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The antibacterial activity of blue-violet light (400nm) against carbapenemase-producing *Enterobacteriaceae* growing as mature biofilms

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Introduction

- Carbapenemase-producing *Enterobacteriaceae* (CPEs) are becoming increasingly prevalent worldwide. These bacteria produce novel β-lactamases (such as NDM-1, Oxa-48 and KPC) which directly hydrolyse carbapenem antibiotics [1], resulting in severely reduced treatment options. Consequently, there is an urgent need to effectively treat, and prevent onward transmission of CPEs.
- Blue light (BL) (~400-405nm) is known to exert antimicrobial effects through the photoexcitation of intracellular porphyrins in bacterial cells, which leads to energy transfer and the production of cytotoxic reactive oxygen species [2,3] **(Figure 1)**.
- Previous work has shown that 400nm BL has a significant antimicrobial effect on biofilms of a panel of 34 bacterial isolates, including 2 CPEs [4].

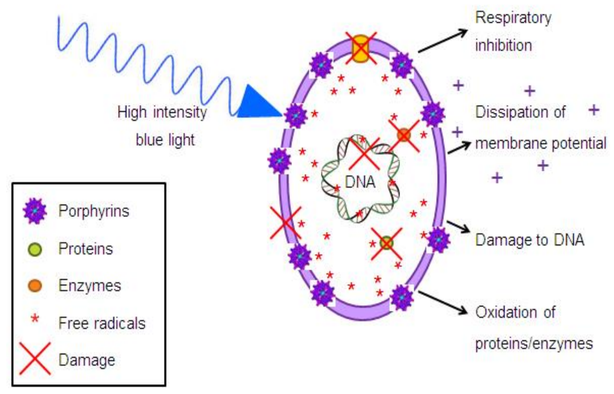

Figure 1: The proposed mechanism of action of blue light on porphyrins (Thwaite JE, 2016).

Aim

To assess the antibacterial effect of 400nm blue light against mature biofilms of a variety of CPEs.

Methods

- *In vitro* experiments were conducted to determine the antibacterial activity of BL against mature biofilms of 12 CPEs **(Figure 2)**. These comprised *Klebsiella* spp and *E.coli* with a variety of resistance mechanisms **(Table 1).**
- All isolates were subjected to testing using a modified version of the minimum biofilm eradication concentration (MBEC) assay **(Figure 3).** In brief, biofilms were grown on peg plates, and then exposed to 400nm BL (irradiance: 60 mW/cm^2) using the Loctite LED Flood array for:
  - 15 minutes at a distance of 15.5 cm (dose: 54 J/cm^2)
  - 30 minutes at a distance of 15.5 cm (dose: 108 J/cm^2)
  - 5 minutes at a distance of 5.2 cm (dose: 162 J/cm^2)
- Biofilm viability post-BL treatment was measured by assessing the seeding of the treated biofilms into a sterile reporter broth, and comparing this to values obtained from the positive and negative controls.

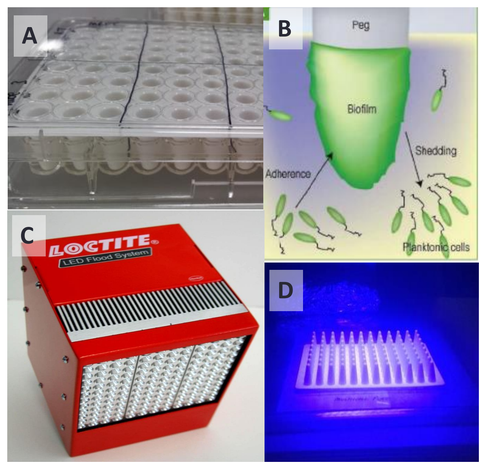

Figure 2: A & B: Biofilms forming on transferable pegs (MBEC), **C:** the Loctite LED flood system, **D:** Biofilm pegs being exposed

Figure 3: Biofilm Eradication

Overnight culture of organism prepared in Lysogeny broth

↓ 24 hrs at 37ºC

Overnight cultures diluted to 0.1 optical density in Muller Hinton broth + peg plate

↓ 72 hrs at 33ºC

Pegs exposed to 400nm BL for 15, 30, and 5 minutes then into reporter broth

↓ 24 hrs at 33ºC

Assessment of OD (seeding) and crystal violet assay to confirm biofilm presence

Results

- All 12 CPE isolates were susceptible to 400nm BL treatment, with a dose-dependent effect (greater reductions in seeding with increasing durations of exposure).
- At 30 minutes (dose of 108 J/cm^2), reductions in biofilm seeding of ≥80% were observed for 11 of the 12 isolates, compared to just 5 of 12 after 15 minutes (dose of 54 J/cm^2). All reductions in seeding were statistically significant when compared to the dark-incubated positive control (Student's t-test, $p<0.05$).
- Following 5 minutes of BL exposure (dose of 162 J/cm^2), 10/12 of the isolates showed a significant reduction in biofilm seeding, however the reductions in biofilm seeding of CPE_6949 and CPE_1798 were not significant at this time point. Furthermore, at this distance and duration, only 2/12 isolates showed a reduction in seeding of >80%.
- Interestingly, one CPE isolate (CPE_8180) was less susceptible than the rest, with a maximum reduction in seeding of 66% at 30 minutes. This *bla*NDM producing *K. pneumoniae* isolate will be investigated further to ascertain why it is less susceptible.

