Inactivation of surrogate coronaviruses on hard surfaces by health care germicides

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Background: In the 2003 severe acute respiratory syndrome outbreak, finding viral nucleic acids on hospital surfaces suggested surfaces could play a role in spread in health care environments. Surface disinfection may interrupt transmission, but few data exist on the effectiveness of health care germicides against coronaviruses on surfaces.

Methods: The efficacy of health care germicides against 2 surrogate coronaviruses, mouse hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV), was tested using the quantitative carrier method on stainless steel surfaces. Germicides were o-phenylphenol/p-tertiary amylphenol) (a phenolic), 70% ethanol, 1:100 sodium hypochlorite, ortho-phthalaldehyde (OPA), instant hand sanitizer (62% ethanol), and hand sanitizing spray (71% ethanol).

Results: After 1-minute contact time, for TGEV, there was a $\log_{10}$ reduction factor of 3.2 for 70% ethanol, 2.0 for phenolic, 2.3 for OPA, 0.35 for 1:100 hypochlorite, 4.0 for 62% ethanol, and 3.5 for 71% ethanol. For MHV, $\log_{10}$ reduction factors were 3.9 for 70% ethanol, 1.3 for phenolic, 1.7 for OPA, 0.62 for 1:100 hypochlorite, 2.7 for 62% ethanol, and 2.0 for 71% ethanol.

Conclusion: Only ethanol reduced infectivity of the 2 coronaviruses by >3-$\log_{10}$ after 1 minute. Germicides must be chosen carefully to ensure they are effective against viruses such as severe acute respiratory syndrome coronavirus.

Key Words: Coronavirus; disinfection; surfaces; severe acute respiratory syndrome; SARS; environmental.

Health care-associated infections are responsible for thousands of deaths worldwide each year.[1] Approximately 5% of all nosocomial infections are because of viral exposure,[2] and, in pediatric wards, viruses account for at least 30% of health care-associated infections. Studies have shown viruses to be common in health care environments and capable of surviving for extended periods of time on environmental surfaces.[3] In these settings, health care workers, medical devices, and environmental surfaces can act as both a reservoir for infection and a mode of transmission of infection to patients and staff.[4,5]

In 2003, the nosocomial transmission of viral disease proved to be a major contributor to a worldwide outbreak of severe acute respiratory syndrome (SARS), caused by a novel human coronavirus (CoV) (SARS-CoV). Outbreaks of SARS occurred in multiple health care facilities, infecting patients, staff, visitors, and volunteers.[6] SARS-CoV was also found on environmental surfaces in hospitals where outbreaks occurred,[7] and studies demonstrated that it could survive on surfaces for 24 to 72 hours.[8] Airborne transmission was the main route of spread; however, Rabenau et al[9] observed that "there are a number of instances when transmission occurred through other means that are often still not well defined," and other studies of outbreak settings showed that providing handwashing facilities reduced transmission in hospitals,[10] suggesting that hands and surfaces could have played a role in transmission. The outbreak highlighted the need for effective and quick evaluation of means for controlling the spread of nosocomial infection.[11]

Disinfection of hospital surfaces is an effective measure for reducing the risk of exposure for health care workers and patients;[12] appropriate disinfection of contaminated surfaces and equipment is crucial in interrupting the spread of viruses such as SARS-CoV.[8,13-15] However, to assist in the selection of appropriate germicidal agents for use against coronaviruses on hospital surfaces and equipment, data are needed on the effectiveness of commonly used hospital germicides against coronaviruses. These data must

accurately reflect disinfectant efficacy against viruses under the conditions in which they occur on surfaces, such as desiccation and embedding in proteinaceous matrices. Many previous disinfection studies have used liquid suspension methods for testing germicide efficacy.[16-18] These studies report greater efficacy against viruses than studies performed with carrier methods. Viruses may be more resistant on surfaces than in suspension because they can adsorb to the surface or become embedded in organic material[19] and may be more difficult to inactivate with chemical germicides than viruses suspended in liquid. Thus, it is possible that suspension tests overestimate the level of antimicrobial activity of germicides against viruses on surfaces. Carrier-based methods may more closely resemble real environmental conditions in which viruses contaminate surfaces and provide a more conservative estimate of germicide activity against viruses that are dried onto environmental surfaces.

