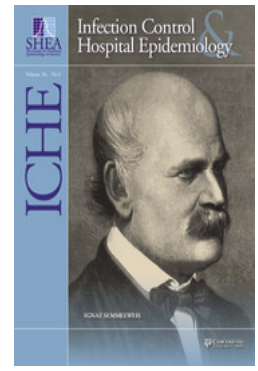

Efficacy of Different Cleaning and Disinfection Methods against *Clostridium difficile* Spores: Importance of Physical Removal versus Sporicidal Inactivation

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CONCISE COMMUNICATION

Efficacy of Different Cleaning and Disinfection Methods against *Clostridium difficile* Spores: Importance of Physical Removal versus Sporicidal Inactivation

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We tested the effectiveness of disinfectants and wipe methods against *Clostridium difficile* spores. Wiping with nonsporicidal agents (physical removal) was effective in removing more than 2.9 $\log_{10}$ *C. difficile* spores. Wiping with sporicidal agents eliminated more than 3.90 $\log_{10}$ *C. difficile* spores (physical removal and/or inactivation). Spraying with a sporicide eliminated more than 3.44 $\log_{10}$ *C. difficile* spores but would not remove debris.

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Clostridium difficile infection (CDI) can be a serious infection and leads to substantial morbidity and mortality, especially among older persons. The Centers for Disease Control and Prevention recently described a dramatic increase in the number of deaths associated with CDI, from 3,000 during 1999–2000 to 14,000 during 2006–2007.[1] A recent publication reported that, among 28 community hospitals, *C. difficile* replaced methicillin-resistant *Staphylococcus aureus* as the most common etiology of nosocomial infection.[2] The epidemiologic evidence strongly supports an important role for environmental contamination in the acquisition of CDI in healthcare facilities.[3,4] For example, environmental surfaces of hospital rooms housing a patient with CDI are frequently contaminated with *C. difficile*, as are the hands of healthcare personnel (HCP) who care for such patients. In addition, the frequency of HCP hand contamination correlates with the frequency of environmental contamination. Patients admitted to a room previously occupied by a patient with CDI have been demonstrated to have an increased risk of CDI.[5] Finally, improved room disinfection with sporicidal agents has led to decreased rates of CDI.[6,7]

Although environmental contamination has been demonstrated to be an important component of patient-to-patient transmission of *C. difficile*, the best method to clean contaminated surfaces has not been completely assessed. We undertook the following set of experiments to determine the relative importance of physical removal of *C. difficile* spores versus sporicidal inactivation.

METHODS

This wipe study was performed by using sheets of Formica on which were diagramed 5 Rodac plate templates (approximately 25 cm^2) on each sheet in the shape of a "Z" pattern. Each template was inoculated with 10 μL of trypticase soy broth (TSB) containing approximately 10^4–10^5 *C. difficile* spores (BI strain provided by D. N. Gerding) using a spread-plate method. The TSB contained 10% fetal calf serum to simulate a more accurate environment (ie, protein contamination) in which *C. difficile* might be found. Once the inoculum was applied, it was allowed to air dry for more than 10 minutes. After drying, the Formica surface was exposed to 1 of 6 solutions or disinfectants (Table 1) using 1 of 6 cleaning or wipe methods. When a wipe was used (Kimberly Clark Nonwoven Spunlace Wiper #6411 or disposable pop-up wipe), it was moved in a smooth motion across the 5 Rodac plate templates with approximately 1 pound of pressure. When spraying was performed, the nozzle of the spray was held approximately 6–8 inches above each template. After each treatment, the surface was allowed to air dry, and the amount of drying time required was recorded. Once the surface had dried, each template was cultured using a Rodac plate containing DE Neutralizing Agar. Rodac plates were incubated anerobically using the Pack-Anaero Anaerobic Gas Generating System (Mitsubishi Gas Chemical) at 37°C for 48 hours. After 48 hours of incubation, the colonies of *C. difficile* (if any) were counted. After each experiment, the Formica surfaces were disinfected with hypochlorite, and residual disinfectants were removed by repeated washings with soap and water (3 times), rinsed with sterile water (5 times), and wiped with 70% isopropyl alcohol.

