Efficacy of decontaminants and disinfectants against *Clostridium difficile*

Prerna Vohra and Ian R. Poxton

Correspondence

Ian R. Poxton

i.r.poxton@ed.ac.uk

Centre for Infectious Diseases, University of Edinburgh College of Medicine and Veterinary Medicine, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK

Clostridium difficile is a common nosocomial pathogen transmitted mainly via its spores. These spores can remain viable on contaminated surfaces for several months and are resistant to most commonly used cleaning agents. Thus, effective decontamination of the environment is essential in preventing the transmission of *C. difficile* in health-care establishments. However, this emphasis on decontamination must also be extended to laboratories due to risk of exposure of staff to potentially virulent strains. Though few cases of laboratory-acquired infection have been reported, the threat of infection by *C. difficile* in the laboratory is real. Our aim was to test the efficacy of four disinfectants, Actichlor, MicroSol 3+, TriGene Advance and Virkon, and one laboratory decontaminant, Decon 90, against vegetative cells and spores of *C. difficile*. Five strains were selected for the study: the three most commonly encountered epidemic strains in Scotland, PCR ribotypes 106, 001 and 027, and control strains 630 and VPI 10463. MICs were determined by agar dilution and broth microdilution. All the agents tested inhibited the growth of vegetative cells of the selected strains at concentrations below the recommended working concentrations. Additionally, their effect on spores was determined by exposing the spores of these strains to different concentrations of the agents for different periods of time. For some of the agents, an exposure of 10 min was required for sporicidal activity. Further, only Actichlor was able to bring about a 3 log₁₀ reduction in spore numbers under clean and dirty conditions. It was also the only agent that decontaminated different hard, non-porous surfaces artificially contaminated with *C. difficile* spores. However, this too required an exposure time of more than 2 min and up to 10 min. In conclusion, only the chlorine-releasing agent Actichlor was found to be suitable for the elimination of *C. difficile* spores from the environment, making it the agent of choice for the decontamination of laboratory surfaces.

Received 14 January 2011

Accepted 6 April 2011

INTRODUCTION

Clostridium difficile is the causative agent of *C. difficile* infection (CDI) (Bartlett *et al.*, 1978; Bartlett, 2008; George *et al.*, 1978). Though CDI is a common nosocomial infection, it is not limited to the hospital environment; community-acquired CDI and asymptomatic carriage are also common (Freeman *et al.*, 2010).

C. difficile is acquired via the faecal–oral route as spores (Lyerly *et al.*, 1988). Symptomatic patients shed large amounts of both vegetative cells and spores into the environment (Wilcox *et al.*, 2003). Though vegetative cells survive under aerobic conditions for only 15 min on dry surfaces, they can survive for up to 6 h on moist surfaces (Weber *et al.*, 2010). The spores of *C. difficile*, however, can persist on hospital floors for up to 5 months (Kim *et al.*, 1981) and are resistant to several cleaning agents, especially in the presence of organic matter (Fawley *et al.*, 2007;

Wheeldon *et al.*, 2008). Surfaces contaminated with *C. difficile* spores can facilitate cross-colonization (Fawley & Wilcox, 2001). It has been suggested that health-care workers (and patients) acquire *C. difficile* from contaminated surfaces (Gerding *et al.*, 1995; McFarland & Stamm, 1986) such as portable commodes, bedpans, blood pressure cuffs, walls, floors, washbasins and furniture (Fekety *et al.*, 1981; Samore *et al.*, 1996; Weber *et al.*, 2010). A correlation between transmission of *C. difficile* among patients and contamination of the hands of health-care workers has been clearly observed (McFarland *et al.*, 1989).

Although *C. difficile* is a dreaded nosocomial pathogen, the risk of laboratory-acquired CDI has not been given much attention, in spite of the exposure of laboratory workers to relatively high inocula of *C. difficile*. However, cases of CDI have been reported in laboratory personnel (Bouza *et al.*, 2008) as well as in health-care workers (Arfons *et al.*, 2005; Hell *et al.*, 2009; Ray & Donskey, 2003). Thus, it is important to ensure proper decontamination of surfaces in the laboratory as well as proper hand-washing technique to

Abbreviation: CDI, *Clostridium difficile* infection.

