Disinfectant Wipes with Nonsporicidal Claims Transfer Clostridioides difficile Spores from Contaminated Surfaces to Uncontaminated Surfaces during the Disinfection Process

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Abstract

Background: Pre-wetted disinfectant wipes are increasingly being used in healthcare facilities to help address the risk of healthcare associated infections (HAI). However, HAIs are still a major problem in the US with Clostridioides difficile being the most common cause, leading to approximately 12,800 deaths annually in the US. An underexplored risk when using disinfectant wipes is that they may cross-contaminate uncontaminated surfaces during the wiping process. The objective of this study was to determine the cross-contamination risk that pre-wetted disinfectant towelettes may pose when challenged with C. difficile spores. We hypothesized that although the tested disinfectant wipes had no sporicidal claims, they will reduce spore loads. We also hypothesized that hydrogen peroxide disinfectant towelettes would present a lower cross-contamination risk than quaternary ammonium products.

Methods: We evaluated the risk of cross-contamination when disinfectant wipes are challenged with C. difficile ATCC 43598 spores on Formica surfaces. A disinfectant wipe was used to wipe a Formica sheet inoculated with C. difficile. After the wiping process, we determined log10 CFU on previously uncontaminated pre-determined distances from the inoculation point and on the used wipes.

Results: We found that the disinfectant wipes transferred C. difficile spores from inoculated surfaces to previously uncontaminated surfaces. We also found that wipes physically removed C. difficile spores and that hydrogen peroxide disinfectants were more sporicidal than the quaternary ammonium disinfectants.

Conclusion: Regardless of the product type, all disinfectant wipes had some sporicidal effect but transferred C. difficile spores from contaminated to otherwise previously uncontaminated surfaces. Disinfectant wipes retain C. difficile spores during and after the wiping process.

Background

Pre-wetted disinfectant wipes are increasingly being used in healthcare facilities to disinfect equipment and environmental surfaces proximal to patients to help address the risk of healthcare associated infections (HAIs) [1]. This may play a significant role in reducing the incidence of certain HAIs [2, 3]. However, despite efforts being made to reduce the incidence of HAIs, one out of 31 patients in the United States (US) acquires one or more HAI on a daily basis [4]. Among pathogens implicated in the incidence of HAI, Clostridioides difficile is the most common in the US [5, 6]. The Center for Disease Control and Prevention (CDC) estimated that in 2017 there were approximately 223,900 hospitalized patients with C. difficile infections in the US with at least 12,800 deaths [6]. In acute care facilities, C. difficile infections result in approximately $4.8 billion in extra healthcare costs [5] due to prolonged hospital stays and readmissions [5, 7].

Contamination of environmental surfaces in healthcare facilities accounts for approximately 20% of HAI [8]. Specifically, hard non-porous environmental surfaces such as bed rails [9, 10, 11], bedside tables [9], and call buttons [10] may harbor C. difficile spores and contribute to transmission resulting in HAI in healthcare facilities [12]. Eradication of C. difficile from environmental surfaces is particularly difficult as
spores can persist on environmental surfaces for months [8, 13]. Specifically, the use of non-sporicidal cleaning agents may risk increasing the sporulation rate for C. difficile [14]. The use of disinfectant wipes with C. difficile sporicidal claims have been a recommended strategy to reduce the incidence of HAIs [15], as wipes increase compliance with standard cleaning and disinfection practices compared to traditional “wipe and bucket” methods [16]. However, pre-wetted disinfectant wipes may pose the risk of cross-contaminating “clean” surfaces during the wiping process [17] as the standard test for the registration of towelette products rarely simulates real world wiping scenarios [3]. In 2015, Satter demonstrated that disinfectant towelettes used to wipe one-centimeter diameter disk inoculated with Staphylococcus aureus or Acinetobacter baumannii transferred about twice as much bacteria onto “clean” or otherwise not previously contaminated surfaces when compared with their bactericidal efficacy [17].

