PHAGE FUTURES CONGRESS

Poster Presentation

Abstract Booklet

Dedicated time for poster viewing has been scheduled within the programme. All authors are requested to be present by their posters to address the delegates' questions.

Posters are displayed all around the meeting rooms.

Modelling *in vivo* infection dynamics of naturally occurring bacteriophages in intestinal *Escherichia coli* and estimating bacteriophage contribution to transmission of antimicrobial resistance genes in the bacteria

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Mathematical modeling of infection dynamics of naturally occurring bacteriophages has been limited. Modeling natural bacteriophage ecology would enable evaluating phages' roles in distribution of bacterial genes in pathogenic bacteria and microbiomes. We developed a mathematical model of infection dynamics of naturally occurring bacteriophages in an enteric bacterial species in mammals, bacterial-gene transduction by the bacteriophages, and relative contribution of transduction vs. plasmid conjugation to spread of a resistance gene in the bacterial species during the intestinal transit.

Modeling the ecological dynamics of enteric bacteriophages suggested that the transfer rate of an antimicrobial-resistance gene in enteric bacteria via transduction is several fold lower than via plasmid conjugation. The model can be adapted to test other hypotheses about natural bacteriophage ecology.

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Combatting Lyme disease: phages for diagnosis and treatment

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Lyme disease (LD) is caused by the bacteria *Borrelia burgdorferi* sensu lato and is transmitted to humans through the bite of infected ticks. LD is the most commonly reported tick-borne disease in the United States with estimation of more than 300 000 cases annually by the CDC. In Europe, the number of LD has increased steadily with an ap`proximation of 85 000 cases every year.

The current diagnosis of LD is based on clinical presentation. The FDA-approved laboratory diagnosis is serological test, which cannot detect early LD (ELD) and is too low in sensitivity, missing around 50% of patients. Early diagnosis is vital because ELD can be treated effectively with antibiotics but is harder to treat if the infection is allowed to develop into late LD (LLD). In addition, studies of *Borrelia* infections in animal models revealed the presence of *Borrelia* after antibiotic therapy, which suggests that antibiotics may be unable to eradicate *Borrelia*.

Phages have been investigated for diagnosis and treatment of many types of bacterial infections, except *Borrelia*. We developed and validated a highly sensitive and reliable qPCR assay targeting *Borrelia* phages to diagnose LD. To evaluate the performance of the qPCR relative to the serological test, 222 LLD samples were examined. The qPCR yielded positive results from 200 (90%) samples. In contrast, a total of 56 (25%) samples were positive by serological test, which were also positive by the qPCR. Additionally, 7 (50%) out of 14 serological negative ELD samples showed positive by the qPCR. Further evaluation was conducted against ~1000 ticks collected from ~200 geographical locations throughout the UK. An average of 38% of ticks were positive by the qPCR. To characterise *Borrelia* phages and phage-encoded holins and endolysin, we established methods for phage isolation and *in vitro* assay to measure lytic activity against *Borrelia*.

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The control effect of phage product NuoAnqing on diarrhea and salpingitis in laying hens

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Diseases caused by bacterial infections are increasingly difficult to treat because of the increasingly serious drug-resistant, causing huge economic losses to poultry, livestock and aquaculture and posing a threat to human health and also the environment. Our research is concentrating on the inhibition of chickens diarrhea and oviduct inflammation caused by *Escherichia coli* and *Salmonella spp.* by phage product NuoAnqing(produced by Qingdao Phagepharm Bio-tech Co., Ltd, Qingdao). The phage titers of the cocktail phage product could reach 10^{11} PFU/ml.

Hen manure samples were collected and sprayed with phage product NuoAnqing, bromogeramineand normal saline (control) respectively. Hen oviduct samples were homogenated and treated with phage product NuoAnqing at room temperature for 30 minutes. Total coliform of all the samples were detected. The results showed that the total coliform of manure and oviduct samples treated with phage product declined 1~2 orders of magnitude. The phage effect was much higher than disinfectant bromogeramine when treated hen manure samples.

Phage product NuoAnqing was used in chicken farms with the purpose of controlling diarrhea caused by colibacillosis and salmonellosis with phage product NuoAnqing at diet water of laying hens. 10000160-day old layer hens with symptoms of diarrhea and 5000 500-day old layer hens with oviduct inflammation in Shandong Province were taken in phage therapy. After 3 connected days of phage treatment(10^8 PFU/day/hen), the symptoms of diarrhea disappeared, simultaneously, the quality of eggshell had obviously improved. The titers of phage in feces were up to 10^4-10^5 PFU/g. Also, the number of poor quality eggs, such as eggs with feces and eggs with light color had been reduced.

Our study showed that phage product NuoAnqing was effective in controlling diarrhea and salpingitis of laying hens caused by colibacillosis and salmonellosis.

