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Major Article

The efficacy of a simulated tunnel washer process on removal and destruction of *Clostridioides difficile* spores from health care textiles

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Background: Research on reducing *Clostridioides difficile* spore contamination of textiles via laundering is needed. We evaluated the sporicidal properties of 5 laundry chemicals and then determined the ability of a peracetic acid (PAA) laundry cycle to inactivate and/or remove spores from cotton swatches during a simulated tunnel washer (TW) process.

Methods: In phase I, spore-inoculated swatches were immersed in alkaline detergent, sodium hypochlorite, hydrogen peroxide, or PAA for 8 minutes. In phase II, inoculated swatches were passed through a simulated 24-minute TW process employing 5 wash liquids. Spore survivors on swatches and in test chemical fluids in both studies were enumerated using standard microbiologic assay methods.

Results: In phase I, hypochlorite solutions achieved >5 $\log_{10}$ spore reductions on swatches and >3 $\log_{10}$ reductions for wash solutions. PAA achieved minimal spore reduction in the wash solution (0.26 $\log_{10}$). In phase II, the PAA equilibrium-containing process achieved a >5 $\log_{10}$ spore reduction on swatches. In wash solution tests, the cumulative spore reduction peaked at >3.08 $\log_{10}$ in the final module.

Conclusions: Sodium hypochlorite as a laundry additive is sporicidal. The cumulative effects of a TW process, coupled with a PAA bleach agent at neutral pH, may render textiles essentially free of *C difficile* spore contamination.

Clostridioides difficile (known as *Clostridium difficile*) infection (CDI) is a serious health care concern in many countries and can lead to a variety of diseases, such as diarrhea, pseudomembranous colitis, toxic megacolon, perforations of the colon, sepsis, and death.[1] In 2015, the annual burden of CDI in the United States was 453,000 cases and 29,000 deaths.[2] Given that *C difficile* spores can be isolated from a CDI patient's skin and that the diarrheal stool from CDI patients will give rise to high numbers of spores, we assume that the gowns and bed linens of patients will become contaminated with this pathogen.

However, the role of porous surfaces (ie, health care textiles [HCTs]) as reservoirs of microbial contamination enabling patient-to-patient transmission of infection is assumed, but not well defined.[3-5] A recent epidemiologic review of infectious disease outbreaks in hospitals that were attributed to a variety of laundered, clean HCTs did not find confirmed reports of patient-to-patient transmission, but instead revealed that patients in these outbreaks were exposed to environmental bacteria or fungi.[6] Nevertheless, when clean HCTs are put into use during the patient's hospitalization, these porous materials will acquire microorganisms from the patient's skin, contact with potentially infectious body substances, the environment, and from hand transfer of microorganisms by health care professionals, visitors, etc.[7-10] Microbial bioburden on HCTs can vary from <1 $\log_{10}$ to

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4-6 $\log_{10}$ colony-forming units (CFU)/100-cm^2 area, depending on the amount of soilage and infectious body substances.[11]

Research conducted by the health care laundry industry to determine process parameters for the production of hygienically clean HCTs is increasing.[12] A driving factor in this trend has been the need to evaluate the ability of modern laundry technology to remove soil and inactivate microbial contaminants. Much of the medical literature addressing the microbial inactivation properties of the laundry process features the use of washer-extractor type washing machines or laboratory equipment that simulates this washing action.[11,13,14] However, large-volume laundry operations in the United States and Canada have transitioned away from washer/extractors to large-scale tunnel washers (TWs), a laundering technology that enables reduced consumption of thermal energy and fresh potable water. Figure 1 shows a diagram of a continuous batch wash TW with 8 modules or compartments. As opposed to the traditional washer/extractor design that utilizes a single drum "water fill/mechanical action/drain" sequence, modern TWs use a screw mechanism in which textiles move through a sequence of connecting modules at a constant rate of speed that employ a gradual exchange of the wash liquors. TWs are typically between 6 and 18 modules long. The speed at which the textiles move through the modules is determined by the desired duration for the laundry process. Modules are used together as needed to lengthen the time that textiles are in during a certain step of the process. For instance, if 6 minutes is needed for the alkali step, this would involve 2 modules in a TW, with a transfer time of 3 minutes for each step. There are many mechanical design variations among different TWs. Some of these features include top versus bottom transfer mechanics, counter-flow versus standing bath, and machine drain configurations, all of which impact the dilution attributes of the wash process. TWs use significantly less water per pound of textiles to wash, compared with washer/extractor equipment. The TW process is one that is highly automated, enabling efficient use of laundry chemicals and water in precise amounts based on the weight of each load, type of fabric, and soil amount.

