Synergistic efficacy of 405 nm light and chlorinated disinfectants for the enhanced decontamination of *Clostridium difficile* spores

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KEY WORDS: *Clostridium difficile*; Spores; 405 nm light; Inactivation; Disinfectants; Synergy

HIGHLIGHTS:

- *Clostridium difficile* vegetative cells and spores were inactivated by 405nm light
- Spores require ~10-fold higher doses of 405nm for inactivation than vegetative cells
- Combined use of 405nm light & chlorinated disinfectants gave synergistic inactivation
- Use of 405nm light might allow use of reduced chlorinated disinfectant concentrations

ABSTRACT

The ability of *Clostridium difficile* to form highly resilient spores which can survive in the environment for prolonged periods causes major contamination problems. Antimicrobial 405nm light is being developed for environmental decontamination within hospitals, however further information relating to its sporicidal efficacy is required. This study aims to establish the efficacy of 405nm light for inactivation of *C. difficile* vegetative cells and spores, and to establish whether spore susceptibility can be enhanced by the combined use of 405nm light with low concentration chlorinated disinfectants. Vegetative cells and spore suspensions were exposed to increasing doses of 405nm light (at 70-225 mW/cm^2) to establish sensitivity. A 99.9% reduction in vegetative cell population was demonstrated with a dose of 252 J/cm^2, however spores demonstrated higher resilience, with a 10-fold increase in required dose. Exposures were repeated with spores suspended in the hospital disinfectants sodium hypochlorite, Actichlor and Tristel at non-lethal concentrations (0.1%, 0.001% and 0.0001%, respectively). Enhanced sporicidal activity was achieved when spores were exposed to 405nm light in the presence of the disinfectants, with a 99.9% reduction achieved following exposure to 33% less light dose than required when exposed to 405nm light alone. In conclusion, *C. difficile* vegetative cells and spores can be successfully inactivated using 405nm light, the sporicidal efficacy can be significantly enhanced when exposed in the presence of low concentration chlorinated disinfectants. Further research may lead to the potential use of 405nm light decontamination in combination with selected hospital disinfectants to enhance *C. difficile* cleaning and infection control procedures.

1. Introduction

Clostridium difficile is a gram positive anaerobe which is the most common cause of antibiotic associated diarrhoea, with the spectrum of illness ranging from asymptomatic colonization, to mild diarrhoea, to life threatening pseudomembranous colitis [1–3]. The ability of *C. difficile* to form highly infectious and resilient spores which can survive in the environment for prolonged periods can result in significant environmental contamination [4,5]. *C. difficile* is spread by the faecal-oral route, however in recent years the environment has been shown to play an important role in the spread of disease [6]. Adequate disinfection is of the upmost importance as the rooms of patients with *C. difficile* infection (CDI) can reach contamination rates of up to 50%, whilst those of asymptomatic patients can reach approximately 25% contamination, with spores surviving in the environment for up to five months [7,8]. The surfaces most frequently contaminated with spores are the floors, bathroom areas and the toilet [7–9]. A study carried out by Barbut et al [9] reported that 74% of rooms that had housed patients with CDI were contaminated with *C. difficile* spores. Furthermore, a 12% increase in risk of nosocomial CDI was reported when patients were in the immediate vicinity, or later occupants, of a room occupied by a patient with CDI [10,11].

Despite the development of a range of novel disinfection and sterilisation technologies, *C. difficile* remains a significant problem within the environment of various healthcare settings. Current guidelines recommend the use of chlorinated disinfectants for the decontamination of surfaces [12]. There are many drawbacks associated with the current decontamination methods used to disinfect the rooms of patients diagnosed with *C. difficile*, with *C. difficile* remaining in the environment upon admission of subsequent patients; samples collected from rooms of patients who are neither infected nor colonized with *C. difficile* have been reported to reach up to 8% contamination, demonstrating the inefficiency of currently used disinfectants at eliminating *C. difficile* [9]. Furthermore, although chlorinated disinfectants currently used for the elimination of *C. difficile* spores have been demonstrated to significantly reduce *C. difficile* contamination rates, drawbacks include their corrosive nature and the release of irritating vapours affecting healthcare workers [7]. Another problem associated with chlorinated disinfectants is the lack of staff compliance with adhering to the correct disinfection methods [6].

A recent technology that has been proposed for environmental decontamination applications is the use of 405 nm violet-blue light [13–16]. Recent studies carried out in hospital isolation rooms have demonstrated the use of this technology for continuous decontamination of occupied environments, with reductions in the levels of bacterial contaminants by up to 80%, over and above that achieved with standard cleaning and infection control procedures alone [13–16]. Although less germicidal

than ultraviolet light, violet-blue light has the advantage that it can be used at levels which provide an antimicrobial effect whilst being non-detrimental to exposed individuals, or indeed to mammalian cell preparations [17–19]. The antimicrobial mechanism of violet-blue light involves the photo-excitation of intracellular porphyrin molecules within the exposed organisms, which then stimulates the production of a range of reactive oxygen species, including singlet oxygen ($^1O_2$), resulting in oxidative damage to cellular components, and ultimately cell death [15]. Porphyrin photo-excitation occurs with light in the region of 400-420 nm, with peak activity being demonstrated at 405 nm [20–22]. Recent studies have demonstrated the efficacy of violet-blue light for inactivation of a range of pathogenic bacteria, fungi, yeasts, and under certain circumstances, viruses [21,23–25]. Successful inactivation of endospore-forming bacteria, (*Clostridium, Bacillus*) and their dormant spores has previously been demonstrated [22], however, results were limited and highlighted the resilience of spores to light inactivation, therefore further information relating to the efficacy of 405 nm light against spores, and how this efficacy can be enhanced, is required.

As discussed, standard cleaning procedures against *C. difficile* involve the use of chlorinated disinfectants and as both disinfectants and 405 nm light induce lethal oxidative damage to microorganisms, their combined use has the potential to deliver enhanced sporicidal effects. This study firstly determines the susceptibility of *C. difficile* vegetative cells and spores to 405 nm light, and progresses to provide the first evidence of the enhanced sporicidal effects of 405 nm light through synergistic action with low concentration chlorinated disinfectants including sodium hypochlorite (NaOCl), Actichlor and Tristel.