Table 1. Agents used in this study

Agent	Active ingredient(s)	Manufacturer	Recommended working concentration
Actichlor	Sodium dichloroisocyanurate	Ecolab	1000 p.p.m. chlorine
Decon 90	Anionic and non-anionic surfactants	Decon Laboratories	1:10 dilution
Microsol 3 +	Tertiary alkyamine and quaternary ammonium compounds	Anachem	1:10 dilution
TriGene Advance	Polymeric biguanide hydrochloride and organic quaternary compounds	Medichem International	1:100 dilution
Virkon	Potassium peroxymonosulfate	Antec International	1:100 dilution

prevent potential transmission of *C. difficile* from the laboratory environment into the community and hospitals. The aim of this study was to test the efficacy of five commonly used agents against both vegetative cells and spores of *C. difficile* using laboratory tests as well as a more real-life surface cleaning approach.

METHODS

Bacterial strains. Five strains of *C. difficile* were used in this study: strain 630 (ribotype 012, obtained from P. Mullany, London, UK), strain VPI 10463 (obtained from Unipath, Bedford, UK), ribotype 027 (obtained from E. J. Kuijper, Leiden, The Netherlands), ribotype 001 and ribotype 106 (local strains from Edinburgh, UK). The strains were purified and maintained as spore suspensions in Robertson's cooked meat medium (Oxoid). All culturing of strains was performed anaerobically (80 % H₂, 10 % N₂, 10 % CO₂) at 37 °C in a Mark III workstation (Don Whitley Scientific).

Agents. Five agents were tested in this study: Actichlor, a disinfectant routinely used in hospitals; Decon 90, a laboratory decontaminant; and three commonly used laboratory disinfectants, Microsol 3 +, TriGene Advance and Virkon. The active ingredients, recommended concentrations and manufacturers are listed in Table 1.

MIC testing. MICs of the agents for the vegetative cells of the different *C. difficile* strains were determined by the Wadsworth agar dilution method (NCCLS, 2004). Strains were cultured anaerobically on blood agar at 37 °C for 24 h, followed by culturing in pre-reduced thioglycollate broth supplemented with 5 µg haemin ml⁻¹ and 1 µg vitamin K₁ ml⁻¹ for 8 h. This ensured that the cultures were in the exponential phase when used for MIC testing, and thus consisted of mainly vegetative cells and very few spores. These cultures were diluted to a 0.5 McFarland standard for inoculation onto pre-reduced

Brucella agar (Oxoid) supplemented with 5 % defibrinated horse blood, 5 µg haemin ml⁻¹ and 1 µg vitamin K₁ ml⁻¹ containing doubling dilutions of one of the five agents ranging from the manufacturer's recommended working concentration to 1/1024 of the same. The plates were incubated anaerobically at 37 °C for 48 h and then examined for growth. The lowest concentration of the tested agent to inhibit visible bacterial growth was recorded as the MIC of that agent. The experiments were performed in triplicate.

MICs were also performed by broth microdilution in a 96-well plate. Doubling dilutions of the five agents ranging from the manufacturer's recommended working concentration to 1/1024 of the same were prepared in pre-reduced anaerobic incubation medium and 100 µl of each dilution was added to wells of the plate in duplicate. To the wells, 10 µl culture adjusted to a 0.5 McFarland standard was added. The plates were incubated anaerobically at 37 °C for 48 h and then examined for growth by eye and by measurement of OD₆₀₀. Negative controls with no added culture and positive controls without the agents were also maintained in duplicate. The lowest concentration of the tested agent to inhibit visible bacterial growth and OD₆₀₀ ≥ 0.1 was recorded as the MIC of that agent. The experiments were performed in triplicate.

