Efficacy of "sporicidal" wipes against *Clostridium difficile*

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Background: Hospital-acquired infections associated with *Clostridium difficile* cause severe morbidity and mortality. The current control of *C difficile* endospores with liquid sporicides might have limited efficacy in the health care environment. Sporicidal wipes might offer additional control of surface bioburden and are now increasingly used, although there is little information about their efficacy against spores in practice.

Methods: Ten wipes were tested for sporicidal efficacy using a recently developed 3-stage protocol that measures the ability of the wipe to remove microbial bioburden from a surface, the potential for microbial transfer from the wipe to other surfaces, and the sporicidal activity of the wipe. Scanning electron microscopy was used to visualize the association of spores with the wipe fibers, and light scattering was used to measure the size of spore aggregates released from the wipes.

Results: The ability of the sporicidal wipes to remove *C difficile* spores from an inanimate surface ranged from 0.22 to 4.09 $\log_{10}$ spores removed within 10 seconds. One wipe did not remove any spores. None of the wipes demonstrated high sporicidal activity (ie, >4 $\log_{10}$ reduction) within 5 minutes of contact time, except for a control wipe soaked in 5,000-ppm sodium hypochlorite. Only one wipe demonstrated some sporicidal activity after 5 minutes, with a 1.50 and a 3.74 $\log_{10}$ reduction in spore number of *C difficile* NCTC12727 and R20291 (ribotype 027), respectively. All but one wipe demonstrated that spores could be repeatedly transferred to other surfaces. Light-scattering data provided evidence that some wipes were able to break up spore aggregates, potentially releasing more spores onto the surface. Electron microscopy micrographs showed that spores might be loosely associated with some wipes, explaining the rapid release.

Conclusion: Although the use of sporicidal wipes might offer additional control of microbial burden on surfaces, current efficacy tests might be inadequate to reflect the activity of these wipes in practice. This can lead to the use of wipes that might not be appropriate for applications in the health care environment. Tighter control of labeling and appropriate efficacy tests are needed before antimicrobial wipes are released to the market.

Key Words: Activity; surface disinfection; impregnated wipes; endospores.

Clostridium difficile has been associated with severe morbidity and mortality in the health care environment. The use of effective cleaning regimens and surface disinfection helps minimize the spread of pathogens and ultimately decrease the risk to patients and staff. It also contributes to decreasing the financial burden associated with treatment and longer hospital stays.[1-3] The persistence of *C difficile* endospores on surfaces after disinfection[2-5] presents an additional obstacle to the successful implementation of cleaning/disinfection procedures. Bacterial endospores are considered among the most resistant life forms to surface disinfection and sterilization[6] because of their structure and properties.[7] Thus, all cleaning/disinfection regimens need to be proven effective to prevent the presence/survival and spread of spore-forming pathogenic bacteria and other microorganisms.[8-10] Although sporicidal properties have been claimed for a number of microbicides, only a few of these have a reportedly rapid action (ie, within seconds).[10] Hypochlorites are among the most widely used disinfectants in the health care environment.[11] Sodium hypochlorite has demonstrated some success in decreasing *C difficile* incidence[12,13] and surface contamination,[14] but the efficacy of liquid disinfectants is severely hampered by the limited contact time that typically can be achieved in a busy health care environment.

Antimicrobial wipes are increasingly used to clean/disinfect surfaces proximal to patients in health care settings. Some antimicrobial wipes purportedly have a dual action, providing both cleaning and disinfection. Such products might be efficacious in removing a microbial bioburden from a surface; however, observations of their use in hospitals indicate that their antimicrobial activity might be limited due to short application times and repeated use on multiple