This study was undertaken using the carrier method to evaluate 6 chemical germicides commonly used in health care settings for their efficacy in reducing infectivity of coronaviruses on environmental surfaces. The germicides selected were 4 surface germicides and 2 hand sanitizers. Although hand sanitizers are not used for surface disinfection, the quantitative carrier test can help determine whether or not the active ingredients are effective against coronaviruses. Germicide evaluation was done using 2 non-human coronaviruses as surrogates for the *Coronaviridae* family and pathogenic human coronavirus such as SARS-CoV. The family *Coronaviridae* is divided into 3 groups. Groups I and II include human, mammalian, and avian coronaviruses, and group III consists of avian coronaviruses. Although SARS is thought to be related to the group 2 coronaviruses,[20] and phylogenetic analyses have indicated it may be closely related to mouse hepatitis virus (MHV),[21] there is still disagreement about the exact placement of SARS-CoV within the coronavirus family.[22] Based on this uncertainty, 1 representative of each group of mammalian coronaviruses was included in the study to determine whether there was any difference in their survival and persistence in water. The 2 viruses included in the study were transmissible gastroenteritis virus (TGEV), a diarrheal pathogen of swine and a member of the group I coronaviruses, and mouse hepatitis virus (MHV), a pathogen of laboratory mice and a member of the group II coronaviruses.[20]

MATERIALS AND METHODS

Preparation of viral stocks

MHV and TGEV were kindly provided by R. Baric, University of North Carolina, Chapel Hill. TGEV was grown in swine testicular cell cultures. MHV was grown in delayed brain tumor cell cultures. Viral stocks were propagated by infecting confluent layers of host cell cultures in flasks, harvesting cell lysates, clarifying by centrifugation ($3,000g$, 30 minutes, 4°C), and storing resulting supernatants as virus stock at -80°C. Viral titers were determined by the plaque assay method on confluent host cell layers in 60-mm Petri dishes with overlay medium consisting of 1% agarose, Eagle's minimum essential medium, 10% bovine serum replacement (Fetal Clone II; Hyclone, Logan, UT), 10% lactalbumin hydrolysate, and gentamicin (0.1 mg/mL)/kanamycin (0.05 mg/mL). Cell layers were stained with a second overlay containing 1% neutral red at 48 hours postinfection, and plaques were visualized at 72 hours postinfection.

Preparation of hard water

Hard water was prepared according to the USEPA OPP microbiology laboratory standard operating procedure for disinfectant sample preparation[23] from 2 stock solutions: solution A (14.01 g of $NaHCO_3$, 250 mL of sterile deionized water) and solution B (16.94 g $MgCL_2$-$6H_2O$, 18.50 g $CaCl_2$, 250 mL sterile deionized water). Solution A was filter sterilized using 0.22-μm pore size filters; solution B was autoclaved at 121°C for 30 minutes.

For hard water preparation 12 mL of solution A and 12 mL of solution B were added to a volumetric flask and brought up to 1 L with sterile deionized water. This solution was diluted with 2 additional liters of sterile deionized water. Final solution was adjusted to pH 7.6 to 8.0 by drop wise addition of sodium hydroxide or citric acid. A hardness testing kit (Hach Model 5-EP mg/L No. 1454-01; Hach Corp, Loveland, CO) was used to confirm that hardness of the prepared water was 380 to 400 mg/L $CaCO_3$.

Germicides

Six hospital-grade germicides were tested. The germicide types, active ingredients, and use-dilutions are summarized in Table 1. Germicides requiring dilution were prepared on the day of the experiment, using hard water as the diluent. All germicides were used by the manufacturer's expiration date.