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The Efficacy of Phage Therapy using Murine Models of Vancomycin-Resistant Enterococci Colonization

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The CDC estimates that 23,000 deaths/year in the United States are caused by antibiotic-resistant bacteria, such as Vancomycin-resistant enterococci (VRE). The inability to treat these infections with common antibiotics necessitates the development of alternative interventions. Bacteriophages (phages) represent one potential alternative therapeutic to combat these infections. Here, we sought to develop and characterize an effective phage therapy against VRE gut colonization using a murine model. Several phages with activity against VRE strains were isolated from sewage. We administered various antibiotics in mice to disrupt the normal gut microbiota at different levels and allow VRE to colonize. Using virulent phages, we designed a cocktail with high activity against VRE. VRE-colonized mice were treated with either the phage cocktail, a single phage, ampicillin (a standard antibiotic intervention), or ampicillin plus the phage cocktail. Depending on the VRE colonization model used, phage therapy success varied. In a model using vancomycin pretreatment to achieve VRE colonization, phage therapy lead to sustained VRE decolonization. This contrasts with ampicillin treatment, which resulted in an initial rapid decrease in VRE colonization that rebounded to high levels after ampicillin treatment ceased. In a model using vancomycin, polymyxin B, and clindamycin to achieve more stable VRE colonization, phage therapy had moderate success, reducing VRE in some mice but not others. Finally, in a germ-free mouse model lacking a gut microbiota, phage therapy did not alter VRE colonization levels. Our data suggests that a phage cocktail could be successfully utilized to decolonize VRE in mice. However, based on the results across three VRE colonization models, we hypothesize that members of the gut microbiota assist in phage-mediated VRE decolonization. These investigations will have a significant impact on the largely understudied field of phage therapy and will contribute to the development of new strategies for treating antibiotic-resistant bacterial infections.

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Therapeutic Phage Pipeline against Wound Infections at the Walter Reed Army Institute of Research

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There is a growing proportion of traumatic and surgical wounds infected by multidrug-resistant (MDR) bacteria to the extent that physicians often have difficulties with therapeutic options. The mission of the Wound Infections Department (WID) in the Bacterial Diseases Branch at the Walter Reed Army Institute of Research is the development of new alternative therapeutics against major causes of bacterial wound infections, including MDR Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, and methicillin-resistant Staphylococcus aureus. One of the main focuses of this work is the use of lytic bacteriophages (phages) to develop durable therapeutic cocktails to cover the vast majority of MDR clinical isolates of the bacteria. We have an established pipeline for therapeutic phage cocktails that are in development. Our P. aeruginosa phage therapeutic cocktail is the most advanced product; a prototype 5-phage cocktail was designed that lyses biofilms and showed significant efficacy in preclinical studies against both septic and wound P. aeruginosa infections in mice. Additionally, more than 50 diverse K. pneumoniae phages were isolated and partially characterized. The panels of lytic phages specific for E. cloacae, A. baumannii, wound E. coli, and S. aureus were also isolated, and their testing is in progress. In a collaboration with AmpliPhi Biosciences Corporation (USA-Australia), a 3-phage cocktail against S. aureus was developed and its safety was demonstrated in two Phase I clinical trials. Finally, WID is working in collaboration with J. Craig Venter Institute (Rockville, MD) on the development of improved engineered phages active against S. aureus and K. pneumoniae as next generation phage therapeutics.These findings will help define nutritional strategies to modulate specific *R. gnavus* strains in health and disease.

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The pangenome of Pseudomonas aeruginosa and the missing link with phage infectivity

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Pseudomonas aeruginosa has a complex population structure where given pairs of strains can differ by hundreds of genes, including entire genetic systems linked to phage infectivity (DISARM, CRISPR-Cas, RM, BREX, etc). Such discrepancies in accessory genome content can be expected to form the bedrock of differences in host-range patterns, but we currently miss much of the link between the specific genome profile of a given strain, and which phages can infect it.

Although anti-phage genetic systems can be identified and functionally annotated in genomics data, the annotation of prophage elements - routinely found in P. aeruginosa - proves to be tedious. However, their presence has been linked to transformative host phenotypes, including modified virulence and antibiotics resistance, and they are crucial determinants of infectivity via superinfection exclusion.

Systematically identifying and classifying prophages, tallying anti-phage genetic systems, and establishing infectivity patterns is a challenge that is appropriately met by combining lab techniques and computational approaches:

1) In silico, we leverage the abundant genomic data available for P. aeruginosa in search for (potentially domesticated) prophages, determinants of phage resistance, and we establish core/accessory genome population structures in the search for organizing patterns.

2) In vitro, we collect host-range information on a wide panel of clinical strains and phages, and we induce prophages from our collection of characterized clinical strains to identify them with nanopore-based, single-plaque sequencing.

Statistically linking genomics data to phage infectivity helps reveal features which can be used to build predictive models of phage infection. Such algorithmic approaches would be useful in therapeutic settings when confronted with the task of selecting specific bacteriophages from a large library and achieve personalized treatment.

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Successful treatment of antibiotic resistant poly-microbial bone infection with bacteriophages

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Infections associated with implants are a major challenge in orthopaedic surgery. The main obstacles in these infections derive from biofilm formation, drug resistance, and poly-microbial etiology. These significantly increase the rate of antibiotic treatment failure. Here, we describe a patient suffering from bacterial osteomyelitis with extremely drug resistant (XDR) Acinetobacter baumannii (Ab) and multi-drug resistant (MDR) Klebsiella pneumoniae (Kp) infection, that was successfully treated with a combination of bacteriophages and antibiotics.