In the US, healthcare facilities commonly use visual evaluations to determine “contamination levels” on hard non-porous surfaces prior to disinfection [18]. Consequently, most disinfectant wipes are used as broad-spectrum antimicrobials on inanimate surfaces in healthcare facilities [19, 20, 21]. However, disinfectant wipes may also present the risk of cross-contaminating otherwise uncontaminated surfaces after a towelette has been used to disinfect a contaminated surface. In addition, the Environmental Protection Agency (EPA) has no recommendation on the maximum surface area that could be disinfected with a towelette in order to optimize bactericidal efficacy, while minimizing the risk of cross-contaminating low risk surfaces. In prior work by our group, we found that the efficacy from pre-wetted disinfectant wipes was related to the ability to keep surfaces wet for the label contact time, so “stretching” a wipe to wipe larger areas than the wipe can disinfect may create risks of suboptimal disinfection and cross-contamination [22].

The risk of pathogen transmission by the hands of healthcare workers and patients has been widely investigated [23, 24, 25]. However, less work [17, 26] has been done to determine the risk of cross-contamination by disinfectant wipes using real world techniques in vitro. The objective of this study was to determine the cross-contamination risk that disinfectant towelettes with no sporicidal claims may pose when challenged with C. difficile spores. We hypothesized that although the tested disinfectant wipes have no sporicidal claims, they will reduce C. difficile spore loads, but cross-contamination may still occur. On a related note, we hypothesized that towelettes with sporicidal claims will present a lower cross-contamination risk than wipes without sporicidal claims. We also hypothesized that compared to quaternary ammonium products, hydrogen peroxide disinfectant towelettes will present a lower risk of cross-contaminating low risk surfaces after wiping down an area inoculated with C. difficile spores.

**Methods**

**Disinfectants and bacterial strain used in this study**

This study investigated the risk of cross-contamination of seven disinfectant towelette products; six with non-sporicidal claims and one product with sporicidal claims (Table 1). Ready-to-use wipes containing 1.312% sodium hypochlorite with an EPA registered sporicidal claim were used as a control. C. difficile
spores ATCC 43598 were produced following EPA MLB SOP-MB-28 [27] and used to study the risk of cross-contamination by disinfectant wipes following a modified version of EPA MLB SOP-MB-31 [27].

Table 1
Active ingredients and contact times for disinfectant towelettes tested in this study

| Disinfectant product a | Disinfectant Active Ingredient(s) c | Dilution at use | Active level at use e | Label contact time (mins) f |
|------------------------|------------------------------------|----------------|----------------------|-----------------------------|
| SH b                   | 1.312% sodium hypochlorite          | RTU d          | 1.25%                | 4                           |
| HP1                    | 1.4% hydrogen peroxide              | RTU            | 1.4%                 | 1                           |
| HP2                    | 0.5% hydrogen peroxide              | RTU            | 0.5%                 | 1                           |
| HP3                    | 0.5% hydrogen peroxide              | RTU            | 0.5%                 | 1                           |
| QA1                    | 0.25% n-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chloride 0.25% n-alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride 55% isopropanol | RTU            | 0.25% + 55%           | 2                           |
| QA2                    | 0.76% didecyldimethyl ammonium chloride 15% isopropanol 7.50% ethanol | RTU            | 0.76% + 22.5%        | 1                           |
| QA3                    | 0.233% disobutylphenolxyethoxyethyl dimethyl benzyl ammonium chloride 14.3% isopropanol | RTU            | 0.233% + 14.3%       | 2                           |

a Abbreviated naming scheme for commercially available EPA registered disinfectants used in this study;

b Control disinfectant with C. difficile claim;

c Active ingredients concentration;

d Ready-to-use;

e Active ingredient concentration after dilution;

f Defined label contact time.

