ORIGINAL ARTICLE

Efficacy of Hospital Cleaning Agents and Germicides Against Epidemic *Clostridium difficile* Strains

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OBJECTIVE.   To compare the effects of hospital cleaning agents and germicides on the survival of epidemic *Clostridium difficile* strains.

METHODS.   We compared the activity of and effects of exposure to 5 cleaning agents and/or germicides (3 containing chlorine, 1 containing only detergent, and 1 containing hydrogen peroxide) on vegetative and spore forms of epidemic and non-epidemic *C. difficile* strains (3 of each). We carried out in vitro exposure experiments using a human fecal emulsion to mimic conditions found in situ.

RESULTS.   Cleaning agent and germicide exposure experiments yielded very different results for *C. difficile* vegetative cells, compared with those for spores. Working-strength concentrations of all of the agents inhibited the growth of *C. difficile* in culture. However, when used at recommended working concentrations, only chlorine-based germicides were able to inactivate *C. difficile* spores. *C. difficile* epidemic strains had a greater sporulation rate than nonepidemic strains. The mean sporulation rate, expressed as the proportion of a cell population that is in spore form, was 13% for all strains not exposed to any cleaning agent or germicide, and it was significantly increased by exposure to cleaning agents or germicides containing detergent alone (34%), a combination of detergent and hypochlorite (24%), or hydrogen peroxide (33%). By contrast, the mean sporulation rate did not change substantially after exposure to germicides containing either a combination of detergent and dichloroisocyanurate (9%) or dichloroisocyanurate alone (15%).

CONCLUSIONS.   These results highlight differences in the activity of cleaning agents and germicides against *C. difficile* spores and the potential for some of these products to promote sporulation.

Clostridium difficile remains the infective agent most commonly associated with hospital-acquired diarrhea. The number of reported cases of *C. difficile* infection in England, Wales, and the United States has continued to increase, and represents a major burden on healthcare resources.[1-4] Contaminated environmental surfaces and transient hand carriage by healthcare workers and patients are important sources for *C. difficile* transmission in hospitals.[5-7] Notably, *C. difficile* spores are known to be resistant to many commonly used germicides and can persist for many months in hospital environments.[8] Despite such evidence, few data exist on how best to decontaminate the hospital environment.[9]

C. difficile strains can be separated into distinct types, by multiple methods of genetic fingerprinting. Crucially, epidemiological studies have identified specific predominant *C. difficile* phenotypes and genotypes. Polymerase chain reaction (PCR) ribotype 001 has been reported to be present in 33 of 58 UK hospitals, and is also prevalent in multiple other countries, including Sweden, the United States and Japan.[10-13] Similarly, serogroup C isolates (corresponding to PCR ribotype

012)[14] have been reported to be strongly associated with outbreaks of *C. difficile*–associated disease in Belgium and France.[15,16] More recently, widespread outbreaks have been associated with a previously uncommon *C. difficile* strain, PCR ribotype 027 (also known as NAP 1, North American PFGE type 1), in the United States, Canada, the United Kingdom, and Europe.[17-21] These findings suggest that some strains of *C. difficile* have an increased propensity to cause disease and possibly an increased propensity to persist in the environment in healthcare institutions.

We have demonstrated that the choice of hospital decontamination protocols can markedly affect the prevalence and environmental distribution of *C. difficile* contamination.[7] Also, the sporulation rate of PCR ribotype 001 can be enhanced when this strain is exposed to some, but not all, hospital cleaning agents or germicides.[22] We aimed to extend these findings by comparing the activity of and effects of exposure to cleaning agents or germicides on the vegetative and spore forms of epidemic and nonepidemic *C. difficile*

strains. To mimic conditions found in situ we carried out the exposure experiments using a human fecal emulsion.

METHODS

Selection of Test Strains

We examined 6 *C. difficile* strains of diverse origin, which were as follows: 2 epidemic PCR ribotype 027 strains from a Canadian-US outbreak (Maine Medical Center; strain A) and a UK outbreak (Stoke Mandeville Hospital; strain B); a UK epidemic PCR ribotype 001 strain (strain C); a nonepidemic UK PCR ribotype 078 strain (strain D); a nonepidemic strain isolated in Canada and the United States (strain E); and a nontoxigenic PCR ribotype 010 strain isolated in UK hospital environments (strain F).