A 42-year-old man was admitted with bilateral open fractures of the lower extremities following a motor vehicle accident. Within weeks after admission, his left tibia was infected with XDR Ab and MDR Kp, leading to serial irrigations and debridements, and to prolonged courses of antibiotics. Despite these aggressive interventions, the wound broke down. Phage therapy was then considered to avoid above knee amputation. The patient received a tailored two-phage combination, found highly effective in-vitro against Ab and Kp, along with IV meropenem and colistin. The bacteriophages were administered for a total of 11 days. Signs of wound recovery, graft healing, and elimination of pain were noted a day after the treatment initiation, and no adverse effects of phage therapy were observed. At a six-month follow-up visit, the patient's wound was found to be closed with no secretions and without positive cultures for either Ab or Kp.

The case represents a unique example of successful clinical application of phage therapy, while specifically highlighting its potential in the setting of resilient infections of orthopaedic implants. This case emphasizes the efficacy of phage therapy in the case of poly-microbial infections, and suggests that the combination of antibiotics and phages can be more effective than either treatment modality alone.

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Bacteriophages: A therapeutic approach in animal health

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Antibiotics have been routinely used in the animal production to promote animal growth or prevent outbreaks of disease. However, its excessive use is associated with the emergence of resistant strains, limiting its therapeutic efficacy and increasing antimicrobial resistance, being a big problem in both human and animal medicine.

Bacteriophages have been used for nearly a century as antimicrobial agents and can be used to reduce the prevalence of pathogenic bacteria. The efficacy of these viruses as antibacterial agents has been amply demonstrated in the literature, both in vivo and in vitro.

Poultry products were undoubtedly the most used meats to study the efficacy of bacteriophage, namely in Salmonella biocontrol in foods. In addition, several reports from case studies investigated the use of virulent bacteriophage to control Salmonella and E. coli infections in birds. These studies have shown that the routine use of phage cocktails can provide an effective and sustainable solution for the control of bacterial populations at various stages of the food chain: from housekeeping to food in food processing facilities.

Nevertheless, in Europe the use of bacteriophages in the control of bacterial infections is still limited mainly due to the lack of legislation that meets the criteria and specificities making it difficult to register and commercialize bacteriophage-based products.

The Phagovet project (funded under the “H2020-EIC-FTI-2018-2020 (FAST TRACK TO INNOVATION (FTI)) proposes the development of a cost-effective, efficient and reliable solution for the control of Salmonella and E. coli infections in poultry farms, based on bacteriophage technology. Phagovet products will consist on a biocide and two technological food additive products based on selected bacteriophages able to kill the target bacteria. These will be the first phage-based solutions to control both bacteria in poultry production while avoiding side effects and residues associated to antibiotic use.

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Phage Therapy in an Experimental Model of Staphylococcus aureus Ventilator Associated Pneumonia

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Background: Ventilator associated pneumonia (VAP) is common in critically ill patients and associated with high morbidity and mortality, especially when caused by antibiotic resistant bacteria. The goal of this study was to compare the efficacy of phage therapy versus antibiotics for the treatment of MRSA in a rat model of VAP.

Methods: Four hours after intubation and protective ventilation, rats were inoculated via the endotracheal tube with 6-9 x 109 CFU (LD100) of the MRSA clinical isolate AW7. The animals were subsequently extubated. Two hours after bacterial challenge, rats were randomised to receive intravenously either teicoplanin, a cocktail of four lytic anti-S. aureus bacteriophages or a combination of both. 10 additional animals served as control (no treatment). Primary outcome was survival at 96 h. Secondary outcomes were bacterial counts in lungs, spleen and blood.

Results: Treatment with either phages, antibiotics or combination of both significantly increased survival (58%, 50%, 45%, respectively compared to 0% survival for controls, ($p<0.01$; Fig.1). While phages were as good as the antibiotic in controlling the infection, combination of both did not further improve survival. There was no significant difference in bacterial count in lungs, spleen and blood between treatment and control groups. However, surviving animals had significantly lower CFU/g in the lungs and a trend towards less CFUs in blood and spleen. While no phages could be isolated from uninfected lungs, we observed phage multiplication upon pneumonia.

Conclusions: Phage therapy was equivalent to antibiotic in controlling MRSA VAP. Further studies are needed to assess whether phages delivered as aerosols further improve the outcome.

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Bacteriophage Bank of Korea

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The Bacteriophage Bank of Korea (http://www.phagebank.or.kr) was established in 2010 and serves as the center of isolation, characterization, stocking, and distribution of bacteriophages. Bacteriophages are viruses infecting bacteria. Since first discovery in early 20th century, they served as antibacterial agents until the emergence of antibiotics. Beside their use as alternatives to antibiotics, phages were main object for understanding molecular biological aspects of life. In addition, phages are used as food additives, feed additives, means for displaying proteins and peptides, and targets for elucidating novel mechanisms such as CRISPR system. The Bacteriophage Bank has collected more than 2200 different phages from various environmental sources. They are characterized for host range, virion protein composition, mass spectral analysis of virion proteins, genomic DNA sequences, and morphological analysis using transmission electron microscope. Host bacteria include Escherichia coli, Salmonella enterica, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella penumonia, Baccilus cereus, Acinetobacter baumanii, Enterococcus faecalis, Enterococcus faecium, Cronobacter sakazaki, Serratia marsescens, Campylobacter jejunii, Pseudomonas syringae, and more entities are being added to the list.

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Phage coverage of ~100 clinical XDR P. aeruginosa isolates

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Pseudomonas aeruginosa (PA), listed as critical in the 2017 WHO pathogen list, is a common cause of infections in hospitalized patients, specifically those with burns, cystic fibrosis, and the immunocompromised. In addition, PA is responsible for almost 10% of hospital-acquired infections.