Test Surface Sterilization, Inoculation And Disinfection
A two-meter square area of Formica sheeting was marked into different lengths and labeled as follows: inoculation zone (i-zone), 0.5 m², 1 m², 1.5 m² and 2 m² (Fig. 1). For the i-zone and for every 0.5 m² area, a 10 cm x 10 cm (100 cm²) area was marked in the center of the defined lengths to recover spores from the surface. The entire Formica surface was sterilized by a three-step process. Progressively, the surface was cleaned with 7.0% hydrogen peroxide, 10% bleach and 70% ethanol. Following each of the first two disinfection processes, three rinses each with 250 ml of sterile distilled water was used to rinse the surface. This was followed by a final application of 70% ethanol. The Formica sheet was left to air-dry on a clean laboratory bench.

The C. difficile spore inoculum was prepared following EPA MLB SOP-MB-31 [EPA, 2017] and used to test the risk of cross-contamination by disinfectant wipes from the “i-zone” to other portions of the Formica sheet. A final spore suspension of 500 µL was prepared with a soil load composed of 25 µL 0.05% bovine serum albumin (BSA; Fisher bioreagents, Ottawa, Canada), 35 µL 0.05% yeast extract (ACROS Organics, New Jersey, US), 100 µL 0.004% mucin stock (Abnova, Walnut, USA), and 340 µL C. difficile spores (prepared following EPA MLB SOP MB-28; stored at -20 ± 5 °C). After sterilizing the entire two-meter square area, a marked 10 cm x 10 cm (100 cm²) area in the i-zone was inoculated with five 10 µl aliquots of the C. difficile spore suspension (approximately 5.0 × 10⁸ colony forming units per ml) following EPA MLB SOP-MB-28. The first two towelettes from each disinfectant were discarded and the third used for testing to ensure enough disinfectant liquid load on the towelettes. This was used to wipe the entire two-meter square Formica sheet from the i-zone of the Formica to the two-meter mark of the Formica. The surface was wiped in a continuous up and down movement (Fig. 1). This was repeated for the entire surface area of the Formica sheet starting with the i-zone. The designated surfaces were left at room temperature for the disinfectants’ defined label contact times (Table 1). At the full contact time, swab samples of 100 cm² were collected from every 0.5 m² starting with the inoculation area using PUR-Blue Swabs (World BioProducts, Libertyville, IL; containing 10 mL sterile HiCap neutralizing buffer). The swab samplers were each vortexed for 30 s to release the bacterial spores from the sponge into the solution. The used wipes were placed in a sterile stomacher bag (Whirl-Pak, Nasco, Fort Atkinson, WI) containing 50 ml of 0.52% neutralizing buffer (BD Difco, Becton, Dickinson and Company, MD, USA), shaken for five min at 230 rpm using a stomacher to detect viable C. difficile spores on the towelette. Ten ml neutralizing buffer from the PUR-Blue swabs and the sterile stomacher bags were vacuum-filtered onto a membrane filter (0.2 µm pore size, 47 mm grid, individual sterile pack; Pall Corporation, Port Washington, NY) following EPA MLB SOP-MB-31. The membrane filters were aseptically transferred to pre-reduced brain-heart infusion agar with yeast extract, horse blood and sodium taurocholate plates (BHIY-HT; Anaerobe Systems, Morgan Hill, CA) and incubated under anaerobic conditions at 36 ± 1 °C for 120 ± 4 h before colonies characteristic of C. difficile spores as stated in EPA-MLB SOP-MB-31 were counted. Anaerobic conditions were achieved using an anaerobic jar (BD BBL GasPak, Becton, Dickinson and Company, Franklin Lakes, NJ) and CO₂ gas generating packs (BD GasPak, Becton, Dickinson and Company, MD, USA). Five biological replicates were conducted for each of the disinfectant products tested and one technical replicate performed for each biological replicate per surface area tested.
Statistical analysis