Currently, there is little information available about the ability of the laundry process to remove and/or inactivate *C difficile* spores from fabric. In addition, despite the current popularity of the TWs in the laundry industry, there is virtually no publicly available research on the microbial inactivation properties of the TW process. Given the fact that washer-extractor machine technology differs significantly compared with that for a TW, this raises the question whether it is reasonable to extrapolate the observations from washer-extractor experiments and apply these to TWs. Therefore, the objectives of this article are to: (1) introduce the reader to basic TW process and function, (2) determine whether existing bacterial laundry biocide methodology can be extrapolated to bacterial spore inoculums, (3) provide evidence of the potency of a selection of laundry wash chemicals against *C difficile* spores, (4) describe an initial effort to evaluate the effectiveness of a simulated TW at microbial removal/inactivation of *C difficile* spores, and (5) heighten the readers' awareness of the chemical complexities associated with peracetic acid (PAA) products.

METHODS

The laboratory experiments were divided into phase I and phase II. Phase I was designed to measure the efficacy of selected laundry chemistries to inactivate and reduce the numbers of *C difficile* spores on fabric swatches. The exposure period incorporated the wash cycle attributes of agitation and temperatures specific for each chemical. Phase II was set up to simulate the passage of *C difficile*-inoculated swatches through the "modules" of a TW. The swatches passed along