Table 1. Composition and label claims for wipes

Wipe	Ingredients listed on label	Claim on label
A	Inorganic peroxygen generator, tetra acetyl ethlenediamine, surfactants	Kills and cleans all microorganisms, including *C difficile*
B	"Impregnated with low-level biocides," 5% cationic surfactant, amphoteric surfactant, EDTA	Kills spores including *C difficile*
C	<1% cationic and nonionic surfactants, acidity modifiers	Cleansing and antimicrobial (no sporicidal claim on label)
D	<1% polymeric biguanide hypochloride, alkyl di-methyl benzyl ammonium chloride, didecyl dimethyl ammonium chloride	Active against *C difficile*
E	QAC, polymeric biguanides and bronopol	Rapid and lasting protection against *C difficile*
F	Didecyl dimethyl ammonium chloride 0.45%	Sporicidal
G	Composition not disclosed	Effective against *C difficile* spores under 30 seconds with mechanical action of cleaning
H	Didecyl ammonium chloride, laurakonium chloride, polyaminoporopyl biguanide, 2-bromo-2-nitro-para1-3-diol	Effective against 99.9% of bacteria, spores, viruses, and fungi, including *C difficile*
J	Composition not disclosed	Sporicidally active; kills *C difficile* spores

surfaces.[15] Indeed, antimicrobial wipes have been shown to transfer microbial contaminants to other surfaces after multiple wipings.[15,16]

Various wipes labeled as having "sporicidal" activity are commercially available. The sporicidal activity of the microbicides present in the wipes might have been tested using standard sporicidal tests, such as the European standard EN13704 (as indicated on some labels), which is a sporicidal suspension test method developed for food hygiene, domestic, and institutional use. By its very nature, this test method is inappropriate for evaluating the sporicidal activity of wipes, and in addition, it is unlikely that this test measures the efficacy of wipes under conditions of actual usage (eg, contact time, soiling) in the health care environment.

The aim of the present study was to test the activity of various commercially available wipes labeled as having "sporicidal" activity against *C difficile* spores, using a modified 3-stage protocol described by Williams et al.[15,16] This protocol measures a wipe's efficacy in removing microbial bioburden from surfaces, its intrinsic microbicidal activity reflecting usage conditions, and its propensity to release and transfer a microbial bioburden to other surfaces.

MATERIALS AND METHODS

Sporicidal wipes

Commercially available wipes from 9 manufacturers were used in this study. Label claims and compositions (where disclosed) are presented in Table 1. In addition, Clinell unmedicated (nonwoven) wipes (GAMA Healthcare, London, UK) soaked in sodium hypochlorite (5,000 ppm available chlorine; test wipe) were used as a positive control. Clinell unmedicated wipes were used as a negative control.

The wipes were cut aseptically into 4 × 4 cm squares for testing. The unmedicated and test (sodium hypochlorite) wipes were "activated" by adding 20 mL of sterile deionized water or sodium hypochlorite (5,000 ppm available chlorine). Excess liquid was manually squeezed out from the wipes, and the wipes were used within 5 minutes as recommended by the manufacturers.

Bacterial strains

C difficile strains NCTC 12727 and R20291, ribotype 027 (kindly obtained from Dr J. Brazier, Anaerobic Reference Laboratory, Cardiff, UK) were used. To prepare the spore stock, a single colony was inoculated to 50 mL of reduced brain heart infusion (BHI) broth (Oxoid, Cambridge, UK) and cultured for 10 days at 37°C under anaerobic conditions (5% H_2:10% CO_2: 85% N_2) in a Electrotek GW200 workstation (Electrotek, Shipley, UK). The broth culture was then centrifuged at 5,000 × *g* (Heraeus Primo R; Thermo Fisher Scientific, Waltham, MA) for 15 minutes at 4°C. The supernatant was discarded, and the pellet was resuspended in 2.5 mL of sterile ice-cold water and 10 mL of absolute ethanol before incubation at room temperature for 1 hour. The suspension was centrifuged twice as before and washed with chilled (4°C) sterile deionized water between centrifugations. Four spore preparations were then pooled, heated to 80°C for 10 minutes before centrifugation at 5,000 × *g* for 10 minutes at 4°C, and resuspended in 1 mL of sterile deionized water.

To enumerate the number of spores in the stock suspension, the spore suspension was diluted in tryptone sodium chloride (TSC; 1 g/L of tryptone [Oxoid] and 8.5 g/L of sodium chloride [Thermo Fisher Scientific]), and dilutions were plated onto BHI agar supplemented with 0.1% (w/v) sodium taurocholate (Thermo Fisher Scientific). All media used for the enumeration of *C difficile* were reduced for 24 hours before use.