2. Material and Methods

2.1 Bacterial Preparation

The bacterial strain used throughout this study was *Clostridium difficile* NCTC 11204 (National Collection of Type Cultures, Collindale, UK). For storage the bacterial culture was inoculated onto Microbank beads and stored at -18°C until required. For preparation of vegetative cells, *C. difficile* was streak-inoculated onto blood agar (BA) plates (Blood Agar Base [Oxoid, UK]; 7% horse blood [E&O laboratories, UK]) and incubated at 37°C for 18 hours under anaerobic conditions (miniMACS Anaerobic Workstation, Don Whitley Scientific UK). Post-incubation, the cells were washed off the plate with phosphate buffered saline (PBS; Oxoid UK) by agitation with an L-shaped spreader. This was then diluted to the desired population for experimental use. The absence of bacterial endospores was confirmed by heat treatment at 80°C for 10 minutes prior to enumeration on BA

plates. For preparation of spores, *C. difficile* was inoculated into 15 ml Brain Heart Infusion (BHI) broth (Oxoid, UK) supplemented with 0.1% L-cysteine (Fisher Scientific, UK), and incubated under anaerobic conditions at 37°C for 7 days. Post-incubation, the spore suspension was centrifuged at 3939 ×g for 10-min and resuspended in PBS. This suspension was heat treated at 80°C for 10-min to ensure only spores were present, and then diluted to the desired population for experimental use.

2.2 405nm light inactivation of bacterial suspensions

The light source used was a 405 nm light emitting diode (LED) array [PhotonStar Technologies, UK], with peak output at approximately 405 nm, and a bandwidth of 14 nm at full-width half-maximum (FWHM). The array was attached to a heat sink and fan, to aid thermal management and prevent heat transfer to the exposed samples.

For exposure of both *C. difficile* vegetative cells and spores, sample volumes of 2ml bacterial suspension were positioned in a sample dish approximately 5 cm directly below the LED array. Suspensions were then exposed to 405 nm light at an irradiance of 70 or 225 $mWcm^{-2}$, for vegetative cells and spores, respectively, with irradiance measured using a radiant power meter and photodiode detector [LOT Oriel, USA]. Samples were exposed to increasing doses of light, with dose calculated as irradiance ($mWcm^{-2}$) × exposure time (s). Controls were subject to identical conditions, but exposed to normal laboratory lighting.

2.3 405 nm light inactivation of spores in the presence of disinfectants

The disinfectants used in this study were sodium hypochlorite (NaOCl; Fisher Scientific, UK), Actichlor (Ecolab Ltd, UK) and Tristel (Tristel solutions Ltd, UK). Disinfectants were prepared according to the manufacturer's instructions with subsequent dilutions made as required. Before experiments investigating the synergistic activity of disinfectants in combination with 405 nm light could be carried out, it was important to firstly establish the disinfectant concentrations which would cause a negligible effect on the spores over the maximum exposure period required for inactivation of the spores using the 405 nm light alone. Spores were exposed to a range of concentrations of each of the three disinfectants and their inactivation effects recorded (Table 1). Based on these results, concentrations of 0.0001%, 0.001% and 0.1% were chosen for Tristel, Actichlor and sodium hypochlorite, respectively. Spores were then suspended in these selected disinfectant concentrations, and exposed to increasing doses of 405 nm light, at an irradiance of 225 $mWcm^{-2}$, as described in Section 2.2. Controls were subject to identical conditions, but exposed to normal laboratory lighting.

Table 1. *Exposure of C. difficile spores to chlorinated disinfectants, at a range of concentrations, over a 3 hour period.*

Disinfectant	Concentration	Initial Spore Count (log_{10} $CFUml^{-1}$ ± SD)	Surviving Spore Count (log_{10} $CFUml^{-1}$ ± SD)	% Inactivation
Sodium hypochlorite	0.01%	3.28 ± 0.37	3.25± 0.41	0.9%
	0.1%	3.91 ± 0.13	3.76 ± 0.19	3.8%
	0.2%	2.90 ± 0.33	1.02 ± 1.03	64.8%
Actichlor	0.001%	3.18 ± 0.35	3.23 ± 0.26	0.0%
	0.01%	2.93 ± 0.38	0.00 ± 0.00	100.0%
Tristel	0.0001%	3.36 ± 0.08	3.34 ± 0.18	0.59%
	0.001%	3.14 ± 0.21	2.67 ± 0.14	15.0%
	0.01%	2.28 ± 0.28	0.00 ± 0.00	100.0%

2.4 Plating and enumeration

Post-exposure, samples were spread plated onto BA plates and incubated at 37°C for 48 hours under anaerobic conditions. Plates were then enumerated, and results reported as colony forming units per millilitre ($CFUml^{-1}$) as a function of dose (Jcm^{-2}). Experimental data are an average of a minimum of triplicate independent experimental results, measured in duplicate ($n \geq 6$), with error bars representing the standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) using Minitab statistical software Version 15, with significant differences accepted at $P \leq 0.05$.

3. Results and Discussion

3.1 Determination of the susceptibility of *C. difficile* vegetative cells and spores

Exposure of *C. difficile* vegetative cells to 405 nm light demonstrated that cells were susceptible to inactivation. As shown in Figure 1, the population remained fairly constant with doses of up to 105 Jcm^{-2}, and after which significant inactivation was observed, with a 1.45 log_{10} reduction after exposure to 189 Jcm^{-2} (P=0.006), and complete inactivation (99.9% reduction) of the 3.3 log_{10} population achieved after exposure to 252 Jcm^{-2} (P=0.000).

Figure 2 shows the comparative results for the inactivation of spores. Inactivation was achieved, but as expected, the spores proved to be significantly more resilient to light treatment, with approximately ten times the dose required to achieve a similar 3.5 log_{10} reduction as that achieved with the vegetative cells. Inactivation kinetics showed a significant 0.5 log_{10} reduction after a dose of 810 Jcm^{-2} (P=0.001), and 3.3 log_{10} reduction after 2.43 $kJcm^{-2}$ (P=0.000).