C. difficile spores were recovered from five test zones of a two-meter square Formica sheet and from used disinfectant wipes; counts were log_{10}-transformed. Average log_{10} CFU were calculated for wipes and defined sampled surfaces to test for statistically significant differences among eight disinfectant products. Specifically, we tested for differences among sampled surfaces by analyzing log_{10} CFU/100 cm^2 counts recovered after disinfection. We also analyzed log_{10} CFU/wipe used to test for the risk of cross-contamination from the i-zone to low risk surfaces. The least squares method of the Proc Glimmix test was used to fit liner models (n = 42, α = 0.05) and to test for interactions between disinfectant log_{10} CFU/100 cm^2 and the surface area sampled. Surface area wiped and product type were treated as variables with continuous effects (repeated measures in Proc Glimmix). Tukey adjustments were used to test for significant differences in mean log_{10} CFU among disinfectant products. The same procedure was also used to test for significant differences among surfaces treated with the same disinfectant wipe. All statistical tests were conducted using SAS version 9.4 (SAS institute, Cary, NC).

Results

Disinfectant wipes transfer varied levels of C. difficile spores to low risk (not previously contaminated) hard non-porous surfaces

Regardless of disinfectant product, both the sporicidal or non-sporicidal disinfectant wipes cross-contaminated low risk or otherwise previously uncontaminated surfaces from the i-zone (Figs. 2 & 3). Overall, a disinfectant wipe transferred a mean of 0.12 ± 0.26 and 0.34 ± 0.50 log_{10} CFU/100 cm^2 from the i-zone to the 0.5 m^2 and 2.0 m^2 risk surfaces respectively. Similarly, each wipe transferred on average, 0.13 ± 0.27 and 0.37 ± 0.47 log_{10} CFU/100 cm^2 from the i-zone to the 1 m^2 and 1.5 m^2 surfaces respectively.

Overall, and regardless of the sampling zone, the surface area wiped was statistically significant (P = 0.0001). On average, the log_{10} CFU/100 cm^2 transferred to 0.5 m^2 and 1 m^2 surfaces from the i-zone were significantly lower compared to the log_{10} CFU/100 cm^2 recovered from the i-zone post disinfection (P < 0.05). However, there were no statistically significant differences among the C. difficile spore log_{10} CFU/100 cm^2 transferred to the 1.5 m^2 and 2.0 m^2 surfaces and the log_{10} CFU/100 cm^2 detected from the i-zone after the wiping process (P ≥ 0.05). There were also no statistically significant differences among the mean log_{10} CFU/100 cm^2 transferred to the 0.5 m^2, 1 m^2, 1.5 m^2, and 2 m^2 surfaces from the i-zone (P ≥ 0.05).
The product type was also statistically relevant \((P = 0.0053)\). Specifically, QA2 and QA3 wipes transferred significantly higher \( \log_{10} \) CFU/100 cm\(^2\) from the i–zone to the 0.5 m\(^2\), 1 m\(^2\), 1.5 m\(^2\), and 2 m\(^2\) surfaces compared to the control, SH \((P < 0.05; \text{Fig. 3})\). There were no statistically significant differences in the cross-contamination levels \((\text{mean } \log_{10} \text{ CFU/100 cm}^2)\) among SH, HP1, HP2, HP3, and QA1 \((P \geq 0.005; \text{Figs. 2 & 3})\).

### High levels of C. difficile spores were recovered from disinfectant towelettes after use

Overall, all disinfectant wipes retained C. difficile spores after surface disinfection \((\text{Fig. 3})\); there were statistically significant differences among products \((P < 0.0001)\). Specifically, \( \log_{10} \) CFU/wipe ranged from 0.70 ± 0.00 (minimum detection level) for SH to 2.43 ± 0.72 for QA2 after use \((\text{Fig. 3})\). After the wiping process, control wipes \((\text{SH})\) were significantly less contaminated than towelettes of QA1, QA2, QA3 \((P < 0.05; \text{Fig. 4})\). However, there were no statistically significant differences among \( \log_{10} \) CFU counts from SH and HP1, HP2 and HP3 wipes \((P \geq 0.05)\). Similarly, there were no statistically significant differences among \( \log_{10} \) CFU/wipe recovered from HP1, HP2 and QA1 wipes \((P \geq 0.05)\). Significantly lower contamination levels were observed on used HP1, HP2, and HP3 wipes compared to QA2 and QA3 \((P < 0.05; \text{Fig. 3})\). There were, however, no statistically significant differences among the \( \log_{10} \) CFU/wipe recovered on wipes of QA1, QA2 and QA3 post disinfection \((P \geq 0.05; \text{Fig. 3})\). Similarly, there were no significant differences among \( \log_{10} \) spore counts on HP1, HP2 and HP3 towelettes after the wiping process \((P \geq 0.05; \text{Fig. 4})\).

