This could provide a limitation to applying the same methods to a large-scale facility. The sample size for this study was 10 nursing staff members rather than the entire staff, which could limit the evaluation; however, these participants still provided us with a better understanding of the rapid viral spread within the facility. The housekeeping and therapy staff and patients were not included in the study, which could limit the identification of who in the facility was spreading the organism.

Future directions

In this study, the increased purposeful use of hygiene products, such as hand sanitizers, wipes, and disinfectant spray, significantly reduced the transmission of virus on fomites and the hands of staff in an LTCF. These results suggest that implementation of similar interventions could reduce the environmental transmission of pathogens in LTC settings. The described intervention model could be used by LTCFs to develop their own successful, cost-effective, pathogen control programs. For example, facilities could simply make hand sanitizer more readily available, as demonstrated in the described intervention, to decrease transmission of pathogens from the hands of staff members. Our previous studies have shown that reduction of viruses on surfaces corresponds to a reduction of exposure probability and overall infections risk.[9]

Although it is intuitive that a hygiene intervention reduces microbial contaminants on hands and fomites, this study has quantified the effect and provides useful data for quantitative risk assessment and cost-benefit analysis. This environmental assessment provides vital input values for future modeling of HAI risks, primarily with respect to enteric viruses and bacteria, associated with transmission via hands and fomites in LCTFs.

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