**Clostridium difficile**

- Clostridium difficile spores are highly infectious and resilient, and can survive in the environment for prolonged periods.
- Rooms of patients with CDI can reach contamination rates of up to 50%.
- Despite the development of a range of novel disinfection and sterilisation technologies, C. difficile remains a significant healthcare problem.

**405 nm light**

- 405nm light is a novel decontamination method which inactivates bacteria by oxidative damage, caused by photoexcitation of porphyrin molecules within microbial cells.
- Previous clinical studies have demonstrated the effectiveness of this technology for environmental decontamination (Maclean et al., 2014).
- Efficacy against C. difficile spores has not yet been fully established.

**Aims**

- To investigate the synergistic effect of 405nm light and the commonly used hospital disinfectants sodium hypochlorite (NaOCl), Actichlor and Tristel for the inactivation of C. difficile spores in suspension and on surfaces.
- Both 405nm light and the selected disinfectants cause lethal oxidative damage to microorganisms, therefore their combined use has the potential to deliver enhanced sporidical effects.

**Methods and Results**

**Methods**

- C. difficile spores were suspended in disinfectants, at concentrations that had negligible effect on spores alone, and exposed to 405 nm light (225 mW/cm²) (Fig. 1).
- For surface exposures, spores were seeded onto the surface, and incubated at 50°C for 25 min to allow drying. 50µl disinfectant was then added to the surface, and this was then light exposed.
- Surviving spores were enumerated by direct plate counts (suspensions) and recovery by swabbing (surfaces).
- Experiments were repeated in triplicate, with a minimum of duplicate samples for each repeated exposure (n=3).
- To establish the sporidical activity of each agent alone, control samples were exposed to:
  - (i) 405 nm light in the absence of disinfectants, and
  - (ii) disinfectants in the absence of 405 nm light
- The inactivation of spores simultaneously exposed to 405nm light and disinfectants would then demonstrate the synergistic activity of the combined sporidical agents.

**Conclusions and Further Research Questions**

- In conclusion, it is evident that 405nm light alone can successfully be used for the inactivation of Clostridium difficile, however high light doses are required.
- This work has demonstrated that a synergistic effect can be achieved with the combined use of 405nm light with disinfectants to enhance the sporidical activity of 405nm light. Within the clinical environment this would enable the use of lower concentrations of chlorinated disinfectants which are potentially harmful to both users and materials.
- Spores on vinyl appear to be particularly susceptible to the combined action of 405nm light and disinfectants. Further clinically-relevant surfaces, such as PVC, stainless steel and aluminium, will be investigated in the future.
- Future work will progress to investigate whether this synergistic sporidical effect can be achieved with the use of lower irradiance 405nm light levels, similar to those successfully utilised for environmental decontamination applications in the clinical environment.

**Fig 1.** Exposure of C. difficile spores to a range of disinfectants in suspension (a) 0.1\% NaOCl, (b) 0.001\% Actichlor, (c) 0.0001\% Tristel) in combination with 405 nm light at an irradiance of 225mW/cm².

**Fig 2.** Exposure of C. difficile spores seeded onto vinyl flooring to 0.0001\% Tristel in combination with 405 nm light at an irradiance of 225mW/cm².

**Fig 3.** Exposure of C. difficile spores seeded onto glass to (a) 0.01\% NaOCl (b) 0.0001\% Tristel and (c) 0.0001\% Actichlor in combination with 405 nm light at an irradiance of 225mW/cm².


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