### The sporicidal efficacy of disinfectant towelettes varies by product type and surface area

Overall, the product type was statistically significant \((P = 0.0053)\). Specifically, SH was significantly more sporicidal than QA2 and QA3 \((P < 0.05)\) as on average, lower \( \log_{10} \) CFU/100 cm\(^2\) were recovered from the i-zone and subsequent surfaces \((0.5 \text{ m}^2, 1 \text{ m}^2, 1.5 \text{ m}^2, \text{ and } 2 \text{ m}^2)\) \((\text{Fig. 3})\). However, there were no statistically significant differences among the sporicidal efficacies of SH, HP1, HP2, HP3 \((\text{Fig. 2})\) and QA1 \((P \geq 0.05; \text{Fig. 3})\) as the mean \( \log_{10} \) CFU/100 cm\(^2\) across the tested surfaces were very similar.

Surface area wiped was statistically significant \((P < 0.0001)\) and overall, the sporicidal efficacy of disinfectant wipes decreased with an increase in the surface area wiped from 0.5–2 m\(^2\) \((\text{Figs. 2 & 3})\). Compared to the 1.5 m\(^2\) and 2 m\(^2\) areas, disinfectant towelettes were statistically more sporicidal when used on the 0.5 m\(^2\) and 1 m\(^2\) surface areas. This was the case as \( \log_{10} \) CFU/100 cm\(^2\) from the 0.5 m\(^2\) and 1 m\(^2\) surfaces were significantly lower relative to the i-zone \((P < 0.05; \text{Figs. 2 & 3})\). Overall, there were no statistically significant differences in the sporicidal efficacy of disinfectant towelettes when the
log\textsubscript{10} CFU/100 cm\textsuperscript{2} from the i-zone, 1.5 m\textsuperscript{2} and 2 m\textsuperscript{2} areas were compared (P \geq 0.05). Similarly, regardless of the active ingredient class, there were no statistically significant differences in the sporicidal efficacies recorded within the 0.5 m\textsuperscript{2}, 1 m\textsuperscript{2}, 1.5 m\textsuperscript{2} and 2 m\textsuperscript{2} surface areas (P \geq 0.05, Figs. 2 & 3).

**Discussion**

In this study, we determined the cross-contamination risk that disinfectant wipes may pose during and after the wiping process. We established that during the wiping process, disinfectant wipes transfer C. difficile spores from a contaminated surface (i-zone) to otherwise uncontaminated during the disinfection process. We also found that among all the used disinfectant wipes tested in this study, viable C. difficile spores were detected on the wipes post disinfection. Overall, we found that after the wiping process, the log\textsubscript{10} CFU/100 cm\textsuperscript{2} detected from the 0.5 m\textsuperscript{2} and 1 m\textsuperscript{2} surfaces were significantly lower compared to those recovered from the i-zone. However, there were no significant differences among the log\textsubscript{10} CFU/100 cm\textsuperscript{2} transferred to the 1.5 m\textsuperscript{2} and 2.0 m\textsuperscript{2} surfaces and the log\textsubscript{10} CFU/100 cm\textsuperscript{2} recovered from the i-zone post-disinfection.