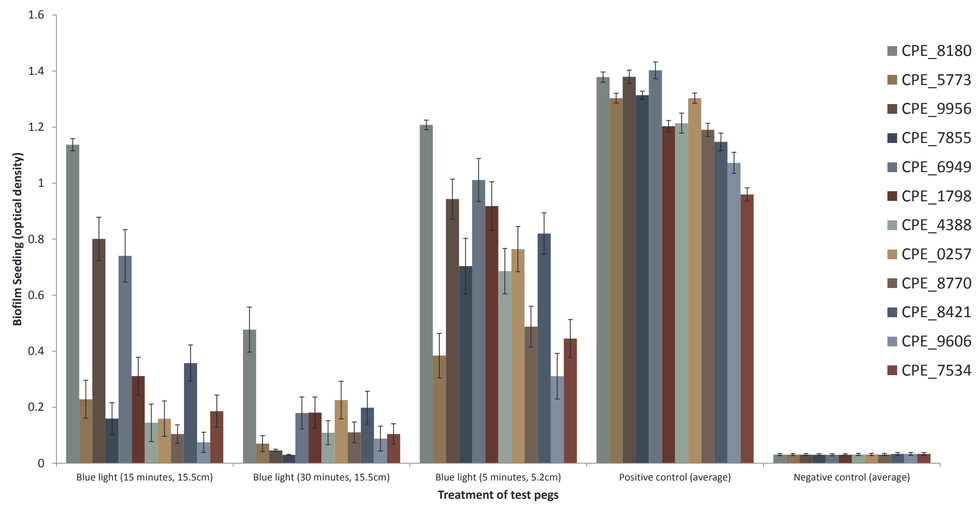

Figure 4: The effect of 15, 30, and 5 minutes of BL exposure on the seeding of mature biofilms of a range of CPEs.

Identifier	Species	Source	Resistance mechanism	% reduction in seeding after duration (dose) of treatment		
				5 minutes	15 minutes	30 minutes
CPE_8180	*K. pneumoniae*	Groin swab	blaNDM	12.2	15.9	66.1
CPE_5773	*K. pneumoniae*	Faeces	blaKPC	87.1	81.9	94.6
CPE_9956	*K. pneumoniae*	Urine	blaNDM and OXA-48	33.2	40.0	96.7
CPE_7855	*K. pneumoniae*	Urine	blaNDM and OXA-48	46.0	87.9	97.7
CPE_6949	*K. pneumoniae*	Urine	blaKPC	24.0	47.7	87.7
CPE_1798	*K. pneumoniae*	Urine	OXA-48	22.4	74.3	85.5
CPE_4388	*K. oxytoca*	Sputum	blaKPC	42.8	79.8	91.2
CPE_0257	*K. pneumoniae*	Urine	blaKPC	40.0	82.9	83.4
CPE_8770	*K. pneumoniae*	Drain	blaNDM	64.9	89.0	90.9
CPE_8421	*E. coli*	Drain	OXA-48	28.9	68.3	82.9
CPE_9606	*E. coli*	Tip of cannula	blaNDM	93.8	93.0	91.8
CPE_7534	*E. coli*	Wound	blaNDM	62.4	76.3	89.1

Table 1: Showing the species, source, resistance mechanism and percentage (%) reductions in seeding for all time points for the 12 CPE isolates tested

Conclusions

BL (400nm) is effective at reducing the seeding of a panel of CPEs growing as mature biofilms *in vitro*. Although the experiments were conducted on plastic surfaces, it is clear that BL offers great promise as a topical decontamination agent for both clinical and environmental applications.
Further research is warranted to fully explore this novel alternative to traditional antimicrobial and decontamination agents.

References

1. Gupta N, Limbago BM, Patel JB, Kallen AJ. 2011. Carbapenem-Resistant *Enterobacteriaceae*: Epidemiology and Prevention. *Clinical Infectious Diseases*, 53: 60–67. **2.** Maclean M, McKenzie K, Anderson JG, Gettinby G, Macgregor SJ. 2014. 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. *Journal of Hospital Infection*, 88: 1-11.**3.** Dai T, Gupta A, Murray CK, Vrahas M, Tegos GP, Hamblin MR. 2012. Blue light for infectious diseases: *Propionibacterium acnes, Helicobacter pylori,* and beyond? *Drug Resistance Updates,* 15: 223-236.**4.** Halstead FD, Thwaite JE, Burt R, Laws TR, Raguse M, Moeller R, Webber MA, Oppenheim BA. 2016. Antibacterial Activity of Blue Light against Nosocomial Wound Pathogens Growing Planktonically and as Mature Biofilms. *Appl. Environ. Microbiol.,* 82: 4006-4016.

Supported by NIHR

This poster presents independent research funded by the **National Institute for Health Research Surgical Reconstruction and Microbiology Research Centre (NIHR SRMRC)**, a partnership between the NIHR, University Hospitals Birmingham NHS Foundation Trust, the University of Birmingham and the Royal Centre for Defence Medicine. *The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.*