All *C. difficile* spore suspensions used in our study consisted of viable spores achieved by heat treatment at 56°C for 10 minutes, which destroys the vegetative forms. The presence of viable spores was verified for each suspension before use with use of a malachite green spore stain and by testing each suspension according to the Association of Official Analytical Chemists hydrochloric acid protocol.[8]

RESULTS

Results are summarized in Table 2. Any method that included wiping the Formica surface resulted in a greater than 2.90 $\log_{10}$ reduction in *C. difficile* spores. Even wiping with a nongermicidal product, QC-53, was effective in eliminating more than 2.90 $\log_{10}$ *C. difficile* spores. Thus, physical removal can eliminate approximately 3 $\log_{10}$ *C. difficile* spores from environmental surfaces.

Disinfectants with activity against *C. difficile* spores (eg, hypochlorite) were highly effective in eliminating *C. difficile* spores even without physical removal (a decrease of more

TABLE 1. Products Evaluated

Disinfectant	Manufacturer	Active ingredients[a]	Dilution tested
QC-53	Ecolab	None	1 : 128
A456-II	Ecolab	6.510% octyl decyl dimethyl ammonium chloride; 2.604% dioctyl dimethyl ammonium chloride; 3.906% didecyl dimethyl ammonium chloride; 8.680% alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chloride	1 : 256
1:10 Bleach	Clorox	6% sodium hypochlorite (household bleach)	1 : 10
Kimtech One-Step Germicidal Wipe	Kimberly Clark Professional	4.4% hydrogen peroxide; 0.23% peracetic acid; excipient ingredients = 4.9% acetic acid	Undiluted
Clorox Germicidal Wipe	Clorox	0.55% sodium hypochlorite	Undiluted
#9255–41–1 and 3	Clorox	4,000 ppm hypochlorous acid	Undiluted

[a] All products, with the exception of QC-53, are classified as disinfectants. QC-53 is a nongermicidal degreaser and detergent. ppm, parts per million.

than 3 $\log_{10}$ for spray only). Products without activity against *C. difficile* spores (QC-53 and A456-II) were ineffective in eliminating *C. difficile* spores (a decrease of less than 2 $\log_{10}$) without physical removal. Wiping surfaces twice compared with wiping them once lead to improved removal of *C. difficile* spores when products without disinfectant activity against *C. difficile* spores (QC-53 and A456-II) were used. Sporicidal disposable wipes were effective in both removing and inactivating the *C. difficile* spores.

Drying times with wiping were generally 2–6 minutes. However, drying times for spraying with no wiping were 28–40 minutes.

DISCUSSION

Environmental contamination of the surfaces in hospital rooms with *C. difficile* has been demonstrated to be both common and associated with patient-to-patient transmission of *C. difficile*.[3,4] *C. difficile* spores are inactivated by hypochlorites, but they are not susceptible to the commonly used hospital disinfectants, phenolics and quaternary ammonium compounds. In epidemic settings, the use of hypochlorites for surface disinfection has been a component of the interventions used to control outbreaks.[3,4] For this reason, the most current infection control guideline on *C. difficile* recommends the use of "chlorine-containing cleaning agents or other sporicidal agents to address environmental contamination in areas associated with increased rates of CDI."[9(p442)] There are recent data that suggest that daily room disinfection with chlorine-containing cleaning agents in rooms housing a patient with CDI should be considered,[6] and at UNC Health Care, we have implemented that practice.