**Disinfectant Wipes Cross-contaminate Hard Non-porous Surfaces**

Cross-contamination is described by the CDC as the transfer of bacteria by contact from one surface to another [28]. Disinfectant wipes were the transfer “agents” between the surface inoculated with C. difficile spores and non-contaminated surfaces. In a similar study, Lopez et al. 2014 found that Bacillus thuringiensis spores inoculated on inanimate surfaces were transferred from wipe-disinfected fomites to fingers [29]. More recently, Becker et al. demonstrated that disinfectant wipes loaded with propanol or quaternary ammonium compounds (QAC) transferred viruses from a 25 cm\textsuperscript{2} inoculated surface onto three other surfaces of the same size in the process of using the wipes [26].

Compared to the i-zone, the log\textsubscript{10}CFU/100 cm\textsuperscript{2} from the 0.5 m\textsuperscript{2} and 1 m\textsuperscript{2} low risk surfaces were significantly lower than the log\textsubscript{10}CFU/100 cm\textsuperscript{2} of the i-zone post disinfection. This could be explained by the observation that more disinfectant liquid was released from the wipe onto the 0.5 m\textsuperscript{2} and 1 m\textsuperscript{2} areas compared to the 1.5 m\textsuperscript{2} and 2 m\textsuperscript{2} areas. This was evident as the 0.5 m\textsuperscript{2} and 1 m\textsuperscript{2} surfaces were visibly wet after the wiping process. In a previous study by our group [22], we found that the percent of liquid released per 0.1 m\textsuperscript{2} of a Formica surface significantly decreased as the surface area wiped increased. However, overall, there were no significant differences in the log\textsubscript{10}CFU/100 cm\textsuperscript{2} of spores transferred to the 0.5 m\textsuperscript{2} 1.0 m\textsuperscript{2}, 1.5 m\textsuperscript{2} and 2.0 m\textsuperscript{2} surface areas. In a similar study, Becker et al. did not find significant differences in the titer of viruses detected from three 25 cm\textsuperscript{2} uncontaminated surfaces after wiping them with a QAC disinfectant wipe previously used on a contaminated surface [26]. The reported risk of cross-
contamination by C. difficile spores is particularly relevant in healthcare settings as C. difficile infections have been associated with contaminated environmental surfaces [30, 31, 32].

**Used Disinfectant Wipes Are Potential Cross-contamination Agents After Use**

Although used disinfectant wipes are typically considered standard medical waste [33], we found that used disinfectant wipes may retain high numbers of C. difficile spores after use. This demonstrates a “mechanical” spore removal mechanism from contaminated surfaces during the wiping process. This finding is similar to those of Gonzales et al. who reported a physical removal of Clostridium sporogenes and Bacillus atrophaeus spores by antimicrobial wipes during the wiping process [34]. Kenters et al. also reported a similar mechanical removal effect by wipes challenged with C. difficile spores [35]. We observed that after wiping down the Formica sheet, all the used disinfectant towelettes were dry to the feel. This suggests that after use, the residual disinfectant liquid on the wipe may be insufficient to kill the spores picked up on the cloth within the product’s label-defined contact time. The ability for disinfectant wipes to retain spores after use may present a considerable cross-contamination risk especially if the same wipe is used on multiple surfaces or pieces of equipment. This may be the case as Siani et al. reported that disinfectant towelettes with no sporicidal claims that harbored C. difficile spores could eventually serve as cross-contamination agents [1].

The wipe design and substrate may also play a significant role in the level of organisms removed by the wipe [37]. Although the specific effects of the wipe materials were not evaluated in this study, differences in the levels of spores retained on the wipe could be associated with the wipe material type and with the amount of disinfectant liquid loaded on the wipe. Some wipe material types may hold more disinfectant liquid, which may be helpful in disinfection. We observed that wipes that had a rough feel probably due to their low cotton content (mostly the QA wipes) retained higher spore loads. In a 2012 study, Masuku et al. reported that the kind of material the wipe was made of, significantly impacted disinfection levels [38].