Three-stage efficacy test protocol

Neutralizing solution. The neutralizing solution used to quench the activity of the antimicrobial wipes used in this study comprised 30 g/L of saponin (Sigma-Aldrich, St. Louis, MO), 1 g/L of L-histidine (Sigma-Aldrich), 30 g/L of polysorbate 80 (Sigma-Aldrich), 3 g/L of azolectin from soybeans (Sigma-Aldrich), and 5 g/L of sodium thiosulphate (Thermo Fisher Scientific) prepared in TSC.

The 3-stage protocol described by Williams et al[16] was adapted for *C difficile* spores. This test consists of 3 separate measurements:

Stage 1: Efficacy of wipes in removing spore contamination from surfaces. Before use, spore preparations were vortexed for at least 1 minute. Spores of *C difficile* (20 µL; ~7 $\log_{10}$ colony-forming units [CFU]) were inoculated in the center of a sterile steel disc (2 cm diameter with a grade 2B finish; Goodfellow Cambridge, Huntingdon, UK) and dried for 1 hour at 37°C. A sporicidal wipe (4 × 4 cm) was attached to a sterile steel rod to allow mechanical rotation with a drill (IKA; Labortechnik, Staufen, Germany). Wipes were mechanically rotated for 10 seconds at 60 rpm against surfaces, exerting a weight of 500 ± 5 g. Steel discs were transferred into bottles containing neutralizer (10 mL) and glass beads (5 g, 3 mm diameter; Sigma-Aldrich). After horizontal shaking (150 rpm for 1 minute) and neutralization for 5 minutes, the suspension was serially diluted and used to inoculate BHI agar containing 0.1% (w/v) sodium taurocholate. Dilution series were performed in microcentrifuge tubes containing 900 µL of TSC. Bacterial colonies were counted after a 48-hour anaerobic incubation at 37°C. The $\log_{10}$ cell removal from the disk surfaces was calculated by subtracting the mean $\log_{10}$ CFU of spores recovered from the disc after using the wipes from the number of spores inoculated onto the disk.

Stage 2: Bacterial transfer from wipes. Following the application of wipes to the contaminated surfaces as described above, adpression tests were performed. Five BHI plates containing 0.1% (w/v) of sodium taurocholate were consecutively inoculated by pressing the wipe onto their surface, exerting a weight of approximately 500 ± 5 g. Bacterial colonies were counted after 48 hours of anaerobic incubation at 37°C.

Stage 3: Sporicidal activity of wipes by direct inoculation. One commercial, one test, and one control (unmedicated) wipe sample were directly inoculated with 20 µL of the test spore suspension (~7 $\log_{10}$ CFU). After 10 seconds or 5 minutes of exposure, wipes were transferred into glass bottles containing neutralizer (10 mL) and glass beads (5 g; 3 mm diameter). These 2 contact times reflect either the in use exposure time (ie, 10 seconds)[15] or the recommended exposure time from a standard test method (eg, EN13704). After vortex mixing for 1 minute and neutralization for 5 minutes, the suspension was serially diluted in TSC and used to inoculate BHI agar plates containing 0.1% (w/v) of sodium taurocholate as described above. Bacterial colonies were counted after 48 hours of anaerobic incubation at 37°C. The sporicidal effect of the wipes was calculated by subtracting the mean $\log_{10}$ number of survivors on the test wipes from the mean $\log_{10}$ number of survivors on unmedicated control wipes.

The procedure for recovery of spores from the unmedicated wipes using this method has been described previously.[15-17]

Scanning electron microscopy

Scanning electron microscopy was used to visualize the association of *C difficile* endospores with the unmedicated wipe and wipes A, B, and D, to explain the results observed with measurements 2 and 3 of the 3-stage protocol. Spore suspensions were prepared as described above, and 20 µL of spore suspension (containing ~10^7 CFU/mL) was spotted directly onto the surface of the sporicidal wipe. Samples were imaged in low-vacuum mode on a Philips XL30 environmental scanning electron microscope (Phillips Healthcare, Andover, MA) using a back-scatter electron detector.