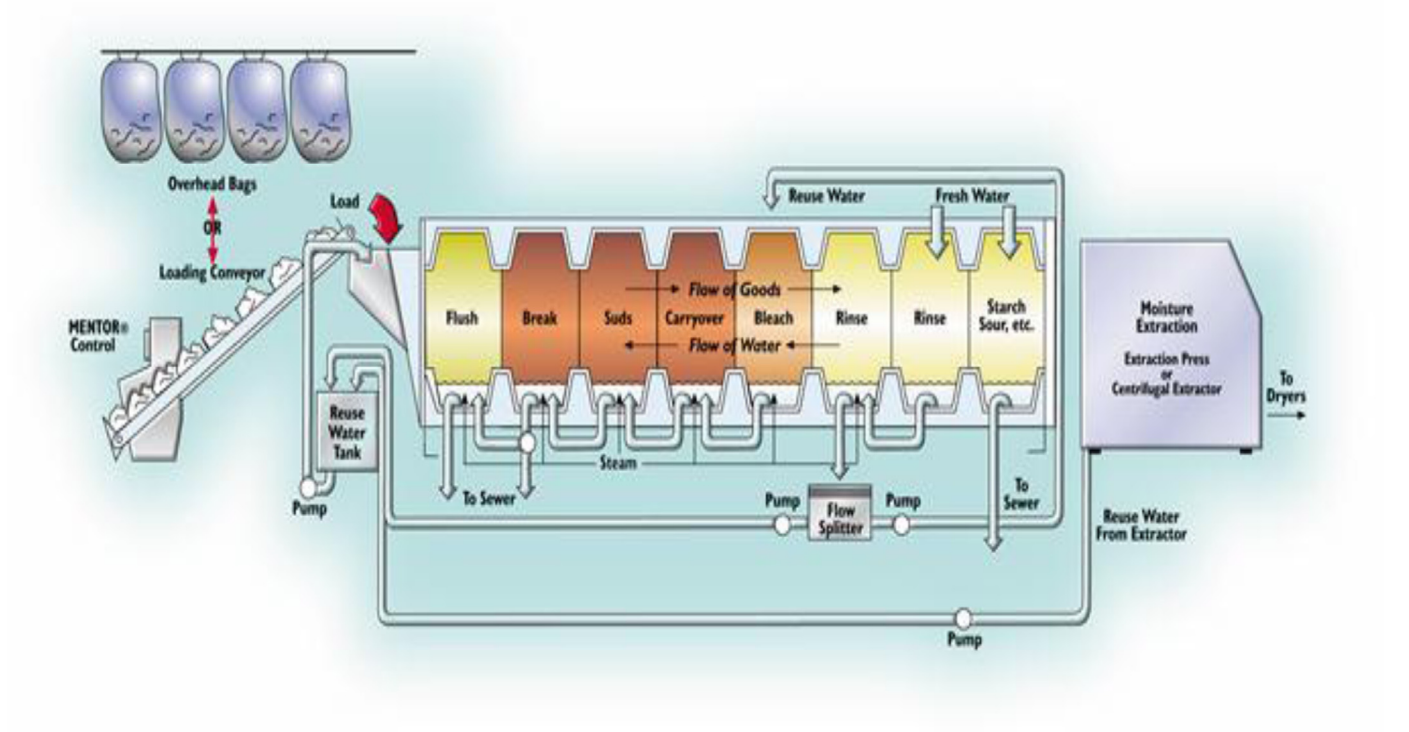


Fig 1. Diagram of a standard CBW TW.
Used with permission from Pellerin Milnor, Kenner, LA. The modules or compartments are usually numbered in ascending order from the start of the process (ie, the flush compartment) to the last stage (ie, the starch and sour compartment). Another version of Milnor TWs, the PulseFlow washer, is available. Other configurations of TWs are available from Braun, Lavatec, Kannegiesser, and other manufacturers. *TW*, tunnel washer.

from the initial alkaline and detergent modules, through exposure to a PAA bleaching agent, followed by a final rinse. The effect of this wash process on the viability and numbers of *C difficile* spores was measured at the end of this process.

Preparation of the C difficile *spore test inoculum and fabric swatches*

The test organism used was *C difficile* (ATCC #43598 [identified as *C difficile* #43598]) provided in spore form from the American Type Culture Collection (Manassas, VA). Working aliquots from this spore stock were prepared for phase I in accordance with American Society for Testing and Materials (ASTM) method E2839-11.[15] The prepared spore suspension was stored at −20°C and was adjusted to the desired test inoculum concentrations prepared for use in accordance with ASTM method E2197-11 (now superseded by E2197-17).[16]

The 100% cotton test fabric swatches for phase I were prepared in accordance with ASTM method E2406-09.[17] A "spindle" support device was prepared from a single length of stainless steel wire bent to contain 3 horizontal extensions and 2 vertical sections, as described in ASTM method E2406-09. Support strips of scoured cotton fabric were cut to fit the spindle, and 1 strip was wrapped around the stainless steel spindle multiple times. Pockets were secured to the fabric strip into which the cotton test swatches would be added during the experiment. The swatches were cut to size (1 x 1.5 inch) from the scoured cotton fabric, and a plastic tag was attached to each swatch to aid in aseptic removal of the swatch from the spindle pocket during the testing. The support strips and swatches were placed into separate containers and steam sterilized.

Protocol for phase I

Four commercially available laundry wash chemicals and 2 concentrations of chlorine bleach were prepared and used within 3 hours in the experiments to measure sporicidal potency against *C difficile* spores. The commercial source for the products used to prepare solutions A-D was Gurtler Industries (South Holland, IL). Test chemicals were prepared per liter of softened potable water as follows: A) an alkaline detergent mix (3 mL of "Power Jolt" + 2 mL of "Ge Blue"); B) chlorine bleach−5 mL 10% sodium hypochlorite; C) chlorine bleach −2 mL 10% sodium hypochlorite (both dilutions were prepared from Liquid Chlorine Bleach); D) hydrogen peroxide−7.5 mL of 1 N sodium hydroxide added to 1 L, to which was added 2 mL of 30% hydrogen peroxide and used promptly (target pH was 11.0) (NDT Oxy Bleach Plus); E) PAA solution−acidic (target pH was 3.0) prepared from 2 mL 15% PAA; and F) PAA solution−alkaline (target pH was 9.0) prepared from 23 mL of 1 N sodium hydroxide and 2 mL 15% PAA, used promptly. Both solution E and solution F were prepared using Persan MP-2 (Enviro Tech Chemical Services, Modesto, CA). Persan-MP 2 has a 15/10/30 proportion of PAA/hydrogen peroxide/acetic acid. The water control was identified as solution G. The test chemical-to-fabric ratio (w/w) of 5:1 was chosen for this experiment. No soil load was used. An aseptic technique was used for all manipulations of the spindle, the fabric pockets, and the fabric swatch carriers. Sterile cotton swatches were inoculated with 30 μL of the spore suspension and allowed to dry. Three inoculated carriers were added to the spindle, 1 carrier per pocket. The test solutions were prepared and pre-equilibrated to set temperatures to match typical use temperatures during the following wash cycles: solution A, 71.1°C; solution B, 65.5°C; solution C, 65.5°C; solution D, 76.7°C; solution E, 60.0°C; solution F, 60.0° C; and solution G, 37.7°C. The spindles with the carriers were placed into sterile jars and fully immersed in the test chemicals, 1 chemical per jar. The jars were then placed onto a laundrometer device in a 45° C incubator and set to simulate tumble washing at 45-60 revolutions per minute for 8 minutes.[18] On completion of the simulated wash/exposure period, spindles were removed from the jars, and the fabric on the spindle was fully wrung to remove as much of the test chemical as possible. The fabric swatches were then removed from the pockets and neutralized in sterile jars containing 10 mL of letheen broth + 0.1% sodium thiosulfate + 0.01% catalase. In addition, 0.5 mL of each test chemical solution was also neutralized as outlined in the Environmental Protection Agency's (EPA's) Office of Chemical Safety and Pollution Prevention 810.2400 performance test guidelines.[19] These containers and the containers with the swatches were vortexed, serial 10-fold dilutions were prepared for both the vortexed liquid for each test chemical and the neutralized swatch liquid, and assayed using duplicate Brain Heart Infusion Agar with Horse Blood and Taurocholate agar plates. These were incubated anaerobically for 5 days at 35°C-37°C and were counted using standard plate count procedures.