**Hydrogen peroxide-based wipes are more sporicidal than wipes with QACs**

All hydrogen peroxide-based disinfectants tested in this study were more sporicidal than most (2/3) of the QAC disinfectant wipes tested. The sporicidal activity of hydrogen peroxides has been associated with their ability to produce free hydroxyl radicals after binding to deoxyribonucleic acids (DNA) [39]. These hydroxyl free radicals damage DNA and cell membrane lipids [39]. Although the tested disinfectants, with the exception of SH, had no sporicidal claims, we found that all the tested disinfectants reduced spore loads. This is likely a joint effect of physical spore removal by the wipe substrate and spore inactivation by the disinfectants [34, 35, 36]. Specifically, Rutala et al. reported that disinfectant wipes with no sporicidal claims had sporicidal effects, and the wipes could physically
remove more than 2.9 logs of C. difficile spores from inoculated surfaces [35]. The US EPA requirements for obtaining a disinfectant label claim for C. difficile require a minimum of a six log$\text{_{10}}$ reduction [40]. But in our study, we found no statistical difference in disinfectant performance between all of the hydrogen peroxide wipes without a C. difficile sporicidal label claim and the sodium hypochlorite-based product with a C. difficile sporicidal label claim. This suggest that the benefits in efficacy in passing the EPA method may not translate to actual differences in efficacy in real world use, as simulated in this study. Thus, there may be no actual clinical benefit from using a sporicidal disinfectant wipe in reducing patient risk of C. difficile infection versus using a hydrogen peroxide (non-sporicidal) disinfectant wipe. This needs further study.

We acknowledge that our study is limited as we did not investigate the effect the different wipe materials could have on the risk of cross-contamination. We also did not study the impact of a prolonged contact time on the inactivation of spores retained by used disinfectant wipes; both warrant further study.

**Conclusion**

Overall, disinfectant wipes may transfer C. difficile spores from contaminated to uncontaminated surfaces and retain high spore loads after the disinfection process, but the rate at which this occurs varies by product and likely is affected by the disinfectant liquid load, chemistry, and wiping material. We determined that non-sporicidal wipes reduce spore load, but the need to conduct similar studies using prevalent HAI pathogens as *Staphylococcus aureus* and *Pseudomonas aeruginosa* remains. We definitively established that when disinfectant wipes are used on large surface areas, they may present a considerable cross-contamination risk, which could put patients at greater risk of HAIs.

**Abbreviations**

ATCC
American type culture collection

BHIY-HT
Brain-heart infusion agar with yeast extract, horse blood and sodium taurocholate

CDC
Center for Disease Control

CFU
Colony forming unit

DNA
Deoxyribonucleic acids

EPA
Environmental Protection Agency

HAI
Healthcare-associated infection

TSB
Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: All quantitative data generated or analysed during this study are included in this published article.

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Authors’ contributions: CAN and GKC performed trials to develop the model, analysed and interpreted the data generated, and wrote the manuscript. XL provided industry experience, designed elements of the experimental protocol, and was a contributor in writing and editing the manuscript. PT also provided industry experience and was a contributor in writing and editing the manuscript. HFO served as the principle investigator for the study and was a contributor in writing and editing the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Schematic diagram of the Formica surface used for wipe testing. Two meters of Formica were delineated into 0.5 m² sections. 5.0 x 10⁸ logCFU C. difficile spores were spotted onto the inoculation zone (i-zone) as represented by red dots. The entire surface was wiped in an up and down motion across the entire surface as indicated by black outlined arrows from left to right. 10 cm x 10 cm (100 cm²) sampling zones (light gray squares) were sampled to recover potentially cross-contaminated spores.
Figure 2

Mean log10 CFU/100 cm² remaining on sampled portions of the Formica sheet post disinfection with SH or hydrogen peroxide disinfectant wipes

Figure 3

Mean log10 CFU/100 cm² remaining on sampled portions of the Formica sheet post disinfection with SH or quaternary ammonium alcohol disinfectant wipes
Figure 4

Mean log10 CFU remaining on used wipes post disinfection with SH, hydrogen peroxide or quaternary ammonium alcohol disinfectant wipes