Enhancing bacteriophage therapeutics through *in situ* production and release of heterologous antimicrobial effectors

Jiemin Du[1#], Susanne Meile[1#], Jasmin Baggenstos[1], Tobias Jäggi[1], Pietro Piffaretti[1], Laura Hunold[1], Cassandra I. Matter[1], Lorenz Leitner[2], Thomas M. Kessler[2], Martin J. Loessner[1], Samuel Kilcher[1*], Matthew Dunne[1*]

1. Institute of Food, Nutrition and Health, ETH Zurich, Switzerland; **2.** Department of Neuro-Urology, Balgrist University Hospital, Switzerland

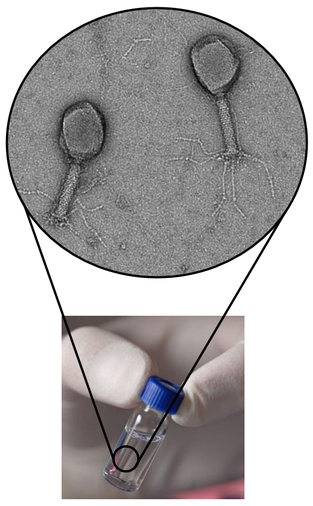
Authors contributed equally to this work; *****Address correspondence to matthew.dunne@hest.ethz.ch and samuel.kilcher@hest.ethz.ch

Background

While conventional antibiotics remain the most effective treatments against bacterial infections, the global emergence and spread of antimicrobial resistance (AMR) highlight the need for developing novel and more pathogen-specific antimicrobial interventions.

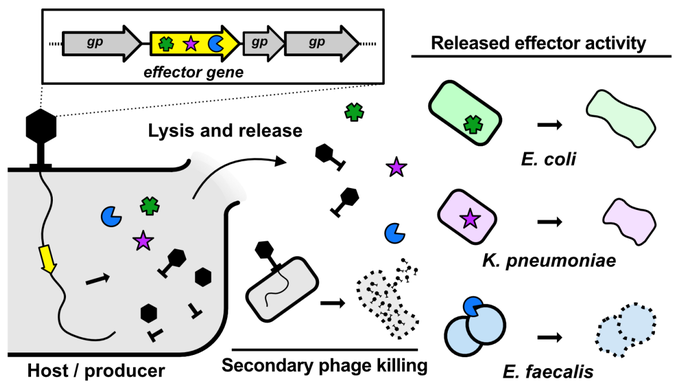
Bacterial viruses – **bacteriophages** – are highly promising alternatives because of their pathogen specificity and ability to self-replicate at infection sites; however, as observed with antibiotics, bacteria also develop resistances to phage therapy.

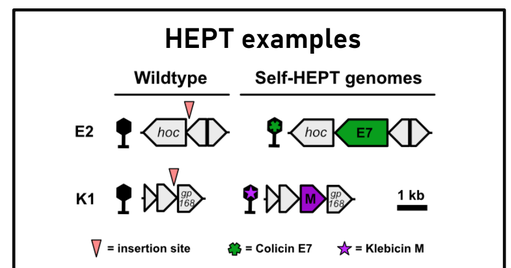
Using **genetic engineering** we can bypass the inherent limitations of natural phages as therapeutics. Here, we describe the development of phages for target-specific effector gene delivery and host-dependent production of colicin-like bacteriocins and cell wall hydrolases (endolysins). Using urinary tract infection (UTI) as a model, we show how **heterologous effector phage therapeutics (HEPTs)** suppress resistance and improve uropathogen killing by dual phage- & effector-mediated targeting.



Read our Publication

The HEPT Principle: A Two-Pronged Attack!

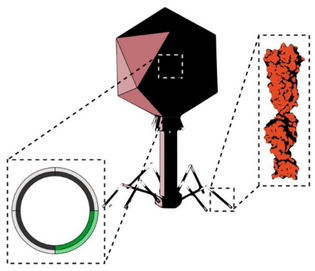


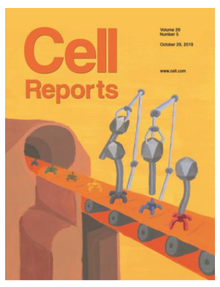


Genes for **colicin E7** or **klebicin M** are engineered into the structural cassette of phages E2 and K1 genomes to generate HEPTs targeting *E. coli* (**E2::*colE7***) or *K. pneumoniae* (**K1::*kvarM***).

