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Major article

Microfiber cloths reduce the transfer of *Clostridium difficile* spores to environmental surfaces compared with cotton cloths

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Background: Environmental surfaces in health care facilities contaminated with *Clostridium difficile* spores can be a reservoir that contribute to transmission of hospital-acquired infections. Microfiber cleaning cloths may improve the effectiveness of surface cleaning. The objective of this study was to assess the removal and transfer of *C difficile* spores on surfaces cleaned by microfiber compared with cotton cloths.

Methods: *C difficile* spores (approximately 4.2 $\log^{10}$/site) were applied to ceramic surfaces. Microfiber or cotton cloths were used to wipe the surfaces that were sprayed with either buffer or a nonsporicidal cleaning agent. To ensure reproducible pressure and surface contact time, a drill apparatus was used. The pressure was 1.5-1.77 N, and the total number of rotations was 10. Viable counts were used to assess the efficiency of microfiber and cotton cloths in removing and transferring spores.

Results: Of 4.4 $\log^{10}$ *C difficile* spores inoculated on a ceramic surface, microfiber and cotton cloths removed 2.4 and 1.7 $\log^{10}$, respectively. Microfiber cloths containing 4.2 $\log^{10}$ *C difficile* spores transferred 1.7 $\log^{10}$ *C difficile* spores when used to wipe a ceramic surface compared with cotton cloths that transferred 2.4 $\log^{10}$. Similarly microfiber wipes transferred fewer spores on consecutive surfaces wiped compared with cotton cloths (0.8 $\log^{10}$ vs 1.80 $\log^{10}$).

Conclusion: The use of microfiber cloths may reduce the risk of *C difficile* spore transfer during surface cleaning.

The role of the environment as a reservoir for transmission of hospital-acquired infections has been well documented.[1-9] Recent research studies have focused on understanding the role of the cloth and chemical agent used for cleaning.[6,7] For the health care setting, an ideal cleaning cloth should minimize or eliminate the transfer of bacteria from one environmental surface to another.[10] This goal might be achieved by the cleaning cloth removing microbes from surfaces but not releasing them. Alternatively, it could be achieved by the combination of the cloth removing microbes combined with a disinfectant that effectively kills the microbes on the cleaning cloth and on the surface wiped. In the past, cotton cloths and mops were used for cleaning the floors and surfaces in the hospital setting. More recently, microfiber has been introduced as an alternative for better cleaning in health care facilities.[11] The structure of microfiber cloths is different from that of cotton because the microfiber has a larger surface area per centimeter.[12] Microfiber has been shown to be an innovative tool to remove and retain dust, particles, and fluids from surfaces[13] more efficiently than cotton wipes.[11] The role of microfiber cloths in environmental cleaning within the health care setting has been reviewed in a Provincial Infectious Diseases Advisory Committee document.[13] They point out the need to ensure the chemicals used for cleaning and disinfection are compatible with the microfiber cloths used and that reprocessing of microfiber cloths does eventually reduce their efficacy.[13]

Recent research demonstrated that microfiber cloth floor mops and cleaning cloths were effective in removing but not killing vegetative bacteria if a disinfectant was not used.[10,11,14–16] As such, a new microfiber cloth should be used in each room to prevent the transmission of microorganisms between different patient care areas.[11] To prevent microbe transfer, it may also be necessary to change cloths between different areas within the same room or use a folding technique as described by Bergen et al.[16]

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To reduce the risk of microbial spread, the current Canadian guidelines[13] recommend using a disinfectant agent for patient care areas. One of the greatest challenges in terms of environmental cleaning is the presence of *Clostridium difficile* spores. Although several studies[12,15,17,18] have assessed disposable wipes using standardized wiping, there have been limited simulated-use studies[12,15] published to evaluate the ability of the microfiber cloths with or without disinfectants to remove and kill *C difficile* spores found on health care surfaces.

The aim of this study was to use a simulated-use protocol with standardized wiping conditions to compare the ability of microfiber and cotton cloths to remove and subsequently transfer *C difficile* spores from one surface to a second surface in the presence or absence of a cleaning agent.

MATERIALS AND METHODS

Microorganisms used

C difficile 765 was isolated from a patient with *C difficile*–associated disease, and previous testing (data not shown) showed that this strain produced high levels of spores. The same spore stock was used for all experimental testing.