Preparation of spores. Strains were cultured in 500 ml pre-reduced anaerobic incubation medium (Brown *et al.*, 1996) anaerobically at 37 °C for 7 days. The cultures were then collected by centrifugation at 4000 g for 10 min. The pellets obtained were washed twice with sterile PBS and then treated with 50 % ethanol for 1 h with constant shaking. The pellets were collected by centrifugation, washed twice in PBS and then resuspended in 1 ml sterile distilled water and stored at 4 °C. Before each experiment, spore counts were determined from 10-fold serial dilutions of the final spore suspension plated on blood agar and incubated for 48 h.

Sporicidal assays. Spore preparations were standardized to 10⁶ spores ml⁻¹. The agents were tested for their sporicidal activity in a

Table 2. MIC of each agent for vegetative cells of five *C. difficile* strains represented as a fraction of the recommended working concentration

Values are the median from both the agar-dilution and the broth microdilution methods for MIC testing.

Agent	Strain 630	Strain VPI 10463	Ribotype 027	Ribotype 001	Ribotype 106
Actichlor	1/8	1/8	1/2	1/8	1/8
Decon 90	1/4	1/4	1/2	1/2	1/4
Microsol 3 +	1/128	1/128	1/128	1/128	1/128
TriGene Advance	1/32	1/32	1/32	1/32	1/32
Virkon	1/8	1/8	1/4	1/4	1/8

Table 3. Minimum sporicidal concentration of the agents for five *C. difficile* strains represented as a fraction of the recommended working concentration after different times of exposure

The tests were performed in 1 ml volumes with 10^5 spores being exposed to the disinfectants.

Agent*	Time of exposure (min)	Minimum sporicidal concentration				
		Strain 630	Strain VPI 10463	Ribotype 027	Ribotype 001	Ribotype 106
Actichlor	2	<1/5	<1/5	1/2	<1/5	1/2
	10	<1/5	<1/5	<1/5	<1/5	<1/5
	30	<1/5	<1/5	<1/5	<1/5	<1/5
Decon 90	2	1	1/2	1/2	1	1/2
	10	1/2	<1/5	<1/5	1/2	<1/5
	30	<1/5	<1/5	<1/5	<1/5	<1/5
Virkon	2	1	1	1	1	1
	10	1	<1/5	1	<1/5	<1/5
	30	1	<1/5	1	<1/5	<1/5

*The minimum sporicidal concentration for Microsol 3+ and TriGene Advance for all the strains was less than 1/5 at 2, 10 and 30 min.

suspension test at the recommended working concentration and 1/2 and 1/5 of the same. For each test, 100 µl spore suspension containing 10^5 spores was added to 900 µl of each of the dilutions and mixed thoroughly. At 2 min, 10 min and 30 min, 100 µl of the test was inoculated into 900 µl pre-reduced anaerobic incubation medium, mixed and incubated at 37 °C for 5 days in duplicate. Positive controls without any disinfectant and negative controls without spores were also maintained in duplicate to check that the medium was sustaining the germination of spores and growth of the resulting vegetative cells and that it was not contaminated, respectively. The tubes were examined for growth and the lowest concentration of the agent showing no growth was recorded as the minimum sporicidal concentration. Samples from tubes with no visible growth were plated to identify cidal or static activity and these were examined for growth after 5 days of anaerobic incubation.

Determination of $\log_{10}$ reduction. To determine the $\log_{10}$ reduction in *C. difficile* spores in suspension tests, spores were treated with the cleaning agents at the recommended working concentration in the presence or absence of organic matter. For the tests in the absence of organic matter, 100 µl spores in distilled water (approx. 10^4 spores ml^{-1}) was added to 900 µl agent. After 10 min, the spores were collected by centrifugation at 16 000 g for 2 min. The spore pellets were washed twice with 1 ml distilled water. The spores were then resuspended in 1 ml distilled water and 100 µl of this suspension was spread onto blood agar plates in duplicate and incubated at 37 °C

anaerobically for 48 h. Untreated positive controls were maintained in duplicate. For the tests in the presence of organic matter, BSA (Sigma) was introduced into the test at a final concentration of 0.27 %. The $\log_{10}$ reduction was calculated as $\log_{10} (N_0/N_{10})$, where N_0 =the number of spores in the positive control and N_{10} =the number of viable spores recovered from the test at 10 min.