The aim of this study was to test the coverage of phages as potential treatment for XDR PA isolates. We screened 102 XDR P. aeruginosa clinical isolates, obtained from the Hadassah Medical Center's microbiology laboratory, for phages sensitivity and lytic activity. For this, we utilized 9 individual phages from a variety of sources (including isolations from sewage and environmental samples). The efficacy of the phages was analyzed both qualitatively – on agar plates – and quantitatively – using growth kinetics.

The 102 clinical isolates, 43% of which had endogenous phages, were screened with 6 lytic phages, resulting in 75% coverage. We then isolated 3 new phages, which raised the coverage of the clinical samples to 95%. The final 5 isolates were resistant to all PA phages in our bank, and we are currently isolating new phages and trying to improve the current ones using a rapid evolution process (“phage training”) in an effort to achieve complete coverage.

We demonstrate here that using a phage bank consisting of just 9 phages can provide a significant level of coverage for XDR PA isolates. Moreover, our results provide the confidence that our stock will be effective against almost any isolate and if not, we will probably be able to isolate new effective phages. Next, we will expand the model size to cover all 600 XDR clinical isolates in our collection.

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Genetic Nature of Host Range Expansion in Staphylococcal Phage Sb-1

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Infections with methicillin-resistant Staphylococcus aureus (MRSA) have become a broad spread problem in current medicine, with limited treatment options. Bacteriophage (phage) therapy is a promising approach to treat MRSA infections in conjunction with standard of care antibiotics. S. aureus-specific phage Sb-1 was isolated in the Republic of Georgia (USSR) in 1977 and has been widely used for treatment of various human S. aureus infections. Sb-1 has a very broad host range within S. aureus that includes MRSA strains, and its host range can be further expanded by adaptation to phage-resistant clinical isolates. We tested Sb-1 phage manufactured by the Eliava Institute on a panel of 25 MRSA isolates from the US Military, and it was able to lyse 23 of them. A host range mutant of Sb-1 designated Sb-1M was then selected and tested in comparison with the parental phage. Sb-1 was active against 78 out of 90 (87%) diverse global S. aureus isolates, while eight additional strains were susceptible to Sb-1M, increasing its activity rate to 86/90 (96%). Eight of twelve phage-resistant strains (67%) isolated on three different continents fell into two closely related clusters based on pulsed field gel electrophoresis. In an effort to better understand the genetic basis for this host range expansion, we sequenced the complete genomes of Sb-1 and Sb-1M. Comparative genomic analysis revealed a hypervariable complex repeat structure in Sb-1 genome wherein a distinct allele correlated with the host range expansion. This hypervariable region previously uncharacterized in Twort-like phages represents a novel candidate host range determinant.

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From the sewage to the patient bed: Phage therapy in the Hebrew University and Hadassah Medical Center in Jerusalem, Israel

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The Hebrew University - Hadassah Medical Center

At the Hebrew University and Hadassah medical campus at Ein - Kerem we have composed a multidisciplinary group which is dedicated for the exploration of novel phage therapy. This group is composed by the Hazan lab at the Hebrew University, Jerusalem, the Department of Clinical Microbiology and Infectious Diseases of Hadassah medical and supported by other clinical departments in the hospital such as Orthopedics and Pulmonology. We explore the use of phages in a variety of fields including, dental medical care, environmental decontamination and the possibility of compassionate treatment of patients. To this end we conduct basic-science studies of various aspects of phage biology and additionally, applicable research on phage therapy. At this stage we have isolated more than 100 phages against some of the most antibiotic resistant bacterial strains such as Staphylococcus aureus, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Propionibacterium acnes, Bacillus anthracis and few more, stored in a newly created "phage bank". We characterize these newly isolated phages in-vitro and ex-vivo and test their ability to combat biofilms, and perform whole genome sequencing. In some cases, phage killing efficacy is tested in-vivo using animal models. We also focus on rapid methods for combatting specific phage-resistant bacteria by “phage training”, use of phage and phage-antibiotics cocktails and isolation of new phages.

Our vision of using phages for treatment is taking the “fully adaptive and personal tailored” approach which, in our view, truly unleashes the full potential of phages. So far, we have treated one patient successfully (For details see our other poster).

Our aim is to materialize this multidisciplinary collaboration between basic science lab and clinicians into a Phage Center of research and treatment in Israel which will hopefully be part of a worldwide network of collaborating such institutes to overcome the crisis of antibiotic resistance.

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Investigating environmental Pseudomonas phages for potential therapy of canine otitis externa

