

# 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% inhibition) 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:** Kruskal-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.

**Awards Won:**