Bacterial spore production

The bacterial spore production was a modification of the method described by Freeman et al.[19] *C difficile* 765 stock preparations were stored as frozen stocks. The microorganism was subcultured twice prior to preparing spores. Fifty blood agar media (5% sheep blood combined with tryptic soy agar) plates were inoculated with *C difficile* 765 and incubated in an anaerobic chamber at 35°C for 7-14 days prior to harvesting the spores. The cultures were checked for the presence of spores by microscopy and malachite green stain method. When the proportion of spores related to vegetative forms of *C difficile* 765 on microscopy reached 80%, the spores were harvested by scraping the bacterial growth from 50 plates into a final volume of 5 mL of sterile reverse osmosis water. An equal volume of ethanol (95%) was added, and the suspension was placed on a platform rocker with gentle mixing for 1 hour to ensure all vegetative forms were killed. Aliquots of the spore suspension were then placed into sterile tubes and stored in the refrigerator at 4°C. The same spore stock containing 2.3×10^6 spores/mL was used for all experiments. The concentration of spores was determined by viable count before performing each experiment to confirm the amount of spores present in the stock suspension.

Test soil

The organic challenge used was artificial test soil (ATS) as described by Alfa et al.[20]

Inoculation method

The surfaces tested were ceramic tiles (2.2 × 2.2 cm) obtained from Home Depot Warehouse (Winnipeg, MB, Canada). Microfiber cloths provided by Johnson Diversey (Oakville, ON, Canada) were compared with cotton cloths (Kushies Baby Bébé, Storey Creek, ON, Canada) for their ability to remove spores from a surface and subsequently transfer the spores to the next surface wiped. Microfiber and cotton cloths were cut into 16 cm^2 pieces. The microfiber and cotton cloths and ceramic pieces were autoclaved for 15 minutes in a gravity cycle. Preliminary testing showed no difference in microbial removal from surfaces using microfiber that was autoclaved compared with nonautoclaved microfiber (data not shown). All cloths tested were used only once.

All inoculation steps of the experiment were conducted under the class II type B biological safety cabinet. *C difficile* 765 spore preparations previously produced were centrifuged (16,000 × g) for 10 minutes at 4°C. After centrifugation, the supernatant was removed and carefully replaced with the same volume of sterile ATS[20] and then thoroughly mixed. The ATS–*C difficile* suspension containing 2.3×10^5 spores/mL was then used to inoculate sterile ceramic carriers or test cloths (100 μL/site to provide 2.3×10^4 spores/site). All carriers were placed on a large piece of sterile aluminum foil for inoculation. The inoculated carriers were dried overnight before the elution procedure was conducted. Before testing, the inoculated ceramic tile was prewetted by spritzing with phosphate buffer saline (PBS) or with a hydrogen peroxide 0.01% cleaning agent (PerDiem; Virox Technologies, Mississauga, ON, Canada) to completely wet the surface.

The transfer testing was performed using the same conditions previously described, except that the inoculum was placed directly onto the test cloth. The level of *C difficile* spores inoculated onto ceramic surfaces or cloths was approximately 2.3×10^4 colony forming units (cfu).

Simulated-use cleaning apparatus

To mimic the manual force and movement used for cleaning surfaces, we developed a test apparatus that combined the same carrier used by Sattar et al[12] with an apparatus similar to that described by Williams et al.[18] A drill and drill bit assembly were fastened to a stand (VWR International, Mississauga, ON, Canada). The rubber stopper attached to the drill bit assembly acted as the carrier for the test cloth. A scale was positioned underneath the stopper to measure the pressure exerted on the test surface (Denver MAXX 2001 Electronic Precision Balance; Denver Instrument, Denver, CO). The pressure was calculated as mass over area, and revolutions per minute were determined by a digital laser tachometer (Model DT2236B photo/contact, Sanpo, Quangdong, China). A drill body (IKA Works, Wilmington, NC) was attached to the drill stand. A Traceable Walkaway digital timer (Control, Friendswood, TX) was used to control the number of cloth rotations used for each experimental test. Preliminary experiments were performed to determine the optimal conditions of pressure and number of rotations (data not shown). For all experiments, the conditions used were 1.5-1.77 N and 10 rotations of the cloth on the surface.

Elution and enumeration of spores from ceramic carriers and microfiber and cotton cloths

Each test cloth or ceramic carrier was placed in a 50 mL sterile conical tube containing 10 mL of sterile PBS. The tube was mixed by vortexing for 1 minute, sonicated 3 times for 5 seconds each time, followed by 1 minute of vortexing. These elution conditions were selected based on preliminary experiments to determine the optimal recovery. The recovery efficiency of this elution method was 65.3%, 24.4%, and 39.0% for microfiber, cotton, and ceramic surfaces, respectively.