Determination of Minimum Inhibitory Concentrations (MICs)

Strains were cultured anaerobically in Schaedler's anaerobic broth at 37°C for 24 hours. Standardized inocula were then inoculated at multiple points onto Wilkins-Chalgren agar that contained doubling dilutions of 1 of 5 commonly-used cleaning chemicals: anionic surfactant and sodium dichloroisocyanurate (NaDCC; Chlor-clean; Guest Medical), nonionic surfactant and phosphate (Hospec; Youngs Detergents), detergent and hypochlorite (NaOCl; Dispatch; Caltech Industries), hydrogen peroxide (H_2O_2; G-Force; JohnsonDiversey), and NaDCC alone (Sanichlor; Ecolab) (all agents were tested at a range of dilutions from 1/1,024 of the manufacturer's recommended working strength to 1/4 of the manufacturer's recommended working strength) (Table). Agar plates were examined following anaerobic incubation at 37°C for 48 hours. The MIC was defined as the lowest concentration of cleaning agent or germicide that prevented visible bacterial growth.

Spore Viability Assay

Mature spores from the 6 *C. difficile* strains tested were prepared as described by Baines et al.[23] and were added to multiple 5-mL aliquots of water containing one of the hospital cleaning agents and/or germicides at a working strength concentration (Table). Duplicate aliquots were sampled after 0, 10, 20, and 30 minutes of contact time by vacuum filtering using 0.22-μm grade nylon filters (Microfil V apparatus; Millipore). The filters were washed with 50 mL of sterile water to remove residual cleaning agent and/or germicide, and then placed onto Brazier's cycloserine cefoxitin egg yolk agar supplemented with 5 mg/L lysozyme and 2% lysed horse blood (CCEYL agar; Bioconnections). We enumerated spore germination after anaerobic incubation at 37°C for 48 hours. A single *C. difficile* colony was considered evidence of germination from a single spore. Results were interpreted with respect to the viability of spores preparations exposed to a cleaning agent and/or germicide, compared nonexposed control preparations.

Sporulation Assay

Fecal samples from 5 healthy volunteers were pooled, emulsified, and centrifuged progressively, until the emulsion passed through a 0.22-μm filter. The 6 *C. difficile* strains were grown overnight in Schaedler's anaerobic broth, and then 100 μL of each culture was transferred into 5 prepared fecal emulsions, each containing a subinhibitory concentration (0.25 × MIC for each strain) of 1 of the 5 cleaning agents and/or germicides (Table). Following incubation at 37°C for 72 hours in an anaerobic environment, duplicate samples were air-dried on glass slides and stained using a malachite green–carbol fuchsin spore stain. Spores were counted by light microscopy and expressed as a percentage of total cells (there were 5 fields per slide, each of 100 cells). Each culture was performed in duplicate. Cultures containing no cleaning agents or germicides were included as experimental controls. The sporulation data were examined by 2-way analysis of variance to determine significant differences.

RESULTS

MICs for *C. difficile* Strains

The MICs of the 5 cleaning agents and/or germicides for *C. difficile* strains A-F are shown in the Table. Growth of *C.*

TABLE.   Cleaning Agents and/or Germicides Used in the Study

Active component(s)	Brand	Manufacturer	Working concentration[a]	MIC, as a proportion of working concentration
Anionic surfactant and NaDCC	Chlor-clean	Guest Medical	1,000 ppm chlorine	1/4
Nonionic surfactant and phosphate	Hospec	Youngs Detergents	0.10%	1/16
Detergent and NaOCl	Dispatch	Caltech Industries	5,500 ppm NaOCl	1/64
Hydrogen peroxide	G-Force	JohnsonDiversey	1 : 64 dilution[b]	1/128
NaDCC	Sanichlor	Ecolab	1,000 ppm chlorine	1/4

