

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 log₁₀ 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, log₁₀ 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-log₁₀ 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.

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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 al⁹ 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 hand-washing 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

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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 material¹⁹ 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 preparation²³ from 2 stock solutions: solution A (14.01 g of NaHCO₃, 250 mL of sterile deionized water) and solution B (16.94 g MgCl₂·6H₂O, 18.50 g CaCl₂, 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 CaCO₃.

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. Vespene Ilse (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

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