The eluent was serially diluted 1:10 from 10^{-1}-10^{-5} in PBS with a pH of 7.5. One hundred μL of the 10^{-2}-10^{-4} dilutions were inoculated onto *Clostridium difficile* Monobactam Norfloxacin (Oxoid, Nepean, ON, Canada) agar using the spread plate technique. *Clostridium difficile* Monobactam Norfloxacin plates were incubated in the anaerobic chamber at 37°C for 48 hours, and then the number of colonies detected were counted. Plates showing between 20 and 200 cfu were counted, and the final results were expressed as the $\log^{10}$ (cfu/cm^2) for both the cloths and ceramic carrier.

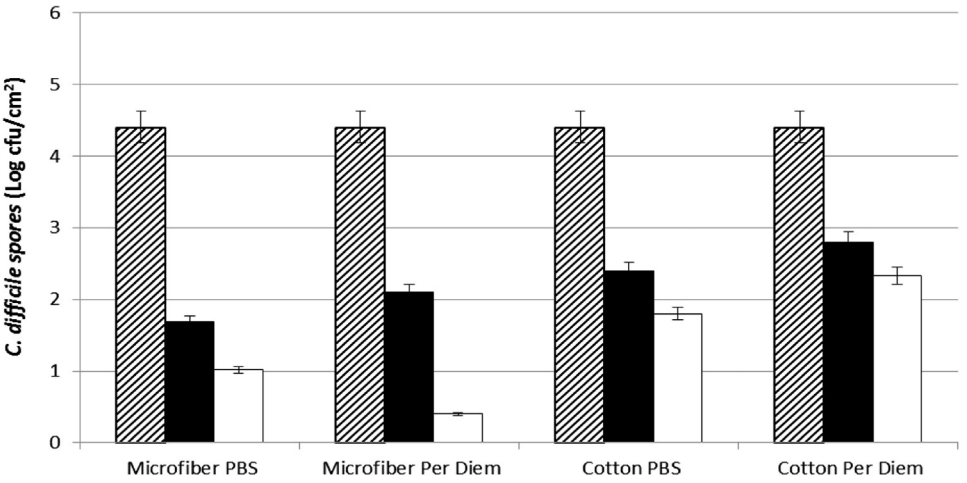

Fig 1. Transfer of *Clostridium difficile* spores from wet ceramic surface 1 to wet ceramic surface 2. The transfer of *C difficile* spores between 2 prewetted surfaces with PBS and hydrogen peroxide 0.01% (PerDiem) was evaluated using the drill apparatus. A 2.2 × 2.2 cm area (ie, 4.2 cm^2 surface area) in the center of the ceramic carrier (total surface area of the carrier was 16 cm^2) was inoculated with a C *difficile* spore suspension in ATS. The final results were expressed as the mean ± SD of triplicate tests. Hatched bars represent the ceramic surface inoculum, black bars represent the level of spores remaining on the ceramic surfaces after wiping with microfiber or cotton cloths, and white bars represent the amount of spores transferred when a second ceramic surface was wiped with either microfiber or cotton cloths that were used to wipe the first ceramic surface. *ATS*, artificial test soil; *PBS*, phosphate buffer saline.

Statistical analysis

The clinical study results were analyzed using a *t* test (unpaired test) at the 95% level of significance. We used Microsoft Excel 2007 software (Microsoft, Redmond, WA) for graphing and calculations on the differences between the means of each group of data.

Each experiment consisted of a set of 3 replicates and was performed at least 3 times (ie, a total of 9 tests). Log transformation of the viable counts was used to compare reduction in the bioburden.

RESULTS

The efficacy of microfiber and cotton cloths to remove *C difficile* spores from one surface and transfer them to another surface was evaluated. We tested ceramic surfaces because these are commonly present in the patient's environment (eg, toilet bowls, sinks). An adaptation of the apparatus described by Williams et al[18] was used to mimic the wiping action used for cleaning surfaces. The spore removal capacity of microfiber versus cotton test cloths is shown in Figure 1. To more closely mimic the daily activities of the housekeeping staff in the health care facilities, the transfer study was repeated using a cleaning agent (0.01% PerDiem) instead of PBS as the wetting agent. The data showed that cotton cloths transfer significantly more spores than microfiber cloths between wet ceramic surfaces regardless of whether a detergent was used or not ($P = .0261$ and $P = .0001$, respectively). The PerDiem at its use dilution did not kill *C difficile* spores. As such, there was no need to use a neutralizing agent for this testing.

We also tested the capability of microfiber and cotton cloths to release spores onto a clean surface. This time the *C difficile* spore inoculum was placed directly onto the cleaning cloths (Fig 2). Our data demonstrated that microfiber cloths released significantly fewer spores onto a clean surface than cotton cloths ($P = .001$).