These results demonstrated that *C. difficile* spores were much more resilient than vegetative cells to 405 nm-light exposure. A 99.9% reduction in vegetative cell population was demonstrated with a dose of 252Jcm^{-2}, whereas, as previously mentioned, spore inactivation required a 10-fold increase in dose. These findings support a previous study which reported that *C. difficile* spores required much higher dose levels than vegetative cells to achieve a similar reduction in population [22]. This is expected

The significant difference in dose required for inactivation of spores compared to that of vegetative cells is expected as spores are resilient, difficult to inactivate structures, developed under stressful environmental conditions, and formed to enable bacteria to survive until suitable conditions (such as transfer to the human body) return. This increased resilience of *C. difficile* spores compared to that of vegetative bacteria has also been demonstrated with other decontamination methods, such as UV-C. A study by Rutala et al., achieved a >99.9% reduction in vegetative bacterial counts on surfaces following a 15 minute exposure to UV-C radiation in comparison to a 99.8% reduction in *C. difficile* spores following a 50 minute exposure to UV-C [26]. In a study carried out by Vohra et al., five disinfectants were successful for inhibition of the growth of *C. difficile* vegetative cells, in comparison to spore inactivation in which only Actichlor was found to achieve 3 log_{10} reduction under clean and dirty conditions [27]. Furthermore, It was also the only agent that decontaminated a range of non-porous surfaces artificially contaminated with *C. difficile* spores [27]. This increased resilience of spores in comparison to vegetative cells is further supported by a study demonstrating that, following induction of spore germination, *C. difficile* becomes much more susceptible to

inactivation by UV-C radiation and heat, due to the restoration of vegetative cell properties and irreversible loss of spore properties [28].

This enhanced resilience of *C. difficile* spores in comparison to its vegetative counterpart is similar to that of other spore forming bacteria, such as the spores of *Bacillus* species [29,30]. *Bacillus* spores exhibit an increased resistance to various chemicals that routinely inactivate vegetative cells including hydrogen peroxide, alcohols, phenols, chlorhexidine, and benzalkonium compounds [29,31,32]. Furthermore, the spores of both *Clostridium* and *Bacillus* species have been reported to be 5-50 times more resistant than their corresponding vegetative cells to 254 nm UV radiation [33–35] .

In comparison to the 3.3 $\log_{10}$ reduction achieved after exposure to 2.4 $kJcm^{-2}$ 405 nm light in the present study, a 1.8-2.9$\log_{10}$ reduction in *C. difficile* spores was achieved when a population of 10^5 CFU was inoculated onto stainless steel carrier disks and placed in several areas within a hospital room, both in the direct and indirect line of UV-C at a dose of 44.9 – 132 Jcm^{-2}[36]. Nerandzic et al. further demonstrated a 2-3 $\log_{10}$ reduction in *C. difficile* counts following exposure to a constant dose of 59.4 Jcm^{-2} [37]. In a study using a handheld device that generates UV in the far UV spectrum (185-230 nm) a 4.4$\log_{10}$ CFU reduction in *C. difficile* spores, following exposure to 100 $mJcm^{-2}$ for 5 seconds, was reported [38]. Although UV-C is highly successful for the inactivation of *C. difficile* spores, there are several drawbacks associated with the use of UV-C. These disadvantages include exclusion of personnel from treatment zones due to harmful effects of UV-C on the skin and eyes, degradation of photosensitive materials, the potential for microbial resistance to develop, and, if used intermittently, the decontamination effective is only short term [16]. In comparison with ozone, 405 nm light is much more efficient for decontamination. A study comparing the efficacy of eight disinfection technologies, including ozone, for decontamination of hospital isolation rooms contaminated with *C. difficile* results demonstrated only a 1.3 $\log_{10}$ reduction following treatment with 25 ppm ozone for 142 min [6].

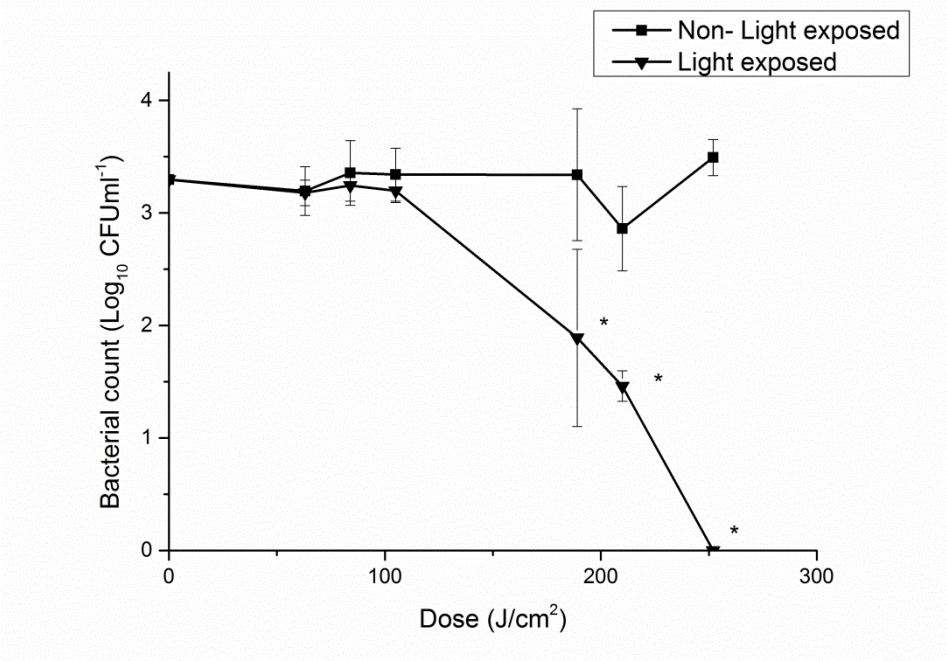

Figure 1. *Inactivation of Clostridium difficile vegetative cells by exposure to 405 nm light. Cells were exposed to an irradiance of 70 mWcm^{-2}, whilst suspended in PBS. * represent significant bacterial inactivation, when compared to the associated non-exposed control (P≤0.05). Each data point is a mean value ± SD (n≥6).*