Heterologous effector phage therapeutics (HEPTs) enable pathogen-specific gene delivery and production of antimicrobial effector genes (yellow). Upon phage-induced host cell lysis, effector proteins (e.g., colicins) are released alongside progeny virions to exert a secondary antimicrobial activity against defined bacterial targets. HEPTs were designed against uropathogens *E. coli*, *K. pneumoniae*, and *E. faecalis* (*not shown here*).

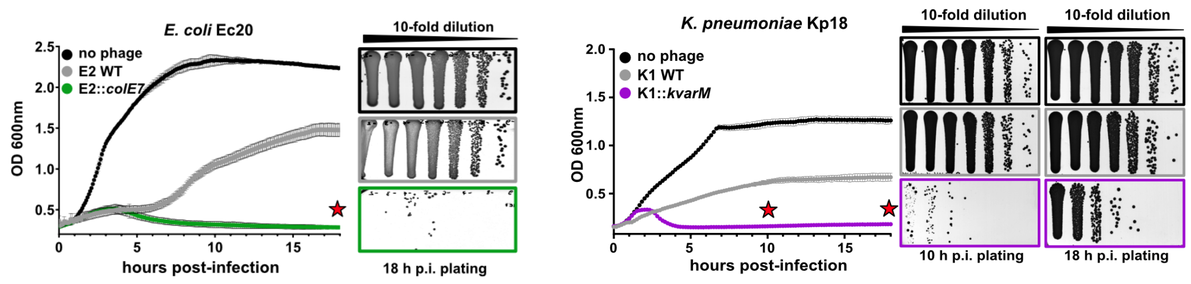
Genetic Engineering Strategies at ETH Zurich



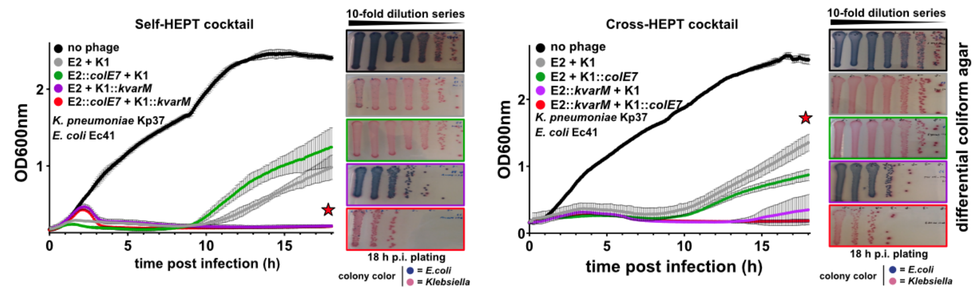


Gene deletion	Gene insertion	Gene modification
Remove toxins, virulence factors, Remove AMR genes Remove lysogenic factors	**Antimicrobial effectors** Immunomodulatory genes Reporter genes (diagnostics)	Structural modifications Host range adaptation Protein-Phage fusions
+ Increased safety **+ Strictly lytic "killer phage" phenotype**	**+ Improve efficacy (e.g., HEPTs)** **+ Cross-genus targeting** **+ Pathogen detection**	**+ Adapt & improve host range** **+ Modulate immunogenicity** **+ Biocontainment**