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Otitis externa due to Pseudomonas aeruginosa infections can be a debilitating ear condition in dogs. Such infections may be recurrent and chronic disease can prove recalcitrant to antibiotic therapy due to evolution of drug resistance in the pathogen. In severe clinical cases this may progress to tympanic rupture and otitis media, eventually necessitating surgical intervention for total ablation of the ear canal. Limited studies in this area suggest that phage therapy could have some clinical utility. We have isolated and characterised a large selection of Pseudomonas aeruginosa phages from various natural environments by direct plating and different enrichment strategies involving the domesticated lab strain, PAO1, and assorted clinical veterinary isolates. We have determined the host ranges of these environmental phages amongst the dog isolates and categorised the phages by various techniques including transmission electron microscopy (morphology/taxonomy), DNA sequencing and genomics. The phage genomic analysis revealed a spectrum of genome sizes from around 40kb to “jumbo” phages of over 200kb. The genome sequences of various canine clinical isolates of the pathogen have also been determined and bioinformatically interrogated. Our previous studies on transducing phages, in Pseudomonas aeruginosa PAO1 and human clinical isolates from cystic fibrosis patients, encouraged us to investigate the new environmental phages for any generalised transduction capacity. These preliminary screens suggested that multiple new phages had the capacity to effect horizontal gene transfer. This trait could make them less attractive as component phages in any therapeutic cocktail, although potentially useful for the molecular genetic analysis of virulence in the canine otitis externa strains. Bioinformatic analysis was also used to screen for candidate phage genes that might play other undesirable roles if used for a phage therapeutic, including any potential temperate features that might lead to lysogenic conversion in the infected pathogen.

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New effective bacteriophages against S. aureus isolated from waste water

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Infection caused by antibiotic resistant Staphylococcus aureus is a serious problem in human and veterinary medicine. Phage therapy is one of the alternatives to treat staphylococcal infections and new phages against resitant bacteria are needed.

A possible method of obtaining new phages is isolation of spontaneous host-range mutants on non-sensitive bacterial strains. Another source of new phages is an enviroment and these phages has to be well chracterized. In cooperation with companies MB Pharma and Fagofarma we isolated several new bacteriophages angainst S. aureus from waste water. Host-spectrum of choosen bacteriophages was tested on clinical strains of S. aureus including MRSA. For determination of phage family electron microscopy was used. Phage genome was sequenced and characterized. Most of phages belong to Myoviridae and some of them to Podoviridae family. One of the isolated phages has very wide host spectrum and it is wider than in case of spontaneous phage mutants. Host spectra of some of the isolated phages are not so wide but these phages often have unique host spectra including resistant bacteria. For this reason this phages are ideal to use in coctails. Isolation of bacteriophages from waste water could be very quick and useful method of obtaining new phages against antibiotic resiatance bacteria.

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The use of a single phage compared to a cocktail to reduce Staphylococcus aureus colonization

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The Eliava Institute in Tbilisi, Georgia, is well known for its bacteriophage cocktails used in treating a variety of infections, yet the composition and in vivo efficacy of these cocktails are not well-defined. To determine the in vivo efficacy of the Eliava cocktail Fersisi in reducing bacterial burden, we used a methicillin-resistant Staphylococcus aureus mouse nasal colonization model and assessed the ability of the cocktail compared to a single bacteriophage, K, to decolonize the mouse nasal area. We found that the Fersisi cocktail significantly reduced MRSA levels in the nose whereas phage K did not. To determine the composition of the cocktail, we isolated individual phage plaques and obtained two S. aureus phages, named P2 and P6, that appeared to be distinct from one another, based on restriction analysis and PCR followed by sequencing. When we assessed the ability of the individually isolated phage to decolonize MRSA from the nasal region of mice, treatment with phage P2 resulted in a statistically significant reduction in bacterial load, whereas treatment with P6 or K did not. We plan to sequence phages P2 and P6 to investigate why P2 decolonizes better than P6 or K. These results demonstrate that a single phage within a cocktail may be responsible for any positive treatment effects observed.

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Impact of virulent bacteriophages on Vibrio cholerae infection and their use in preventing cholera

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Cholera is a severe human diarrheal disease caused by the bacterium Vibrio cholerae and is a substantial health burden on the developing world. It has been hypothesized that virulent bacteriophages (phages) play a crucial role in modulating the dynamics of cholera epidemics and may impact the evolution of epidemic strains. We have isolated three distinct species of virulent phages that prey extensively on V. cholerae within the human gastrointestinal tract. From genetic and phenotypic analyses, we have determined the receptors to be the LPS O1 antigen and the major outer membrane porin OmpU. By combining the three phages, we have developed a cocktail that can be retained in the small intestine of infant mice and infant rabbits for at least 24 hours following oral administration. Prophylactic treatment of animals for at least 24 hours prior to V. cholerae challenge can prevent colonization in the infant mouse model and the onset of cholera symptoms in the infant rabbit model. None of the surviving V. cholerae colonies from either model are resistant to all three phages. Genome sequencing and variant analysis of the surviving colonies indicate that resistance to the phages is largely conferred by mutations in genes required for the production of the phage receptors. In addition, 16S sequencing suggests there is no severe impact on the adult murine microbiome following oral administration of the phage cocktail. We hypothesize prophylactic use of this cocktail in humans could reduce the severity of the cholera burden in developing countries. For acute infections, such as cholera, phage prophylaxis could provide a strategy to limit the impact of bacterial disease on human health. PhagePro is currently pursuing product development to take this phage cocktail to market in countries suffering from cholera epidemics.