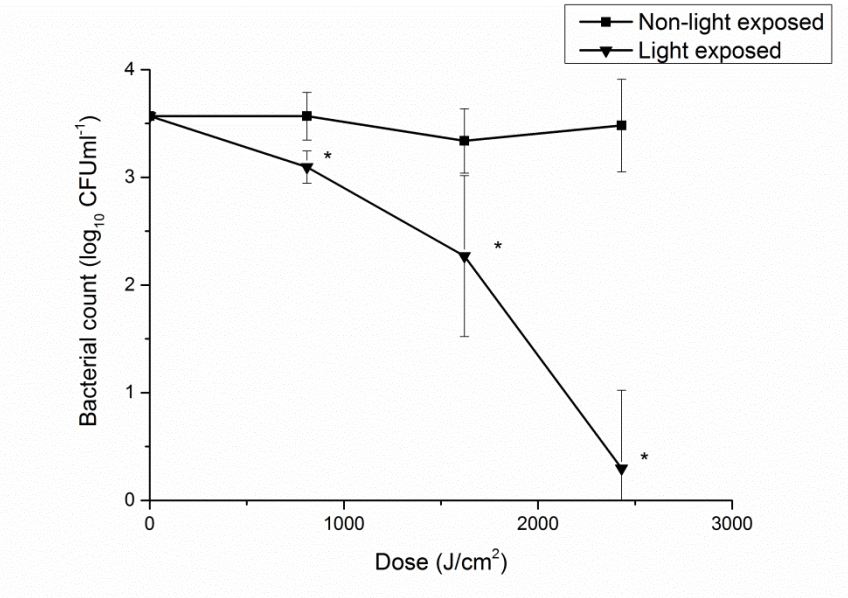

Figure 2*. Inactivation of Clostridium difficile spores by exposure to 405 nm light. Spores were exposed to an irradiance of 225 mWcm^{-2}, whilst suspended in PBS. * represent significant bacterial inactivation, when compared to the associated non-exposed control (P≤0.05). Each data point is a mean value ± SD (n≥6).*

3.2 Synergistic sporicidal activity of 405 nm light and disinfectants

Results demonstrating the synergistic activity of 405 nm light and low concentration chlorinated disinfectants are shown in Figures 3-5. First looking at the control data, *C. difficile* spores suspended in 0.1% NaOCl, 0.001% Actichlor and 0.0001% Tristel alone over the 3-hr period showed no significant reduction in population (P=>0.05). In addition to this, the baseline sporicidal activity of 405 nm light when the spores were suspended in PBS demonstrated a requirement for doses of 0.81, 1.62 and 2.43 $kJcm^{-2}$ to achieve reductions of 0.5,1.3 and 3.5log_{10} $CFUml^{-1}$, respectively. Enhanced sporicidal activity was observed in all cases when the spores were exposed to 405 nm light whilst suspended in the three disinfectants. In the case of NaOCl (Figure 3), a significant 1 log_{10} reduction was achieved following exposure to a dose of 0.8 $kJcm^{-2}$ in comparison to a 0.5 log_{10} reduction when exposed to 405nm light alone (P=0.007) (Figure 3). A 3.2log_{10} reduction was achieved following exposure to a dose of 1.62 $kJcm^{-2}$ in comparison to a 1.3 log_{10} reduction when exposed to 405 nm light alone (Figure 3). When exposed to 405 nm light whilst suspended in Actichlor, a significant 1.6 log_{10} reduction was achieved following exposure to a dose of 0.8 $kJcm^{-2}$ in comparison to a 0.5 log_{10} reduction when exposed to 405nm light alone (P=0.001). Near complete inactivation (3.2 log_{10} reduction) was achieved with a dose of 1.62 $kJcm^{-2}$, compared to approximately 1.5 times this dose being required when using 405 nm light exposure alone (Figure 4). Similar results were also shown with Tristel, with a 3.3 log_{10} reduction observed after exposure to a dose of 1.62 $kJcm^{-2}$ compared to 405 nm light alone, again requiring approximately 1.5 this dose to achieve near complete reduction (Figure 5).

The overall pattern of results achieved in these experiments was fully consistent with the demonstration of a synergistic inactivation effect caused by the combined use of 405 nm light and the tested chlorinated disinfectants. Whilst the pattern was apparent, and this included statistically significant results, not all of the comparative results reached statistical significance and this is most likely due to the low population in densities used. Further work will investigate the enhanced inactivation of 405nm light in combination with disinfectants against higher population densities.

