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What impact does cold air atmospheric plasma have on bacterial biofilms formed on hospital surfaces?

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Background: Healthcare associated infections continue to affect patients around the world. Considering the increasing numbers of multi-resistant micro-organisms it is imperative that more effective methods of decontamination are evaluated in order to protect patients. Previously, our laboratory has demonstrated that cold air atmospheric plasma (CAPP) exhibited efficient bactericidal and sporicidal activity. Anti-microbial effects of CAPP are due to a cocktail of different reactive species including ultraviolet radiation, reactive oxygen and nitrogen species (ROS, RON), and charged particles. This study expands on this to evaluate the effects of CAPP on bacterial biofilms formed by *Escherichia coli*, methicillin resistant Staphylococcus aureus (MRSA) and *Klebsiella pneumoniae* on hospital surfaces and to examine the mode of action of CAPP on bacterial inactivation.

Material/methods: Bacterial biofilms were formed on various surfaces found in clinical wards such as stainless steel, powder coated steel, glass etc. They were treated with CAPP for 90 seconds and analysed using traditional crystal violet assays to determine effects on biomass, confocal laser scanning microscopy (CLSM) to assess the ratio of live and dead cells and scanning electron microscopy (SEM) to examine the morphology of the cells present after treatment. Bacterial biofilms were also exposed to a fluorescent probe (2',7'-dichlorodihydrofluorescein diacetate - H2DCFDA) in order to measure ROS production between treated and untreated bacterial biofilms.

Results: The anti-microbial effects of CAPP on biofilm biomass were species and surface specific. However, CAPP treatments lead to an increase in dead cells within the bacterial biofilms analysed by confocal microscopy and affected the numbers of cells present and morphology of cells observed by SEM compared to the untreated controls. ROS production was measured following CAPP treatment for 90 seconds. The concentration of ROS was increased for all species, especially *E.coli* (P<0.05) and MRSA (P<0.05) compared to the untreated controls (Fig 1.0).

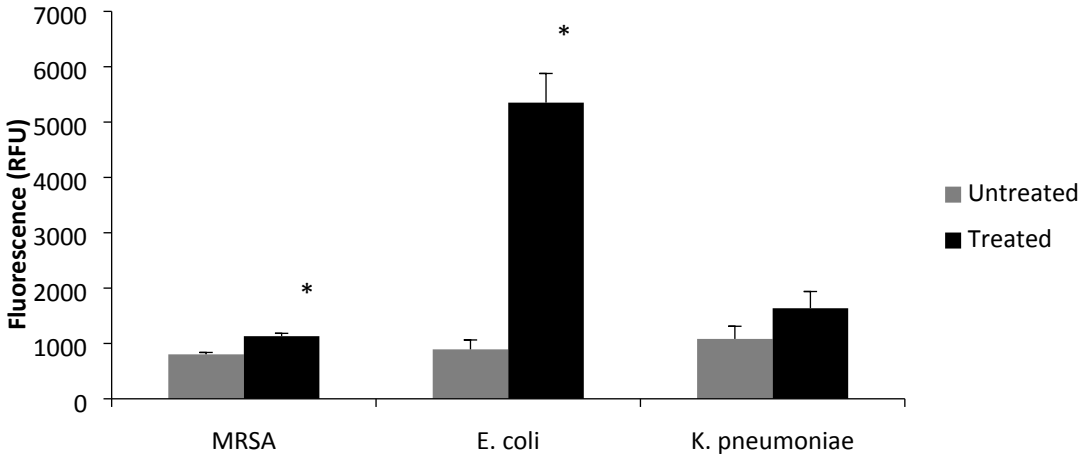

Figure 1.0 Measurement of reactive oxygen species using H2DCFDA. ROS were measured following CAPP treatment for 90 seconds using the H2DCFDA fluorescent probe. Results demonstrate that ROS are increased significantly for MRSA (P<0.05) and *E.coli* (P<0.05).

Conclusions: CAPP applied to hospital surfaces leads to increased numbers of dead cells, disrupts cellular morphology and increases ROS. However, further research is required to confirm effectiveness in the clinical setting.