Neutralizing solutions

Neutralizing solutions were used to inactivate germicide activity after the experimental contact time. Vesphene IIse (Steris Corp, Mentor, OH), 70% ethanol, Clorox Anywhere spray (Clorox Co, Oakland, CA), and Purell Sanitizing Hand Gel (Johnson & Johnson Inc, New Brunswick, NJ) were neutralized using 3% glycine. Chlorine bleach was neutralized with 0.1% thiosulfate and Cidex-OPA (Johnson & Johnson Inc, New

Table 1. Germicides tested

Germicide	Type	Active ingredient	Use-dilution
Steris Vesphene IIse Non-sterile disinfectant cleaner	Phenol	9.09% O-phenylphenol, 7.66% P-tertiary amylphenol	1:128
Chlorine bleach	Halogen	6% Sodium hypochlorite	1:100 (~600 mg/L)
Cidex OPA	Aldehyde	0.55% Ortho-phthalaldehyde	Undiluted
70% Ethanol	Alcohol	70% Ethanol	Undiluted
Purell hand sanitizer	Alcohol	62% Ethanol	Undiluted
Clorox Anywhere hand sanitizing spray	Alcohol	71% Ethanol	Undiluted

Brunswick, NJ) with 0.5% sodium bisulfite. To prevent cytotoxicity in cell culture assays, each neutralizing solution, with the exception of 3% glycine, was prepared as a stock solution and diluted to the use concentration with cell culture medium. Glycine was prepared by adding 2.5 mL cell culture medium to 47.5 mL of a 3% glycine solution. Sodium thiosulfate was prepared as a 10% (wt/vol) stock solution and diluted with cell culture medium to a use concentration of 0.1%. Sodium bisulfite was prepared as a 5% stock solution and diluted in cell culture medium to a use concentration of 0.5%.

Disc-based quantitative carrier test method for virus disinfection

Test surfaces were 1-cm^2 stainless steel carriers with a No. 4 polish. The quantitative carrier test method used was adapted from Sattar et al.[19] Each germicide experiment used 3 control carriers (no germicide applied) and 3 test carriers (germicide applied). Each experiment was performed in duplicate. Each carrier was placed in a 24-well plate, and 20 μL of virus suspension was applied. The virus was allowed to dry for 2 hours. After drying, 50 μL of use-dilution germicide was placed on the dried virus suspension on 3 test carriers, and 50 μL of cell culture medium was placed on 3 control carriers. After 1-minute contact time, 950 μL of neutralizing solution was added to the 3 test carriers to halt virucidal activity, and 950 μL of cell culture medium was added to the control carriers. To elute viruses from carriers, 150 μL of 15% beef extract (pH 7.5) was then added to all 6 carriers. Carriers were agitated on a shaking platform (60 rpm) for 20 minutes. The liquid from each well was then recovered, diluted, and assayed for virus infectivity as previously described. To determine the reduction in virus infectivity, the concentration of virus per 20-μL sample volume was calculated. Reduction in viral titer was calculated using difference in virus concentration between test carriers and control carriers. Log$_{10}$ reductions were calculated for each germicide based on 6 independent exposure trials.

Statistical analysis

Statistical analysis was performed using SAS 9.1 (2008; SAS Institute Inc, Cary, NC). A 1-way analysis of variance (ANOVA) was used to compare the log$_{10}$ reduction among germicides. Additionally, a 2-way ANOVA was performed to compare the efficacy of all surface germicides between the 2 virus types.

RESULTS

Reductions of MHV and TGEV infectivity on surfaces by hospital germicides are shown in Table 2. For MHV, only 70% ethanol and Purell Hand Gel (62% ethanol) produced a log$_{10}$ infectious virus titer reduction factor >2.5 after 1-minute contact time. Hypochlorite (1:100 use dilution) was least effective, producing a log$_{10}$ reduction factor <1. Hypochlorite, Vesphene IIse, Cidex-OPA, and Clorox Anywhere spray each produced a log$_{10}$ reduction factor of <2.5, with log$_{10}$ reduction factors of 0.62, 1.33, 1.71, and 1.98, respectively. Statistical analysis using 1-way ANOVA showed that the mean log$_{10}$ reduction factors for the 6 germicides differed significantly ($P < .0001$).

For TGEV, infectivity reduction factors of >3-log$_{10}$ were observed for 70% ethanol (3.19), Purell Hand Gel (4.04), and Clorox Anywhere spray (3.57). Vesphene and Cidex against TGEV produced intermediate log$_{10}$ infectivity reduction factors of 2.03 and 2.27, respectively. As seen with MHV, hypochlorite exposure resulted in an infectious TGEV titer log$_{10}$ reduction factor <1 (0.35). Statistical analysis using 1-way ANOVA showed that the mean log$_{10}$ reduction factors were significantly different ($P < .0001$) among the 6 germicides.

