Novel Combinational Therapy Targets *Pseudomonas aeruginosa* Biofilm Infections in a Flow Model

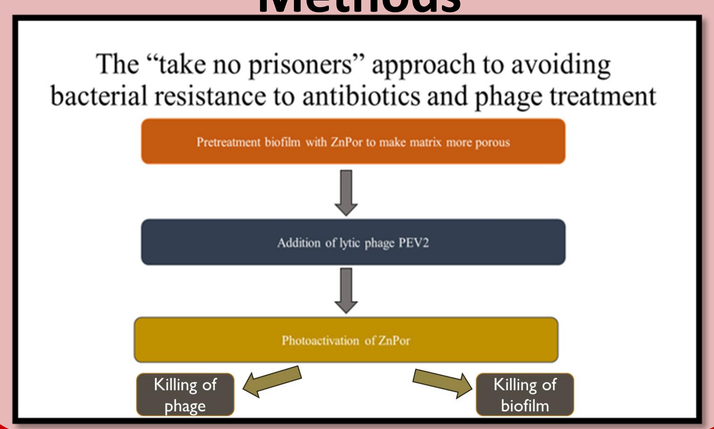
Jessica Geyer, Dr. Jayne Robinson

University of Dayton

Introduction

The rise of multidrug resistant (MDR) pathogens is one of the greatest medical concerns of the century because the use of antibiotics in these infections is almost entirely useless, leaving patients at a higher risk of mortality. In addition, almost 80% of human infections involve biofilms which can be up to 1000-fold more resistant to antibiotics than planktonic cells, such as the biofilm-associated cells of *Pseudomonas aeruginosa*. The resistance of these pathogens has initiated an urgent need to look for alternative antimicrobial therapies such as phage therapy. In Phage therapy, lytic bacteriophages eliminate bacteria that cause infection, even those caused my MDR pathogens. Our lab has examined a successful combinational therapy that treats biofilms *of P. aeruginosa* using a lytic bacteriophage (PEV2) and our novel patented porphyrin (ZnPor).

Methods



Significance

The significance of our novel approach of antimicrobial management is that, unlike other protocols involving biofilm eradication, our strategy addresses the issue of resistance.

Results

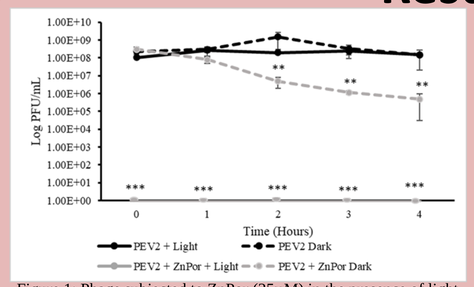


Figure 1: Phage subjected to ZnPor (25µM) in the presence of light (300W) showed complete and immediate viricidal activity. Phage subjected to ZnPor (25µM) without the presence of light still showed a significant decrease from the control. (** > 2 log difference of respective control, ***> 3 log difference of respective control)

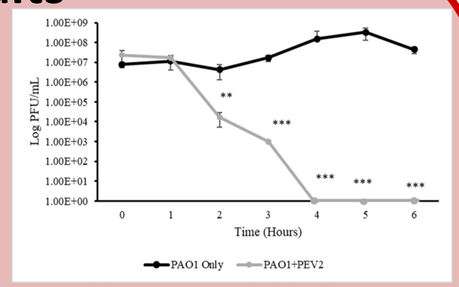


Figure 2: Planktonic PAO1 is completely lysed by PEV2 (MOI 10:1) at 4 hours. (** > 2 log difference respective of control, ***> 3 log difference respective of control)