Surface decontamination testing. Five different surfaces were used in this study: aluminium, glass, plastic, self-adhesive vinyl tiles and white ceramic tiles. The surfaces were autoclaved and dried in a hot air oven before use: the self-adhesive vinyl tiles could not be autoclaved and were cleaned with 70 % ethanol before use. On all the clean surfaces, squares of approximately 1 cm^2 were marked out with a wax crayon. These were then artificially contaminated with spores of the five *C. difficile* strains and tested for cleaning efficiency with all the cleaning agents. Each square was contaminated with 10 µl spore suspension (10^5 spores ml^{-1}) and left to air dry for 2 h. Fifty microlitres of cleaning agent at the manufacturer's recommended concentration was then added to the square on top of the dried spore suspension. After 2 min and 10 min, the area was scratched with a pipette tip 10 times in an attempt to mimic scrubbing and the agent was aspirated. The area was then washed with 100 µl distilled water. The aspirated agent and wash were added to 850 µl distilled water to obtain a final volume of 1 ml. One hundred microlitres of this was plated on blood agar and incubated anaerobically for 48 h. The surfaces were also cleaned only with water and these served as positive

Table 4. Mean $\log_{10}$ reduction in *C. difficile* spores of five strains in the absence and presence of organic matter

The $\log_{10}$ reduction is a mean of the values obtained for all five *C. difficile* strains.

Agent	$\log_{10}$ reduction (mean $\pm$ SEM)	
	Organic matter negative	Organic matter positive
Actichlor	3.093 ± 0.2239	3.076 ± 0.2429
Decon 90	0.5159 ± 0.06722	0.3384 ± 0.101
Microsol 3+	1.519 ± 0.1025	0.7288 ± 0.1294
TriGene Advance	1.698 ± 0.0806	0.5399 ± 0.09384
Virkon	1.171 ± 0.1705	0.1657 ± 0.04113

controls. The $\log_{10}$ reduction was calculated as above from two independent experiments.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 4.0 software. In order to compare agents and strains, one-way analysis of variance (ANOVA) was performed.

RESULTS AND DISCUSSION

Efficacy against vegetative cells

The growth of vegetative *C. difficile* was effectively suppressed by all the agents tested. The MICs of all agents for the strains were found to be the same by agar dilution and broth microdilution and these values were lower than the manufacturer's recommended concentration (Table 2). However, for Actichlor, Decon 90 and Virkon, higher concentrations seemed to be required to destroy vegetative cells of the epidemic ribotypes 027, 001 and 106. Microsol 3+ appeared to be the most effective agent, active at a 128-fold dilution of the recommended concentration, followed by TriGene, which was effective at a 32-fold dilution of the recommended concentration. Actichlor, used for routinely cleaning hospitals, was effective at its recommended concentration, but less than a 2-fold dilution was unable to inhibit the growth of the hypervirulent ribotype 027. These observations suggest that the epidemic ribotypes may have a greater resistance to commonly used cleaning agents. Also, they emphasize the importance of preparing these agents at the correct concentration.

Efficacy against spores

The agents tested were also found to be sporicidal at the recommended concentration in suspension tests at 2, 10 and 30 min of testing (Table 3). Once again it was observed that ribotype 027 required a greater concentration of Actichlor for destruction of its spores. This was true for the epidemic ribotype 106 as well. For Virkon, any dilution below the recommended concentration was not sporicidal at 2 min, making it a less effective disinfectant than Actichlor. Interestingly, Microsol 3+ and TriGene Advance were found to be sporicidal at a fivefold dilution of the recommended concentration even at 2 min of exposure. However, a neutralizer not used in these experiments, and the agents were not removed by washing: they were diluted 10-fold into the recovery medium. Thus, it is possible that there was a prolonged exposure of the spores to low but effective concentrations of the agents tested.