NOTE.   MIC, minimum inhibitory concentration; NaDCC, sodium dichloroisocyanurate; NaOCl, hypochlorite.
[a] According to manufacturer's guidelines.
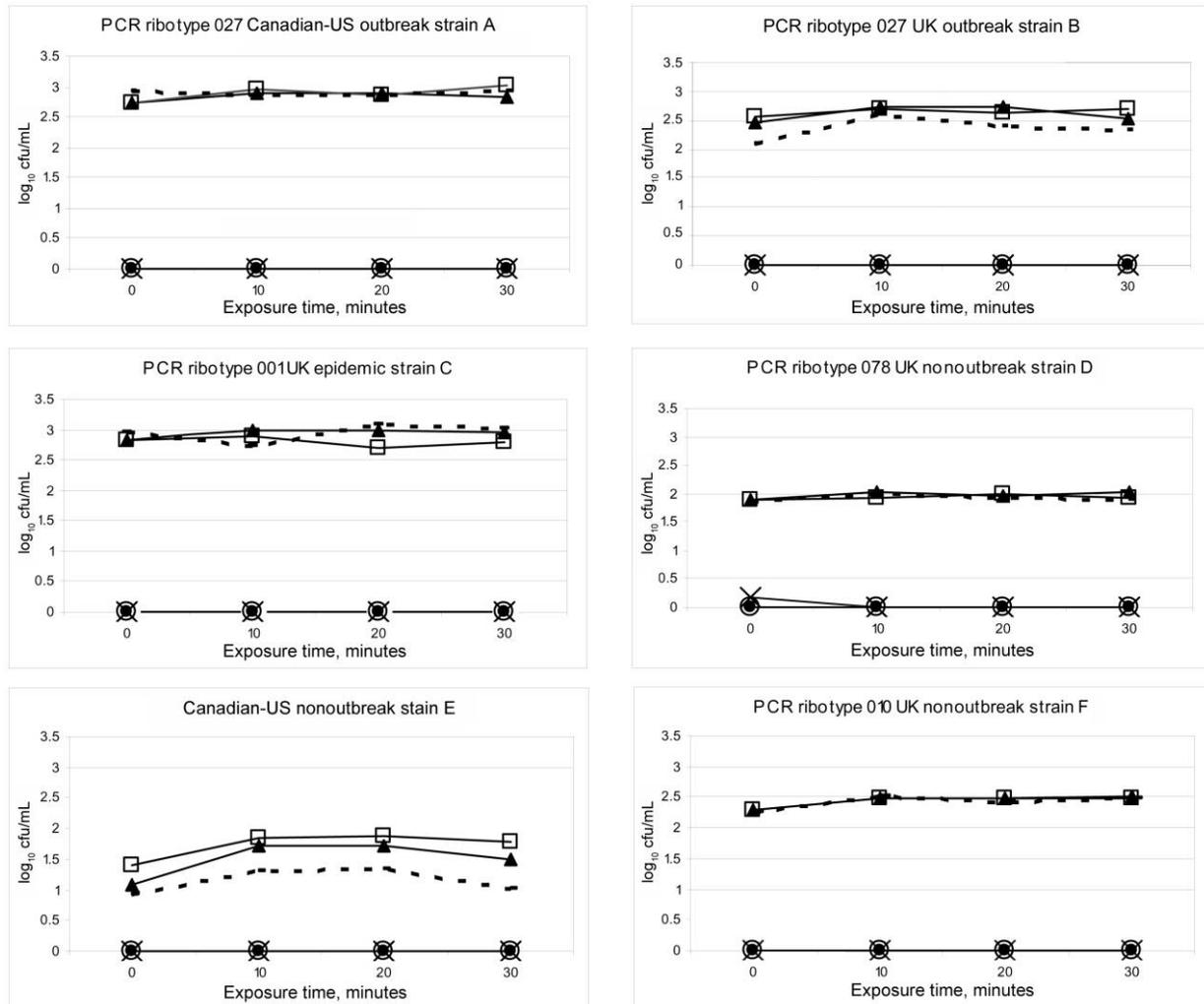[b] Concentration of hydrogen peroxide not stated by the manufacturer.

FIGURE 1. Mean spore germination rates for 5 strains of *Clostridium difficile* exposed to 5 commonly used hospital cleaning agents and/or germicides at manufacturer's recommended concentrations. *X,* anionic surfactant and sodium dichloroisocyanurate; *triangles,* nonionic surfactant and phosphate; *open circles,* detergent and hypochlorite; *squares,* hydrogen peroxide; *closed circles,* sodium dichloroisocyanurate; *dashed line,* control (no cleaning agent). CFU, colony-forming units; PCR, polymerase chain reaction.

difficile vegetative cells was inhibited by all of the cleaning agents and/or germicides tested at concentrations markedly below the working strength recommended by their respective manufacturers. The agent containing hypochlorite (Dispatch) was found to be the most active antimicrobial among the chlorine-containing products; it was active against vegetative *C. difficile* at an equivalent of 86 ppm of available chlorine; for both agents containing dichloroisocyanurate (Sanichlor and Chlor-clean), approximately 250 ppm of available chlorine was required to inhibit *C. difficile* growth. The nonanionic surfactant cleaning agent (Hospec) prevented *C. difficile* vegetative growth at a 16-fold dilution of the recommended working strength, while the agent containing hydrogen peroxide (G-Force) remained inhibitory at a 128-fold dilution of its recommended working strength.