Analysis of the mean log$_{10}$ reductions of MHV and TGEV by germicides was done using the Tukey multiple comparison test to determine whether reductions by individual germicides significantly differed from one another ($P < .05$) (Table 3). In addition, 2-way ANOVA was used to compare 2 independent variables, germicide and virus type, and their influence on the log$_{10}$ virus reduction factor. This analysis aids in determining how much of the variability in reduction is explained by each of these 2 variables, as well as potential

Table 2. Disinfection efficacy against MHV and TGEV on carrier surfaces

Germicide	Log$_{10}$ infectivity reduction (95% CI)	
	MHV	**TGEV**
Chlorine bleach	0.62 (0.52-0.72)	0.35 (0.24-0.45)
Vesphene IIse	1.33 (1.16-1.51)	2.03 (0.89-3.17)
Cidex-OPA	1.71 (1.35-20.7)	2.27 (2.09-2.45)
70% Ethanol	3.92 (3.32-4.53)	3.19 (2.97-3.40)
Purell hand sanitizer	2.66 (1.77-3.56)	4.04 (3.57-4.51)
Clorox Anywhere hand sanitizing spray	1.98 (1.68-2.27)	3.51 (3.29-3.73)

CI, confidence interval.

Table 3. Statistical comparison of disinfectant efficacy for TGEV and MHV

Comparison	Significant (*P* < .05)	
	MHV	**TGEV**
Bleach vs Vesphene	No	Yes
Bleach vs Cidex	Yes	Yes
Bleach vs 70% ethanol	Yes	Yes
Bleach vs Purell	Yes	Yes
Bleach vs Clorox spray	Yes	Yes
Vesphene vs Cidex	No	No
Vesphene vs 70% ethanol	Yes	Yes
Vesphene vs Purell	Yes	Yes
Vesphene vs Clorox spray	No	Yes
Cidex vs 70% ethanol	Yes	No
Cidex vs Purell	No	Yes
Cidex vs Clorox spray	No	Yes
70% Ethanol vs Purell	Yes	No
70% Ethanol vs Clorox spray	Yes	No
Purell vs Clorox spray	No	No

interaction between them. Using 2-way ANOVA, infectivity reduction results are statistically significant (*P* < .0001). A type III sum of squares test was used to examine variation among mean reduction results. Germicide had a greater influence on log$_{10}$ viral reduction (*P* < .0001) than did the virus type (TGEV vs MHV) (*P* = .0008). Additionally, when interaction between germicide and virus type was evaluated using the ANOVA test, it was determined that there is a statistically significant interaction between virus type and germicide type (*P* = .0001).

DISCUSSION

Health care-associated transmission can play an important role in the spread of coronavirus infection, and coronavirus nucleic acids have been found on hospital surfaces in outbreak settings.[7] Data from surrogate coronaviruses suggest that these viruses can survive for long periods on hard surfaces, potentially posing a continued risk of infection if health care surfaces are not adequately disinfected. The efficacy of 6 hospital surface germicides was tested against 2 coronaviruses, MHV and TGEV, used as surrogates for SARS-CoV. These findings expand the available data on disinfection beyond what has been previously studied using other surrogates such as human coronavirus 229E. Although 229E shares some tissue tropism with SARS, there are important differences. Studies of SARS patients and outbreaks demonstrated that SARS-CoV is also a fecally shed virus, with intestinal tissue tropism; 229E lacks this. This may indicate important differences in resistance to environmental stressors because fecally shed viruses must be able to survive the conditions in the gastrointestinal tract, including extremes of pH, abundance of other microbes, and bile salts. This gastrointestinal tropism has played an important role in at least 1 major outbreak; this suggests that surrogates such as MHV (a virus with multiple tropisms) and TGEV (an enteric virus) that reflect this diversity in tissue tropism are necessary.

