Modified Appelmans protocol for in vitro *Klebsiella pneumoniae* phage host range expansion

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Summary: Multi-antibiotic resistant *Klebsiella pneumoniae* (Kp), also known as MRGN (multi-resistant Gram negative) organisms, are one of the consequences of inappropriate use of antibiotics in patients and livestock (One-Health). MRGN Kp can be found in many places in the environment, including surface water and sewage treatment plants. In view of high case fatality rates in intensive care patients, alternative therapeutic methods must be considered. Adjuvant therapy with bacteriophage cocktails in combination with antibiotics might be a suitable therapeutic approach.

Methods-Results: In the phage working group of the Bundeswehr Institute of Microbiology (IMB), we isolate and characterize bacteriophages from wastewater on highly resistant clinical isolates of 3 to 4 MRGN Kp (carbapenem resistance). We currently hold one of the most extensive collections of 3/4 MRGN Kp and their specific phages in Europe. Our Kp isolates show a large variety of capsule types (80+) and are difficult to treat due to the formation of biofilms. Bacteriophage treatment of infections with MRGN Kp would be more effective using Kp phages with broad host ranges. We currently use a modified 'Appelmans protocol' [1], to create phages with a broader host range via in vitro DNA recombination between different phages in targeted phage host combinations. Three phages were selected, combined with eight selected bacterial strains, and grown over multiple cycles in the Varioskan (LUX Multimode microplate reader; Thermo Fisher, Waltham, Massachusetts) at 37°C in 96-well plates overnight. After each cycle, products were pooled, centrifugated and filtrated (0.2 µm). The resulting phage cocktail was then used again to start the next cycle.

Conclusion: Phages with expanded host range were identified and whole genome sequence analysis of the phages from the modified Applemans protocol is in progress. The underlying mechanisms will be used for the design of recombinant phage with extended host range for therapeutic purposes in ongoing cooperations with clinical partners [2] and in our *Phage4-1Health* program [3].

References:

1. Burrowes BH, Molineux IJ, Fralick JA. Directed in Vitro Evolution of Therapeutic Bacteriophages: The Appelmans Protocol. Viruses. 2019 Mar 11;11(3):241. doi: 10.3390/v11030241. PMID: 30862096; PMCID: PMC6466182.

2. Eckstein S, Stender J, Mzoughi S, Vogele K, Wölfel R, Ben Moussa M , and Bugert JJ. Towards bacteriophage therapy in infections with multidrug resistant Klebsiella pneumonia. - Poster submitted to the 44. ICMM, Brüssels, September 5-9, 2022.
3. https://phage4-1health.org/