Effect of organic matter on efficacy

All the selected agents except Decon 90 are marketed as sporicidal agents. Thus, it was of interest to determine the $\log_{10}$ reduction in spore numbers brought about by them at the recommended concentration. Under clean conditions, Actichlor was found to be the most effective

Fig. 1. Efficacy of Actichlor (A), Decon 90 (D), Microsol 3+ (M), TriGene Advance (T) and Virkon (V) ($\log_{10}$ reduction) against the spores of five strains of *C. difficile* in the absence and presence of organic matter. Only Actichlor effectively destroyed spores of all the strains in the absence or presence of organic matter, causing 3 $\log_{10}$ reduction in spores. The efficacy of all the other agents decreased significantly in the presence of organic matter, especially in destroying spores of the epidemic ribotypes 027, 001 and 106. Bars indicate $\pm$ SEM of two experiments.

Fig. 2. Decontamination of different surfaces contaminated with spores of five *C. difficile* strains by Actichlor (A), Decon 90 (D), Microsol 3+ (M), TriGene Advance (T) and Virkon (V) tested at 2 min and 10 min of exposure. Actichlor (A) was the most effective decontaminant at both the time points and destroyed 100 % of spores of all the strains by 10 min. The other agents fared poorly, especially against the epidemic ribotypes 027, 001 and 106. Bars indicate $\pm$ SEM of two experiments.

sporicidal agent ($P < 0.001$), bringing about a 3 $\log_{10}$ reduction of *C. difficile* spores at 10 min, at which time Microsol 3+, TriGene Advance and Virkon only caused approximately 1.5, 1.7 and 1.2 $\log_{10}$ reduction (Table 4). However, these disinfectants were still significantly more

effective than Decon 90 ($P < 0.001$ compared to Microsol 3+ and TriGene Advance; $P < 0.01$ compared to Virkon). The superior efficacy of chlorine-releasing agents as compared to peroxy compounds against the spores of *C. difficile* has been observed previously (Lawley *et al.*, 2010).

No inter-strain differences were observed, except when Decon 90 was used; the least $\log_{10}$ reduction was observed for ribotype 027 ($P < 0.05$). In the presence of organic matter, the efficacy of all the cleaning agents, except Actichlor, dropped considerably. Actichlor was the most effective disinfectant even in the presence of organic matter ($P < 0.001$). Of note was the drop in efficacy of Virkon from approximately 1.2 $\log_{10}$ in the absence of organic matter to approximately 0.2 $\log_{10}$ in the presence of it. Also, interestingly, in the presence of organic matter, for all the agents except Actichlor, the $\log_{10}$ reduction for the epidemic strains was more markedly reduced as compared to that for the non-epidemic strains (Fig. 1). Although for Actichlor, Microsol 3+ and TriGene Advance this difference was not significant, when using Virkon, ribotype 001 was the least effectively destroyed strain ($P < 0.05$), and when using Decon 90, strain 630 was the most effectively destroyed ($P < 0.001$). As contamination with organic matter is common in the environment, the efficacy of cleaning agents in its presence is of greater practical significance. From the observations above, it is clear that chlorine-based agents are a better choice for disinfection, especially to eliminate the currently epidemic *C. difficile* strains.

Surface decontamination

To test the decontamination of surfaces using the selected agents, 1 cm² areas were artificially contaminated with 10³ spores of each strain, allowed to dry and then cleaned, in an attempt to mimic a real-life situation. Further, washing of the surface was performed by aspiration of disinfectant and subsequent washes. It was observed that only the chlorine-releasing Actichlor was able to completely decontaminate all the surfaces tested; however, it required more than 2 min and up to 10 min to see this effect (Fig. 2). For all the other agents, although there was a greater $\log_{10}$ reduction in spore numbers after 10 min of treatment, it was not significant. Here too it was found that the epidemic strains were less effectively cleaned. Spores of strain 630, which was previously epidemic, were also more resistant to the cleaning, while spores of VPI 10463, which is rarely isolated from patients, were the most effectively destroyed. Thus, though chlorine-releasing agents destroy *C. difficile* spores, the contact times required for surfaces to be decontaminated might be unrealistic due to time constraints as well as the odour, corrosive effects on surfaces and irritation to users (Block, 2004; Ungurs *et al.*, 2011). However, the natural environment might not be as heavily contaminated as the surfaces in these experiments and thus may be more efficiently cleaned. In the laboratory, however, contamination of surfaces by high concentrations of spores is a possibility, and they must be cleaned and decontaminated carefully and thoroughly.