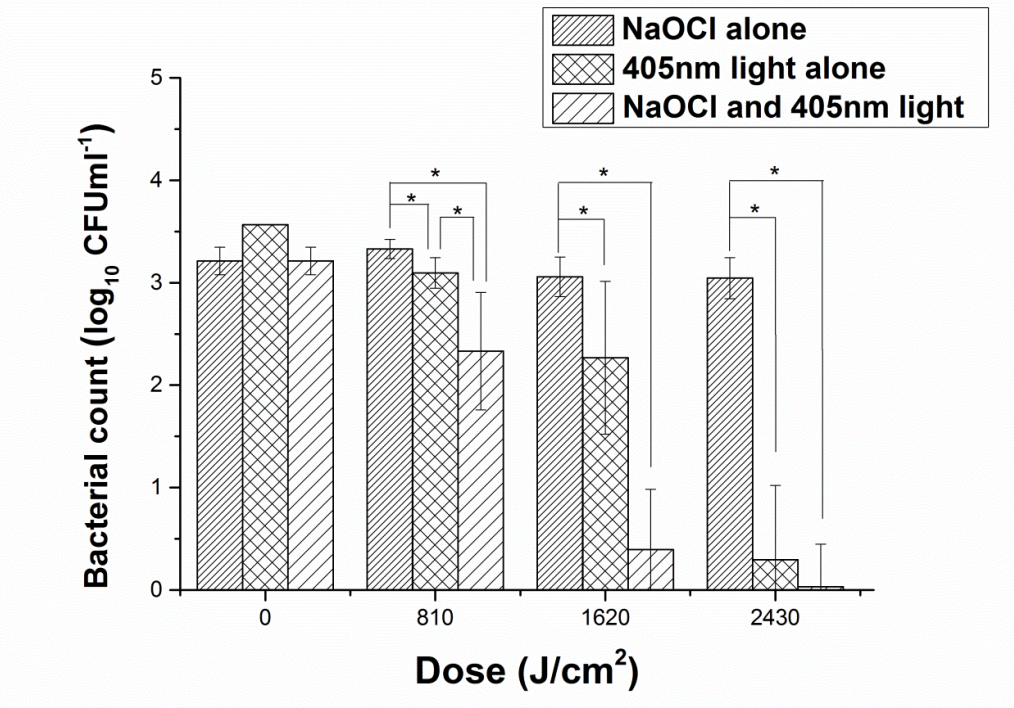

Figure 3. *405 nm light inactivation of Clostridium difficile spores whilst suspended in 0.1% NaOCl. Spores were exposed to an irradiance of 225 mWcm^{-2}. * represent significant bacterial inactivation (P≤0.05). Each data point is a mean value ± SD (n=6).*

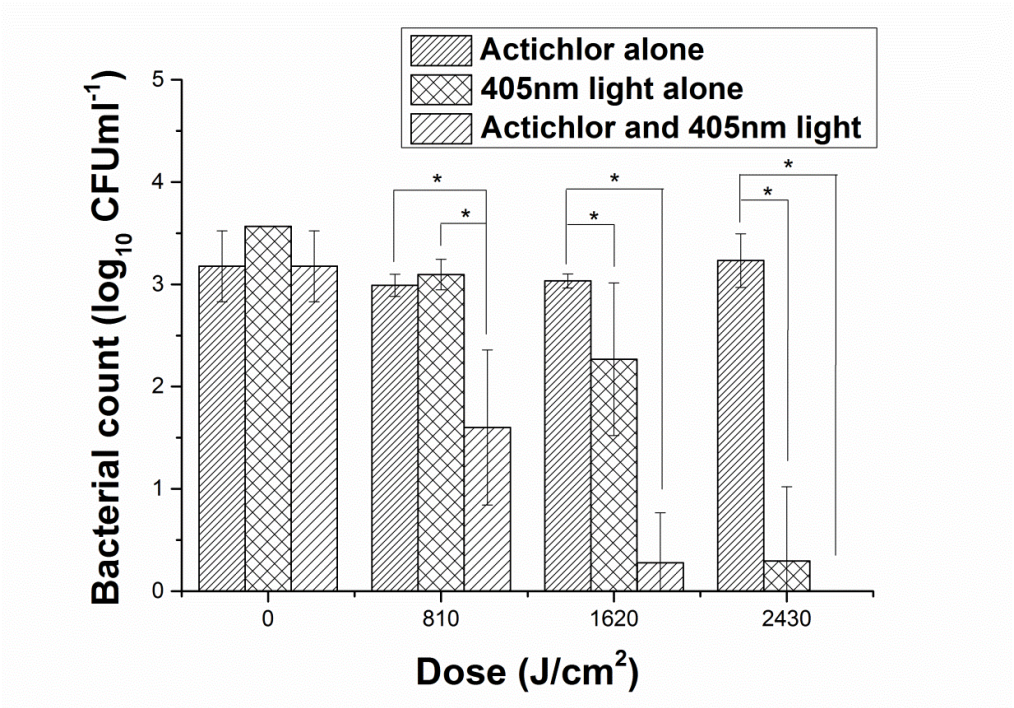

Figure 4. *405 nm light inactivation of Clostridium difficile spores whilst suspended in 0.001% Actichlor. Spores were exposed to an irradiance of 225 mWcm^{-2}. * represent significant bacterial inactivation (P≤0.05). Each data point is a mean value ± SD (n=6).*

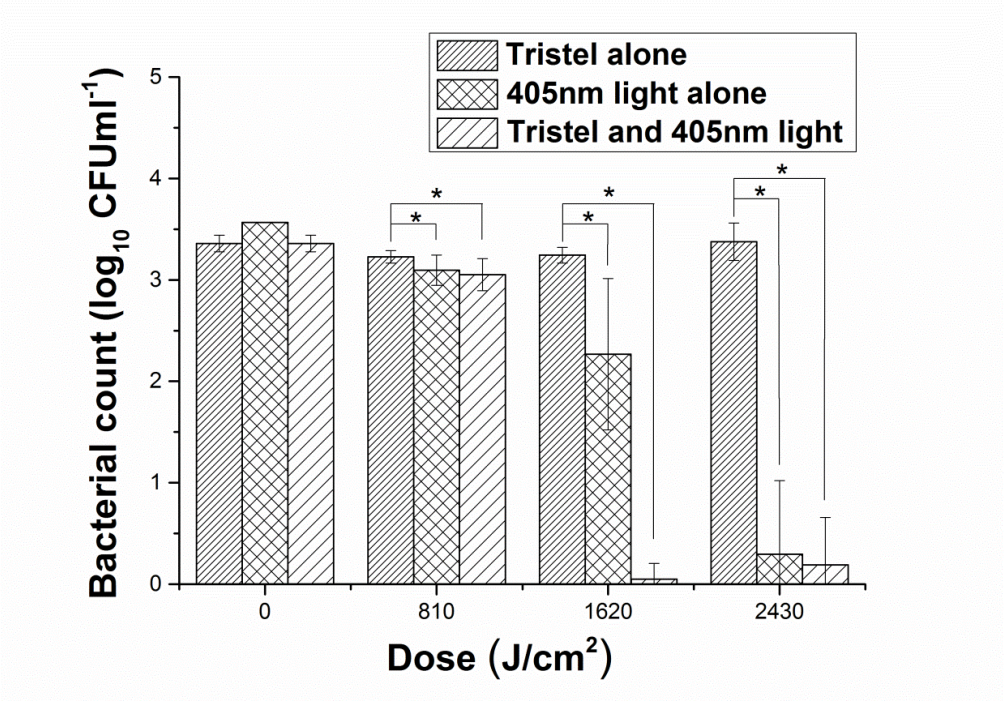

Figure 5. *405 nm light inactivation of Clostridium difficile spores whilst suspended in 0.0001% Tristel. Spores were exposed to an irradiance of 225 mWcm^{-2}. * represent significant bacterial inactivation (P≤0.05). Each data point is a mean value ± SD (n=6).*