Dynamic light scattering

An N4Plus submicron particle sizer (Beckman Coulter, Fullerton, CA) was used to measure the release of potential spore aggregates and their size from 4 wipes (the unmedicated control wipe, wipe A, wipe B and wipe D) to explain the results observed with measurements 2 and 3 of the 3-stage protocol. The dynamic light-scattering measurement ran for 200 seconds, used a laser angle of 90°, and was repeated 3 times. The mean of 3 measurements was used to quantify the size of aggregates.

Twenty µL of spore suspension (~ 10^7 CFU/mL) was spotted directly onto the surface of each sporicidal wipe. After a 10-second contact time, samples were added to a flat-bottomed flask containing glass beads (5 g, 3 mm diameter) and 10 mL of sterile distilled water. The bottles were vortexed for 1 minute to remove spores from the wipe surface, after which 2 mL of the sample was removed, transferred to a clear cuvette, and processed with the N4Plus submicron particle sizer in accordance with the manufacturer's instructions.

Statistical analysis

At least 3 replicates were performed for each experiment. Statistically significant differences were tested

for by one-way analysis of variance (ANOVA) at the 95% confidence interval using the Minitab 15 statistical software package (Minitab, Coventry, UK).

RESULTS

This study used 2 strains of *C difficile*, a culture collection strain (NCTC 12727) and an outbreak strain (R20291, ribotype 027). A high concentration of the spore test inoculum was used to ensure that a $>5 \log_{10}$ removal and kill could be measured. This high concentration might not be representative of the spore concentration found on surfaces. In addition, the inocula for both strains contained large spore aggregates, with an average aggregate size of 2128 ± 958 nm for *C difficile* NCTC 12727 and 2207 ± 523 nm for *C difficile* R20291. In retrospect, the application of thorough vortexing for at least 1 minute was not sufficient to provide a homogeneous inoculum. The 10-second wiping time used in this investigation was based on the observations of Williams et al[15] on the use of wipes in the health care setting.

The various wipes tested in this study exhibited different properties. Wipe A removed significantly more spores of *C difficile* NCTC 12727 from the stainless steel disk compared with the other wipes tested ($P \leq .05$, ANOVA), including the unmedicated wipe soaked in sodium hypochlorite (5,000 ppm AvCl) (Table 2). The control wipe and wipes E and H removed ~1 $\log_{10}$ (ie, 90%) of spores from the surfaces, and the hypochlorite-soaked wipe removed 2 $\log_{10}$ (Table 2). The spores' origin was found to be an important variable affecting a wipe's efficacy in removing spores from surfaces, with differing results obtained for each strain investigated (Table 3). With *C difficile* R20291, wipes E and H performed best, removing $>1.90 \log_{10}$ spores from the surfaces. Wipes C, D, F, G, and J failed to remove *C difficile* NCTC 12727 from the surfaces, but removed $>1 \log_{10}$ of *C difficile* R20291. Wipe B was equally poor at removing *C difficile* NCTC 12727 and R20291. It also should be noted that some wipes did not reduce spore numbers and some even produced a small increase in spore numbers (indicated by a "+" in Tables 2 and 3), likely reflecting the breakup of spore aggregates.

C difficile R20291 spores were very resilient to the microbicide formulation in the wipes within the 10-second contact time, even sodium hypochlorite (Table 3). The 5-minute contact time dramatically improved the sporicidal activity of the unmedicated wipe soaked in sodium hypochlorite and of wipe A, both of which achieved at least a 3 $\log_{10}$ reduction in number (Table 3). Wipes E, F, and H demonstrated some sporicidal activity against *C difficile* NCTC 12727 spores within 10 seconds ($<2 \log_{10}$ reduction), but

this activity seemed to be lost after 5 minutes of contact (Table 2). Efficacy testing was repeated in triplicate for each wipe (same batch) and contact time, and the standard deviations for the results obtained were small overall. These wipes' lack of activity after 5 minutes is difficult to explain, but might be associated with variability in the wipe material itself. The quality of the wipes (eg, wetness) was not checked before the start of the experiment. The wipes used were not taken randomly from the packet, and although the packets were sealed after use, the same batch was used throughout the testing period. In addition, the top 3 wipes were always discarded as they showed signs of drying out. More importantly, the investigations of sporicidal activity after 10 seconds and 5 minutes were not performed at the same time, possibly introducing some in-laboratory variability.