Controls for phase I

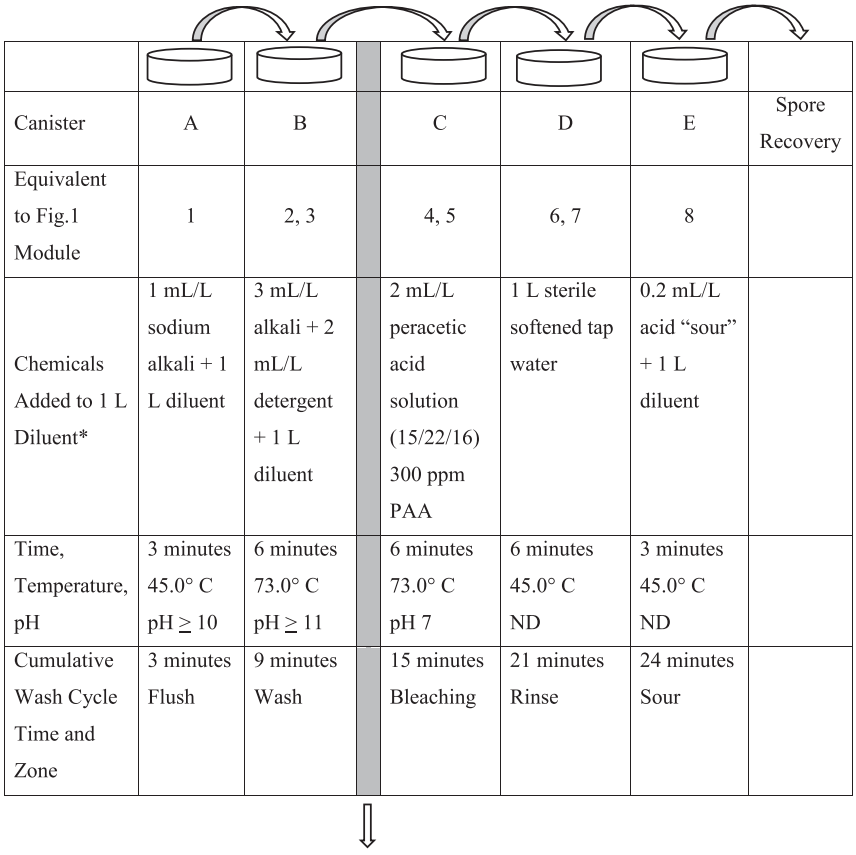
Controls for phase I included (1) spore culture purity, (2) carrier sterility, (3) neutralizer subculture medium sterility, (4) viability of inoculated fabric carrier, (5) initial spore suspension confirmation, (6) neutralization confirmation, and (7) a numbers control. Following the exposure period, the numbers control carriers were wrung out, placed in the neutralizer, serial diluted, and dilutions plated as described previously. Minimum geometric mean value of 1×10^4 CFU/carrier and 1×10^4 CFU/mL in the wash water were minimal accepted values for this control.

Protocol for phase II

Phase II was designed to evaluate the sporicidal efficacy of a simulated TW process using a PAA laundry additive as the bleaching agent in a 24-minute wash cycle. Potential spore removal attributed to the action of a laundry detergent is expected to contribute to the overall spore reduction capability of the cycle. The test organism, its preparation, fabric preparation, carrier and spindle preparation, the contamination of the carriers, the neutralization step, and the preparation of dilutions and plating for spore survivors were identical to those detailed in the first experimental phase. No soil load was used.