Results of this study demonstrate that the use of 405 nm light in combination with the three selected disinfectants can lead to a considerable reduction in exposure time and concentrations of disinfectant required to eliminate *C. difficile* spores. Results demonstrated that 3-3.5 $\log_{10}$ reductions could be achieved following exposure to a dose of 1.62 kJcm^{-2}: an approximate 33% reduction in dose than that required when using 405 nm light exposure alone. Significantly, the levels of disinfectants that were used were deliberately selected so that they exerted negligible effects on the spores when applied alone – thus demonstrating that even low concentration disinfectants can induce the synergistic inactivation effect. Together, the oxidative effects exerted by both the disinfectants [39–42] and the 405 nm light exposure [13,21,43] when combined, prove to be more lethal than when applied independently, resulting in accelerated spore inactivation.

An important aspect for consideration in this study is the concentrations of disinfectants used to assess the synergistic activity with 405 nm light. As discussed, the concentrations used were selected based on the concentrations that induced no significant effect on the spores over the maximum exposure period required to achieve complete inactivation of the *C. difficile* spores using 405 nm light alone, i.e. 3-hr (Table 1). These concentrations proved to be different for each

disinfectant (0.1% NaOCl; 0.001% Actichlor; 0.0001% Tristel), and in all cases, were lower than the recommended concentrations for use within the clinical environment. For hospital use, concentrations of 0.1% sodium hypochlorite (1,000 ppm available chlorine), 0.1% Actichlor (1000 ppm available chlorine), and 0.01% Tristel (100-120 ppm available chlorine) are recommended for use [27,41-45]. However, in the case of NaOCl, although a concentration of at least 1,000 ppm is currently recommended for high level disinfection, several reports have recommended concentrations of 1,000-100,000 ppm for high level disinfection and sporicidal activities [9,46–48] and this is likely to be the case with other chlorinated disinfectants. Although the concentrations used in this study were up to 100 times more dilute than recommended for use in the clinical environment, when used in combination with 405 nm light significant sporicidal activity was achieved.

This study has established that 405 nm light alone can successfully inactivate *C. difficile* spores (Figure 2), however, the demonstration that sporicidal activity can be enhanced when used in combination with hospital disinfectants is of significant interest. The results presented in this paper provide proof-of-concept validation that synergistic sporicidal activity can be achieved using spore suspensions and high irradiance levels, however it would be of great interest in future studies to replicate the results with contaminated surfaces representative of what would be expected within the clinical environment, and lower 405 nm light irradiance levels such as those used for environmental decontamination applications [13,14]. A limiting factor of 405 nm light over other technologies such as UV-light, hydrogen peroxide vapour or ozone, is the lower sporicidal activity, however the ability to enhance this activity through its combined use with disinfectants – which would already be in routine use within the hospital environment – coupled with the ability to apply this technology safely in the presence of patients and staff [17–19] gives this technology attractive features for provision of continuous environmental decontamination against *C. difficile*.

In addition to enhancing the sporicidal effects of 405 nm light, the combined use of 405 nm light and disinfectants has demonstrated that there is the potential to use lower concentration disinfectants whilst still achieving sporicidal activity. This could have significant advantages due to the recognised handling concerns, such as cutaneous and respiratory irritation associated with long term use as well as material damage, such as corrosion and pitting on equipment and surfaces, associated with the use of high strength chlorinated disinfectants [7,45].

4. Conclusions

In conclusion, this study has demonstrated for the first time that the sporicidal activity of 405 nm light against *C. difficile* can be significantly enhanced when combined with low strength chlorinated disinfectants including sodium hypochlorite, Actichlor and Tristel. Further work is required to investigate whether similar synergistic activity can be achieved when spores are seeded on surfaces, and whether this effect can be achieved within the clinical environment using irradiance levels appropriate for environmental decontamination applications, but the results of this study provide an excellent basis for future work in the area. The results presented suggest that there may be potential benefits in using disinfectants in combination with antimicrobial 405 nm light, as together they have enhanced sporicidal activity, whilst enabling use of lower concentrations of chlorinated disinfectants and thereby reducing potentially harmful effects on both users and materials. Further research may lead to the potential use of 405 nm light decontamination in combination with hospital disinfectants to enhance *C. difficile* cleaning and infection control procedures.

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References

[1] D.A. Burns, J.T. Heap, N.P. Minton, SleC is essential for germination of Clostridium difficile spores in nutrient-rich medium supplemented with the bile salt taurocholate, J. Bacteriol. 192 (2010) 657–64. doi:10.1128/JB.01209-09.

[2] S. Johnson, D.N. Gerding, Clostridium difficile-Associated Diarrhea, Clin. Infect. Dis. 26 (1998) 1027–1036.

[3] E.R. Dubberke, A.I. Wertheimer, Review of current literature on the economic burden of Clostridium difficile infection, Infect. Control Hosp. Epidemiol. 30 (2009) 57–66. doi:10.1086/592981.

[4] C. Landelle, M. Verachten, P. Legrand, E. Girou, F. Barbut, C.B. Buisson, Contamination of Healthcare Workers' Hands with Clostridium difficile Spores after Caring for Patients with C. difficile Infection, Infect. Control Hosp. Epidemiol. 35 (2014) 10–15. doi:10.1086/674396.