The unmedicated wipe soaked in sodium hypochlorite performed well (5 $\log_{10}$ reduction in spore number within 5 minutes), and wipe A achieved $>1.50 \log_{10}$ inactivation within 5 minutes (Table 2). Interestingly, wipes B, C, D, E, and G released significantly higher numbers of spores ($P \leq .05$, ANOVA) than the number of spores in the test inoculum (Table 3), suggesting the presence of significant clumping in the initial spore inoculum. This study used a high spore inoculum concentration ($\sim 10^7$ CFU/mL), and even though care was taken to not produce aggregates, the results demonstrated the presence of some large spore aggregates ($>2,000$ nm) in the test inoculum. It is interesting to note that the spore aggregates from both isolates released from wipe A (791 ± 45 nm for NCTC12727 and 659 ± 99 nm for R20291), wipe B (792 ± 77 nm for NCTC12727 and 744 ± 17 nm for R20291), and wipe D ($1,040 \pm 86$ nm for NCTC12727 and 821 ± 85 nm for R20291) were significantly smaller ($P \leq .05$, ANOVA) than the aggregates in the initial inoculum. The presence of large aggregates on the wipe fibers was visualized by scanning electron microscopy (data not shown).

Differences in spore binding to the wipes were found as well. Although spores appeared to uniformly cover the fibers of wipe A and of the unmedicated wipe (data not shown), large aggregates that appeared "loosely" bound to the fibers were observed in wipes B and D (data not shown). Such differences in apparent binding might explain earlier results suggested greater spore release from some wipes (Tables 2 and 3). These results also are concordant with the transfer of spores following a series of adpressions (Tables 2 and 3). Indeed, only wipe A was shown to prevent the transfer of spores after 5 consecutive adpressions. All of the other wipes, including the unmedicated wipe soaked in sodium hypochlorite, released spores in 5 consecutive transfers. Particularly in wipes B, D, E and F, an

Table 2. Summary of results using the 3-stage method examining the efficacy of wipes against *C difficile* NCTC 12727 (n = 3)

	Bacterial removal, $\log_{10}$ CFU/disk $\pm$ SD, at 500 $\pm$ 5 g surface pressure	Sporicidal effect, $\log_{10}$ reduction $\pm$ SD		Bacterial transfer after 10 seconds of wiping at 500 $\pm$ 5 g surface pressure
		10-second contact	5-minute contact	
Unmedicated wipe	1.13 ± 0.36	0.85 ± 0.62	−0.84 ± 0.11	5 consecutive transfers; TNTC
Hypochlorite-soaked wipe	2.02 ± 0.21	+0.14 ± 0.49	5.39 ± 0.00	5 consecutive transfers; TNTC
Wipe A	4.09 ± 0.79	0.11 ± 0.15	1.54 ± 0.84	No spores transferred
Wipe B	0.67 ± 0.11	0.37 ± 0.23	+0.50 ± 0.19	5 consecutive transfers of ≤43 spores
Wipe C	0.84 ± 0.66	0.31 ± 0.15	+0.82 ± 0.14	5 consecutive transfers; from 40 to TNTC
Wipe D	0.22 ± 0.07	0.04 ± 0.05	+0.84 ± 0.03	**5 consecutive transfers; from 0 to TNTC**
Wipe E	1.30 ± 0.33	1.41 ± 0.14	+0.92 ± 0.15	**5 consecutive transfers; from 0 to TNTC**
Wipe F	0.57 ± 0.07	1.77 ± 0.27	0.01 ± 0.44	**5 consecutive transfers; from 1 to TNTC**
Wipe G	+0.08 ± 0.08[1]	0.99 ± 0.14	+0.70 ± 0.15	5 consecutive transfers; TNTC
Wipe H	1.14 ± 0.65	1.96 ± 0.09	+0.66 ± 0.13	5 consecutive transfers; from 83 to TNTC
Wipe J	0.88 ± 0.13	0.41 ± 0.10	+0.66 ± 0.10	5 consecutive transfers; from 2 to TNTC

NOTE. Bold type indicates increasing number of spores transferred with consecutive adpressions. + indicates no reduction in number.
TNTC, too numerous to count.