A preliminary simulated "walk-through" was conducted to determine the pH profile of the bleaching solution. The test substances for phase II were selected to represent the typical laundry solution (ie, "wash liquor") present in each canister (ie, "TW module"). Substances #1 to #3 were prepared in sterile softened tap water. From start to finish of the simulated wash cycle, these included (1) a sodium alkali solution, (2) an alkali + detergent solution, (3) a PAA solution, (4) sterile softened tap water, and (5) sterile softened tap water + sour. The PAA chemical used in phase II was a 15% PAA with high peroxide and lower free acid (15/22/16); the pH of this chemical solution was adjusted to pH 7.0 (NDT Oxcellerate; Gurtler Industries, South Holland, IL). Each test substance/solution was assigned individually to a canister designated A-E, and 100 mL of each test substance/solution was added to its designated canister such that a substance-to-fabric test ratio of 6:1 was achieved. Sterile stainless steel balls were added to each canister to provide agitation during the experiment.

Carrier swatches in triplicate were inoculated with 30 μL of the spore suspension and dried as before. Two water baths were set to temperatures selected for the simulated wash cycle, with some of the test substances being run at 73°C, whereas others were run at 45°C. Use of neutralizers and recovery of the swatches and each wash water were performed at the end of the simulated cycle as described previously. The stainless steel balls and containers of sterile softened tap water were also equilibrated to these temperatures by holding them in the water baths. Figure 2 summarizes the details of the wash solutions preparation, the cycle time, temperature and pH, the cycle zone designation, and the cumulative time of this simulated wash



Canister	A	B	C	D	E	Spore Recovery
Equivalent to Fig.1 Module	1	2, 3	4, 5	6, 7	8	
Chemicals Added to 1 L Diluent*	1 mL/L sodium alkali + 1 L diluent	3 mL/L alkali + 2 mL/L detergent + 1 L diluent	2 mL/L peracetic acid solution (15/22/16) 300 ppm PAA	1 L sterile softened tap water	0.2 mL/L acid "sour" + 1 L diluent	
Time, Temperature, pH	3 minutes 45.0° C pH ≥ 10	6 minutes 73.0° C pH ≥ 11	6 minutes 73.0° C pH 7	6 minutes 45.0° C ND	3 minutes 45.0° C ND	
Cumulative Wash Cycle Time and Zone	3 minutes Flush	9 minutes Wash	15 minutes Bleaching	21 minutes Rinse	24 minutes Sour	

Fig 2. Phase II study experimental design elements: test substance preparation; exposure time, temperature, and pH; cycle zone, cumulative time, and direction of spindle/swatches transfer. *ND*, not determined; *PAA*, peracetic acid.

* Diluent for all solutions was sterilized, softened tap water.

process. Experimental design features that were common to all of the canisters and the spindle were as follows: (1) a spindle with the carriers was added to each replicate canister for each test substance, a "dummy" canister with a thermometer for temperature measurement, a neutralizer control canister, and a control canister containing softened sterile water, all of which had been pre-equilibrated to the temperature specified for each was cycle stage; (2) each set of 4 canisters was placed in the laundrometer in a chamber set for the same pre-equilibrated temperature; (3) agitation was initiated and the wash cycle was run for the duration for each stage as specified; and (4) at the completion of each phase, the temperature in the chamber was recorded, and the spindles were removed from all canisters and transferred to pre-equilibrated canisters prepared for the next stage of the cycle. At the completion of the last stage, the ending temperature was recorded, as was the pH of test substance/solution (the PAA bleach solution) in canister C. The spindles were removed from all canisters, and the carrier swatches were aseptically removed from the spindles. Each carrier was placed into a jar containing 10 mL of the neutralizer. A 0.5-mL sample from each wash liquor was added to 10 mL of neutralizer in separate containers. The steps to assay the swatches and the wash waters for spore survivors were identical to those in phase I.

There were a few unique design features in this protocol. Whereas the solutions for most of the test chemicals/solutions could be prepared in advance, test substance/solution #3 (PAA) was freshly prepared toward the end of the test substance/solution #2 phase of the wash cycle, added to canisters and equilibrated to the appropriate temperature. Additionally, the transfer of the spindle with the carriers between canisters B and C required the fabric carrier and the swatches be wrung out (using sterilized tweezers to express the free liquid) to reduce transfer of alkali into canister C, thereby approximating the drainage of the wash liquor from the "B module" in a TW.