A log$_{10}$ viral reduction factor of >3 has been previously suggested as a benchmark for effective virucidal activity against coronaviruses and other viruses on surfaces.[3,15,17,24] The results of this study show that, of the commonly used hospital germicides tested, only the ethanol-based germicides were able to achieve this level of reduction of infectious virus after 1 minute of contact time. For MHV, the 3 ethanol-based germicides (71%, 70%, and 62% ethanol) gave the greatest reduction in infectivity, with log$_{10}$ reduction factors ranging from 1.5 to 4.9. This was greater than the reductions observed with hypochlorite, phenolic, and orthophthalaldehyde based germicides. These same performance trends are evident in tests of these germicides against TGEV. The ethanol-based germicides gave log$_{10}$ infectivity reduction factors ranging from 2.9 to 4.6, greater than those observed for hypochlorite, phenolic, and orthophthalaldehyde germicides. The phenolic and orthophthalaldehyde germicides had greater virucidal activity against TGEV than against MHV. However, only the reductions in infectivity by orthophthalaldehyde were significantly different between MHV and TGEV. Statistical analysis indicates that mean log$_{10}$ viral reductions differed significantly based on both the type of germicide and the type of virus tested and that there are interaction effects between germicide and virus type. Hence, both the selection of germicide and the type of virus will influence the resultant magnitude of reduction of virus infectivity titer.

Several previous studies have determined that surface disinfection is an important method for preventing viral transmission from surfaces to humans.[17] The risk of acquiring infection decreases proportionately to the amount of viral agent present on surfaces.[3] This study shows that ethanol-based germicides achieve the

greatest reduction in viral titer on surfaces. These findings are consistent with previous studies of coronavirus disinfection, but this study provides more precise estimates of inactivation on surfaces than have been previously observed with other human coronaviruses, such as 229E.

Some previous studies of chemical disinfection of 229E have been limited by cytotoxicity problems that limited the ability to measure infectious virus reduction.[25] Sattar et al[26] found that 70% ethanol reduced human coronavirus 229E dried onto a stainless steel surface by >99.9%. This study shows that the actual reduction is slightly greater than $3-\log_{10}$ (3.9 for TGEV and 3.19 for MHV). The available data on disinfection of SARS-CoV itself are not extensive, partly because of the challenges of working with SARS. Much of the available data focuses on alcohols. Seventy percent ethanol was found in 1 study to inactivate SARS by $>3 \log_{10}$.[27] Rabenau et al[9] reported a $\log_{10}$ reduction factor of >5 after 30-second contact time using 78% ethanol and 70% propoanol, a reduction that was actually greater than what they observed with other germicides classified as chemical steriliants. These results support the findings of this study that ethanol-based disinfectants are efficacious against coronaviruses. However, flammability limits the use of ethanol for spills or contamination events involving large surface areas.

Other studies show that disinfectant efficacy can vary by virus type, with nonenveloped viruses such as adenovirus differing from enveloped viruses such as the coronaviruses. Studies of hospital germicide efficacy against adenovirus 8 using the same carrier-based method with 1-minute contact times found greater $\log_{10}$ reduction factors by OPA (4.37) than were observed in this study for TGEV (2.27) and MHV (1.71). In contrast, Vesphene IIse reduced adenovirus 8 by a $\log_{10}$ reduction factor of only 0.41, compared with 1.33 for MHV and 2.03 for TGEV in this study. Reduction of adenovirus 8 by 70% ethanol was similar to that observed for coronavirus in this study, but 0.12% hypochlorite reduced adenovirus 8 approximately $4-\log_{10}$ greater than reductions observed for coronaviruses with 0.06% hypochlorite.[5] This suggests that disinfection efficacy may differ greatly by virus type and that nonenveloped viruses may not be appropriate surrogates for predicting the effects of disinfectants on enveloped viruses such as coronavirus and influenza.

Hypochlorite demonstrated a $\log_{10}$ reduction factor <1 after 1 minute for both TGEV and MHV when applied at the 1:100 (0.06%) use-dilution prescribed by the manufacturer. Previous studies of coronavirus disinfection have found higher reductions with concentrations of hypochlorite greater than the recommended use-dilution, suggesting these results are consistent with a concentration-dependent effect. Sattar et al[26] reported

99.9% reduction of viral titer for human coronavirus 229E when 0.10% and 0.50% sodium hypochlorite solutions were tested with 1-minute contact times. Commercial marketers of sodium hypochlorite recommend contact time of 5 minutes. In actual use, it is likely that contact times are shorter than this, and increases in concentration may be necessary to offset the use of shorter contact times. The results of this study suggest that the 1:100 use-dilution should not be recommended for use on surfaces with suspected contamination by coronaviruses. Hypochlorite is an important environmental surface disinfectant in health care; without the flammability and rapid evaporation of ethanol, it is suitable for large surface area spills. Increases in both sodium hypochlorite concentration and contact time should be evaluated to determine whether these factors would improve virucidal activity of hypochlorite on hard surfaces to achieve a $>3-\log_{10}$ reduction performance target.