Table 3. Summary of results using the 3-stage method examining the efficacy of wipes against *C difficile* R20291 ribotype 027 (n = 3)

	Bacterial removal, $\log_{10}$ CFU/disk $\pm$ SD, at 500 $\pm$ 5 g surface pressure	Sporicidal effect, $\log_{10}$ reduction $\pm$ SD		Bacterial transfer after 10 seconds of wiping at 500 $\pm$ 5 g surface pressure
		10-second contact	5-minute contact	
Unmedicated wipe	0.94 ± 0.22	0.14 ± 0.07	+0.42 ± 0.07	5 consecutive transfers; TNTC
Hypochlorite-soaked wipe	0.86 ± 0.37	+0.50 ± 0.83	4.64 ± 0.00	5 consecutive transfers; TNTC
Wipe A	0.68 ± 0.36	0.72 ± 0.32	3.74 ± 2.26	<11 spore transfers in the first adpression
Wipe B	0.67 ± 0.92	+2.38 ± 0.03	+0.05 ± 0.10	**5 consecutive transfers; TNTC**
Wipe C	1.29 ± 0.08	+1.55 ± 0.09	+0.11 ± 0.10	5 consecutive transfers; TNTC
Wipe D	1.80 ± 1.00	+1.90 ± 0.23	+0.20 ± 0.04	**5 consecutive transfers; from 0 to TNTC**
Wipe E	1.97 ± 0.21	+1.20 ± 0.05	+0.26 ± 0.08	5 consecutive transfers; from 0 to TNTC
Wipe F	2.32 ± 0.38	+0.28 ± 0.10	+0.41 ± 0.20	**5 consecutive transfers; from 3 to TNTC**
Wipe G	1.45 ± 0.09	+1.02 ± 0.41	+0.32 ± 0.04	5 consecutive transfers; TNTC
Wipe H	1.92 ± 0.25	0.01 ± 0.14	+0.30 ± 0.05	5 consecutive transfers; TNTC
Wipe J	1.98 ± 0.32	+0.17 ± 0.32	+0.12 ± 0.08	5 consecutive transfers; TNTC

NOTE. Bold type indicates increasing number of spores transferred with consecutive adpressions. + indicates no reduction in number.
TNTC, too numerous to count.

increased number of spores were released from the wipes with an increasing number of transfers (highlighted in bold in Tables 2 and 3). This finding correlates well with the breakup of spore aggregates within the wipes, as observed for wipe B and D, as well as with the loose interaction of the spores with the wipe materials.

DISCUSSION

The limited sporicidal activity of the wipes within a short contact time of 10 seconds, reflecting conditions of use in practice,[15] is not surprising, even for the wipe soaked in sodium hypochlorite. All known sporicides have been shown to be effective within minutes, but not within seconds, after application.[10] Thus, it is unrealistic to expect a 4 or 5 $\log_{10}$ reduction within the 10-second contact time. With this in mind, the addition of a sporicide to these wipes serves to ensure safe disposal of these wipes, not to provide spore inactivation on wiped surfaces. The most active sporicides are oxidizing agents and chlorine-releasing agents.[10] Thus, the low sporicidal activity of most of the wipes tested in this study after direct inoculation was not surprising, given the wipes' microbicidal content (Table 1). Most of the wipes tested contained quaternary ammonium compounds (QACs), biguanides, and/or other surfactants, which have been reported to be sporostatic, but

not sporicidal.[7] Thus, it is truly remarkable that the formulations of these wipes passed standard sporicidal tests as claimed on the packaging. Some wipes demonstrated some modest sporicidal activity within 10 seconds that seemed to be lost with longer contact times. These surprising results might be explained by the nature of the QAC–spore interactions, as well as by the formulations. QACs interact strongly with the cell surface through chemical or ionic binding.[18] Despite the use of a neutralizer and notably Tween 80 to desorb QACs from the spore surface, any residual QACs could inhibit spore germination and result in a lower colony count. QAC concentrations remaining on the spores were not measured in this study. The nature of the formulation itself might increase the activity of a microbicide. Without disclosure of the full formulation (which is likely proprietary information), determining the synergistic activity of the QAC formulations contained in these wipes is difficult. The discrepant efficacies observed at the 2 contact times investigated might be better explained by the different wipes used from the same pack. As noted earlier, the 10-second and 5-minute contact time testing was done on different occasions. Although the wipe packs were sealed, the qualities of the wipes (eg, wetness) were not measured, and differences in wipe quality could account for the differing results.

