CHALLENGES FACED IN THE PLANNING, DEPLOYMENT, AND ADOPTION OF A LAYERED MEDICAL DEFENSE STRATEGY

Discovery Of Burkholderia Pseudomallei Bacteriophages That Mediate Antimicrobial Susceptibility Through Efflux Pump Dependent Infection

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Burkholderia pseudomallei is a significant public health and biothreat pathogen that is recognized as a potential Weapon of Mass Destruction (WMD) due to its resistance to treatment. Moreover, its natural prevalence means that it can infect deployed Warfighters by acquisition from natural environments or by nefarious means. Antibiotic treatment of B. pseudomallei infections and post-exposure prophylaxis are hampered by the bacterium's intrinsic and acquired antimicrobial resistance (AMR). Efflux pumps, specifically those of the resistance nodulation cell division (RND) family, are major AMR factors and the sole known multidrug resistance (MDR) determinants in B. pseudomallei. These pumps often compromise the therapeutic use of current drugs and those in pre-clinical or clinical development. As such, novel strategies aimed at disarming efflux pumps hold much promise for greatly improving existing but rather limited therapeutic regimens, or perhaps even affording new treatment strategies, thus providing novel Counter WMD (C-WMD) approaches. The objective of our project is to examine the feasibility of exploiting B. pseudomallei-specific bacteriophages that use surface exposed outer membrane (OM) channel proteins of RND efflux pumps. We anticipate that mutations in the efflux pumps enabling evasion of phage infection will potentiate the activity of previously ineffective antibiotics. The overall goal of the initial work was to identify phages that bind to the OM channel proteins - OprA and OprC - of the clinically significant AmrAB-OprA and BpeEF-OprC efflux pumps. This required the construction of isogenic mutants expressing or lacking AmrAB-OprA and mutants expressing or lacking BpeEF-OprC in the Select Agent excluded B. pseudomallei strain Bp82. The presence and integrity of all mutations was verified by PCR and Sanger sequencing, and Etest antibiotic susceptibility profiles. These strains were then employed for phage screening and characterization. In one approach, mutant strains that overexpress the OprA or OprC channel proteins were used to isolate phages from soil samples that utilize these proteins as receptors to attach to the bacteria. Phages discovered by this approach will be counter screened against mutants that lack these protein channels to demonstrate dependency on these channels for infection. In another approach, we have evaluated 140 phage isolates that are known to infect B. pseudomallei, 19 of which were able to form plaques on a lawn culture of Bp82. Phage infectivity was further evaluated in two Bp82 derivative mutants, Bp82.410 (ΔamrR; AmrAB-OprAUP) and Bp82.500 (bpeSP29S; BpeEF-OprCUP), that overexpress the OprA and OprC channel proteins, respectively. So far, we have observed differences in phage infectivity between both host strains. Most phages tended to use OprC as a receptor for binding rather than OprA. These phages have been classified as members of family Myoviridae. The results of this project are expected to advance C-WMD science in that they move forward a novel aspect of phage therapy for B. pseudomallei infections that can potentially be developed into a specific therapy immune to traditional AMR mechanisms for infected Warfighters.

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