Efficacy Against Spore Germination

The reduction in the germination rate of spores in *C. difficile* strains A-F after exposure to each of the 5 cleaning agents and/or germicides was measured in comparison with controls (strains which had no exposure to cleaning agents or germicides). The results are shown in Figure 1. All of the chlorine-containing agents (Chlor-clean, Dispatch, and Sanichlor) caused an immediate, complete prevention of spore germination. By contrast, exposure to either neutral detergent (Hospec) or hydrogen peroxide (G-Force) was not associated a with significant reduction in spore germination; hence, the numbers of viable spores after 30 minutes of exposure to either of these agents did not differ significantly from those for controls. No discernible differences in the spore survival
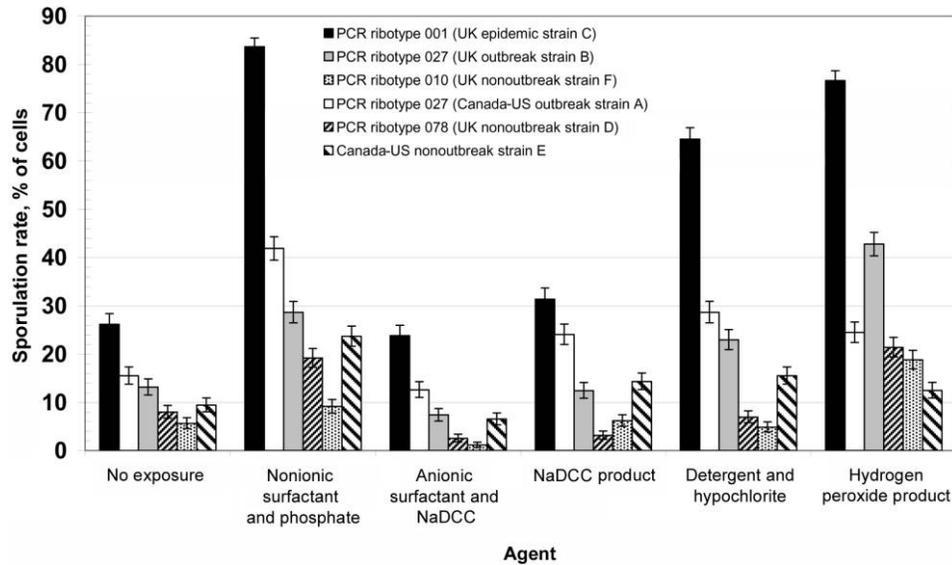
FIGURE 2.   Mean sporulation rates for 6 *Clostridium difficile* strains exposed to subinhibitory concentrations of 5 commonly used hospital cleaning agents and/or germicides. Two-way analysis of variance was performed after angular transformation of data, followed by unplanned comparison. Whiskers indicate minimum significant difference; no overlap between sets of whiskers represents a significant difference at the 5% level. NaDCC, sodium dichloroisocyanurate.

rate of *C. difficile* epidemic strains were observed in comparison with that of nonepidemic strains.

Effect of Subinhibitory Concentrations on Sporulation Rates

When cultured in fecal emulsion in the absence of a cleaning agent or germicide, the sporulation rate of each epidemic *C. difficile* strain (A, B, and C) was significantly greater than that of the other strains tested. However, the sporulation rate of strain C (PCR ribotype 001) was markedly greater than that of all the other strains tested, both in the absence and in the presence of a cleaning agent and/or germicide (Figure 2). Mean sporulation capacity, expressed as the proportion of a cell population that is in spore form, was 13% for all strains not exposed to a cleaning agent or germicide, and it was significantly increased by exposure to neutral detergent (Hospec; 34%), a combination of detergent and hypochlorite (Dispatch; 24%), and hydrogen peroxide (G-Force; 33%). By contrast, exposure to dichloroisocyanurate-containing agents (either Chlor-clean [9%] or Sanichlor [15%]) was generally not associated with significant changes in sporulation. Sporulation in response to cleaning agents or germicides was higher for the *C. difficile* epidemic strains (especially in strain C, PCR ribotype 001), compared with the other strains tested.

DISCUSSION

The contribution of the healthcare environment to infection transmission is controversial. The best evidence that the environment is an important source of nosocomial infection concerns bacteria that resist desiccation.[24] The foremost pathogens, in terms of survival on environmental surfaces, are spore-forming bacteria such as *C. difficile.* Although fecal soiling of the environment may include vegetative cells, spores of *C. difficile* predominate, especially after exposure to air and drying.[25] The activity of cleaning agents and/or germicides on *C. difficile* epidemic strains, and particularly on spores, has been poorly studied, and the few studies in this area have usually only examined single strains.[26-28]