The unmedicated wipe soaked in sodium hypochlorite demonstrated sporicidal activity (>4 log_{10} reduction) after 5 minutes of contact time, in agreement with previous findings with liquid sporicides.[10] The 5-minute contact time reflects the exposure time recommended in some European test protocols (eg, EN1307). In this study, this contact time was used to determine whether or not the wipes had sporicidal activity, which would ultimately make them safe to dispose of after usage. Sodium hypochlorite (5,000 ppm AvCl) has been shown to be sporicidal (6 log_{10} reduction in CFU/mL) after a minimum of 3 minutes of contact with "acidified" bleach (pH 5.3) and 10 minutes of contact with "regular" bleach (pH 10).[19] Wipe A showed some remarkable activity against the spores of C difficile R20291, but much lower activity against C difficile NCTC 12727 ($\sim$1.5 log_{10} reduction). Particularly variable results were observed with these wipes, possibly due to the presence of aggregates and the nature of the spores in the 2 isolates tested. The other formulated wipes demonstrated little or no activity after 5 minutes of contact time. This may be linked to various factors (eg, absorbency, multicomponent, nonhomogeneous precursor formulation in dry powder), as well as to the very different construction of wipe A compared with the other wipes tested. These factors were not investigated in the present study. More importantly, these wipes seemed to shed more spores than originally inoculated. These wipes are labeled as containing surfactants to help remove bioburden from surfaces (Table 1). These surfactants likely contributed to the breakup of clumps; spore aggregates also seemed loosely associated with the fibers of certain wipes. These findings provide an explanation for the increasing number of spores released from some wipes after multiple adpressions.

Liquid sporicides used in the health care setting might not be highly efficacious within short contact times and in the presence of soiling. Even with highly reactive chemistry, such as oxidizers, a spore kill (>5 log_{10}) might be achieved only after several minutes.[10] With that in mind, the removal of pathogenic spores proximal to patients, and thus the use of wipes providing that they can remove a high bioburden from a surface, might be a valuable additional control measure in these environments. However, these wipes must demonstrate the ability to remove a high spore concentration from surfaces, prevent the transfer of spores to other surfaces, and to kill spores, making them safe for disposal after use. As illustrated in this study and mentioned by others,[15-17] antimicrobial wipes might not effectively inactivate microorganisms within 10 seconds after application. Furthermore, the microbicide formulation of the wipes must be carefully evaluated to ensure that surfactant activity (in most wipes) is balanced with the inability to break up clumps, which would encourage the release of microorganisms. It also would be safer to ensure a "one wipe–one application–one direction" approach as recommended by Williams et al.[15] This would require the manufacturer to supply appropriate instructions on the use of the wipes. Given the increasing use of antimicrobial wipes in the health care settings, traditional standard sporicidal tests might not be appropriate, because they do not reflect conditions found in practice. A standardized wipe efficacy test needs to be adopted to ensure the usefulness of wipe applications in the health care settings, as well as more accurate product labeling.

In conclusion, the results of the present study are consistent with the findings of previous investigations using the 3-stage protocol.[15-17] The sporicidal activity of antimicrobial wipes might be limited within a 10-second application time, although some wipes demonstrated good efficacy against C difficile spores within 5 minutes, making them safe to dispose of. The "one wipe–one application–one direction" recommendation is paramount, given that most of the wipes tested were shown to release spores after multiple applications (ie, adpressions). The use of sporicidal wipes in the health care setting might prove to be an important addition to cleaning/disinfection control measures currently in place, provided that their efficacy is appropriately tested and that clear instructions are provided to the end user.

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