[5] L.J. Wheeldon, T. Worthington, P.A. Lambert, A.C. Hilton, C.J. Lowden, T.S. Elliott, Antimicrobial efficacy of copper surfaces against spores and vegetative cells of Clostridium difficile: the germination theory, J. Antimicrob. Chemother. 62 (2008) 522–525. doi:10.1093/jac/dkn219.

[6] L. Doan, H. Forrest, A. Fakis, J. Craig, L. Claxton, M. Khare, Clinical and cost effectiveness of eight disinfection methods for terminal disinfection of hospital isolation rooms contaminated with Clostridium difficile 027, J. Hosp. Infect. 82 (2012) 114–121. doi:10.1016/j.jhin.2012.06.014.

[7] M. Rupnik, M.H. Wilcox, D.N. Gerding, Clostridium difficile infection: new developments in epidemiology and pathogenesis, Nat. Rev. Microbiol. 7 (2009) 526–536. doi:10.1038/nrmicro2164.

[8] L.V. McFarland, M.E. Mulligan, R.Y.Y. Kwok, W.E. Stamm, Nosocomial acquisition of Clostridium difficile infection, 320 (1989) 204–210. doi:10.1056/NEJM198901263200402.

[9] F. Barbut, D. Menuet, M. Verachten, E. Girou, Comparison of the efficacy of a hydrogen peroxide dry-mist disinfection system and sodium hypochlorite solution for eradication of Clostridium difficile spores, Infect. Control Hosp. Epidemiol. 30 (2009) 507–514. doi:10.1086/597232.

[10] M.K. Shaughnessy, R.L. Micielli, D.D. DePestel, J. Arndt, C.L. Strachan, K.B. Welch, C.E Chenoweth, Evaluation of hospital room assignment and acquisition of Clostridium difficile infection, Infect. Control Hosp. Epidemiol. 32 (2011) 201–206. doi:10.1086/658669.

[11] J.G. Bartlett, D.N. Gerding, Clinical recognition and diagnosis of Clostridium difficile infection, Clin. Infect. Dis. 46 Suppl 1 (2008) S12–18. doi:10.1086/521863.

[12] M.H. Wilcox, A.P. Fraise, C.R. Bradley, J. Walker, R.G. Finch, Sporicides for Clostridium difficile: the devil is in the detail, J. Hosp. Infect. 77 (2011) 187–188. doi:10.1016/j.jhin.2010.10.017.

[13] M. Maclean, S.J. Macgregor, J.G. Anderson, G.A. Woolsey, J.E. Coia, K. Hamilton, I. Taggart et al., Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light, J. Hosp. Infect. 76 (2010) 247–251. doi:10.1016/j.jhin.2010.07.010.

[14] S.E. Bache, M. Maclean, S.J. MacGregor, J.G. Anderson, G. Gettinby, J.E. Coia, I. Taggart, Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings, Burns. 38 (2012) 69–76. doi:10.1016/j.burns.2011.03.008.

[15] M. Maclean, K. McKenzie, J.G. Anderson, G. Gettinby, S.J. MacGregor, 405 Nm Light Technology for the Inactivation of Pathogens and Its Potential Role for Environmental Disinfection and Infection Control, J. Hosp. Infect. 88 (2014) 1–11. doi:10.1016/j.jhin.2014.06.004.

[16] M. Maclean, K. McKenzie, S. Moorhead, R.M. Tomb, J.E. Coia, S.J. MacGregor, J.G. Anderson, Decontamination of the Hospital Environment: New Technologies for Infection Control, Curr. Treat. Options Infect. Dis. 7 (2015) 39-51. doi:10.1007/s40506-015-0037-5.

[17] R. Yin, T. Dai, P. Avci, A.E. Jorge, W.C. de Melo, D. Vecchio, Y.Y. Huang et al., Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond, Curr. Opin. Pharmacol. 13 (2013) 731–762. doi:10.1016/j.coph.2013.08.009.

[18] R.S. McDonald, S. Gupta, M. Maclean, P. Ramakrishnan, J.G. Anderson, S.J. MacGregor, R.M. Meek et al., 405 nm Light exposure of osteoblasts and inactivation of bacterial isolates from arthroplasty patients: potential for new disinfection applications?, Eur. Cell. Mater. 25 (2013) 204–214.

[19] M.R. Hamblin, T. Hasan, Photodynamic therapy: a new antimicrobial approach to infectious disease?, Photochem. Photobiol. Sci. 3 (2004) 436–450. doi:10.1039/b311900a.

[20] M. Maclean, S.J. MacGregor, J.G. Anderson, G. Woolsey, High-intensity narrow-spectrum light inactivation and wavelength sensitivity of Staphylococcus aureus, FEMS Microbiol. Lett. 285 (2008) 227–232. doi:10.1111/j.1574-6968.2008.01233.x.

[21] M. Maclean, S.J. MacGregor, J.G. Anderson, G. Woolsey, Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array, Appl. Environ. Microbiol. 75 (2009) 1932–1937. doi:10.1128/AEM.01892-08.

[22] M. Maclean, L.E. Murdoch, S.J. MacGregor, J.G. Anderson, Sporicidal effects of high-intensity 405 nm visible light on endospore-forming bacteria, Photochem. Photobiol. 89 (2013) 120–126. doi:10.1111/j.1751-1097.2012.01202.x.

[23] K. McKenzie, M. Maclean, I.V. Timoshkin, E. Endarko, S.J. MacGregor, J.G. Anderson, Photoinactivation of bacteria attached to glass and acrylic surfaces by 405 nm light: potential application for biofilm decontamination, Photochem. Photobiol. 89 (2013) 927–935. doi:10.1111/php.12077.

[24] L.E. Murdoch, K. McKenzie, M. Maclean, S.J. Macgregor, J.G. Anderson, Lethal effects of high-intensity violet 405-nm light on Saccharomyces cerevisiae, Candida albicans, and on dormant and germinating spores of Aspergillus niger, Fungal Biol. 117 (2013) 519–527. doi:10.1016/j.funbio.2013.05.004.