DISCUSSION

There have been limited published investigations to determine if removal or transfer of *C difficile* spores from surfaces was altered by using microfiber cloths instead of cotton cloths.[11,12,14] Our study is the first, to our knowledge, to use a standardized apparatus to assess the risk of *C difficile* spore transfer, not just the ability of the cloth to remove spores from a surface. Smith et al[15] reported that all 9 types of microfiber cloths tested showed similar ability to remove methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *C difficile* spores from various surfaces. Wren et al[14] found that ultramicrofiber cloths were superior to conventional cleaning cloths for removing microbes from test surfaces. Both of these studies used research staff to perform the surface wiping. Our simulated-use study extends the findings of Wren,[14] Smith,[15] and colleagues because it is the first study to use standardized wipe conditions and show that there was significantly better removal of *C difficile* spores from surfaces wiped with microfiber versus cotton cloths.

A previous investigation by Bergen et al[16] demonstrated that the spread of *C difficile* spores and other bacteria from microfiber cloths may occur when using the 16-side folding method for cleaning environmental surfaces in health care facilities.[16] Our data extend their findings by demonstrating that a single-layer microfiber cloth released significantly fewer spores when used to wipe a second clean surface compared with a single layer of a cotton cloth.

Siani et al[17] used multiple spore types to demonstrate that 10 seconds of contact time is not enough for a cleaning wipe soaked in a sporicidal agent to produce a 4 log^{10} spore reduction on inoculated surfaces. Our data demonstrated that microfiber cloths could remove 2.4 log^{10} spores from an inoculated ceramic surface when a cleaning agent that had no sporicidal activity was used to wet the surface and the contact wiping time was 5 seconds.

A limitation of our study was that we did not evaluate the combined effect of a sporicidal disinfectant with the microfiber cloth to determine the potential for spore transfer from one surface to another.

The laundry processing of microfiber and cotton cloths was also tested in preliminary experiments (data not shown). Our data correspond with a study conducted by Diab-Elschalawi et al,[21] in that microfiber cloths could not be as readily decontaminated as cotton cloths by laundering. A cleaning and disposal protocol should be introduced in every health care setting to choose the proper laundry conditions, such as washing and drying times, in optimal temperature, adequate launder detergents, and number of reprocessing cycles.[21]

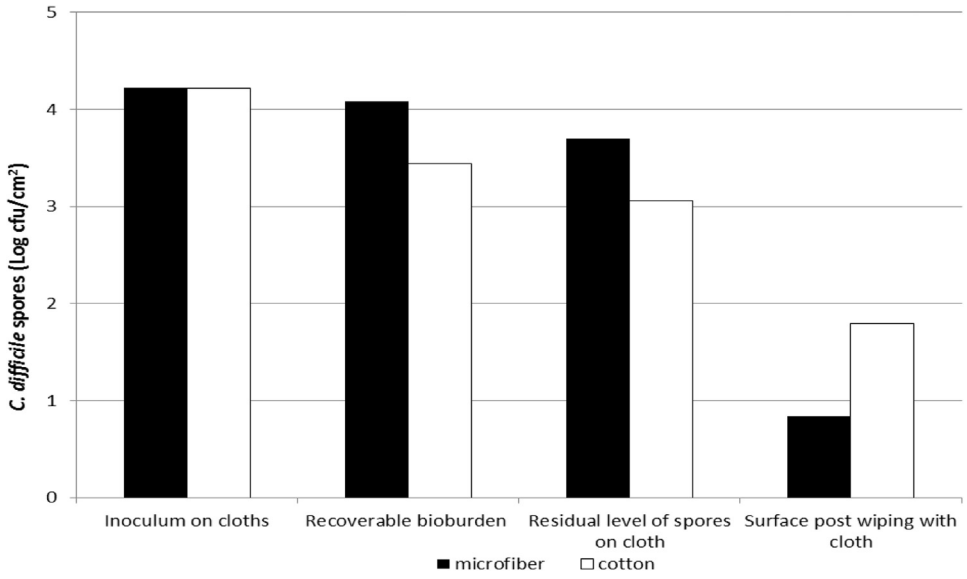

Fig 2. Release of *Clostridium difficile* spores from an inoculated microfiber and cotton cloths to a clean ceramic surface. Inoculated microfiber and cotton cloths were used to evaluate the transfer of spores from the cleaning cloths to ceramic surfaces using the drill apparatus. The results for microfiber cloths are represented by black bars, and those for cotton cloths are shown as white bars. *cfu*, colony forming units.

In summary, microfiber cloths can remove significantly more *C difficile* spores from surfaces compared with cotton cloths. In addition, the ability of microfiber cloths to retain spores provides convincing evidence that this cleaning approach could reduce transfer of microorganisms. Utilization of microfiber cleaning cloths in the health care environment may diminish the risk of spread of antibiotic-resistant organisms, but additional clinical studies using a sporicidal agent are needed.

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