RESULTS

The results for the phase I and phase II studies are presented in Tables 1–3b. Phase I was intended to evaluate sporicidal activity during a fixed exposure time for laundry chemicals that may be used in the different steps of a wash cycle. Both sodium hypochlorite solutions demonstrated sporicidal activity (>5.0 $\log_{10}$ reduction) on cotton swatches and for the wash solutions. Hot alkaline washing solutions typical of the laundry "break" operation were not found effective for spore inactivation in short-exposure periods. Both alkaline hydrogen peroxide and the PAA test solution F showed no reduction of viable spores on the cotton swatches. Of the non-chlorine oxidative chemicals, only the PAA test solution E, at pH 3.0, was able to effect minimal reduction (0.29 $\log_{10}$) of *C difficile* spores in its wash solution. The results for the PAA product are consistent with the historical observations of PAA performance–bleaching activity is optimized when the pH of PAA is in the alkaline range, whereas antimicrobial action is enhanced when the pH is adjusted to the acidic range.

The results of phase II (simulation of a TW) illustrate that *C difficile* spores can be destroyed/removed at a >5.0 $\log_{10}$ level when exposed to a specific laundry process/sequence, including an oxygenated bleach (PAA 15/22/16 [PAA/hydrogen peroxide/acetic acid] solution adjusted to pH 7.0). These results also confirm that the hot alkaline "break" bath is not effective at spore destruction. Additionally,

Table 1
Phase I study results: measurements of log_{10} reductions of viable *Clostridioides difficile* spores on cotton swatches and in wash solutions for various laundry chemical additives

		Nos. control – starting log_{10} spore CFU/swatch = 5.26				Nos. control – starting log_{10} spore CFU/mL = 3.36	
	Test substance	pH	Log_{10} spore CFU/swatch, recovered	Average log_{10} spore CFU/swatch	Log_{10} reduction	Log_{10} spore CFU/wash solution, recovered	Log_{10} reduction
A	Power Jolt GE Blue (alkaline detergent)	11.0-12.0	5.85, 6.07, 6.13	6.02	NR	3.52	NR
B	10% sodium hypochlorite, 500 ppm	10.0-10.5	<1.00, <1.00, <1.00	<1.00	>5.00 (>99.999%)	<1.00	>3.00 (>99.9%)
C	10% sodium hypochlorite, 200 ppm	10.0-10.5	<1.00, <1.00, <1.00	<1.00	>5.00 (>99.999%)	<1.00	>3.00 (>99.9%)
D	32% hydrogen peroxide, 640 ppm	11.0-12.0	6.14, 5.96, 6.16	6.09	NR	4.48	NR
E	PAA (acidic) Persan MP-2 15/10/30, 300 ppm	3.0	5.58, 5.59, 5.09	5.42	NR	3.07	0.29 (48.7%)
F	PAA (pH adjusted) Persan MP-2 15/10/30, 300 ppm	9.0 +/- 0.25	5.92, 5.91, 6.16	5.99	NR	4.33	NR
G	Water control	7.0 ± 1.0	6.15, 5.09, 6.23	5.82	NR	3.70	NR

NOTE. Percentage expression of log reductions are included for the benefit of readers who may be more familiar with these values.
Exposure period for all chemicals and the water control was 8 min, with additional exposure time of approximately 1 minute needed for the removal of the spindle with the swatches from the test apparatus.
CFU, colony-forming units; *NR*, no reduction; *PAA*, peracetic acid; *ppm*, parts per million.

dilution and rinsing alone would appear insufficient to completely remove *C difficile* spores from fabric.

The results of both phase I and phase II experiments suggest that the laundry chemicals sodium hypochlorite and a PAA formulation adjusted to pH 7.0, when used in simulated wash processes, exhibit antimicrobial activity such that the log_{10} reduction levels may meet the EPA benchmark for a laundry disinfectant (≥4 log_{10}) or that for a laundry sanitizer (≥3 log_{10}).[19]

DISCUSSION

To our knowledge, our study evaluating the microbial inactivation/removal of *C difficile* spores from fabric in a simulated TW process experiment is the first research to conduct the sequential movement of fabric from compartment to compartment, the sequence of the wash chemistries/liquors, and the total exposure and transfer times as are used in the industrial TW cycles. The phase II results suggest that the cumulative effects of the tunnel wash process in the presence of a PAA bleaching agent at pH 7.0 can provide spore inactivation/removal such that contaminated HCTs may be rendered essentially free of *C difficile* spores. This is encouraging, as the health care laundry market is moving in the direction of using non-chlorine oxidative laundry chemicals (eg, PAA) as the bleaching alternative.[6] Whereas our phase I results confirm that chlorine bleach is a potent sporicidal laundry chemical, we know that chlorine bleach interacts with chlorhexidine gluconate residue on fabrics to produce orange/reddish stains that are permanent, and as a result many health care institutions will discard such HCTs, an action which increases costs of maintaining adequate par levels of clean, visually acceptable HCTs.[20]