The contaminated environment is important in the transmission of *C. difficile* (Fawley & Wilcox, 2001; Weber *et al.*, 2010), and just as in hospitals, laboratories

can be a source of transmission of CDI, due to both the virulent types of strains and the high concentrations of *C. difficile* routinely used. Reports of laboratory-acquired CDI led us to evaluate the type of disinfectants and cleaning agents used in our laboratory in this study. From these experiments, which are preliminary and do not strictly follow the guidelines for disinfectant testing, we can conclude that vegetative cells of *C. difficile* as well as spores can be destroyed by a variety of agents in suspension tests with long contact times. However, hard non-porous surfaces were only decontaminated by the use of a chlorine-releasing agent. Even then, a contact time of up to 10 min was required to eliminate high concentrations of *C. difficile* spores. Thus, chlorine-releasing agents should be the decontaminants of choice, not only in hospitals (Fraise, 2011), but also in laboratories.

ACKNOWLEDGEMENTS

This work is part of a PhD funded by the Overseas Research Students Awards Scheme and the University of Edinburgh Centre for Infectious Diseases PhD Studentship. We would like to thank Alexander Phythian-Adams and Dr Surekha Reddy for providing some of the agents, and Malcolm Baldock for laboratory assistance. This work was presented as a poster at the Third International *Clostridium difficile* Symposium, Bled, Slovenia, September 2010.

REFERENCES

- Arfons, L., Ray, A. J. & Donskey, C. J. (2005). *Clostridium difficile* infection among health care workers receiving antibiotic therapy. *Clin Infect Dis* **40**, 1384–1385.
- Bartlett, J. G. (2008). Historical perspectives on studies of *Clostridium difficile* and *C. difficile* infection. *Clin Infect Dis* **46** (Suppl. 1), S4–S11.
- Bartlett, J. G., Moon, N., Chang, T. W., Taylor, N. & Onderdonk, A. B. (1978). Role of *Clostridium difficile* in antibiotic-associated pseudo-membranous colitis. *Gastroenterology* **75**, 778–782.
- Block, C. (2004). The effect of Perasafe and sodium dichloroisocyanurate (NaDCC) against spores of *Clostridium difficile* and *Bacillus atrophaeus* on stainless steel and polyvinyl chloride surfaces. *J Hosp Infect* **57**, 144–148.
- Bouza, E., Martin, A., Van den Berg, R. J. & Kuijper, E. J. (2008). Laboratory-acquired *Clostridium difficile* polymerase chain reaction ribotype 027: a new risk for laboratory workers? *Clin Infect Dis* **47**, 1493–1494.
- Brown, R., Collee, J. G. & Poxton, I. R. (1996). Bacteroides, Fusobacterium and other Gram-negative anaerobic rods; anaerobic cocci; identification of anaerobes. In *Mackie and McCartney Practical Medical Microbiology*, pp. 501–519. Edited by J. G. Collee, A. G. Fraser, B. P. Marmion & A. Simmons. London: Churchill Livingstone.
- Fawley, W. N. & Wilcox, M. H. (2001). Molecular epidemiology of endemic *Clostridium difficile* infection. *Epidemiol Infect* **126**, 343–350.
- Fawley, W. N., Underwood, S., Freeman, J., Baines, S. D., Saxton, K., Stephenson, K., Owens, R. C., Jr & Wilcox, M. H. (2007). Efficacy of hospital cleaning agents and germicides against epidemic *Clostridium difficile* strains. *Infect Control Hosp Epidemiol* **28**, 920–925.
- Fekety, R., Kim, K. H., Brown, D., Batts, D. H., Cudmore, M. & Silva, J., Jr (1981). Epidemiology of antibiotic-associated colitis;

isolation of *Clostridium difficile* from the hospital environment. *Am J Med* 70, 906–908.

