Evaluating the potential synergy between atmospheric non-thermal plasma to modulate and antibiotics in resistant microbes

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Abstract: We report the ability of atmospheric non-thermal plasma (ANPT) to modulate the antibiotic sensitivity of resistant microorganisms. We treated two antibiotic resistant microbes, *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus*, with ANPT, then subjected the treated microbes to an antibiotic screen. Findings show that ANPT can increase antibiotic susceptibility and synergistically affect resistant microbes.

1. Introduction

Chronic and surgical wounds cost an estimated $30-100 billion dollars a year in healthcare costs in the US alone with a reported 2.5% of the American population suffering reduced quality of life due to chronic wounds[1]. Chronic and surgical wounds are extremely prone to bacterial infections, and with the rise in antibiotic resistance the prevalence of these wounds is rising with *Staphylococcus* and *Pseudomonas* being the most common microbes in these infections[2]. Non-thermal plasma has been shown to decrease the healing time of chronic and surgical wounds by activating and decreased bacterial load[3]. Plasma has been shown to neutralize bacteria[3], however there is a gap in research regarding the interactions with plasma treatment and standard healthcare measures for bacterial infections such as antibiotics.

Here we use ANTP to treat the resistant bacteria *S. aureus* and *P. aeruginosa*, then exposed the bacteria to a panel of 48 different antibiotics. We find that ANTP overall increased antibiotic sensitivity, but not for all antibiotics.

2. Methods

A 2D dielectric barrier discharge (DBD) plasma device[4] connected to an AC power supply using humidified atmospheric air with a flow rate of 10 standard liters per minute and an average power output of ~5W was used in this study. Bacteria were cultured ~16hrs in LB media at 37°C, then diluted to a standard concentration in 1% saline solution, then placed on a pedestal 2cm away from the plasma. Treatment times were 3 min for *P. aeruginosa*, and 5 min for *S. aureus* (to compensate for the differing plasma resistance of each respective microbe). Plasma treated microbes, and control untreated bacteria were cultured in triplicate in Biolog antibiotic screen plates PM11C and PM12B for 12 hours to determine their antibiotic sensitivity for 48 different antibiotics. Growth is measured via spectrometry at OD590.

3. Results and Discussion

Figure 1 shows the changes in resistance to a diverse subset of 10 antibiotics of varying classes of the 48 antibiotics tested. The results are reported in a heatmap, where the green cells show synergy between plasma and the respective antibiotics as the relative growth is below 1, whereas the red cells show antagonism where the microbes grow better in the presence of the antibiotic after plasma treatment compared to the untreated microbes. Results show that *S. aureus* and *P. aeruginosa* generally become more resistant to antibiotics after plasma treatment, but this isn't true for all antibiotics tested, and variance is even seen within concentrations of the same antibiotic.

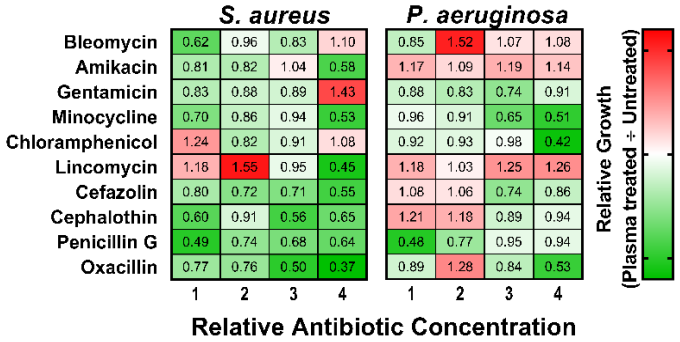

Fig. 1. Changes is resistance of *S. aureus* and *P. aeruginosa* towards 10 different antibiotics at 4 different concentrations. Results are reported are reported as "relative growth" which is the growth of the plasma treated microbes divided by the growth of the untreated

4. Conclusion

With the increasing rise of antibiotic resistance, new treatments are desperately needed. Here we show the ability of plasma to increase antibiotic sensitivity of resistant microbes, with other antibiotics decreasing sensitivity. These findings warrant more high-resolution testing to tease apart the interactions of ANPT and antibiotic sensitivity.

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