We found markedly different effects when *C. difficile* was exposed to commonly used hospital cleaning agents and germicides. Notably, tests involving exposure of *C. difficile* vegetative cells to cleaning agents and germicides yielded results very different from those obtained in experiments with spores. Working-strength concentrations of all of the cleaning agents and germicides inhibited growth of *C. difficile* in culture. However, only chlorine-containing germicides inactivated *C. difficile* spores. Neither hydrogen peroxide (G-Force) nor nonionic surfactant with phosphate (Hospec) exerted any discernible effect on *C. difficile* spores, despite prevention of vegetative growth at 1/128 and 1/16 of the recommended working strength, respectively. The resistance of bacterial spores to nonionic surfactants is well established, and therefore the lack of activity against *C. difficile* spores was not surprising.[8] However, the poor efficacy of the hydrogen peroxide–containing agent demonstrated in the present study was unexpected. Vaporized hydrogen peroxide has been described for use as a sporicide,[29] and therefore its lack of activity against *C. difficile* spores is perhaps contradictory. The concentration of active hydrogen peroxide in the working strength solution used in the present study is not stated by

the manufacturer. It is, therefore, possible that the concentration recommended for use contains insufficient germicide to kill spores, and/or other chemicals present in G-Force may reduce its sporicidal effectiveness.

These findings concur with the results of previous experimental and ward-based studies. Wilcox et al.[7] demonstrated a significant correlation between the use of a chlorine-containing (dichloroisocyanurate) germicide and reduction in C. difficile infection incidence on 1 of 2 hospital wards that were examined using a cross-over study design. Mayfield et al.[30] reported a significant decrease in C. difficile infection cases on a bone marrow transplant unit following implementation of a hypochlorite-based cleaning regimen. Although environmental prevalence of C. difficile was not measured, it is interesting to note that the incidence of C. difficile infection returned almost to the original level following the reintroduction of the original quaternary ammonium–compound germicide.[30] Kaatz and colleagues[31] found that phosphate-buffered hypochlorite (1,600 ppm of available chlorine; pH 7.6) was more effective than unbuffered hypochlorite (500 ppm of available chlorine) at reducing environmental contamination by C. difficile.

We previously described the enhanced sporulation rate of the epidemic C. difficile PCR ribotype 001 strain in response to exposure to cleaning agents that did not contain chlorine.[22] The clinical significance of this finding is unknown. However, we have shown that in some settings, the environmental prevalence of C. difficile correlates with incidence of C. difficile infection.[22,32] In view of these findings and the environmental decontamination intervention studies discussed above, it is plausible that increased sporulation capacity, in response to certain environmental stresses, may be associated with greater spread or persistence in some C. difficile strains. Such stresses include drying, exposure to air, and exposure to cleaning agents and/or germicides. In the present study, we aimed to simulate these stresses by exposing 6 C. difficile strains in fecal emulsions to subinhibitory concentrations of cleaning agents and/or germicides. The results confirm our earlier findings,[22] and furthermore show that other C. difficile epidemic strains also had greater sporulation capacity than nonepidemic strains—notably when exposed to cleaning agents and/or germicides that did not contain chlorine. Dichloroisocyanurate-containing agents generally did not promote sporulation beyond that observed in control experiments. The poor activity of the hydrogen peroxide–containing agent (G-Force) against C. difficile spores was compounded by its propensity to promote sporulation in epidemic strains when they were exposed to a subinhibitory concentration of this germicide.

Our results support the use of dichloroisocyanurate-containing germicides to control C. difficile in healthcare institutions, in preference to the other agents tested. In our spore viability assay, all 3 chlorine-containing agents inactivated both vegetative cells and spores at the concentrations recommended by manufacturers. However, the hypochlorite-containing compound (Dispatch) was the only chlorine-containing agent tested that was associated with an increase in mean sporulation capacity, when compared with the dichloroisocyanurate-containing compounds (Chlor-clean and Sanichlor). Bloomfield and Uso demonstrated that dichloroisocyanurate-containing agents are superior to hypochlorite-containing compounds, being less susceptible to inactivation by organic material.[33] The use of chlorine-containing products presents health and safety, cleaning, and materials compatibility challenges, the risks of which need to be assessed. However, the combined body of evidence suggests that dichloroisocyanurate (ie, chlorine-release) germicides currently represent the optimum choice for the removal of C. difficile from healthcare environments.

Removal or inactivation of C. difficile spores in the healthcare setting is believed to be an important control measure for this increasingly prevalent pathogen. Our results suggest that compounds that do not kill C. difficile spores at working concentrations, such as general-purpose detergents and hydrogen peroxide, may promote the persistence and accumulation of spores in healthcare environments. The environmental cleaning of healthcare premises needs to be both timely and efficient. Additionally, healthcare institutions should ensure that cleaning agents and/or germicides with adequate microbiological effectiveness are employed.

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