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Phage Loaded Magnetic Nanoparticles to Remove Multidrug Resistant Bacterial Biofilms: A Combined Experimental and Computational

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Abstract: Multidrug-resistant bacterial biofilms can shield pathogenic bacteria and their existence in both medical and environmental settings poses a significant health threat. Biofilms are refractory to antimicrobial treatment because of the physical hindrance and bacterial heterogeneity. Therefore, there is an urgent need for effective biofilm eradication while avoiding undesirable health risks. Here, natural existing phages and magnetic nanoparticles were conjugated to achieve effective biofilm removal regardless of bacterial antibiotic resistance. Polyvalent phages infecting Escherichia coli NDM-1 and Pseudomonas aeruginosa PA01 were isolated from environmental samples by the sequential multiple-host approach. The broad host ranges of polyvalent phages allowed them to reproduce in a variety of biofilm bacteria enhanced their penetration in biofilm conditions. In addition, the ability to produce depolymerase enzymes further facilitated biofilm matrix disruption. To accelerate biofilm removal, a “Trojan Horse” strategy was proposed to eradicate well-established biofilms. Magnetic phage clusters (MPCs) could penetrate the biofilms with a weak magnetic field, which significantly improved biofilm removal efficiency relative to free phages. The nanoparticles were modified with amine functional group to mitigate NPs aggregation and to optimize phage orientation after conjugation. After six-hour treatment, the smaller MPCs (more infectious centers but weaker physical disruption) eradicated the 2-day mixed-species biofilms to a greater extent compared to the larger MPCs (fewer infectious centers but stronger physical disruption). Accordingly, a mathematical model was developed to simulate the phage-biofilm interactions under different treatments (e.g., free phage, larger MPCs, and smaller MPCs). The model indicated that larger MPCs worked better in intact and thick biofilm conditions while smaller MPCs were preferred in scatted and thin biofilm conditions. Overall, MPCs enhanced phage infection in biofilms and hold the promise as an alternative or supplement in biofilm removal.

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Therapeutic potential of phages for mycobacterial infections

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Mycobacterium tuberculosis represents a global health threat and kills 1.7 million people each year. The widespread emergence of multiple drug resistant (MDR-TB) and extensively drug resistant (XDR-TB) strains complicates treatment and there is an urgent need for new therapeutic approaches. Nontuberculosis mycobacteria (NTM) infections – common in cystic fibrosis (CF) patients – also present a substantial clinical challenge and are often broadly antibiotic resistant and nonresponsive to treatment. Through integrated research-education programs such as the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) we have assembled a collection of over 10,000 individual phages using Mycobacterium smegmatis as a host, 1,600 of which are completely sequenced (phagesdb.org). These span considerable genetic diversity and can be grouped into clusters (e.g. Cluster A, B, C etc) and subclusters (e.g. Subcluster A1, A2, A3 etc) based on their sequence relationships. All but one cluster are morphologically siphoviral, and half of the clusters are temperate. We have screened subsets of these phages for their ability to infect M. tuberculosis and other NTM strains. Several groups were identified that efficiently infect a wide spectrum of M. tuberculosis clinical isolates and could potentially constitute a phage cocktail for TB control. In contrast, clinical isolates of NTM strains differ substantially in their phage susceptibility profiles, and many are infected by few if any phages in the collection. However, we were able to identify three phages that infect one strain of Mycobacterium abscessus. One of these is a naturally occurring phage isolate, one is an engineered lytic derivative of a Cluster K phage, and the third is an engineered host range mutant that efficiently infects both M. smegmatis and the M. abscessus strain. Together, a three-phage cocktail of engineered but non-recombinant phages has potential for a personalized intervention with minimal incidence of phage resistance.

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Spontaneous Kayvirus host-range mutants are safe for phage therapy of staphylococcal infections

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Bacteriophages of the genus Kayvirus are valuable therapeutic agents against staphylococcal infections. Due to the emergence of phage-resistant strains, it is necessary to update the composition of phage preparations and to evaluate their safety. In this work we describe the selection, lytic properties, comparative genomics and safety of spontaneous mutants of polyvalent bacteriophage 812 of the genus Kayvirus. Their lytic ability was tested on a set of methicillin-resistant Staphylococcus aureus (MRSA) representing the globally circulating clones. Single- and multi-step mutants were isolated as rare plaques on strains resistant to the parental phage. The mutant 812h1 showed the broadest host range and lysed almost 90% of MRSA strains while the wild-type phage 812 lysed only 67%. A considerable host-range extension was observed in phages containing a truncated endolysin gene and in the mutant 812h1 that showed multiple changes in the host-takeover module and in the genes upstream of the right long terminal repeat compared to the wild-type phage. In addition, comparative genomics revealed that single-nucleotide polymorphisms from the parental phage 812 population were fixed in next-step mutants, mainly in the genes for structural proteins, receptor binding proteins, and the nucleic acid metabolism. In the genomes of studied mutants, no genes for antimicrobial resistance, bacterial virulence, or lysogeny were found. The phages were not able to package host antimicrobial resistance genes effectively. The approach used for host-range mutant isolation is an effective and safe way to update the preparations against newly emerged phage-resistant strains. The work was supported by the Czech Science Foundation (18-13064S) and the Grant Agency of the Masaryk University (MUNI/A/0958/2018).