Table 2
Phase II study results: log_{10} reductions of viable *Clostridioides difficile* spores on cotton swatches at the end of a simulated tunnel washer process

	Nos. control–starting log_{10} spore CFU/swatch = 5.09	
	Test solution exposure log_{10} spore CFU/swatch, recovered	Sterile, softened water exposure log_{10} spore CFU/swatch, recovered
Log_{10} spore CFU/swatch, recovered	<1.00, <1.00, <1.00	5.33, 5.15, 5.25
Average log_{10} CFU/swatch, recovered	<1.00	5.24
Log_{10} reduction	>5.00 (>99.999%)	NR

NOTE. Percentage expression of log reductions are included for the benefit of readers who may be more familiar with these values.
CFU, colony-forming units; *NR*, no reduction.

Use of chlorhexidine gluconate to help prevent infection is a clear benefit in health care, but the stain problem on the HCTs with the use of chlorine bleach necessitates an alternative resource for laundry disinfection and/or bleaching.[21-24]

Phase I results using PAA (showing no reduction of *C difficile* spores) appear to contradict the phase II results. First, 2 different formulations of PAA /hydrogen peroxide/acetic acid products were used, and the equilibrium mixture differs between the products. The phase I product (test substance/solutions E and F [15/10/30 PAA/hydrogen peroxide/acetic acid]) was more acidic with 30% acetic acid, which is typical for a particular class of PAA products. The phase II PAA solution (PAA 15/22/16 [PAA/hydrogen peroxide/acetic acid] solution at pH 7.0) was more acidic compared with the phase I test substance/solution F, but not as acidic as the test substance/solution E.

PAA demonstrates greater antimicrobial activity at lower pH, as does sodium hypochlorite. Fukuzaki noted the optimal pH region (at neutral or slightly acidic pH) for the germicidal activity of sodium hypochlorite differs from that of its cleaning activity, which is in the alkaline pH range.[25] Organic matter has a less adverse effect on PAA's antimicrobial activity compared with that for other disinfectants.[26,27] PAA products are equilibrium mixtures consisting of PAA, hydrogen peroxide, and acetic acid. The synergistic activity of hydrogen peroxide and PAA are believed to be central to the sporicidal property of the mixture. Hydrogen peroxide action damages the spore's surface, thereby allowing PAA to penetrate into the spore interior to denature proteins, enzymes, and the inner membrane.[28]

We intentionally selected pH 7.0 for the PAA/hydrogen peroxide/acetic acid solution used in phase II for the bleach bath based on the attributes of PAA chemistry, including: (1) enhanced biocidal activity of PAA in the acidic range, (2) awareness of the potential for corrosion of metal TW components should an acidic bath remain standing in the equipment for prolonged periods of time, and (3) the fact that PAA has a textile bleaching optimum, approximating pH 9.0. By clarifying the compositional make-up of the 2 PAA products used in these 2 studies, we hope to enlighten the readers' awareness of the availability of multiple grades of PAA in the laundry marketplace. Not all grades of PAA should be expected to exhibit equivalent antimicrobial efficacy. This current research's format should be expanded to address other known grades of PAA, including those consisting of 5% active PAA and perhaps others consisting of highly acidic 22% PAA known to contain low compositional percentages of free hydrogen peroxide.

Research is needed to determine whether modern laundry chemicals and different washer technologies can reliably produce hygienically clean HCTs as they relate to bacterial spores.[6,11,12,29] Previous laundry process research involved washer-extractor technology and

Table 3a and 3b
Log_{10} reductions of viable *Clostridioides difficile* spores in wash solutions in a simulated tunnel washer process

3a.

Nos. control – Starting log_{10} spore CFU/mL = 4.38					
	Test solution in canister				
Canister/time	1 3 min	2 6 min	3 6 min	4 6 min	5 3 min
Canister/test Solution	Alkali	Alkali + detergent	PAA (15/22/16) solution 300 ppm PAA, pH 7.0	Sterile, softened tap water rinse	Acid sour
Log_{10} spore CFU/mL, recovered	4.23	3.68	<1.3	<1.3	<1.3
Log_{10} reduction	0.15 (28.9%)	0.70 (79.9%)	>3.08 (>99.9%)	>3.08 (>99.9%)	>3.08 (>99.9%)

3b.

