Timing-Dependent Phage-Antibiotic Effects on the Survival of the Non-Target Bacillus subtilis in an Escherichia coli Co-Culture

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Purpose: Antibiotic resistance is growing. Bacteriophages (phages) are viruses that infect bacteria; using phages and antibiotics together has a synergistic effect. This study applied phages and antibiotics in different orders to determine which has greatest reduction of non-target Bacillus subtilis species in an Escherichia coli co-culture. Procedure: B. subtilis and E. coli were incubated on LB agar for 24 hours. Bacteria were harvested with isotonic buffer at 10^6 CFU/ml, confirmed via spectrophotometry. A concentration causing ~42.8% inhibition in E. coli for the Aztreonam antibiotic and a MOI of 0.7 (yielding ~50% inhibiton) for the T4 phage was chosen. After the bacterial solution was plated on LB agar via spread plate, T4 and Aztreonam were applied in three orders: Phage first (P, A), Antibiotic first (A,P), and Simultaneous addition (SA), with 1 hour gap in sequential treatments. Aliquots were plated on selective agar at 0, 1, 3, and 6 hours. Results: Kruskall-Wallis tests showed significant differences in treatment groups ($p<0.01$). The A,P group had the most statistically significant reduction in E. coli, and both the A,P and SA groups had the most reduction in B. subtilis. Aztreonam possibly caused E. coli to elongate (filamentation), allowing phages to produce more offspring per infection. This resulted in a 1.9x advantage for virus in filamented cells (based on Berg-Purcell capacitance equation with dimensions based on microscopy). An inverse correlation between E. coli lysis and B. subtilis survival ($p<0.001$) suggested a bystander effect. Biophysical modeling was utilized alongside proposed mechanisms. Conclusion: The results showed that A,P had the most reduction in microbial load (57.50%), in comparison to the P,A (36.61%) and SA (45.55%) groups.

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