Fraise, A. (2011). Currently available sporicides for use in healthcare, and their limitations. *J Hosp Infect* 77, 210–212.

Freeman, J., Bauer, M. P., Baines, S. D., Corver, J., Fawley, W. N., Goorhuis, B., Kuijper, E. J. & Wilcox, M. H. (2010). The changing epidemiology of *Clostridium difficile* infections. *Clin Microbiol Rev* 23, 529–549.

George, R. H., Symonds, J. M., Dimock, F., Brown, J. D., Arabi, Y., Shinagawa, N., Keighley, M. R., Alexander-Williams, J. & Burdon, D. W. (1978). Identification of *Clostridium difficile* as a cause of pseudomembranous colitis. *BMJ* 1, 695.

Gerding, D. N., Johnson, S., Peterson, L. R., Mulligan, M. E. & Silva, J., Jr (1995). *Clostridium difficile*-associated diarrhea and colitis. *Infect Control Hosp Epidemiol* 16, 459–477.

Hell, M., Indra, A., Huhulescu, S. & Allerberger, F. (2009). *Clostridium difficile* infection in a health care worker. *Clin Infect Dis* 48, 1329.

Kim, K. H., Fekety, R., Batts, D. H., Brown, D., Cudmore, M., Silva, J., Jr & Waters, D. (1981). Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *J Infect Dis* 143, 42–50.

Lawley, T. D., Clare, S., Deakin, L. J., Goulding, D., Yen, J. L., Raisen, C., Brandt, C., Lovell, J., Cooke, F. & other authors (2010). Use of purified *Clostridium difficile* spores to facilitate evaluation of health care disinfection regimens. *Appl Environ Microbiol* 76, 6895–6900.

Lyerly, D. M., Krivan, H. C. & Wilkins, T. D. (1988). *Clostridium difficile*: its disease and toxins. *Clin Microbiol Rev* 1, 1–18.

McFarland, L. V. & Stamm, W. E. (1986). Review of *Clostridium difficile*-associated diseases. *Am J Infect Control* 14, 99–109.

McFarland, L. V., Mulligan, M. E., Kwok, R. Y. & Stamm, W. E. (1989). Nosocomial acquisition of *Clostridium difficile* infection. *N Engl J Med* 320, 204–210.

NCCLS (2004). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*, Approved Standard, 6th edn. NCCLS document M11-A6. Wayne, PA: National Committee for Clinical Laboratory Standards.

Ray, A. J. & Donskey, C. J. (2003). *Clostridium difficile* infection and concurrent vancomycin-resistant *Enterococcus* stool colonization in a health care worker: case report and review of the literature. *Am J Infect Control* 31, 54–56.

Samore, M. H., Venkataraman, L., DeGirolami, P. C., Arbeit, R. D. & Karchmer, A. W. (1996). Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhea. *Am J Med* 100, 32–40.

Ungurs, M., Wand, M., Vassey, M., O'Brien, S., Dixon, D., Walker, J. & Sutton, J. M. (2011). The effectiveness of sodium dichloroisocyanurate treatments against *Clostridium difficile* spores contaminating stainless steel. *Am J Infect Control* 39, 199–205.

Weber, D. J., Rutala, W. A., Miller, M. B., Huslage, K. & Sickbert-Bennett, E. (2010). Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infect Control* 38 (Suppl 1), S25–S33.

Wheeldon, L. J., Worthington, T., Hilton, A. C., Lambert, P. A. & Elliott, T. S. J. (2008). Sporocidal activity of two disinfectants against *Clostridium difficile* spores. *Br J Nurs* 17, 316–320.

Wilcox, M. H., Fawley, W. N., Wigglesworth, N., Parnell, P., Verity, P. & Freeman, J. (2003). Comparison of the effect of detergent versus hypochlorite cleaning on environmental contamination and incidence of *Clostridium difficile* infection. *J Hosp Infect* 54, 109–114.