Organic matter may play an important role in the poor performance of hypochlorite on surfaces observed in this study. A concentration of 600 mg/L on a surface produced a $\log_{10}$ reduction factor <1 of MHV and TGEV in this study. The poor performance of hypochlorite against viruses dried into surfaces may be due to the high oxidant demand exerted by the proteinaceous cell culture medium matrix in which the viruses were suspended. This results in consumption of available hypochlorite by the proteins and other organic compounds (eg, amino acids) present in the matrix, rendering it unavailable for disinfection. Use of viruses suspended in a proteinaceous matrix simulates the real world conditions under which viruses shed by human hosts occur in health care environments. Viruses are not shed by an infected host as single purified particles; they occur as aggregates, surrounded by membranes and embedded in feces, mucus, and other proteinaceous matrices. Together, these results suggest that changing the recommended use-dilution of hypochlorite may address the problem of oxidant demand exerted by proteinaceous material such as body fluids and result in effective inactivation of coronaviruses shed from human hosts.

Hypochlorite, phenolic, and OPA disinfectants tested reduced infectious viral titer by $<3 \log_{10}$ after 1-minute contact time. OPA is used as a high-level disinfectant for semicritical equipment such as endoscopes;[28] viruses can be deposited on the surfaces of semicritical equipment items during patient care. These results suggest that sufficient contact time is crucial to ensure that inactivation of viruses on the surfaces of semicritical equipment items takes place. According to the manufacturer, the phenolic disinfectant tested demonstrated a 3- to $4-\log_{10}$ reduction in viral titer after 10 minutes contact time when tested against another surrogate

coronavirus, avian infectious bronchitis virus. The results from this study, using 1-minute contact time, suggest that virucidal efficacy is greatly compromised if germicides are not used according to label directions and contact time is shorter than manufacturer recommended contact times.

Enveloped viruses of great nosocomial importance and pandemic potential, such as SARS CoV and avian influenza, are extremely challenging to work with in the laboratory. In the event of re-emergence of SARS-CoV, and in the context of pandemic influenza control, data are needed to guide decisions on appropriate disinfection of health care surfaces for control of viral transmission. TGEV and MHV are nonpathogenic to humans and easily propagated and assayed in cell culture by plaque and quantal MPN assays.[29,30] This study shows that these types of surrogate viruses can help expand our knowledge of practical aspects of virus control, such as inactivation by disinfectants, for viruses of public health importance.

It also increases the available data both for disinfectants that have previously been studied with SARS CoV and disinfectants that have not. Previous studies using SARS have evaluated benzalkonium chloride and magnesium monoperphthalate-based products,[16] povidone iodine,[27] formalin, and glutaraldehyde.[31] This current study included sodium hypochlorite and a phenolic, which have not been evaluated in previous studies. Previous investigators have tested several types and concentrations of alcohols, including isopropanol, propanol, and ethanol, for which varying results have been observed in studies of SARS. The data from this study can add to existing knowledge to help clarify how effective alcohols are against coronaviruses on surfaces.

Four of the 6 germicides tested showed greater reductions of TGEV compared with MHV. This suggests that MHV is potentially a more conservative surrogate for evaluating disinfectant efficacy against SARS-CoV. These studies should be replicated using SARS-CoV to determine which surrogate virus is a more suitable model for the response of SARS to these germicides. There is still an important role to be played by surrogate viruses, especially for the evaluation of new disinfectants or the re-evaluation of use of current disinfectants (such as changes in dose and contact time); the available data suggest that both TGEV and MHV may serve as conservative surrogates for modeling control of SARS-CoV by health care germicides in worst case scenarios.

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