COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Synergistic Effects Of Novel Phage Cocktails With Carbapenem Antibiotics Against Multi-drug Resistant Pseudomonas Aeruginosa As A Layered Medical Countermeasure

Beth Bachert USMA **Christopher Kovacs** United States Military Academy **Erika Rapp** United States Military Academy **William Rankin** United States Military Academy **Sophia McKenzie** United States Military Academy **Brianna Brasko** United States Military Academy **Katherine Hebert** United States Military Academy **Andrew Kick** United States Military Academy **F. John Burpo** United States Military Academy **Jason Barnhill** United States Military Academy

Bacteriophage (phage) therapy is an emerging alternative to antibiotics for multi-drug resistant (MDR) pathogens such as Pseudomonas aeruginosa. P. aeruginosa is commonly associated with hospital-acquired infections as well as war-related wound infections and is a serious threat due to its capacity for antimicrobial resistance, including the carbapenems that target the cell wall. Combination therapies utilizing multi-phage cocktails or phage in conjunction with antibiotics are being evaluated for their efficacy against P. aeruginosa. Several studies have shown that exposure to phage may promote enhanced susceptibility of MDR bacteria to antibiotics. Bacteria may acquire mutations in surface proteins, especially efflux pumps, because of the selective pressure of phage exposure, rendering them susceptible to certain antibiotics. In this study, we explored the synergistic effect of unique phage cocktails in combination with carbapenems against P. aeruginosa. To identify novel phages against P. aeruginosa, we screened sewage samples from wastewater treatment facilities for the presence of phage. Three unique phages were identified, PaC1Φ, PaWP1Φ, and PaWP2Φ, all of which were capable of lysing the host P. aerugosinosa strain PAO1. Growth curves of PAO1 with individual phages showed a delay in growth of PAO1, though phage resistance developed over time. In contrast, a cocktail consisting of all three phages completely abolished the growth of PAO1. To test whether each phage could restore carbapenem sensitivity to PAO1, bacteria was grown in the presence of phage and and/ or subinhibitory concentrations of ertapenem, imipenem, and meropenem. PAO1 exhibited resistance to all three antibiotics, but when used in combination with each phage, significant reductions of bacterial growth were observed, with PaWP1Φ and PaWP2Φ completely abolishing growth of PAO1 in combination with all three antibiotics. In addition, each phage was shown to disrupt pre-formed biofilms of PAO1. Whole genome sequencing was performed to characterize each phage and determine their similarity to existing phages. PaWP1Φ represents a novel phage, with less than 95% similarity to existing phages, while PaC1Φ and PaWP2Φ were found to be highly similar to Pseudomonas phage Aergia and phage Chuck, respectively. Current efforts are aimed at understanding the mechanism of viral entry that drives re-sensitization of P. aeruginosa to carbapanems and leveraging the phage/ carbapenem combination for multilayered countermeasures against this pathogen.