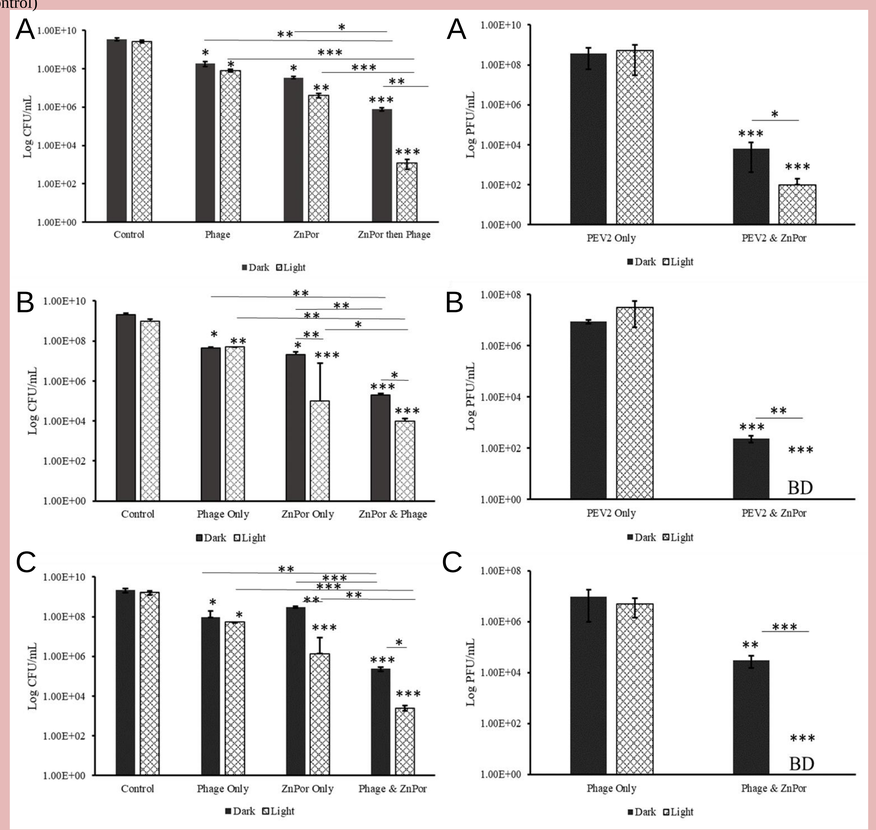


Figure 3: Biofilms grown and subjected to a combination treatment consisting of ZnPor (25µM) and phage PEV2 (MOI 10:1) then treated for 20 mins with and without light. on (A) Polyethylene (Found in catheters and other plastics) (B) Titanium (Found in biomedical implants) (C) Hydroxyapatite substrata (Alternative to bone)

Figure 4: Phage quantifications after combination treatment residing on coupons of (A) Polyethylene (B) Titanium (C) Hydroxyapatite substrata (significance determined by comparison to relative control)

Funding Graduate Student Summer Fellowship

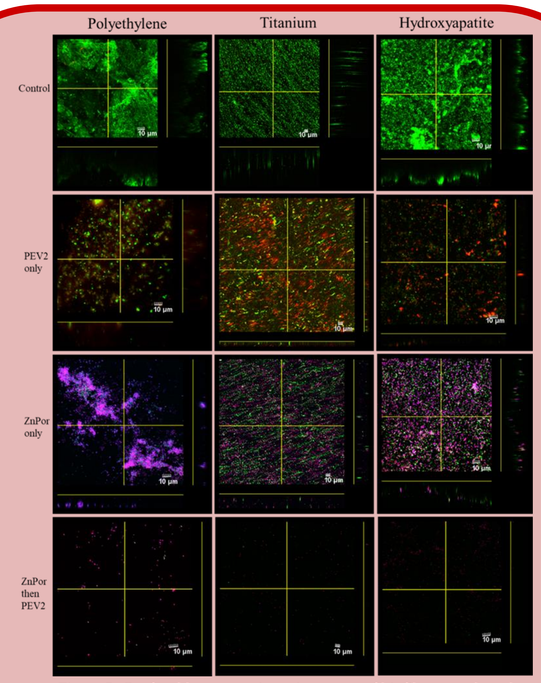


Figure 5: Confocal laser scanning microscopy of biofilms on selected substrata imaged using a LIVE/DEAD stain. Green=LIVE, Red=DEAD, Blue=ZnPor localization, Purple=overlap of ZnPor and Dead stains

Discussion

- While ZnPor retains significant dark toxicity, photoactivation of ZnPor has complete viricidal activity.
- Obligately lytic bacteriophage PEV2 has significant bactericidal activity against *Pseudomonas aeruginosa*.
- There is a significant synergistic effect against *P. aeruginosa* biofilms when ZnPor is used in combination with PEV2 that can be seen quantitatively & qualitatively (confocal laser scanning microscopy).
- The significance of the combination therapy is increased when photoactivated.
- Phage PEV2 can be eliminated from the system when in the presence of ZnPor and can be completely eradicated off of Titanium and Hydroxyapatite when ZnPor is photoactivated.