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Characterization of Phage for the Rational Design of a Phage Cocktail against Vancomycin-Resistant Enterococci

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Bacteriophages are viruses that infect bacteria, and as such play a critical role in regulating bacterial populations. As widespread antibiotic use has led to increased multidrug-resistant bacteria, including vancomycin-resistant Enterococci (VRE), phage present a potential alternative for antimicrobial defense. In order to develop an effective phage therapy against VRE, we sought to characterize isolated phage before assessment in mice. We acquired a cohort of 20 naturally-occurring phage isolated against either vancomycin-resistant Enterococcus faecium or E. faecalis. These phages were assessed for host range, specificity, and virulence using phage spot plating and cross-streak assays. Ten phages were chosen for genome sequencing. We found several strictly lytic phages, which are potential candidates for therapeutic use. Phages showed specificity for either E. faecium or E. faecalis, with some exceptions. Seven sequenced phages were Myoviridae and three were Siphoviridae. Interestingly, two sequenced phages had identical genomes apart from two single nucleotide polymorphisms (SNPs). These SNPs caused nonsynonymous mutations in the phage major tail sheath protein and in a capsid and scaffold protein. One or both SNPs resulted in a significant change in host range between these two phages. We intend to introduce these SNPs individually in two phage mutants to identify the contribution of each SNP to the phage's host range. In doing so we will better understand phage tropism and possibly bacterial receptors. This work will help inform a rationally designed phage cocktail for efficacious VRE treatment.

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The impact of Horizontal Gene Transfer on the evolution and function of cellulose-degrading microbial communities

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Horizontal Gene Transfer (HGT) drives the evolution of microbial communities through the dissemination and amplification of DNA. To date most studies have investigated HGT using a comparative genomic approach of individual isolates against previously sequenced genomes to construct phylogenetic networks of HGT events. While these studies have provided invaluable insight into our understanding of the ecology and evolution of HGT, direct experimentation investigating the process and functional impact of HGT on microbial communities is lacking. Here we designed an experimental regime to explore the impact of HGT on the dynamic of molecular and phenotypic evolution in cellulose-degrading microbial communities over the course of one year. Ten founding communities were established by incubating compost with minimal media and providing cellulose paper as the sole carbon source. Following establishment, the founding communities were split into two transfer regimes: vertical and horizontal. In the vertical regime each of the ten communities were homogenized every two weeks and transferred to fresh medium with a new piece of cellulose paper. In the horizontal regime each transfer involved the founding microbial community as well as an “HGT cocktail” sampled from all ten bottles, providing the opportunity for genes to move between horizontal but not vertical communities. Using comparative metagenomics, we provide evidence for large-scale movement of genetic material between horizontal bottles that involves genes with various predicted functions including iron acquisition, virulence factors, transcription, and individual phage genomes. As a general proxy for community function we also measured the ammonia concentrations during the course of the two-week regime. Surprisingly, we found that the majority of horizontal communities had significantly higher ammonia production compared to their vertical counterparts. To our knowledge these data describe for the first time the emergence of a functional impact of HGT on a complex microbial community through direct experimentation.

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Local phage therapy by intravitreous bacteriophage for the treatment of intraocular bacterial infection in mice

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Introduction: Bacterial endophthalmitis is caused by trauma, endogenous, and complication associated with surgery or intravitreal injections and often results in significant vision loss and blindness. The majority of cases with postoperative endophthalmitis are caused by Gram-positive pathogens such as Enterococcus spp. Furthermore, cases with endophthalmitis due to vancomycin-resistant enterococci (VRE) have reported. We examined that the therapeutic effects of intravitreous bacteriophage on VRE endophthalmitis in mice.

Material & Methods: Endophthalmitis was induced by injection of 10000 VRE into the vitreous. Bacteriophage ΦEF24C-P2 was injected intravitreously 6 h later. The clinical score was evaluated 24 h after bacterial injection, and eyeballs were isolated for enumeration of viable bacteria, quantitation of inflammatory cell infiltration by assay of myeloperoxidase (MPO) activity, and pathological examination.

Results: The bacteria could grow in the presence of vancomycin (2 mg/ml) in vitro. Endophthalmitis were occurred after 24 hours by intravitreaously injection of VRE, and the fundus of mice was invisible at 24 h after bacterial injection as a result of fibrin precipitation or bleeding in the anterior chamber or of vitreous opacity. The number of viable bacteria increased to ~1 × 107 CFU and MPO activity was elevated in vehicle-treated eyes. The clinical score, number of viable bacteria, and MPO activity were significantly decreased by injection of ΦEF24C-P2. Pathological examination revealed inflammatory cell infiltration and retinal detachment in vehicle-treated eyes, whereas cell infiltration was attenuated and retinal structure maintained in phage-treated eyes.

Conclusion: Intravitreous administration of a bacteriophage is effective for treatment of VRE endophthalmitis and local phage therapy may be one of the candidates for the treatment of intraocular bacterial infection.

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Evolution of the Cluster A mycobacteriophage immunity system and the impact on phage virulence and host range

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Widespread use of bacteriophages to control bacterial infections requires knowledge of factors that impact phage susceptibility patterns among clinical isolates. Bacteria can alter phage susceptibility using diverse defense mechanisms, including some derived from temperate phages. Temperate phages are common, and many bacterial strains carry at least one prophage, typically integrated into the host genome. Prophages express a repressor that down-regulates lytic gene expression and also confers immunity to superinfection by identical, or closely-related, phages. These immunity systems thus substantially impact the host ranges of phages with therapeutic potential, and understanding how immunity systems evolve and how phages escape immunity can inform phage therapy strategies. By examining the infection profiles of Cluster A temperate phages of Mycobacterium smegmatis, we have found that rather than distributing into distinct groups with well-defined immunity profiles, these phages and their lysogenic strains encompass a broad spectrum of asymmetric and incomplete immunity phenotypes. The immunity profiles are conferred by the phage repressors and recombinant strains expressing the repressors phenotypically mirror the susceptibility patterns of their cognate lysogens. Isolation of lytic defense escape mutants that are no longer subject to superinfection immunity reveals that within this network, mutations confer varying degrees of virulence, such that phages may exhibit virulence against some, but not all, lysogens. The complexity of these immunity systems and the spectrum of lytic and virulent phenotypes have significant implications in the choice of phages for inclusion in therapeutic cocktails.