HEPTs are More Effective than Wildtype Phages

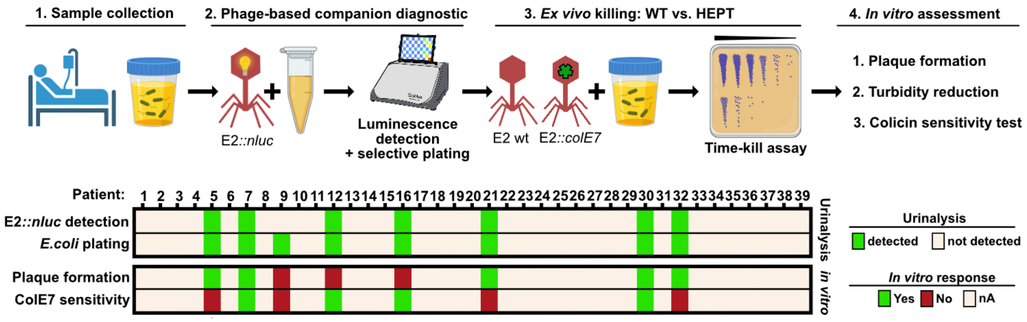


Versus mono-cultures. Turbidity reduction assays combined with timepoint plating (★) demonstrates improved antimicrobial activity (i.e., bacterial regrowth is avoided or delayed) for E2::*colE7* (left) and K1::*kvarM* (right) compared to WT phage treatment of uropathogenic *E. coli* and *K. pneumoniae* monocultures, respectively.

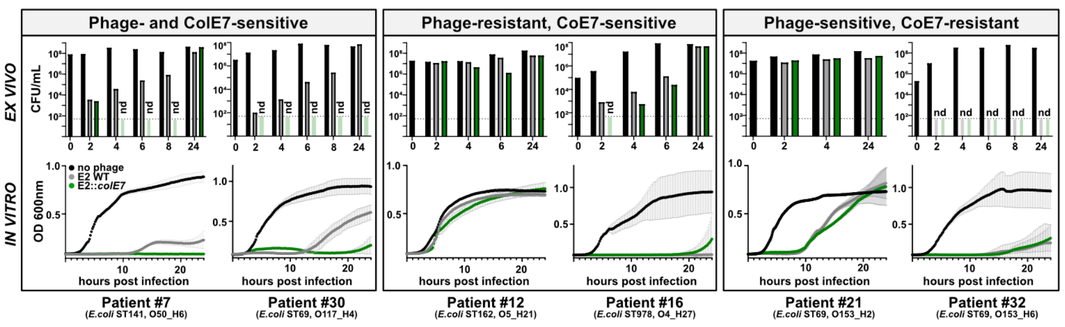


Combination treatment of E. coli / K. pneumoniae co-cultures using combinations of self-targeting HEPTs (left) or cross-targeting HEPTs (right). Cultures of *E. coli* and *K. pneumoniae* were adjusted to $1x10^8$ CFU/mL, mixed at a ratio of 1:1, and infected with the indicated WT phages and/or HEPTs ($5x10^7$ PFU/mL). Optical density was monitored over 18 h, followed by differential plating on chromogenic coliform agar (matching box and curve colors).

Combined with Companion Diagnostics



39 urine samples were subjected to a bioluminescence-based (E2::*nluc*) reporter phage assay to identify phage E2-sensitive *E. coli* in patient urine within 4.5 h. Killing of patient isolates was assessed *in vitro* (synthetic human urine; SHU) using E2::*colE7* or E2 WT.



The colicin-E7 carrying HEPT phage presents enhanced killing of *E. coli* in fresh patient urine as well as in SHU, provided that the isolate is susceptible to both phage and effector, e.g., colicin E7.

The Future of HEPTs as Therapeutics

CAUTIphage is an SNF-funded (Sinergia) collaboration between ETH Zurich, the Balgrist University Hospital, Zurich and multiple project partners. We are developing genetic engineering tools to enhance the therapeutic capabilities of phages to be assessed in future clinical trials. We have engineered libraries of highly effective phages against *E. coli, Klebsiella* spp., and *Enterococcus* spp. Our primary focus is to develop engineered phages as precision antimicrobials for patients suffering with urinary tract infections (UTIs) and catheter-associated UTIs (CAUTIs).

This therapeutic approach is unique and may lead to a breakthrough in the fight against antibiotic resistance worldwide by providing an alternative and effective treatment for UTIs, catheter-associated UTIs (CAUTIs), and other important bacterial infections.