Our data demonstrated that wiping environmental surfaces, even with a nonsporicidal product, can eliminate approximately 3 $\log_{10}$ *C. difficile* spores. Most studies that have quantitated the level of *C. difficile* surface contamination have reported levels below 1 $\log_{10}$ (<10 colony-forming units per Rodac). Thus, the level of *C. difficile* elimination demonstrated by our study would be sufficient to remove the expected level of contamination. Unfortunately, studies have repeatedly demonstrated that less than 50% of room surfaces are adequately cleaned.[10] Improved training of environmental service workers, use of checklists, and use of a measure to assess the adequacy of cleaning (eg, fluorescent dye) can improve cleaning thoroughness.[3,4,10] Alternatively, the data presented in this article demonstrate that spraying a sporicidal disinfectant without wiping can eliminate 3.4 $\log_{10}$–6 $\log_{10}$ spores. Finally, one can use a no-touch method, such as UV light or a hydrogen peroxide system, to eliminate *C. difficile* contamination during terminal room cleaning and disinfection, but these methods require that the room be emptied of both patients and personnel.[10] A limitation of this study is the restricted area cleaned by the wipes, which is not representative of the larger areas in healthcare facilities; in addition, we used a single common surface material (ie, Formica) that may not be representative of other surface materials. In addition, the use of a nonsporicidal wipe that becomes contaminated with *C. difficile* spores would potentially allow the spread of spores to other surfaces,[11] but this issue was not evaluated.

In summary, our data demonstrate that *C. difficile* can be eliminated from the environment via physical action (wiping), by the use of a sporicidal wipe, or by spraying a sporicide without wiping. The prolonged drying times associated with spraying a sporicidal product without wiping were unacceptably high for routine hospital use. Furthermore, spraying alone would not remove dirt and debris and, for these reasons, is not recommended. We believe the use of a wiping

TABLE 2. Effectiveness of Different Wipe and Spray Methods as Measured by Reduction in Bacterial Count and Drying Time

Product	Wipe and/or spray method					
	Saturated cloth[a]	Spray (10 s) and wipe	Spray, wipe, spray (1 min), wipe	Disposable pop-up wipes	Spray, wipe, spray, air dry	Spray and air dry
Ecolab QC-53, detergent						
Reduction	3.38 (1.61–5.16)	3.28 (2.18–4.38)	4.02 (3.68–4.35)	NT	2.90 (1.34–4.45)	<2.00 (1.78–2.21)
Drying time, min:s	2:09	4:18	3:34	NT	24:26	28:11
Ecolab A456-II						
Reduction	3.14 (2.01–4.27)	2.98 (1.92–4.04)	4.18 (3.46–4.90)	NT	2.90 (1.52–4.27)	<2.00 (1.78–2.21)
Drying time, min:s	2:26	6:18	4:44	NT	24:00	30:14
1 : 10 Bleach						
Reduction	3.90 (2.87–4.92)	4.48 (4.26–4.69)	4.48 (4.26–4.69)	NT	4.48 (4.26–4.69)	3.44 (1.65–5.22)
Drying time, min:s	1:45	5:18	5:21	NT	51:08	39:40
Kimtech One-Step Germicidal Wipe						
Reduction	NT	NT	NT	4.18 (4.18–4.18)	NT	NT
Drying time, min:s	NT	NT	NT	4:06	NT	NT
Clorox Germicidal Wipe						
Reduction	NT	NT	NT	3.98 (3.23–4.72)	NT	NT
Drying time, min:s	NT	NT	NT	1:47	NT	NT
Clorox #9255–41–1 and 3						
Reduction	NT	6.14 (6.14–6.14)	NT	NT	NT	5.96 (5.22–6.70)
Drying time, min:s	NT	2:49	NT	NT	NT	40:14

NOTE. Data are mean $\log_{10}$ reduction in bacterial count (95% confidence interval [CI]) unless otherwise indication. Nonoverlapping 95% CIs between any two products or wipe and/or spray methods indicates a significant difference ($P < .05$). Drying time represents the time required to achieve a completely dry Formica surface. NT, not tested.

[a] Kimberly Clark Nonwoven Spunlace Wiper #6411 squeezed until not dripping.

procedure with a sporicidal agent provides excellent removal and inactivation of spores and is an integral part of *C. difficile* control measures.

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