[25] R.M. Tomb, M. Maclean, P.R. Herron, P.A. Hoskisson, S.J. Macgregor, J.G. Anderson, Inactivation of Streptomyces phage φ C31 by 405 nm light, Bacteriophage 4 (2014) 1–6. doi:10.4161/ bact.32129

[26] W.A. Rutala, M.F. Gergen, D.J. Weber, Room decontamination with UV radiation, Infect. Control Hosp. Epidemiol. 31 (2010) 1025–1029. doi:10.1086/656244.

[27] P. Vohra, I.R. Poxton, Efficacy of decontaminants and disinfectants against Clostridium difficile, J. Med. Microbiol. 60 (2011) 1218–1224. doi:10.1099/jmm.0.030288-0.

[28] M.M. Nerandzic, C.J. Donskey, Triggering germination represents a novel strategy to enhance killing of Clostridium difficile spores, PLoS One. 5 (2010) e12285. doi:10.1371/journal.pone.0012285.

[29] E. Melly, A.E. Cowan, P. Setlow, Studies on the mechanism of killing of Bacillus subtilis spores by hydrogen peroxide, J. Appl. Microbiol. 93 (2002) 316–325. doi:10.1046/j.1365-2672.2002.01687.x.

[30] M.P. Silva, C.A. Pereira, J.C. Junqueira, A.O.C. Jorge, Methods of destroying bacterial spores. In: Mendez-Vilas A. Microbial pathogens and strategies for combating them: science, technology and education, Spain: Formatex research center; 2013, p. 490-496.

[31] B. Setlow, P. Setlow, Binding of small, acid-soluble spore proteins to DNA plays a significant role in the resistance of Bacillus subtilis spores to hydrogen peroxide, Appl. Environ. Microbiol. 59 (1993) 3418–3423.

[32] T.N. Demidova, M.R. Hamblin, Photodynamic Inactivation of Bacillus Spores , Mediated by Phenothiazinium Dyes Photodynamic Inactivation of Bacillus Spores , Mediated by Phenothiazinium Dyes, 71 (2005) 6918–6925. doi:10.1128/AEM.71.11.6918.

[33] P. Setlow, Resistance of spores of Bacillus species to ultraviolet light, Environ. Mol. Mutagen. 38 (2001) 97–104. doi: 10.1002/em.1058.

[34] W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, P. Setlow, Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments, Microbiol. Mol. Biol. Rev. 64 (2000) 548–572. doi: 10.1128/MMBR.64.3.548-572.2000

[35] B. Setlow, P. Setlow, Decreased UV light resistance of spores of Bacillus subtilis strains deficient in pyrimidine dimer repair and small, acid-soluble spore proteins, Appl. Environ. Microbiol. 54 (1988) 1275–1276.

[36] J.M. Boyce, N.L. Havill, B.A. Moore, Terminal decontamination of patient rooms using an automated mobile UV light unit, Infect. Control Hosp. Epidemiol. 32 (2011) 737–742. doi:10.1086/661222.

[37] M.M. Nerandzic, J.L. Cadnum, M.J. Pultz, C.J. Donskey, Evaluation of an automated ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens in hospital rooms, BMC Infect. Dis. 10 (2010) 197. doi:10.1186/1471-2334-10-197.

[38] M.M. Nerandzic, J.L. Cadnum, K.E. Eckart, C.J. Donskey, Evaluation of a hand-held far-ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens, BMC Infect. Dis. 12 (2012) 120. doi:10.1186/1471-2334-12-120.

[39] D.E. Cortezzo, K. Koziol-Dube, B. Setlow, P. Setlow, Treatment with oxidizing agents damages the inner membrane of spores of Bacillus subtilis and sensitizes spores to subsequent stress, J. Appl. Microbiol. 97 (2004) 838–852. doi:10.1111/j.1365-2672.2004.02370.x.

[40] G. McDonnell, A.D. Russell, Antiseptics and disinfectants: activity, action, and resistance, Clin. Microbiol. Rev. 12 (1999) 147–179.

[41] L.F. Dawson, E. Valiente, E.H. Donahue, G. Birchenough, B.W. Wren, Hypervirulent Clostridium difficile PCR-ribotypes exhibit resistance to widely used disinfectants, PLoS One. 6 (2011) e25754. doi:10.1371/journal.pone.0025754.

[42] Tristel Product Brochure. Tristel for Surfaces. Updated October 2011 (Revision 9). Available at : http://www.tristel.com/wp-content/uploads/2012/07/Brochure-Tristel-for-Surfaces-Rev-9-Oct-2011.pdf. Accessed 21 July 2015.

[43] L.E. Murdoch, M. Maclean, E. Endarko, S.J. MacGregor, J.G. Anderson, Bactericidal effects of 405 nm light exposure demonstrated by inactivation of Escherichia, Salmonella, Shigella, Listeria, and Mycobacterium species in liquid suspensions and on exposed surfaces, ScientificWorldJournal. 2012 (2012) 137805. doi:10.1100/2012/137805.

[44] P.A. Nagaraja. Hospital Sterilisation. New Delhi: Jaypee Brothers Publishing; 2011.

[45] W.R. Jarbis. The inanimate environment. In: J.V. Bennett, W.R. Jarvis, and P.S. Brachman. Bennett & Brachman's Hospital Infections, Philadelphia: Lippincott Williams and Wilkins; 2007, p. 293.

[46] I. Heling, I. Rotstein, T. Dinur, Y. Szwec-Levine, D. Steinberg, Bactericidal and cytotoxic effects of sodium hypochlorite and sodium dichloroisocyanurate solutions in vitro, J. Endod. 27 (2001) 278–280. doi:10.1097/00004770-200104000-00009.

[47] J.D. Siegel, E. Rhinehart, M. Jackson, L,chiarello and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions : Preventing Transmission of Infectious Agents in Healthcare Settings, (2007). Available at: http://www.cdc.gov/hicpac/pdf/isolation/Isolation2007.pdf. Accessed 29/07/2015.

[48] D.N. Gerding, C.A. Muto, R.C. Owens, Measures to control and prevent Clostridium difficile infection, Clin. Infect. Dis. 46 Suppl 1 (2008) S43–S49. doi:10.1086/521861.