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Fast automated analysis of phage cocktail integrity with MiniTEM

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The alarming rise of antibiotic resistance reported in the past years has triggered the search for alternatives to treat bacterial infections, such as bacteriophages (viruses of bacteria). Due to a high host specificity, phages have no harmful effect on the normal microbial flora; but their use in treatment requires previous knowledge of the bacterial target. Mixtures of phages can be used to overcome this limitation, and also reduce the emergence of phage resistance and overall increase the efficacy of the treatment. These so called phage cocktails, however, have been shown to be less stable than single phage preparations. Therefore, it is important to have a simple method to determine the stability and integrity of the phage cocktails overtime to guarantee the success of the phage cocktail treatment. Here we propose the use of MiniTEM for the characterization of phage cocktail integrity. MiniTEM is a desktop low-voltage (25keV) transmission electron microscope together with its analysis platform allows a high degree of automation in the microscope operation, image acquisition and analysis process. MiniTEM was used to image phage cocktail samples of different integrities, using negative stained standard methods. Images were automatically acquired and analyzed using an optimized script that detects and provides morphological measurements of phage particles. The direct visual information about sample content and particle morphology was key for understanding the number and intactness of the different phage particles composing the cocktail. Imaging results were validated experimentally by determining the efficiency of the phage cocktails of different integrities against target bacteria, using medical standards. Overall, the automated imaging and quantitative morphological analysis which MiniTEM offers allows for simple and fast (20-40 min) analysis of phage cocktail integrity. This analysis will guarantee the use of an efficient phage cocktail in therapy.

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Phage cocktail SalmoFREE® reduces Salmonella in a commercial broiler farm

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The WHO states that Salmonella is one of the most important zoonotic, foodborne pathogens. The main source of Salmonella infections is associated to the consumption of poultry products. This fact enforces the necessity to control Salmonella at the pre and post-harvest stage. Using this motivation, a bacteriophage cocktail against Salmonella was designed by Universidad de los Andes (Universidad de los Andes, patent 15-281747). Development of this cocktail has included, In Vitro characterization, Innocuity assays. The next step in this development was to evaluate the effectiveness of the bacteriophage cocktail in a commercial broiler farm. Additionally, we assessed the relationship between the use of phages and productivity parameters as feed conversion, weight gain and homogeneity. Two field trials under commercial rearing conditions (animals in trial 1 n= 34,986; trial 2 n=34,680) were carried out in a broiler farm in Colombia, which had a record of presence of Salmonella. Each trial comprised two control farmhouses and two experimental ones. SalmoFREE ® and control suspension were delivered in drinking water in three different moments of the production cycle and the presence of Salmonella and phages were detected in cloacal swabs and cecum one day before and after the treatments. Results revealed that the phage cocktail does not affect the animals or the production parameters, demonstrating its innocuity for broilers, and also its effect on the Salmonella presence in the farm achievement a reduction of Salmonella incidence for both trials up to 100%. Additionally, throughout metagenomic approaches using cecum samples, the abundance reduction of the pathogen and the effect of phages on chicken microbiota were analyzed.

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Use of Drosophila melanogaster infection models to evaluate phage therapy against Pseudomonas aeruginosa

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Non-mammalian model hosts have been exploited to understand the various aspects of host-pathogen interactions and also provided innovative research platforms for identification of virulence factors, screening for antimicrobial hits, and evaluation of antimicrobial efficacy. We here describe our optimized protocols and the relevant results to assess the antibacterial therapeutic efficacy of various bacteriophages (phages) that infect the opportunistic human pathogen, Pseudomonas aeruginosa. This can be done based on the systemic infection model using the fruit fly, Drosophila melanogaster. Since phages, unlike antibacterial chemicals, can be easily and sensitively enumerated by simple assays, it is also possible to address the pharmacokinetic properties of administered phages even in this small-scale infection model.

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In Vivo Anti-Bacterial Phage Therapy through Immunological Cloaking

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Phage therapy can serve as an alternative treatment to overcome antibiotic resistance, but the use of phage for anti-bacterial treatments is limited by rapid clearance from systemic circulation. Here we introduce immunologically cloaked T7 phage to reduce phagocytosis through the expression of self-peptide, derived from human CD47, on the major capsid. Self-peptide expressing T7 phage (Self-T7) suppresses up to 70% phagocytosis in vitro compared to wild-type T7 phage (WT-T7). Real-time in vivo image analysis demonstrates that Self-T7 exhibits a markedly longer blood circulation compared to WT-T7. In a mouse model of bacterial infection of intestines, the survival rate of mice is greatly increased by the intraperitoneal or intravenous injection of Self-T7, while WT-T7 only exhibited limited effects on mouse survival. In addition, injected Self-T7 did not induce inflammatory cytokine expressions of IL-1 and IL-6 as well as not affect the distribution of gut microbiota. This work highlights the enhanced in vivo efficacy of anti-bacterial phage therapy through the immunological cloaking of phage for suppressed phagocytosis and prolonged blood circulation.

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