Nos. control – Starting log_{10} spore CFU/mL = 4.38					
	Control solution in canister				
Canister/time	1 3 min	2 6 min	3 6 min	4 6 min	5 3 min
Canister/test solution	Sterile, softened tap water	Sterile, softened tap water	Sterile, softened tap water	Sterile, softened tap water	Sterile, softened tap water
Log_{10} spore CFU/mL, recovered	4.11	3.00	2.85	2.77	2.74
Log_{10} reduction	0.27 (45.6%)	1.38 (95.8%)	1.53 (97.0%)	1.61 (97.5%)	1.64 (97.7%)

NOTE. Percentage expression of log reductions are included for the benefit of readers who may be more familiar with these values.
CFU, colony-forming units; *PAA*, peracetic acid; *ppm*, parts per million.

equipment, the most recent of which reported on the efficacy of modern washer-extractor equipment to reduce the *C difficile* contamination on HCTs.[11,13,14,30,31] In 2010, Carbone[30] stated that current laundry processes may be ineffective in inactivating *C difficile* spores on HCTs. A recent study using washer-extractor technology in a commercial laundry facility reported that despite the use of an industrial bleach agent (15% sodium hypochlorite) and a PAA sour, *C difficile* spores survived this wash cycle.[31] To our knowledge, there have been only 2 reports in the literature that concern TWs, neither of which reported on the microbial reduction properties of the wash cycle. One report discussed an outbreak of *Bacillus cereus* bacteremia among hospitalized patients that was attributed to contaminated HCTs and a contaminated washer.[32] The second report evaluated the use of MS2 phage-charged bioindicators for testing the efficacy of low-temperature wash processes to inactivate virus contamination.[33] A logical question to raise is whether our phase II results are comparable to those from the washer-extractor literature. We agree that a direct comparison of the washer-extractor process to that of the TW is desirable, but this comparison cannot be made at this time for several reasons: (1) laboratory-based studies are the foundation for future research, (2) published laundry efficacy studies involve diverse laundry cycle parameters and chemistries that make direct comparisons problematic, and (3) laundry process studies do need to be performed at full-scale levels in the future, which is important for process validation.

There are several limitations to these 2 studies. In the absence of an approved EPA sporicidal test method for laundry, we chose to use a test method that is used to evaluate the laundry process for antimicrobial efficacy against vegetative bacteria on fabric (ASTM method E2406-11) and modified the method to substitute use of *C difficile* spores as the challenge organism. Phase I results revealed extensive clumping of the spore inoculum, as noted in the spore count controls. In future studies, it would be helpful to modify the suspending solution to include Tween-80 to minimize spore clumping. Additionally, sequential transfer of *C difficile* spore-contaminated fabric carriers through dilution water is insufficient to remove all spores from the fabric in phase II, as viable spores can be recovered from the last laundry bath. Centers for Disease Control and Prevention guidelines discuss the point that dilution in the wash cycle is an important mechanism to the overall performance of the laundry process.[34]

Our studies were necessarily limited in scale and scope (ie, laboratory bench scale,). We chose 100% cotton for our swatch carriers because this fiber is known to release vegetative bacteria easily and has a history of acceptance by the EPA in evaluating laundry biocide efficacy claims. However, 100% cotton is not representative of the majority of HCTs, therefore, it would be important to repeat these studies using swatch carriers of other types of fabric (ie, polyester and various cotton/polyester blends). It is also possible that the inoculum used on the cotton swatches may not be an accurate representation of the level of actual *C difficile* spore concentrations on HCTs contaminated with fecal matter. Furthermore, we assume that microbial contamination on HCTs is unlikely to be evenly distributed over the entire textile item. Additionally, only 1 bleaching agent was evaluated in phase II. Results obtained using this chemical may not be representative for other PAA equilibrium mixtures, let alone other laundry chemicals. Again, more research is needed to evaluate the complex matrix of industrial laundry processes, chemicals, fabrics, and challenge microbes for consistent production of hygienically clean HCTs. Validation studies are needed to establish standard applied research procedures for future health care laundry comparisons to evaluate the complex matrix of industrial laundry processes, chemicals, fabrics, and challenge microbes for consistent production of hygienically clean HCTs. A move in this direction would be beneficial to both the laundry industry and the health care community.

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